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Editorial:

CONTRIBUTION OF FOOD ANTIOXIDANTS TO HEALTH.

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Version española

During the last decades, there has been a lot of research work on oxidative stress both due to its involvement in homeostasis of normal cells and its implications in the development of a large number of degenerative diseases such as cardiovascular disease, neurodegenerative disease, chronic inflammation, cancer etc. Although the role of RSON (reactive species of oxygen and nitrogen) in the pathogenesis of different diseases was originally attributed to oxidative damage exerted on the different molecules by altering their function, it is now also known to be involved as an intracellular messenger in gene regulation and in signal transduction pathways. On the other hand, in a very narrow range of concentration The RSONs can lead to the opposite effects such as proliferation or apoptosis.

As a reaction to this increase of free radicals, living organisms have endogenous defense mechanisms consisting of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase) or non-enzymatic antioxidants (glutathione, thioredoxin, etc). Their function is to eliminate free radicals like superoxide, hydroxyl and peroxides before they react and interact with different biomoléculas (lipids, proteins, DNA), inducing cellular damage.

As reinforcement for this endogenous antioxidant capacity, there are foods with antioxidant compounds of nutritional interest, with a chemical structure compatible with the in vivo antioxidants /antioxidant properties. Among food compounds with antioxidant properties are polyphenols, vitamins, etc., which can modulate the cellular response to RSON through different mechanisms: stabilizing reactive oxygen species, suppressing their formation by inhibiting enzymes or acting as metal chelators. In this way, these foods can restor the endogenous antioxidant defense or regulate intracellular signals resulting from the cellular antioxidant response.

There are more than 2000 epidemiological studies showing a relationship between the protective effect against various diseases and the consumption of foods with antioxidant capacity. This protective effect has been observed against different diseases (mainly cardiovascular), and is correlated with a high intake of fruits and vegetables. According to WHO reports, if not remedied before, in the year 2020 some diseases (cardiovascular diseases, diabetes, hypertension and some cancers) will be the cause of 73% of deaths and of 60% of the global diseases (WHO, 2001). Therefore, in order to prevent the diseases associated with free radicals, the WHO recommendS an intake of 400 g of fruit and vegetables a day.

Regarding the contribution to health of the antioxidant compounds present in food, others factors should also be considered such as:

1) the presence of other non-antioxidant compounds that contribute indirectly to the reduction of these pathologies (folate, fiber, etc.),

2) the antioxidant capacity of the diet depends on the absorption or the metabolic changes that may alter the antioxidant activity of the original molecule.

3) These compounds can perform their function independently of their ability to act as antioxidant, as they can interact with enzymes, by binding to membrane or nuclear receptors, altering gene expression, etc.

4) The effect of the isolated or pure compound is not the same as when it forms part of the food matrix, which can be synergistic with other components of the food.

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Editorial:

CONTRIBUCIÓN A LA SALUD DE LOS ALIMENTOS CON COMPUESTOS ANTIOXIDANTES.

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English version

En las últimas decadas, son muchos los trabajos realizado en el campo de las especies reactivas del oxigeno y del nitrógeno (ERON) tanto por su implicación en las homeostasis de las células normales como por su implicación en el desarrollo de un número elevado de patologías degenerativas, como enfermedades cardiovasculares, neurodegenerativas, inflamación crónica, cáncer, etc. El papel de las ERON en la patogénesis de las diferentes enfermedades aunque en un principio fue atribuido al daño oxidativo que podrían ejercer sobre las diferentes biomoléculas alterando su función hoy se sabe que además participan como mensajeros intracelulares participando en la regulación génica así como en vías de transducción de señal. Estos ERON en un rango muy estrecho de concentración pueden generar efectos opuestos como por ejemplo, proliferación o apoptosis.

Frente a este incremento de los radicales libres los organismos vivos disponen de mecanismos de defensa endógena que consta de los antioxidantes endógenos como las enzimas superóxido dismutasa, catalasa, glutation peroxidasa, o los

antioxidantes tiolicos no enzimáticos glutatión y tiorredoxina. Su función es eliminar los radicales libres como el superóxido, y peróxidos antes de que ellos reaccionen e interaccionen con distintas biomoléculas induciendo el daño celular.

Como refuerzo a esta actividad antioxidante endógena existen varias moléculas, de interés nutricional, con una estructura química compatible con las propiedades antioxidantes in vivo entre ellos se encuentran compuestos como los polifenoles, vitaminas, etc que son capaces de modular la respuesta celular a las ERON a través de diferentes mecanismos, estabilizando las especies oxigénicas reactivas, suprimiendo su formación al inhibir enzimas o actuando como quelantes de metales, regenerando la defensa antioxidante endógena o regulando señales intracelulares resultado en la respuesta antioxidante celular.

Son más de 2000 los estudios epidemiológicos que muestran una relación entre efecto protector frente a diferentes enfermedades y el consumo de alimentos con capacidad antioxidante. Este efecto protector se ha observado contra diferentes enfermedades (principalmente cardiovasculares y cáncer), y está correlacionados con una elevada ingestión de frutas y verduras. Teniendo en cuenta que a Organización Mundial de la Salud (OMS) refleja en sus documentos como, de no actuar adecuadamente, en el año 2.020 las enfermedades no transmisibles (patologías cardiovasculares, diabetes, hipertensión arterial y ciertos tipos de cáncer) serán la causa del 73% de las defunciones y del 60% de la carga mundial de enfermedad (OMS, 2001). De esta forma, la OMS en base a estos estudios recomienda una ingesta de 400 g de frutas y verduras al día, para poder prevenir las patologías asociadas a los radicales libres.

En relación a esta contribución de los compuestos antioxidantes presente sen los alimentos sobre la salud además deben considerarse factores como:

1) la presencia de otros compuestos de naturaleza no antioxidante que contribuyen de forma indirecta a la reducción de estas patologías (folatos, fibra, etc.);

2) la capacidad antioxidante de los alimentos ingeridos depende de la absorción o de las transformaciones metabólicas que pueden modificar la actividad antioxidante original de la molécula.

3) Estos compuestos pueden llevar a cabo su función independiente de su capacidad de actuar como antioxidante al poder interactuar con enzimas, uniéndose a receptores nucleares o de membrana, modificando la expresión de genes, etc.

4) El efecto del compuesto puro o aislado, no es el mismo que cuando forma parte de la matriz alimentaría, donde se pueden establecer efectos sinérgicos con otros componentes del alimento.

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DEPOSITION STUDIES USING MULTIPURPOSE SOLUTION ON HYDROPHILIC CONTACT LENSES

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SUMMARY

In the tears lysozyme and albumin are also present besides other constituents. All these constituents form a biofilm on the hydrophilic contact lenses - minutes after the lens is placed in the eye. These deposits if not removed make the contact lens translucent and impair visual acuity. For the removal of deposit multipurpose solution is used.

In the study, deposits of lysozyme and albumin were made on hydrophilic contact lenses deliberately. These deposits laden contact lenses were then treated with multipurpose solution for 12 hrs. The extent of removal of these deposits by the action of sodium citrate present in multipurpose solution was assessed by measuring albumin and lysozyme quantitatively by using standard analytical procedures.

It was observed that 0.1% of sodium citrate could remove lysozyme and albumin efficiently. Albumin deposited more as compared to lysozyme and non ionic hydrophilic contact lenses are less prone to deposition than ionic. Any further increase in sodium citrate was not desirable.

KEY WORDS: Lysozyme. Albumin. Sodium citrate. Standard tear fluid (STF). Non enzymatic cleaner

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RESUMEN

En las lágrimas están presentes lisozima y albúmina, además de otros constituyentes. Todos estos componentes forman una

biopelícula en las lentes de contacto hidrofílicas y minutos después de la lente se coloca en el ojo. Estos depósitos, si no se eliminan, hacen el contacto con la lente translúcida y afectan la agudeza visual. Para eliminar los depósitos se utiliza una solución multiuso.

En el estudio, se hicieron deliberadamente depósitos de lisozima y albúmina en lentes de contacto hidrofílicas. Estos depósitos sobre los lentes de contacto fueron tratados con la solución multiuso durante 12 horas. El grado de eliminación de estos depósitos por la acción del citrato de sodio en solución multiuso se evaluó mediante la medición de la albúmina y la lisozima cuantitativamente, mediante procedimientos analíticos.

Se observó que el citrato de sodio al 0,1% podría eliminar la lisozima y albúmina de manera eficiente. La Albúmina se depositó más en comparación con la lisozima y las lentes de contacto hidrofílicas no iónicas son menos proclives al depósito que las iónicas. Cualquier aumento posterior de citrato de sodio es indeseable.

PALABRAS CLAVE: Lisocima. Albúmina. Citrato sódico. Fluído standard de lágrimas (STF). Limpiador no enzimático.

INTRODUCTION

In the eye besides tear, other constituents are also present like proteins, lysozyme, albumin and salts including calcium. All these form a lipoprotein surface film on the hydrophilic contact lenses and other contaminants are adsorbed on this film further. The contaminants may be the environmental pollutants such as nicotine, cosmetic ingredients, finger dirt, chemical vapors, water impurities and preservatives/active ingredient from ophthalmic products[1].

Certain other lipid secretions from the eye glands (meibomian glands) can also bind to the lens surface, forming a lipoprotein film that is very difficult to remove. All such deposits if not removed then may cause discomfort and impair visual acuity. Microorganisms may further build up on these deposits and the situation further worsens. To remove such deposits, the lenses are to be treated every day with multipurpose solution (MPS) containing a deproteiniser. The deposits not only cause discomfort but also increase the risk of infection causing giant papillary conjunctivitis (GPC)[2].

The enzyme cleaners provide effective cleaning but leave around 25% of the lens surface area still coated. One of the functions of multipurpose solution (MPS) is to remove lens deposits when lenses are soaked in the solution overnight. In this way, it extends the useful life of the lens and keeps the lens free from deposits and thereby provides clear vision, comfort and maintain normal eye health[3].

During day time the lenses which are previously rinsed with MPS before being worn and during night, the lenses when not in use are soaked in MPS for 7-8 hours. Lens can be worn continuously for 7-8 hours in a day, after this again rinsed and soaked in MPS for 7-8 hours before being worn again[4].

MATERIAL AND METHODS

1. Materials

Polyhexanide hydrochloride (PHMB.HCl) was procured from Avecia Biocides, Manchester, U.K. Sodium citrate was obtained from Merck, Mumbai, India and Lysozyme from SRL, Mumbai, India. Albumin was obtained from E Merck, Mumbai, India. FDA group I (Netrafilcon A) hydrophilic contact lenses were used. All other materials were used as received.

2. Preparation of standard tear fluid (STF) containing deposition constituents

STF of pH 7.4 was prepared (Table I).

Table I: Standard tear fluid (STF) pH 7.4

S. No.	Ingredient	Concentration (%)
1	Boric acid	0.2
2	Sodium tetraborate	0.02
3	Sodium chloride	0.8
4	Purified water q. s. to	100.0 ml

Albumin and lysozyme (Table II) were added into isotonic STF of pH 7.4 by shaking the flask until a clear solution was obtained.

The volume was made and pH was adjusted up to 7.4 using pHmeter.

Table II: Artificial tear fluid containing deposition constituents

S. No.	Ingredient	Concentration
1	Albumin	20µg/ml
2	Lysozyme	20µg/ml
3	STF (pH 7.4) q. s. to	100 ml

3. Selection of hydrophilic contact lenses and MPS

Group I (Netrafilcon A) hydrophilic contact lenses were used for the study. For one MPS, six contact lenses were used. The total contact lenses were 42.

Container used: Transparent vial of 10.0 ml capacity were used for the study.

MPS tested: Seven selected MPS were subjected to deposition studies i.e. MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11

4. Method

In the deposition studies, two main constituents, which are generally deposited on the surface of the contact lens, are lysozyme and albumin. In the present study the removal of lysozyme and albumin from the deposited hydrophilic contact lenses (CL's) by the action of MPS was studied in order to assess the formulation. Eleven preparations of MPS were prepared and coded (Table III).

Table III: Formulae of different multipurpose solutions (MPS) concentration of ingredients in formula code (in %)

S. No.	Ingredients	MPS-1	MPS-2	MPS-3	MPS-4	MPS-5	MPS-6	MPS-7	MPS-8	MPS-9	MPS-10	MPS-11
1	PHMB.HCl	0.0001	0.0002	0.0003	0.0004	0.0005	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
2	Sodium Citrate	0.1	0.1	0.1	0.1	0.1	0.05	0.07	0.15	0.17	0.2	0.3
3	Isotonic STF (pH 7.4) q.s.	100	100	100	100	100	100	100	100	100	100	100

All these preparations contain polyhexanide hydrochloride (PMHB.HCl) as the drug and sodium citrate as the deproteiniser and the concentration of the drug varied from 0.0002 to 0.0005% and the concentration of sodium citrate (deproteiniser) varied from 0.05 to 0.30%. In the deposition studies, lysozyme and albumin were added into an isotonic simulated tear fluid (STF) of pH 7.4 in known concentration and the hydrophilic contact lenses were soaked in it for 24 hours at 37°C in order to make coatings of lysozyme and albumin on them i.e. deposits were made on the lenses deliberately and according to the composition as given in tables (Table II and Table III). These lenses were then soaked in MPS for 12 hours and the lysozyme and albumin were estimated in order to assess the deposit removing capacity of MPS [5].

In 42 vials, the artificial tear fluid containing lysozyme and albumin were added (5.0 ml in each vial). In each of 42 vials the hydrophilic contact lens was placed. All the vials were stopper with their respective caps and placed in biological shaker at 37°C. These were shaken for 24 hours. After 24 hours, the lenses were removed with the help of contact lens lifter and placed in separate vial containing 5.0 ml of MPS.

For one MPS, six vials were used and in each vial one lens was placed. These vials were left for 12 hours at room temperature i.e. 25°C. After this these vials were shaken for 5 minutes and lenses were removed. The treated MPS were analyzed for the deposition of lysozyme and albumin, removed from the lenses, by the following methods.

4.1. Estimation of lysozyme

It was determined as per the method of Hu et al[7]. Different concentrations of lysozyme were prepared in STF of pH 7.4 i.e. 2.0 µg/ml to 20.0 µg/ml. The absorbance of the solutions were determined at λ_{max} 280 nm using UV spectrophotometer. From the readings (Table IV), a standard plot was prepared.

Table IV: Absorbance of different concentration of lysozyme at λ_{max} 280 nm

S. No.	Concentration (µg/ml)	Absorbance	SD±
1	2	0.112	0.002
2	4	0.120	0.001
3	6	0.128	0.002
4	8	0.136	0.003
5	10	0.144	0.001
6	12	0.152	0.002
7	14	0.160	0.002
8	16	0.168	0.002
9	18	0.176	0.001
10	20	0.184	0.002

n = 6, results are the mean of 6 readings.

In deposition studies, the treated MPS preparations were taken and the absorbance were determined at 280 nm. The amount of lysozyme was determined by using the standard plot as per the method. From the readings, a bar chart was plotted for showing the effect of MPS on removal of lysozyme from hydrophilic contact lenses (CL's)[6].

4.2. Estimation of albumin

Albumin was determined as per modified Lowry method[7]. A stock solution of albumin 100 µg/ml was prepared in STF of pH 7.4. From this stock solution, an appropriate volume was transferred into a 10.0 ml capacity volumetric flask. To this 1.0 ml of biuret reagent and 1.0 ml of phenol were added. After 5 minutes, volume was adjusted up to 10.0 ml. In this manner, all reaction mixture were prepared containing different concentrations of albumin i.e. 3.88 µg/ml to 42.68 µg/ml. Albumin gave intense red color in the presence of biuret agent and phenol. The absorbance of these solutions was measured spectrophotometrically at 700 nm (Table VI).

Table VI: Absorbance of different concentration of albumin at λ_{max} 700 nm

S. No.	Concentration (µg/ml)	Absorbance	SD±
1	3.88	0.105	0.002
2	7.76	0.113	0.003
3	11.64	0.121	0.003
4	15.52	0.129	0.003
5	19.4	0.137	0.002
6	23.28	0.145	0.001
7	27.16	0.153	0.002
8	31.04	0.161	0.001
9	34.92	0.169	0.003
10	38.8	0.177	0.002
11	42.68	0.185	0.002

From the absorbance and concentration value, a standard plot was drawn. In deposition studies, the treated MPS was taken and to this the biuret agent and phenol were added as the method given above. The absorbance was determined and the amount of albumin was calculated using the standard plot.

From the readings a bar chart was plotted, showing the effect of MPS solution on removal of albumin (Table VII).

Table VII: Amount of albumin removed (in µg) by multipurpose solutions (i.e. Albumin removed from contact lenses)

S. No.	Multipurpose solution	Lens No. 1	2	3	4	5	6	Average amount (µg)	SD±
1	MPS-2	16.88	16.85	17.04	17.08	17.25	17.15	17.04167	0.1546
2	MPS-6	8.2	8.55	8.15	8.5	8.13	8.1	8.271667	0.1995
3	MPS-7	10.58	11.12	11.35	10.68	11.03	11.02	10.96333	0.286
4	MPS-8	17.11	17.23	17.87	17.88	17.79	17.92	17.63333	0.3634
5	MPS-9	17.5	17.62	17.65	17.55	17.43	17.5	17.54167	0.0823
6	MPS-10	17.8	17.85	17.9	17.83	17.8	17.79	17.82833	0.0417
7	MPS-11	17.95	17.96	18.01	17.91	17.89	17.96	17.94667	0.0423

RESULTS:

Seven MPS coded as MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were tested for their deposits removal capacity and efficiency upon treatment of the deposits laden hydrophilic contact lenses (Table III).

In the study, the deposits of lysozyme and albumin were made on the contact lenses deliberately by soaking them in STF of pH 7.4 containing the above constituents for 24 hours at 37 ± 0.5°C. Hydrophilic contact lenses of group I (Netrafilcon A) were used. The deposits laden contact lenses were then treated with MPS for 12 hours. Sodium citrate (deproteiniser) present in MPS in concentration of 0.1%, 0.05%, 0.07%, 0.15%, 0.17%, 0.20% and 0.30% in MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 respectively removed lysozyme. The average amount of lysozyme in µg removed per lens by MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were 7.52, 3.63, 5.31, 7.48, 7.87, 8.06 and 8.21 respectively (Table V).

Table V: Amount of lysozyme removed (in µg) by multipurpose solution from contact lenses

S. No.	Multipurpose solution	Lens No. 1	2	3	4	5	6	Average amount (µg)	SD±
1	MPS-2	7.13	7.71	7.75	7.25	7.69	7.6	7.52	0.2643
2	MPS-6	3.69	3.45	3.4	3.6	3.8	3.85	3.63	0.1828
3	MPS-7	5.01	5.2	5.31	5.45	5.3	5.6	5.31	0.2029
4	MPS-8	7.25	7.81	7.68	7.4	7.3	7.45	7.48	0.2198
5	MPS-9	7.8	7.9	7.98	7.86	7.69	7.97	7.87	0.1098
6	MPS-10	8.1	8.13	7.99	7.98	8.01	8.12	8.06	0.0689
7	MPS-11	8.2	8.16	8.22	8.31	8.27	8.1	8.21	0.0754

Similarly average amount of albumin removed per lens in µg by MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were 17.04, 8.27, 10.96, 17.63, 17.54, 17.83 and 17.95 respectively (Table VII).

The removal occurred on the surfaces of non-ionic hydrophilic contact lenses, which are FDA approved, group I and group II types[8]. The albumin was deposited more than lysozyme and the capability of the MPS with sodium citrate used as deproteiniser was more towards lysozyme.

The lysozyme could be removed easily than albumin by the MPS for hydrophilic contact lenses.

0.1% of sodium citrate in MPS for hydrophilic contact lenses is upto the mark. Sodium citrate 0.1% can be used as deproteiniser for removal of lysozyme and albumin present on the surface of hydrophilic contact lenses.

DISCUSSION:

One reason for removing contact lens deposits is to extend the useful life of the contact lens. The more important reasons for cleaning hydrophilic contact lenses are to maintain clear vision, good comfort, and most importantly normal eye health. Undesirable organic substances within the tear film layer, such as lipids, mucoproteins, albumin, immunoglobulin, glycoproteins, mucin and lysozyme combine with inorganic compounds, bacteria and microorganisms to form a complex biofilm deposit on contact lens surface within minutes of placing the lens on the eye. These deposits continue to build on the contact lens surface with successive wearing period, eventually causing discomfort from mechanical irritation of the ocular tissues, as well as blurred vision as the optical quality of the contact lens surface degrades. This biofilm can also act as an antigenic stimulus causing allergic lid reactions such as giant papillary conjunctivitis (GPC).

GPC causes blurred vision, reduced wearing time, redness, itching, stinging, ocular discomfort and mucous discharge. GPC used to be a frequent occurrence with hydrophilic contact lenses but with the advent of multipurpose solution with non enzymatic cleaners like citrate, tris, hydranateTM the incidence of GPC in hydrophilic contact lens wearer has decreased considerably.

Sodium citrate is a non enzymatic deproteiniser used in MPS. It's a trivalent anionic molecule with chelating properties. It is effective in removing protein, lipids and polysaccharide deposits from contact lenses surface and breaks calcium bridges which link protein deposits to each other and to the lens. The cleaning activity and no ocular toxicity promote longer lens life. Citrate is therefore used in MPS for the overnight storage of the lens and for rinsing and soaking of the contact lenses. Citrate is a non enzymatic deproteiniser in the MPS.

Tear proteins such as lysozyme and albumin are large multivalent molecules containing both positive and negative local areas of charge. The positively charged sites on protein molecule can form ionic bonds with the negatively charged surface of the ionic contact lens and thereby binding proteins to the surface. One protein layer on to the other therefore builds up within no time, this ionic building is strong type hence to remove such bindings one has to store the lenses overnight and also to clean, rinse the lens by mechanically rubbing with MPS. This cause the removal of deposit coupled by weekly cleaning using an enzymatic cleaner as well.

In the experiment, the non ionic lenses are used because here the binding is not that strong and non enzymatic cleaner which are less allergy prone like sodium citrate has been used. There is no strong binding hence no need of enzymatic cleaner like papain which induce allergy. Hence sodium citrate could solve the purpose and is therefore used as deproteiniser. Binding occurs but not to that extent as in ionic type and this binding is broken by the compound like citrate[8].

In order to ascertain the efficiency of MPS preparation towards the removal of deposits from the contact lenses, a deposition study was performed. In the study, lysozyme and albumin were measured quantitatively i.e. removal of these deposits from the surface of the lens has been studied. Seven MPS preparations i.e. MPS-2, MPS-6, MPS-7, MPS-8, MPS-9, MPS-10 and MPS-11 were tested for their deposits removal efficiency upon treatment of the used lenses by them. These preparations differ in the sodium citrate concentration- the deproteiniser. The concentration of sodium citrate varied from 0.05% to 0.3% in these formulations.

In the study, the deposits of lysozyme and albumin were made on the contact lenses deliberately by soaking them in STF of 7.4 containing these components for 24 hours at 37±0.5°C. Group I (Netrafilcon A) were used for the reasons given above. These lenses are non ionic type. These deposits laden contact lenses were then treated with MPS preparation for 12 hours. During the treatment period, the deposits of lysozyme and albumin were removed by the action of sodium citrate present in the MPS. The extent of removal of these deposits was assessed by measuring albumin and lysozyme quantitatively by using standard analytical procedures discussed in the section. It was observed that the preparation containing less amount of sodium citrate i.e. 0.05% in MPS-6 removed less amount of lysozyme and albumin.

It was further ofserved that(the preparation containing more amount of sodium citrate i.e. MPS-7 and MPS-2 (0.07% of sodium citrate MPS-7 and 0.1% in MPS-2) removeÐ these deposits in increasing orders. However, further increase of sodium citrate i.e. beyond 0.1% in the preparation of MPS-8, MPS-9, MPS-10 and MPS-11 remove the deposits, slightly more as sompared to MPS-12ˆbut not significantly. The result of MPS-2 preparation was comparable with other preparations i.e. MPS-8, MPS-9, MPS-10 and MPS-11. This is due to the fact that entire deposits were removed by MPS-2 preparation containing 0.1% of sodium citrate; therefore further increase in the sodium citrate concentration was not desirable i.e. the concentration of sodium citrate in the preparation MPS-8, MPS-9, MPS-10 and MPS-11 were in excess. Hence MPS-2 is an optimized preparation as far as quantity of sodium citrate is concerned.

It was further observed that deposits due to albumin were more as compared to lysozyme. The solution with 0.1% of sodium citrate could remove lysozyme more efficiently than albumin. Non ionic contact lenses - group I (Netrafilcon A) were used because these lenses actually discourage binding within the polymeric network of hydrophilic contact lenses. The binding is weak enough that non enzymatic cleaner like sodium citrate can break it easily.

From the deposition studies it was concluded that MPS-2, the multipurpose solution preparation, gave better results in the removal of deposits from the surface of the hydrophilic contact lens as compared to the other prepa

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In this paper, the authors present the results of a study on the effect of different concentrations of sodium citrate present in a multipurpose solution commonly used for cleaning hydrophilic contact lenses. In the study, the authors observed that a concentration of 0.1% of sodium citrate was optimal in the removal of lisozyme and albumin.

Although the study was in some ways limited, the results obtained are interesting because of their usefulness to both the prolongation of the life of the contact lenses as well as on the eye health.

Comment of the reviewer Victoria Valls Bellver PhD. Biochemistry. Department of Pediatry and Ginecology. University of Valencia. España

The authors describe a method for treating lypophilic contact lenses using the multipurpose solutions (MPS). MPS are the

solutions most prescribed with all lenses because of their benefits of convenience, simplicity, and disinfections properties.

It is know that among the organic substances that could deposit on contact lens, are the albumin or lysozyme that combination with bacteria or microorganism to form a biofilm on contact lens. In this work, the authors compare MRP with different concentrations of sodium citrate, showing the best results to the concentration of 0.1% where the release of albumin and lisozyme from the lens was higher.

The applicability of this study could to help prevent complications associated to eye allergies.

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PURIFICATION COLUMN ON SILICA AND CHEMICAL CHARACTERIZATION OF A COUMARIN ISOLATED FROM METHANOL EXCERPT OF THE STEMS OF PLANT *SECAMONE AFZELII (ACLEPIEDACEAE)* FROM ABIDJAN - IVORY COAST

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Comment of the reviewer Pedro del Río Pérez. Community pharmacists. La Quintana de Rueda. León. España.

SUMMARY

The purpose of our study is to isolate the first molecule of *Secamone afzelii* and caracterize the family molecular of pure product. The research component is distinguished by a TLC, a luminescence to 366 nm with a front, Rf = 0.6. This molecule is not visible to the naked eye, nor to 254 nm, on the TLC.

The column chromatography on silica helped isolate the product search with a yield of purification equal to 18,67 ± 0.72%. The various tests carried out on the phytochemical extracted. The molecule isolated could be considered to coumarin.

KEY WORDS: *Secamone afzelii*, silica column, purification, coumarin, TLC.

RESUMEN:

La finalidad de nuestro estudio es aislar una molécula de *Secamone afzelii* y la caracterización de la familia molecular del producto puro. El componente estudiado es identificado por TLC, con luminiscencia a 366nm y con un Rf= 0,6. Esta molécula no es visible a simple vista, ni a 254 nm en el TLC.

La columna de cromatografía de silica permitió aislar el producto, con un rendimiento del 18,67 ± 0.72%. Se realizaron distintas pruebas sobre el extracto fitoquímico La molécula aislada puede ser la cumarina.

PALABRAS CLAVE: *Secamone afzelii,* columna de sílice, purificación, cumarina,

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INTRODUCTION

In the world, 80% of people use medicinal plants for medicine, lack of access to medicines prescribed by modern medicine but also because these plants often have a real impact. Today, traditional knowledge is less and less transmitted and tends to disappear. That is why ethnobotany and Ethnopharmacology working to identify anywhere in the world, plants deemed active and it belongs to modern research to clarify the properties and validate the use[1-4].

The search for new molecules should be undertaken within the plant and animal biodiversity using data Ethnopharmacology. This approach allows you to select plants potentially active and increase significantly the number of discovery of new products assets. Interest chemists relate to natural molecules extracted from plants and animals, is increasingly growing. Several authors have studied compounds isolated from plants with multiple interests [5-7]. In recent years we are particularly interested in plants recognized by users as having antioxidant properties. Among these plants are *Secamone afzelii* the family *ASCLEPIEDACEAE*. The methanol extract was tested and found to have antioxidant properties. The molecules responsible for this important quality would be bioactive flavonoids[8-9]. So far no author has isolated molecules of this plant.

The originality of this study is to isolate and characterize the chemical family of the first molecule of *Secamone afzelii* from a methanol extract

MATERIAL AND METHODS

1. Vegetal material:

The vines of *Secamone afzelii* we studied were harvested in Abidjan in small bush of the University of Abobo-Adjame, which is an extension of the Banco forest. The bodies were washed under running water continuously for fifteen (15) minutes. Then the leaves were separated from the stems. These were dried in an oven at 70 ° C for one week. The body was dry pulverized by a grinder (type RETSCH 811,100) for a powder which were conducted all studies

2. Method:

Extraction by maceration

The extraction method by macerating material in a solvent is to leave a certain amount of plant material in a suitable solvent for a sufficiently long period so that the solvent reaches out molecules based on their polarity. Figure 1 summarizes the extraction method that we used.

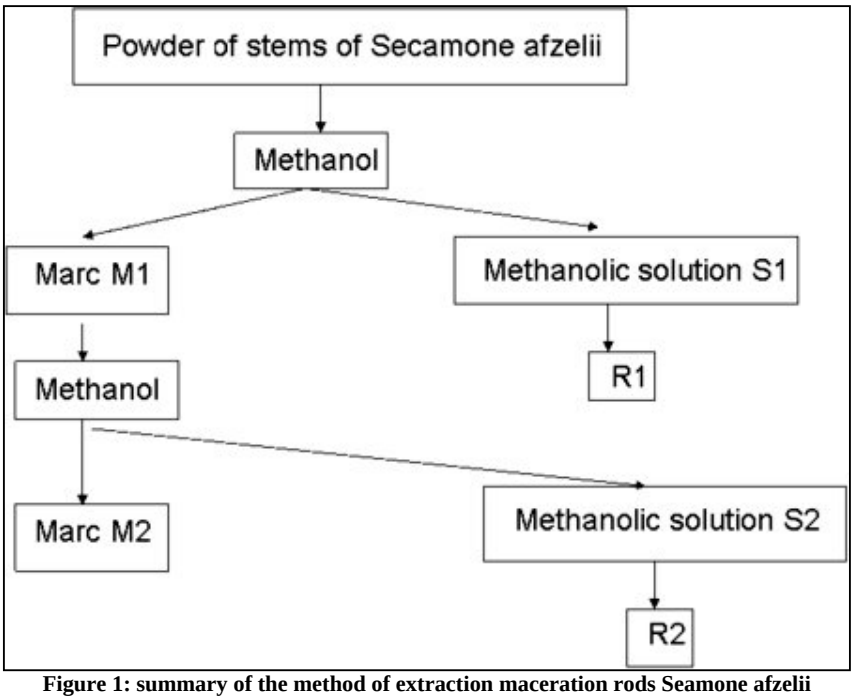

Figure 1: summary of the method of extraction maceration rods Seamone afzelii

100 g powder rod is macerated in distilled methanol (500 ml) for a week, then filtered by Büchner. The solution raw methanol S1 is vacuum distilled through an evaporator (Buchi R110 type MKE 6540 / 2) until a solid gross dry R1. The maceration is resumed with M1 to have a solid gross dry R2. Finally mass of R1 and mass R2 gives 9 g.

Purification column of silica.

The thin-layer chromatography (TLC) with developing the mixture chloroform / methanol (9.5 / 0.5) was conducted on the gross extract methanol. This reveals CCM to 366 nm and Rf = 0.6 luminescent a product that is not visible to the naked eye, nor visible to 254 nm. The methanol extract was purified on a column chromatography with silica, in order to isolate the compound luminescent at UV 366 nm. The column is mounted with hexane. The height of silica is 15 cm and the inner diameter of the column is 4 cm.

Phytochemical tests.

Several tests described in the literature[6] have been made to characterize the chemical family to which it would be possible to include this molecule.

RESULTS:

1. Purification

Purification column silica is first elected to the well-hexane mixture and then with hexane / chloroform (50 / 50).

The desired product is obtained with pure 100% chloroform. Purification yields are co-signed in Table 1.

Experiment	1	2	3
Mass of crude (g)	2	1,5	3,8
Pure Product (g)	0,38	0,30	0,71
Performance of individual experience (%)	19	20	17
Yield (%)		18,67 ± 0,72	

Table 1: Values returns to purify the desired product

The yield purification 18.67 ± 0.72% shows that in the methanol extract, this compound appears with a remarkable rate.

2. Characterization

The various tests have been made[6, 10] to find the chemical family to which the isolated molecules are summarized in Table 2.

Classe of compound	Quinones	Alcaloids	Terpenoids	Coumarins	Flavonoids	Tanin
Reaction observed	No reaction	No reaction	No reaction	Experience N° 1 : Fluorescent stain to 366 nm with or without NH3 (Positive). Experience N°2 : The cycle lactone reaction test (positive)	No reaction	No reaction

Table 2: Phytohemical Screening realized into pure product

The first test to see whether the isolated molecule is a coumarin was positive. We conducted another experience on the molecule belonging to the family of coumarin.

DISCUSSION

This test is that the cycle of coumarin lactone[6, 10]. This test was positive. In view of the two experiences, we can say that the isolated molecule may belong to the family of coumarin.

It is worth noting that spectroscopic analysis in 1H and 13C NMR, then SDM, we can confirm that the first approach and infer the structure of this molecule. But already these initial results will help the interpretation of spectra.

In conclusion, a study of thin-layer chromatography of a methanol extract of stem *Secamone afzelii*, led us to purify the extract methanol. The yield 18.67 ± 0.78% of the product sought to characterize the fact that this compound is revealing that UV visible to 366 nm, was purified.

The phytochemical screening done on the pure compound permits conclude that the isolated molecule is a coumarin.

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***Secamone afzelii* is a plant used in traditional medicine against various pains. The antioxidant capacity of methanol extracts obtained from this plant was described previously by Mensah et al (2004) and Houghton et al (2005). In this work, the authors show a simple and rapid method for purify and characterized partially one of the components with antioxidant capacity present in the metanolic extract of *S. afzelii*. This component was identified as belonging to the family of coumarins. The importance of this study is the potential use of the pure extract by their antioxidant capacity. It is known that compounds of the family of coumarins to act as antioxidants in biological systems. _____**

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The coumarins are compounds very abundant in nature. Chemically derived from the cyclization of O-hydroxycinnamic acid and it have different radicals in the 6- and 7-position. The coumarins are synthesized in the roots and it accumulates in young tissues. They are abundant in dicotyledonous (Rutaceae, Umbelliferae, legumes, solanaceae, ...). One of their functions in plants is antigerminative.

From a chemical point of view the coumarins can be simple (umbelliferone, esculetol, fraxetol,...) or compounds (furocoumarins [psolareno, imperatorine, bergapten ...], 7.8-furocoumarins [pimpinellin, angelicin, ...], 3-pyranocoumarins [samidine, visnadine, ...]). Plants with simple coumarins: tonka beans, meliloto, horse-chestnuts. Plants with compound coumarins bergamot, angelica,

Keller ... Its pathway is complex, starting from shikimic acid through of cinnamic acid and resulting in various coumarins.

The coumarins has diverse pharmacological properties (which is not synonymous with therapeutic application): sedatives (tonka bean), antispasmodic (Kella), phlebotonic (Indian horse chestnut).

The furocoumarins are photosensitives (bergamot, angelica, Ammi majus). At present, some plant species are used in therapeutic in form of the dry extract (capsules) or as oral solution.

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RELATION BETWEEN GLUCOLIPID PROFILE AND SMALL INTESTINE HISTOLOGICAL PATTERNS IN DIABETIC RATS EXPOSED TO AN INTERMITTENT DIETARY RESTRICTION

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SUMMARY

The effects of an intermittent and prolonged dietary restriction on biochemical variables and histological small intestinal patterns in 12-month-old male eSMT rats are examined. These spontaneously diabetic animals were separated in two groups after weaning: 10 rats fed *ad libitum* with standard rat chow and 10 rats fed a restricted diet by deprivation of the same food for 24 hours every 72. At 12 months of age, animals were weighed and euthanized after tail vein bleeding for plasma analysis (glycemia- both fasting and 120 minutes after an oral glucose challenge-, triglyceridemia and total cholesterolemia). Small intestines were removed, weighed and measured in length.

Intestinal specimens were fixed, embedded in paraffin, semi serially cut at 6 µm and stained with PAS-Hematoxilyn and Hematoxilyn-Eosin. Histometry was performed through a linear devise attached to ocular lens and lectin histochemistry was accomplished employing *Canavalis ensiformis, Dolichos biflorus, Arachis hypogea, Ulex europaeus-I, Triticum vulgaris, Ricinus communis* and *Soy Bean (Glicine Max) Agglutinin*. Essentially, eSMT rats, a suitable animal model for studying diabetes and/or its complications, revealed at 12 months of age after undergoing the dietary restriction: 1.- An expected improvement in body weight and determined biochemical variables (fasting and after glucose overload glycemias, triglyceridemia and total cholesterolemia) without reaching euglycemic values. 2.- Changes in most of the analyzed histometric patterns with no relevant reflection on morphometric ones, and 3.- No modifications in lectinhistochemical patterns.

KEY WORDS:Diabetes. Rat. Diet. Digestive tube. Biochemistry. Histology

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RESUMEN:

Se analizaron los efectos de una restricción dietética intermitente y prolongada sobre variables bioquímicas y patrones histológicos intestinales en ratas macho eSMT. Estos animales, diabéticos espontáneos, fueron separados en dos grupos luego de su destete: 10 fueron alimentados *ad libitum* con dieta estándar para ratas de laboratorio y 10, sobrellevaron una privación del mismo alimento de 24 horas cada 72. A los 12 meses de edad, los animales fueron pesados y sometidos a eutanasia tras de extraerles sangre de la vena de la cola para análisis plasmáticos (glucemias en ayuno y tras 120 minutos de sobrecarga glúcida, trigliceridemia y colesterolemia total). El intestino delgado fue removido, pesado y medida su longitud.

Los especímenes fueron fijados, incluidos en parafina, cortados a 6 µm de modo semiserial y coloreados con PAS-Hematoxilina y Hematoxilina-Eosina. La histometría fue llevada a cabo mediante un dispositivo lineal ligado al ocular y la lectin histoquímica, empleando *Canavalis ensiformis, Dolichos biflorus, Arachis hypogea, Ulex europaeus-I, Triticum vulgaris, Ricinus communis and Soy Bean (Glicine Max) Agglutinin*. Esencialmente, las ratas eSMT, un modelo adecuado para el estudio de la diabetes y/o sus complicaciones, revelaron a los 12 meses tras la restricción dietaria seguida: 1.- Una esperada mejoría en el peso corporal, en las glucemias en ayuno y tras sobrecarga glúcida, en la trigliceridemia y en la colesterolemia total aunque sin alcanzar valores euglucémicos. 2.- Cambios en la mayoría de las lectinas analizadas pero sin reflejo sustancial en las variables morfométricas, y 3.- Ausencia de modificaciones en lo que se refiere a la lectinhistoquímica.

PALABRAS CLAVE:Diabetes. Rata. Dieta. Tubo digestivo. Bioquímica. Histología

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INTRODUCTION

Diabetes mellitus, long considered a disease of minor significance to world health, is now taking its place as one of the main threats in the 21th.century. The diabetes epidemic, both in developed and developing nations, particularly refers to type 2 diabetes, a metabolic disorder primarily characterized by insulin resistance, relative insulin deficiency and hyperglycemia. Usually associated with overweight and obesity, its prevalence has risen at an alarming pace in the last twenty five years leading to foresee a number of diabetic persons near 366 million for 2030. Consequently, new studies on this topic become strictly necessary[1-2].

Distinct animal species have been widely used for investigating the different diabetic types and contributed to the current knowledge on this metabolopathy[3-8].

Exceeding induced diabetic animals through diet, alloxan, streptozotocin, surgery and transgenic procedures, those spontaneous or genetically ones (mice and rats) keep being relevant for analyzing the diabetic syndrome[6].

Among the spontaneous or genetically animal models for diabetes, the eSMT rat was developed in our laboratory of Biology by crossing eSS rats (a non obese model of type 2 diabetes) with ßrats (a fertile obese model revealing normocholesterolemia, hypertriglyceridemia, and type 2 diabetes). This synthetic line evidences overweight like ß and shows an early beginning of the diabetic syndrome, an enhanced evolution of fasting hyperglycemia and glucose intolerance like eSS[9-10].

Continuous or intermittent dietary restriction (DR) diminishes the expression of the diabetic syndrome and has been employed for managing type 2 diabetes and treating obesity[11]. Likewise, DR also affected the mucosal growth, the morphology and the cell cytokinetics in the small intestine[12]. Furthermore, DR diminished body and intestinal weights in adult male Lewis rats, put into evidence lower body and liver weights in adult control rats, prevented duodenal hyperplasia and augmentation of ileal villus cell number in 20 months-old -rats, reduced fasting glucose concentrations, triglycerides and cholesterol and increased apoptosis in the small intestine of aging rats[13-15].

Although the effects of limiting caloric input depend on the initial age of restriction[16-17], chronic caloric restriction augmented longevity, improved insulin sensitivity and lowered lifetime glycemia.

Extending former experiences in eSS and eSMT[18-20], the aim of this study was to study the relation between the glucolipid profile and some small intestine histological patterns in male eSMT rats exposed to an intermittent dietary restriction.

MATERIAL AND METHODS

Animals were housed in a room with standard environmental conditions (24ºC, 12 hours light/12 hours dark schedule cycle, air exchange, tap water *ad libitum* and standard rat chow). 20 eSMT rats were separated in two groups after weaning: 10 rats fed *ad libitum* (AL) with standard rat chow and 10 rats fed a restricted feeding schedule (R) by deprivation of food for 24 hours every 72.

At 11 months of age, animals were placed in metabolism cages. After a 5 - day adaptive period and during 10 days, food intake and body weight were determined for calculating the total food intake, the mean body weight and the relative food intake (mg/100 g body weight).

At 12 months of age, animals were weighed (BW) and euthanized after tail vein bleeding for plasma analysis (glycemia- both fasting - G0 - and 120 min after an oral glucose overload - G120 -, triglyceridemia - TG - and total cholesterolemia - TC -). Abdomen was cut and opened along the midline and the small intestine (from pylorus to the ileocecal junction) was immediately dissected, removed, flushed with PBS (phosphate buffered saline) at 4ºC and subsequently trimmed of fat and mesentery. Weight (SIW) and length (SIL) of the small intestine as well as SIW/BW x100 were then calculated.

Plasma Analysis

Fasting glycemia (18-h fast) and 120 min glycemia after an overload of 10% glucose (200 mg/100 g body weight) via stomach tube were determined by the glucose-oxidase enzymatic method using a commercial kit (Wiener Laboratories, Argentina).

Triglyceridemia and Total Cholesterolemia (both 18-h fast) were registered through an enzymatic-colorimetric method using commercial kits (Wiener Laboratories, Argentina).

Every dosage was carried out at the same hour in the morning for avoiding possible variations due to circadian rhythms.

Histomorphometric Study

Segments of small intestine (25 cm from pylorus) were removed. These portions were cut along the mesentery border, pinned in balsa wood, fixed in Carnoy's fluid, embedded in paraffin, semi serially cut at 6 µm mounted 1 out 40 section and stained with Peryodic Acid Schiff + Hematoxylin (PAS+H). A calibrated eye objective micrometer was employed. Villi heights were measured in those sections showing the entire villus from base to tip and villi width, at the middle of each villus. Total wall thickness, mucosa thickness (distance from villus tip to *muscularis mucosae*), crypt depth (distance from villous base to *muscularis mucosae*), goblet cells/villus and enterocytes/villus were complementarily measured. A minimum of ten measurements / rat was achieved.

Lectinhistochemical Analysis

Considering the interactions among lectins, histomorphometry and/or biochemical variables, specimens were successively fixed in Bouin's fluid for 45 min. and 10% formalin in 0.01 M phosphate-buffered saline (PBS-pH 7.2) for 3 hours. Paraffin embedded tissues were cut at 6 µm, stuck on slides with Vectabond, deparaffinised with xylol and hydrated with acetone-alcohol. These slices were incubated in 3% hydrogen peroxide in absolute methanol during 20-30 min for inactivating endogenous peroxidase. Specimens were subsequently rinsed several times in 0.01 M phosphate-buffered saline (PBS-pH 7.2), dried, treated with 0.1% bovine serum albumin in PBS for 15 min and incubated overnight at 4º C with the following lectins: *Canavalis ensiformis* (Con-A) (α-D-mannose and α-D-glucose residues), *Dolichos biflorus* (DBA) (N-Acetyl Galactosamine α-3-N-Acetyl Galactosamine and N-Acetyl Galactosamine α-3-Galactose), *Arachis hypogea* (PNA) (Galactose ß3-N-Acetyl Galactosamine), *Ulex europaeus*-I (UEA-I) (Fucose α 2- Galactose-ß), *Triticum vulgaris* (WGA) (N-Acetylglucosamine/sialic acid group), *Ricinus communis* (RCA) (N-Acetylgalactosamine/galactose group), and *Soy Bean (Glicine Max) Agglutinin* (SBA) (αD-N-Acetyl Galactosamine and ß-3-N-Acetyl Galactosamine). Lectins were employed as horseradish-peroxidase (HRP)-labelled form.

The day after, slices were rinsed with PBS, covered with the Avidin-Biotin Complex (ABC) and incubated in a substrate medium consisting of diaminobenzidine (DAB)-hydrogen-peroxide substrate medium. Reagents and lectins were purchased from Vector Labs. Finally, slices were washed with tap water, counterstained with hematoxylin, dehydrated, mounted and observed in a Zeiss investigation microscope with a color digital video camera (Sony Exwavehad model).

Digital photos in real color were selected and transformed in gray scale images for determining optical densities (OD) through an Image-proPlus Program for Windows 1.1 Version (Media Cibernetics).

For establishing the scale detailed below, structures were measured at 400X (eight counts/structure/animal). The program automatically provided the mean ± standard deviation of lectin fixation for each lectin. Lastly, a mean related with lectin fixation resulting from the total counts/ structures/ rats was determined. Zero was established from a non lectin-reactive zone whilst its maximal value, near to 250, emerged from the higher lectin- reactive zone.

Taking into account the aforesaid means, the lectin OD was graduated in accordance with the following semi-quantitative scale: OD > 200 = negative -0-, OD between 200 and 150 = weakly to moderately positive -1 to 2- and OD <150 = strongly positive -3-.

Statistical Analysis

Data were analyzed applying conventional statistical techniques as the GraphPad Prism Program, version 4.0, April 2003.

Bioethical Evaluation

Our School of Medicine Ethical Committee previously examined and approved this project.

RESULTS:

At 11 months of age, mean body weight in R animals was significantly lower than AL ones (R: 318±42 g vs. AL: 427±31 g, p<0.001) exceeding that the total food intake was equivalent during the studied period (R: 225±19 g vs. AL: 230±36 g, p>0.05). In contrast, when the total food intake was compared with body weight (relative food intake), R values were higher (R: 0.70±0.11 vs. AL: 0.53±0.12, p<0.05).

Biochemical and morphometric variables in R and AL male eSMT rats at 12 months of age are registered in Table 1.

TABLE 1
Body weight and biochemical and morphometric variables in R and AL male eSMT rats at 12 months of age

Group	*G0 (mg/dl)*	*G120 (mg/dl)*	*TG (mg/dl)*	*TC (mg/dl)*	*BW (g)*	*SIW (g)*	*SIW/BW x100 (g/g)*	*SIL (cm)*
R	135±18	204±61	174±38	109±14	350±55	11.10±1.67	3.16±0.14	120.46±7.62
	**	**	*	**	***	ns	***	ns
AL	253±69	473±111	213±58	180±59	420±41	11.01±1.61	2.13±0.11	129.00±14.88

Values are expressed as mean ± standard deviation . Unpaired "t" test: ns: no significant; *: p< 0.05; **: p< 0.01; ***: p< 0.001
G0: Fasting glycemia (18-h fast); G120: 120' Glycemia after an overload of 10% glucose (200 mg/ 100 g body weight); TG: Triglyceridemia; TC: Total Cholesterolemia; BW: Body Weight; SIW: Small Intestine Weight; SIW/BW x100: Small Intestine Weight/Body Weight x 100; SIL: Small Intestine Length

Glycemias, triglyceridemia, total cholesterolemia and body weight resulted significantly lower in the restricted group. Following the American Diabetes Association (ADA) criteria[5], both groups were classified as diabetic (G0 $\geq$ 126 mg/dl and G120 $\geq$ 200 mg/dl). Nevertheless, AL values were notoriously higher than those of R ones.

No significant differences were detected in SIW and SIL. However, R values appeared significantly higher in SIW/BW x 100.

As summarized in Table 2, villi width at ½ height, total wall thickness and crypt depth were significantly higher in R animals whilst longer villi heights and more numerous enterocytes/villus were put into evidence in AL rats. Conversely, mucosal thickness and goblet cells/villus showed no significant values between both feeding plans.

TABLE 2
Small intestine histometry in R and AL male eSMT rats at 12 months of age

Group	*Villi height (µm)*	*Villi width at ½ height (µm)*	*Total wall thickness (µm)*	*Mucosal thickness (µm)*	*Crypt depth (µm)*	*Goblet cells/villus*	*Enterocytes/ Villus*
R	469 ± 42	92±9	926±65	746±37	277±38	31±4	160±12
	**	**	*	ns	**	ns	***
AL	521±34	82±6	857±59	737±37	216±35	34±4	192±6

Values are expressed as mean ± standard deviation
Unpaired "t" test; ns: no significant; *: p< 0.05; **: p< 0.01; ***: p< 0.001

Lectin histochemical results

No differential reactivity to lectins was found in villi and crypt enterocytes and goblet cells of AL rats when compared with R animals (Figures 1 and 2). However, differences appeared among lectins. Hence, a qualitative reactivity (++) with a semi-quantitative one near to 150 (2) were registered in villi enterocytes and goblet cells for every lectin (Figures 1 y 2). The only exception (+++ /<150 = 3) was evidenced in goblet cells for UEA-I. In the crypts, lectin reactivity varied from - to + (higher and near to 200 = 0/1) for every lectin except in goblet cells for RCA (++/near to 150=2) and in enterocytes for UEA-I (+++/lower than 150 = 3).

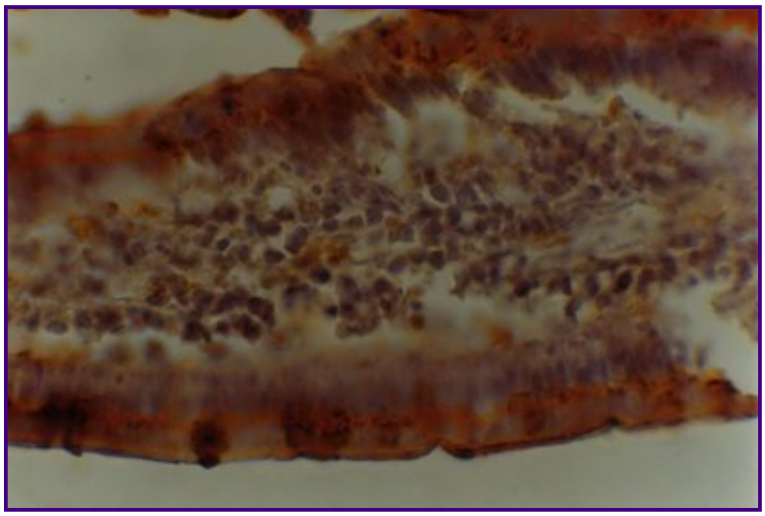

Figure 1: Histochemical reaction to Arachis hypogea (PNA) lectin in the small intestine of 12-month-old male eSMT rats exposed to ad libitum feeding schedule. Enterocytes and goblet cells are qualitatively (++) and semi-quantitatively (Optical Density = 2). The same occurs with the thin sheath of mucus located on the brush border of the enterocytes. The image is seen at a magnification of 400 X.

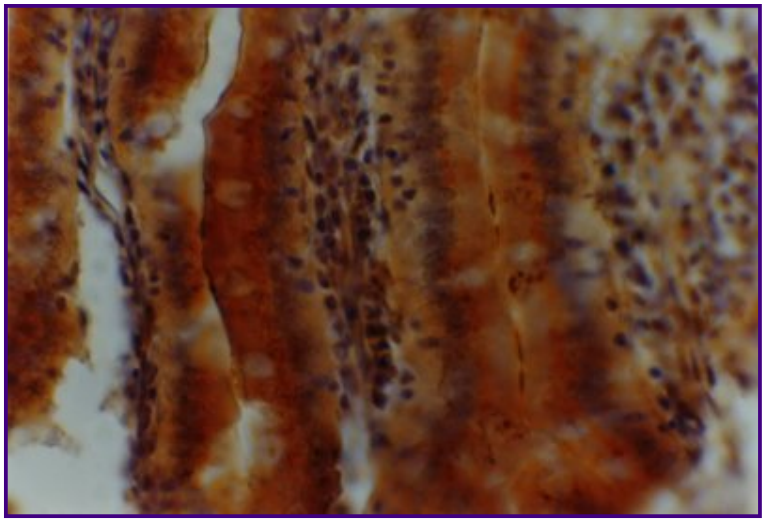

Figure 2: Histochemical reaction to Arachis hypogea (PNA) lectin in the small intestine of 12-month-old male eSMT rats exposed to restricted feeding schedule. Enterocytes and the adjacent mucus sheath are qualitatively (++) and semi-quantitatively (Optical Density = 2). The goblet cells undergo a particular functional stage after secreting the mucus which constitutes the aforesaid sheath [false negativity to PNA lectin in goblet cells with qualitative (++) and semi-quantitative (OD = 2) lectin reaction in mucus sheath]. The image is seen at a magnification of 400 X.

DISCUSSION

Considering our results, alimentary restriction ameliorated the diabetic syndrome as revealed by the decreased BW, G0, G120, TG and TC, exceeding that G0 and G120 kept showing diabetic values in restricted eSMT rats according to the ADA criteria[5]. These data appeared in congruence with a better performance of the diabetic genotype in "poor" environments[21,22]. and agreed with the more benign course of the metabolic syndrome already demonstrated in restricted eSS and OLEFT rats[18,23].

On the other hand, the higher relative food intake in R animals pointed out a compensatory consume after the fasting lapse, as opportunely suggested[19]. This could be particularly involved in the higher relative SIW (joined to a lower BW and a similar SIL-SIW) and the greater total wall thickness registered in R rats. In this regard, Kujalova and Fabry (1960) found that the small intestine became hypertrophic when food was fed intermittently[24] whilst Jervis and Levin (1966) reported lower BW and higher SIW and SIL in the small intestine of 1-year-old white rats with severe chronic-alloxan diabetes fed *ad libitum*[25].

R animals also showed shorter villi heights, lesser enterocytes/villus, higher villi width at ½ heights and deeper crypts, In this sense, changes in crypt depth have been associated with alterations in the maturity of cells[26].

The presence of distinct quantities of nutrients in the lumen, the endocrine imbalance of chronic diabetes and/or the intestinal hormones (incretins and others)[27] could be interacting to produce the aforesaid results.

In turn, the histometric differences between eSMT and STZ-induced diabetic rats[7,8,26] may be supported in the frequently higher fasting glycemia (400 mg/dl or more) of the last ones, capable of altering the relations between the glucolipid profile and the small intestine histological patterns when long-term effects of type 2 diabetes are taken into account.

Both feeding schedules did not seem to affect lectin binding. Thus, differences detected in villi and crypt enterocytes and goblet cells could be attributed to distinct glucidic constitution of those structures and could be reflecting variations in intestinal function and differentiation[28].

Most of these results in eSMT rats appeared coincident with those formerly reported by other workers in non diabetic rats suggesting that, at this age, glucidic residues were similar to those studied in our diabetic line[29,30]. Conversely, our results were not coincident with obtained in mice where distinct feeding schedules produced different lectin bindings[31]. In this regard, species-dependent reasons may be put forward.

Whatever the achieved results be, the physiological levels of the epithelial mucins appear necessary for the normal intestinal uptake and the absorption of nutrients.

While the two feeding schedules here employed are in a straight line related with biochemical variables and body weight, its relation with morphometric and histometric findings in the small intestine could be neither confirm nor denied. Exceeding the reasonable interactions among lectins, histomorphometry and/or biochemical variables, other ways of relations remain to be established.

To sum up, eSMT rats, a suitable animal model for studying diabetes and/or its complications, revealed at 12 months of age after undergoing a restricted feeding schedule (R animals):

1. A predictable improvement in body weight and defined biochemical variables related with the diabetic syndrome (G0, G120, TG and TC). Although no category modification could be established according to ADA criteria since GO and G120 did not reach euglycemic values, both showed a eye-catching nearness to the border to impaired fasting glucose (125 mg/dl) and impaired glucose tolerance (199 mg/dl)

2. Changes in certain histometric patterns with the exception of those morphometric related with small intestine length and weight. In this regard, a non-published report[32] evidenced that the histometric patterns studied in the small intestine of Wistar rats (a common euglycemic control) did not significantly differ at this age with those of AL- eSMT ones. This could suggest that at this age no adaptive diabetic impacts are still detected in the small intestine of eSMT rats fed *ad libitum*

3. No modifications in lectinhistochemical patterns between AL and R eSMT rats

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Comment of the reviewer Noemí Gómez Manero MD. PhD. Servicio de Medicina Interna. Complejo Asistencial de Burgos. España

In the last years, the global incidence of type 2 diabetes mellitus has increased exponentially, becoming a prevalent problem of health.

Classically, type 2 diabetes mellitus has been considered a problem in middle-aged and older people, but lately, many cases have been diagnosed in people younger than 30 and even in children. These data are specially relevant in developed and developing countries. Different factors contribute to this called "type 2 diabetic epidemic", but obesity,overweight, and sedentarism play and undoubtely role in this scene.

In this experimental paper, Hisano et al., show the favourable metabolic effect of intermittent dietary restriction in eSMT rats, diminishing body weight, basal glycemia, glycemia after an overload of glucose, and serum cholesterol and trygliceride levels. These favourable metabolic changes were accompanied with some changes in histologic patterns between the two groups, although no significant differences in lecitinhistochemical patterns were found.

These results will help us to understand the physiopathology of type 2 diabetes mellitus,and focus on the importance of diet in the management of this prevalent disease.

Comment of the reviewer Carlos G. Musso, MD. Nephrology Department. Hospital Italiano de Buenos Aires. Buenos Aires. Argentina

In this paper Hisan et al. show biochemical and histological effects of an intestinal intermittent and prolonged dietary restriction on diabetic rats.

Their findings shed new and valuable information on a topic of great interest is that of food restriction and its favorable consequences.

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OXCARBAZEPINE INDUCED HYPONATREMIA: A POTENTIAL EXPLANATION OF ITS PHYSIOPATHOLOGY.

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SUMMARY

Carbamazepine and oxcarbazepine are antiepileptic drugs which can induce hyponatremia, being this disorder more frequent with the latter.

In this report we present a clinical case regarding an oxcarbazepine induced hyponatremia, and we hypothesized that oxcarbazepine could induce hyponatremia as a consequence of its influence on distal nephron where it could promote free water retention, urinary sodium loss, or both of them.

Besides, we proposed that a greater tubular sesitivity to oxcarbazepine than to carbamazepine could explain the different hyponatremia inducing capability between these two drugs

KEY WORDS: Oxcarbazepine. Hyponatremia. Physiopathology

RESUMEN

La carbamazepina y la oxcarbazepina son drogas antiepilépticas potencialmente inductoras de hiponatremia, siendo este disturbio más frecuente con ésta última.

En este reporte presentamos un caso de hiponatremia inducida por oxcarbazepina, y proponemos una potencial explicación fisiopatológica para esta entidad, a la cual se la considera consecuencia de un aumento a nivel de los túmulos colectores de la

reabsorción de agua libre, de la pérdida urinaria de sodio, o de ambos fenómenos combinados.

Además, postulamos que la mayor propensión a la aparición de hiponatremia con el uso de oxcarbazepina respecto del empleo de carbamazepina podría ser consecuencia de una mayor sensibilidad de los túmulos colectores a la primera de las drogas mencionadas.

PALABRAS CLAVE: Oxicarbazepina. Hiponatremia. Fisiopatología

INTRODUCTION

Hyponatremia is one of the most frequent electrolyte disorders associated to medication, being those drugs prescribed for psychological or neurological disorders the most dangerous ones in this sense[1].

Carbamazepine and oxcarbazepine are antiepileptic drugs which can induce hyponatremia, an adverse effect usually asymptomatic in the setting of these dugs[2,3].

Even though, both drugs are chemically related, since oxcarbazepine is a ketoderivative of carbamazepine, hyponatremia seems to be more associated to oxcarbazepine[3-5]. There is no explicative hypothesis for such difference in the literature[6], then we present a case of oxcarbazepine induced hyponatremia, and potential explanation for its physiopathology in the present clinical report.

CASE REPORT:

Female patient, 71 years old, who had the following antecedents:

- **Epilepsy under treatment with carbamazepine (600 mg/day)**
- **Hypertension treated with enalapril (10 mg/day) plus atenolol (25 mg/day)**
- **Pulmonary thromboembolism treated with warfarin (10 mg/day)**

Since it was difficult to reach an adequate anticoagulation status in this patient, it was interpreted that the inductor enzimatic effect of carbamazepine could explain this refractory to anticoagulation. Then, it was decided to change the antiepileptic drug from carbamazepine (600 mg/day) to oxcarbazepine (600 mg/day) which has a lesser enzimatic induction effect.

Fourty eight hours after this change, the patient developed an asymptomatic hypotonic hyponatremia, with normal extracellular fluid, lowering her serum sodium level from 138 mmol/l progressively to 120 mmol/l.

Since, it was intended to keep the oxacarbazepine treatment, water restriction was effectively implemented (documented by a reduced fractional excretion of urea), but there was no amelioration in her serum sodium level (Table 1).

Table 1: laboratory values before and after water restriction

	Basal	After water restriction
Serum sodium (mmol/l)	120	122
Serum creatinine (mg/dl)	0.7	0.8
Serun urea (mg/dl)	33	47
Serum glucose (mg/dl)	110	100
FENa (%)	1.3	1.9
FEU (%)	63	55

FENa: fractional excretion of sodium. FEU: fractional excretion of urea

Then, enalapril, another potetial cause of hyponatremia, was discontinued and hypertension was controlled increasing her previous atenolol dose (25 mg/day to 50 mg/day). However, despite the above described therapeutical changes, her hyponatremia was not solved.

Finally, oxacarbazepine was stopped and carbamazepine was reintroduced as her antiepileptic treatment, while anticoagulation was handled using subcutaneous low weight heparin. Fourty eight hours after these changes serum sodium levels went back to

normal range.

DISCUSSION:

Although structurally related to carbamazepine, oxcarbazepine has several clinically relevant advantages over the former, including a more favorable pharmacokinetic profile and a better tolerability.

Because the metabolism of oxcarbazepine follows non-oxidative pathways, it has a lower propensity to induce hepatic oxidative enzymes and a reduced potential for drug-drug interactions. Additionally, oxcarbazepine does not undergo autoinduction[3,7].

Even though, early reports suggested that carbamazepine could induce excessive release of vasopressin and lead to inappropriate secretion of antidiuretic hormone, more recent studies have not found such hormonal increase[8].

It was documented in another study that after a water load, serum sodium and free water clearance were diminished in persons (patients or volunteers) who were on oxcarbazepine. Besides, oxcarbazepine induced hyponatremia was not associated with significant elevation in serum vasopressin levels. These findings indicate that oxcarbazepine-induced hyponatremia is not attributable to the syndrome of inappropiate secretion of antidiuretic hormone[7-8].

Moreover, it was also reported that serum natriuretic peptides levels were decreased in these hyponatremic patients[7]. Actually, in a study performed in patients on oxcarbazepine, serum aldosterone levels were increased in six normonatremic patients while these hormonal levels remained stable in 4 patients who were suffering from hyponatremia, suggesting that an increase in aldosterone levels could involved a compensatory mechanism to prevent hyponatremia in these patients[3].

Besides, it is already known from Edelman equation (natremia = free water / sodium + potassium) that hypotonic hyponatremia can be basically a consequence of an increase in free water body content, sodium deplection or a combination of both mechanisms[9].

Then, based on the above provided information, it could be hypothesized that the modification of collecting tubules induced by oxcarbazepine could lead them to an increased water reabsorption or/and sodium loss. Besides, a greater induction of these tubular changes in patients on oxcarbazepine respect to the cabamazepine induced ones, could explain the reported higher incidence of hyponatremia in patients on oxcarbazepine.

CONCLUSION:

Oxcarbazepine could induce hyponatremia as a consequence of its influence on distal nephron where it could promote free water retention, urinary sodium loss, or both of them.

A greater tubular sesitivity to oxcarbazepine influence respect to carbamazepine one could explain the different hyponatremia inducing capability between these two drugs.

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HIPONATREMIA INDUCIDA POR OXCARBACEPINA: UNA HIPÓTESIS FISIOPATOLÓGICA

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RESUMEN

La carbamazepina y la oxcarbazepina son drogas antiepilépticas potencialmente inductoras de hiponatremia, siendo este disturbio más frecuente con ésta última.

En este reporte presentamos un caso de hiponatremia inducida por oxcarbazepina, y proponemos una potencial explicación fisiopatológica para esta entidad, a la cual se la considera consecuencia de un aumento a nivel de los túmulos colectores de la reabsorción de agua libre, de la pérdida urinaria de sodio, o de ambos fenómenos combinados.

Además, postulamos que la mayor propensión a la aparición de hiponatremia con el uso de oxcarbazepina respecto del empleo de carbamazepina podría ser consecuencia de una mayor sensibilidad de los túmulos colectores a la primera de las drogas mencionadas.

PALABRAS CLAVE: Oxicarbazepina. Hiponatremia. Fisiopatología

SUMMARY

Carbamazepine and oxcarbazepine are antiepileptic drugs which can induce hyponatremia, being this disorder more frequent with the latter.

In this report we present a clinical case regarding an oxcarbazepine induced hyponatremia, and we hypothesized that oxcarbazepine could induce hyponatremia as a consequence of its influence on distal nephron where it could promote free water retention, urinary sodium loss, or both of them.

Besides, we proposed that a greater tubular sesitivity to oxcarbazepine than to carbamazepine could explain the different hyponatremia inducing capability between these two drugs

KEY WORDS: Oxcarbazepine. Hyponatremia. Physiopathology

INTRODUCCIÓN

La hiponatremia es uno de los trastornos electrolíticos más frecuentes asociados a medicación, siendo las drogas prescriptas para desórdenes psicológicos y/o neurológicos aquellas de mayor riesgo en este sentido[1].

La carbamazepina y la oxcarbazepina son drogas antiepilépticas potencialmente inductoras de hiponatremia, aunque en general de corte asintomático[2,3].

Si bien ambos antiepilépticos están químicamente emparentados, la oxcarbazepina es un ceto derivado de la carbamazepina, la incidencia de hiponatremia es mayor con la oxcarbazepina, desconociéndose la razón de esta diferencia[3-6]. En este reporte presentamos un caso de hiponatremia inducida por oxcabazepina, y postulamos su posible etiopatogenia

CASO CLÍNICO:

Paciente de sexo femenino, de 71 años de edad, portadora de los siguientes antecedentes:

- **Epilepsia en tratamiento con carbamazepina (600 mg/día)**
- **Hipertensión arterial tratada con enalapril 10 mg/día y atenolol 25 mg/día.**
- **Tromboembolismo pulmonar en tratamiento con warfarina 10 mg/día.**

A raíz de no haberse podido alcanzar un estado de anticoagulación, pese a haberse usado dosis adecuadas de warfarina, y que se sospechaba que dicha refractariedad era causada por el efecto inductor enzimático hepático propio de la carbamazepina, se decidió rotar el esquema antiepiléptico de carbamazepina (600 mg/día) a oxcarbazepina (600 mg/día), debido a su menor accionar en este sentido.

Tras 48 horas de realizado el cambio de medicación, la paciente desarrolló una hiponatremia hipo-osmolar, asintomática y con líquido extracelular normal. Su natremia había descendido de un valor inicial de 138 mmol/l a 120 mmol/l.

Dado que se deseaba mantener el tratamiento con oxcarbazepina, se le indicó a la paciente restricción hídrica, sin embargo no hubo mejoría en su natremia, pese a haberse realizado dicha restricción hídrica correctamente (como lo demostró el haber documentado un ascenso de la uremia y descenso de la excreción fraccional de urea (EFU) de la paciente durante la misma (Tabla 1).

Tabla 1: Valores de laboratorio antes y después de la restricción de agua.

	Basal	Tras restricción hidrica
Sodio Sérico (mmol/l)	120	122
Creatinina sérica (mg/dl)	0.7	0.8
Urea sérica (mg/dl)	33	47
Glucosa sérica (mg/dl)	110	100
FENa (%)	1.3	1.9
FEU (%)	63	55

FENa: Excreción Fraccional de sodio, FEU: Excreción Fraccional de urea.

Con el objetivo de resolver la hiponatremia de la paciente sin remover la oxcarbazepina, y dado que el enalapril es una droga potencialmente inductora de hiponatremia, se suspendió el enalapril como antihipertensivo, y se lo reemplazó mediante un ascenso de su dosis de atenolol: de 25 mg/día a 50 mg/día.

Sin embargo, a pesar todos de los cambios realizados al esquema terapéutico, su hiponatremia no resolvió, razón por la cual finalmente se decidió suspender la oxcarbazepina, reiniciar la carbamazepina e iniciar heparina de bajo peso molecular subcutánea como anticoagulante. Cuarenta y ocho horas después de estos cambios medicamentosos la paciente retornó a su

natremia basal normal: 138 mmol/l

DISCUSIÓN:

Aunque estructuralmente la oxcarbazepina está relacionada con la carbamazepina, la primera posee ventajas clínicamente relevantes sobre la segunda, incluyendo su mejor perfil farmacocinético y mejor tolerancia.

A raíz de que el metabolismo de la oxcarbazepina sigue vías no oxidativas, tiene una baja propensión a ejercer un efecto inductor sobre las enzimas oxidativas hepáticas y menor número de interacciones farmacológicas. Además, tampoco posee efecto autoinductor[3,7].

Aunque reportes tempranos habían sugerido que la carbamazepina podía llegar a inducir liberación de vasopresina y provocar síndrome de secreción inapropiada de hormona antidiurética, otros estudios han desestimado este mecanismo[8].

Estudios más recientes han documentado que luego de la sobrecarga de agua, el sodio sérico y el clearence de agua libre disminuyó en personas (sanas o enfermas) medicadas con oxcarbamazepina, sin que hubiese en éstos últimos elevación de la vasopresinemia[7,8].

Estos hallazgos indican que la hiponatremia inducida por oxcarbazepina no es atribuible a un síndrome de secreción inadecuada de hormona antidiurética.

También fue documentado que los niveles de péptido natriurético auricular estaban disminuidos en pacientes tratados con oxcarbazepina, y que una respuesta compensadora del sistema renina angiotensina aldosterona podría prevenir la aparición de hiponatremia. En un estudio realizado en pacientes recibiendo este antiepiléptico, los niveles séricos de aldosterona estaban aumentados en 6 pacientes con natremia normal, y permanecieron estables en 4 pacientes en quienes la natremia había caído, sugiriendo que un aumento en los niveles de aldosterona podría involucrar un mecanismo compensatorio para prevenir la hiponatremia en estos pacientes[3].

Dado que según la ecuación de Edelman (natremia = agua libre /sodio + potasio) una hiponatremia hipo-osmolar, puede ser consecuencia de un aumento relativo de agua libre, una depleción relativa de sodio, o una combinación de ambas[9]; en base a la información provista por los estudios antes presentados, podríamos proponer que la etiopatogenia de la hiponatremia secundaria a oxcarbazepina podría deberse a cambios que este fármaco produce a nivel de los túmulos colectores haciéndolos:

- **más permeables a la recuperación de agua libre (sensibles al accionar de la vasopresina circulante): esto explicaría la instalación de hiponatremia por aumento en la recuperación distal de agua libre en ausencia de vasopresinemia elevada.**
- **más permeables a la pérdida urinaria de sodio: esto explicaría la instalación de hiponatremia con bajos niveles séricos de factores atriales y la activación del sistema renina-angiotensina-aldosterona, así como el hecho de que el uso de diuréticos (aumento de soduria) contribuya a su instalación.**
- **un mecanismo combinado**

Que éstos mecanismos se den en forma aislada o combinada, pero de forma más marcada en pacientes bajo tratamiento con oxcarbazepina que bajo carbamazepina, podría explicar la mayor incidencia de hiponatremia en esta primer grupo.

CONCLUSIÓN:

La hiponatremia inducida por oxcarbazepina podría producirse por un aumento de la reabsorción distal de agua libre, de su pérdida urinaria de sodio o una combinación de estos factores.

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GANGLIO CENTINELA Y CÁNCER DE MAMA

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RESUMEN

La biopsia selectiva del ganglio centinela actualmente es una técnica comprobada que permite evaluar y estadificar a las pacientes con cáncer de mama, así como seleccionar a las candidatas a disección radical axilar. El valor clínico del ganglio centinela reside en su valor predictivo sobre el estado ganglionar axilar, ya que si no contiene células tumorales, tampoco el resto de ganglios axilares presentarán metástasis.

PALABRAS CLAVE: Cáncer de mama, biopsia de ganglio centinela, ganglio centinela

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SUMMARY

The sentinel node selective biopsy is currently a proven technique that permits assessment and stating the patients that suffers breast cancer, and allows selecting patients to radical axillary lymphadenectomy. The clinical value of this technique reside in its predictive value about sentinel node status, since if doesn't contain tumor cells; the rest of axillary nodes would be clean.

KEY WORDS: Breast cancer, sentinel node biopsy, sentinel node

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INTRODUCCIÓN

La incidencia de cáncer de mama se ha incrementado a nivel mundial, parte de este incremento se debe una detección masiva con el uso rutinario de la mamografía[1], detectando casos en estadios tempranos de la enfermedad. El factor pronóstico más

importante en el cáncer de mama temprano continua siendo el estado de los ganglios axilares[2], que es una de las mayores determinantes para el tratamiento neoadyuvante. La biopsia selectiva del ganglio centinela (BSGC) actualmente es una técnica comprobada que permite evaluar y estadificar a las pacientes con cáncer de mama, así como seleccionar a las candidatas a disección radical axilar[3] (DRA). El valor clínico del ganglio centinela (GC) reside en su valor predictivo sobre el estado ganglionar axilar, ya que si no contiene células tumorales, tampoco el resto de ganglios axilares presentarán metástasis[4].

Se define como ganglio centinela al primer ganglio de una cadena linfática que recibe el drenaje desde el tumor primario, y el primero en acoger las células tumorales diseminadas por el sistema linfático[4,5]. El concepto de ganglio centinela en el cáncer de mama se basa en que las células tumorales se diseminan ordenadamente a través del sistema linfático y de esta forma, la afectación de los ganglios linfáticos se rige por un orden mecánico determinado por el flujo linfático entre el tumor y su primera estación ganglionar[4]. La BSGC ha emergido como un método mínimamente invasivo, cuyo objetivo es identificar al ganglio que recibe en primer lugar la linfa de la zona afectada, del cual su resultado histopatológico predice el estado de los demás ganglios en la zona linfoportadora[6], reemplazando a la DRA, que queda reservada a las pacientes en las que el ganglio centinela es positivo o no se localiza[2].

PRINCIPIOS Y LEYES DEL GANGLIO CENTINELA

Siguiendo los principios de la teoría de Halsted, que dictan que el flujo linfático es dinámico, ordenado y previsible[5,6] y que el primer relevo ganglionar del sistema linfático sirve de filtro para la diseminación tumoral[6], será considerado GC todo aquel ganglio que en vía directa de drenaje linfático del tumor, capte el colorante y/o presente cuentas elevadas de radiactividad en el gammagrama transoperatorio[3]. Cabe aclarar que el GC no es necesariamente el ganglio más cercano al tumor, ya que ganglios más alejados del tumor pueden recibir la linfa antes que los más cercanos si no existe drenaje directo entre éstos y el tumor[3], y que además, puede existir más de un GC.

POBLACIÓN IDEAL DE ESTUDIO, INDICACIONES Y CONTRAINDICACIONES

La población ideal para la realización de BSGC debe cumplir con algunas características, entre las que destacan; mujeres con cáncer de mama invasivo comprobado por biopsia[7] y sin clínica de afección de ganglio axilar[8,9], en estas existe una eficacia de 97-99% en la predicción del estatus del los ganglios axilares[10].

Anteriormente se consideraban como contraindicaciones absolutas para la realización de la BSGC a mujeres con evidencia clínica o histopatológica de afección axilar y alergia a la tinción o al radiocoloide11, y como contraindicaciones relativas a este procedimiento, el hecho que existiera una biopsia previa de la lesión tumoral, antecedentes de cirugía no oncológica en mama y cirugía axilar, enfermedad avanzada, quimioterapia neoadyuvante, pacientes en estadio T_3 y T_4, lesiones multifocales y multicéntricas, carcinoma ductal "in situ", edad mayor de 50 años, índice de masa corporal mayor de 30, embarazo[11], cáncer de mama inflamatorio.

En la actualidad, la realización de BSGC es posible en la mayoría de los escenarios clínicos de pacientes con cáncer de mama sin afección clínica de ganglios axilares, el tamaño del tumor la localización, la terapia neoadyuvante, incluso se puede realizar en pacientes con sospecha clínica de afección nodular con biopsia guiada por ultrasonido con resultado no diagnóstico[10].

LA IDENTIFICACIÓN GANGLIO CENTINELA

De la gran variedad de técnicas para la realización de la BSGC, no existe una técnica única que las agrupe a todas[12], tanto en tinción, lugar de inyección, como en otros aspectos. La identificación del GC se puede efectuar con la inyección de una colorante para linfografía (azul de metileno, azul patente[13], azul isosulfan, sulfuro coloide, albumina coloide, verde indocianin[14]), Las desventajas de usar únicamente tinción para la identificación del GC son que el rango de detección del GC depende de las habilidades del cirujano, y que no es posible un mapeo preoperativo del GC[14].

La inyección de radioisópos (tecnecio) permite localizar el GC preoperativamente dentro y fuera de la cadena axilar[6,14]. Las desventajas del uso de radioisotópos es que su uso es regulado, la preparación de los radioisotópo es laboriosa y se requiere de un equipo de detección gamma[14]. Se usa también la combinación de gammagrafía y biopsia por aspiración con aguja fina dirigida por ultrasonido, y se describe como un procedimiento simple, accesible y mínimamente invasivo, razones por las cuales tiene un uso con potencial dentro de las detecciones preoperatorias de GC[14]. Según las guías de la Sociedad Americana de Oncología Clínica y las guías de la Sociedad Americana de Cirujanos de Mama, la combinación de tinción y radioisotópos alcanza los más altos rangos de identificación, y los más índices de falsos negativos[14], e identifica los sitios de drenaje linfático axilar y extra-axilar[6].

La inyección de las sustancias identificadoras puede ser intratumoral, peritumoral, intradérmica o subdérmica suprayacente al tumor, subareolar[3] y periareolar[14]. La inyección subareolar tiene un índice de éxito mayor aunque en teoría no identifica el GC específico de la zona del tumor y tiene la desventaja de no identificar sitios de drenaje extraxilar. Está basada en el concepto de que tanto el parenquima mamario como la piel de la glándula tienen al ectodermo como origen embriológico común, el drenaje linfático mamario parenquimatoso y dérmico confluye en el plexo subdérmico-subareolar de Sappey y drena a un mismo GC en

la axila[3], esto ha sido demostrado al encontrarse el GC teñido azul y radiactivo cuando se inyecta el coloide intratumoralmente y el colorante vía subdérmica[15].

Los estudios de los microlinfáticos de la piel han mostrado que existe una vasta red linfática que inicia en los prelinfáticos capilares de la capa papilar, que se interconectan con capilares en la dermis y drenan en una extensa red de colectores subdérmicos, el flujo de esta red es alto, se estima en alrededor de 2.8 cm/min, lo que explica en parte porque el GC es más fácilmente localizable cuando la inyección es subareolar que cuando es intratumoral[15].

La inyección intratumoral o peritumoral se basa en que se identifican con precisión los sitios de drenaje extraaxilar, sin embargo, tiene la desventaja de no identificar los conductos aferentes hasta en 40% de los casos, el porcentaje de ganglio centinela localizado es menor y disminuye aún más en pacientes con antecedente de biopsia del sitio a inyectar debido a la fibrosis secundaria y a la falta de difusión del colorante[3].

En un estudio publicado por Madun y cols. la identificación del ganglio centinela en la gammagrafía fue de un 95% para el grupo con inyecciones simultaneas intradérmica periareolar y peritumoral, obteniendo una identificación del 98% para el drenaje axilar y un 22% para el drenaje mamario interno[16]. El escaneo nuclear se realiza idealmente en el preoperativo (1 día antes), para identificar el número de ganglios y su posición[9]. Se inyectan de 50-80 MBq de tecnecio 99m, inyectando volúmenes pequeños de 0.3-0.4 ml[9].

Durante el transoperatorio, después de la inducción de la anestesia general, se inyecta azul de metileno o azul patente9. Después de la inyección del colorante se da masaje a la mama durante 10 minutos, lo que facilita la difusión del colorante[15]. Durante la cirugía, el canal linfático que fue teñido se identifica y nos indica el nódulo centinela[9]. Si el nódulo centinela no se tiñe, la gammagrafía sola puede indicar el ganglio con radioactividad[9]. Casi la totalidad de los GC extirpados después de la BSGC se los había visualizado en la imagen previa, lo cual facilitó considerablemente su detección intraoperatoria. Esto nos lleva a pensar que la realización de linfogammagrafía prequirúrgica es imprescindible, como señalan muchos autores y parece indiscutible en la actualidad[2]. Se puede usar una biopsia múltiple dirigida a 3 ganglios centinela para predecir el estatus del resto de la cadena linfática, aunque sin resultados concluyentes[14].

Los ganglios extirpados son estudiados mediante congelamiento y tinción citológica[9,14]. Es importante el uso de inmunohistoquímica para la mejor identificación de micrometastasis[17]. El estudio anatomopatológico se puede llevar a cabo mediante la realización de cortes seriados y su tinción con hematoxilina-eosina[5] (H/E). Cortes a 3 diferentes niveles con intervalos de 3 micras de espesor[6]. Adicionalmente se puede utilizar inmunohistoquímica usando anticuerpos monoclonales contra la citoqueratina en el transoperatorio aumentado la detección de micromestastasis[14] en lo casos en que la tinción con H/E sea negativa, sobre todo si se plantea una cirugía axilar conservadora[17].

USOS, VENTAJAS Y DESVENTAJAS

Un diagnóstico preciso del estatus del GC permite la selección de los pacientes que necesitan una disección axilar linfática durante el intraoperatorio[14]. Si el GC es positivo, existe seis veces más posibilidades de encontrar metástasis en el vaciamiento axilar17 que si fuera negativo a metástasis.

Entre las ventajas de la BSGC se encuentran: morbilidad quirúrgica prácticamente inexistente[7], la incisión axilar es menor, el tiempo operatorio es menor, la exposición a los tejidos es mínima, no se produce linfedema, se puede realizar con anestesia local y se puede plantear en régimen ambulatorio[7].

La DRA debe ser realizada cuando el procedimiento de BSGC falle, sea técnicamente insatisfactoria o cuando exista sospecha clínica que existen ganglios no centinela afectados.

METÁSTASIS Y MICROMETÁSTASIS

El valor predictivo de la detección y análisis histopatológico del GC es el de revelar la presencia de metástasis en los ganglios de la axila, sin recurrir a la disección total de la misma[5], este no sólo depende de su correcta identificación, de la uni-multifocalidad del tumor primario o de la posibilidad de metástasis "en salto" a otros ganglios posteriores al centinela en el drenaje linfático, sino también, y de manera decisiva, del protocolo utilizado para el estudio intraoperatorio del GC[17].

El factor pronóstico más importante en cáncer de mama es el estado histológico ganglionar[6] y el factor que más se relaciona con la presencia de metástasis axilares es el tamaño tumoral[7]; en general, en carcinomas iguales o menores de 5 mm (T1a) la posibilidad de enfermedad metastásica es de aproximadamente 3% al 5%; para tumores T1b varía entre 6% y 17%; para tumores T1c 38%; en tumores T2 entre 23% y 48%, y en tumores T3 del 29% al 64%[1].

Además de un tamaño tumoral pequeño, ciertos tipos histológicos favorables (coloide, tubular, papilar, cribiforme y adenoideo quístico), un bajo grado nuclear e histológico y una edad mayor de 65 años parecen disminuir la posibilidad de afectación ganglionar axilar1. Se ha sugerido que la enfermedad metastásica oculta o micrometástasis puede ser un predictor de recurrencia en el 25% de los casos sin metástasis ganglionares que desarrollan recurrencia a lo largo del tiempo, lo cual refuerza

la necesidad de un estudio minucioso y de un diagnóstico preciso17.

Se han encontrado metástasis en ganglios no centinelas en un 10% de los pacientes con metástasis en el GC, y en un 20 a un 35% en pacientes con micrometástasis en el GC. No hay evidencia definitiva de que un GC negativo se correlacione invariablemente con una axila negativa, excepto tal vez en los tumores T1a-b17.

DISCUSIÓN Y CONCLUSIONES

Anterior a la aceptación universal de la BSGC en la practica clínica, la DRA se ofrecía a prácticamente todas las pacientes con cáncer de mama sin distinción, y la remoción de los nódulos linfáticos era aceptada con gras satisfacción por médicos y pacientes.

Con la implementación de la BSGC, el tratamiento del cáncer de mama se modifico fuertemente, reduciendo dramáticamente el número de SRA, y mejorando la calidad de vida de muchas pacientes con cáncer18. Dos factores importantes que afectan el éxito de la BSGC son la experiencia y entrenamiento del cirujano y del patólogo en este tipo de procedimientos. Comparado con la DRA, la BSGC no siempre refleja el verdadero estatus de la axilar13. En series largas, los falsos negativos alcanzan un 11%9.

La incidencia reportada de metástasis axilares en tumores mamarios invasores menores o iguales a 2 cm en su diámetro mayor ha sido reportada de alrededor de un 20%1. La incidencia de metástasis axilares en carcinoma mamario está directamente relacionada al tamaño del tumor mamario.

Continúan vigentes muchos aspectos controvertidos, sobre todo la elección de la vía óptima de inyección, el tipo de radiocoloide, la intervención de cadenas linfáticas extraaxilares, la inclusión de pacientes con cirugía mamaria previa o tras tratamiento neoadyuvante2, etc. Se insiste en la necesidad de validar el método en cada institución en particular para logra una mejor correlación diagnóstica1.

La ventaja fundamental de la BSGC es la de evitar la linfadenectomía axilar en aquellas pacientes donde la biopsia del ganglio indique que no existe afectación tumoral de éste18. La linfadenectomía solo sería necesaria en el caso de afección ganglionar y existen criterios de indicación de tratamiento adyuvante que no dependen del estado axilar7 (factores clínicos dependientes de la paciente o del propio tumor).

Actualmente la BSGC es el método estándar para la estadificación del cáncer de mama operable, y la DRA debe ser limitada a aquellas pacientes con metástasis a los ganglios axilares y debe ser considerada inapropiada e innecesaria cuando los ganglios no estén afectados12. Si el GC es positivo, la axila ya no podrá ser considerada negativa5, aunque no siempre existe metástasis en ganglios no centinela en un paciente con GC positivo.

Debemos siempre considerar que la diseminación hematógena del cáncer de mama puede preceder o coincidir con la diseminación linfática11, aunque esto es en menor proporción, no obstante la BSGC se ha probado como un método seguro para proveer información de los ganglios linfáticos y de ser necesario convertir electivamente o profilácticamente el procedimiento en una DRA11.

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El autor realiza con el presente trabajo una revisión global sobre el ganglio centinela y el cáncer de mama.

Es una revisión que incluye todos los aspectos, desde el concepto a su utilidad pronóstica, está bien estructurada, fácil de leer y bastante completa, con lo que puede ser de gran utilidad para aquellos que quieran tener una visión general de lo que significa el ganglio centinela y sus implicaciones.

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El tratamiento conservador del cáncer de mama implica obtener el máximo de curabilidad con el mínimo de mutilación. Se acepta que el objetivo quirúrgico es la eliminación total del tumor con márgenes suficientes, conservando una morfología mamaria y simetría lo mejor posible. El vaciamiento axilar, la irradiación y terapias farmacológicas u hormonales completan el tratamiento. El estudio del linfonodo centinela significó una nueva posibilidad en la disminución de la agresión quirúrgica. Esta técnica tiene una curva de aprendizaje que obliga al entrenamiento de los equipos, cirujanos y patólogos.

En muchos centros se ha alcanzado una importante experiencia en la técnica de BSGC, lo que ha llevado a reducir la controversia acerca de su uso. Sin embargo, se mantiene vigente la recomendación de validar el método en cada institución, especialmente al utilizarse distintas técnicas en centros diferentes o incluso entre equipos específicos de cirujanos y patólogos en un mismo centro.

El presente artículo representa una interesante revisión de los aspectos teóricos que fundamentan su uso y de aspectos prácticos de su aplicación. La colaboración entre cirujanos, oncólogos y patólogos, incluyendo el seguimiento de los casos y la discusión de

los resultados, es de primordial importancia para el desarrollo y consolidación las de nuevas técnicas terapeúticas.

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HIPOTESIS MEDICA

USO DEL TAMOXIFENO PARA TRATAMIENTO DE LA FIBROSIS SISTÉMICA NEFROGÉNICA

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RESUMEN

La fibrosis sistémica nefrogénica (FSN) es una entidad infrecuente, que se desarrolla en pacientes insuficientes renales en diálisis, vinculándose su etiopatogenia con diversos factores tales como el uso de gadolinio intravenoso, el antecedente de cirugías vasculares, etc.

El Tamoxifeno, es un modulador selectivo (inhibición competitiva) de los receptores estrogénicos, que posee propiedades antifibróticas las cuales se emplean para el tratamiento de entidades tales como: fibrosis retroperitoneal, esclerosis peritoneal asociada a diálisis peritoneal, tumores desmoides y en algunos casos de esclerodermia.

En el presente articulo presentamos la hipótesis original de que el tamoxifeno, por su propiedades fibrinolíticas, podría ser una terapia no inmunosupresora potencialmente útil para el tratamiento de la fibrosis sistémica nefrogénica, evitándose desde ya su uso en pacientes portadores de contraindicaciones para recibirlo.

PALABRAS CLAVE: Tamoxifeno. fibrosis sistémica nefrogénica

SUMMARY

Nephrogenic systemic fibrosis is (NSF) a rare entity which develops in patients with renal failure undergoing dialysis, and its etiology is related to several factors such as the use of intravenous gadolinium, vascular surgeries, etc.

Tamoxifen, is a selective modulator (competitive inhibition) of estrogen receptors, has antifibrotic properties which are used for treating retroperitoneal fibrosis, peritoneal sclerosis peritoneal associated to peritoneal dialysis, desmoids tumors and in some cases of sclerodermia.

In the present article, it is presented the hypothesis of a potential use of tamoxifen as a non immunosuppressant treatment for NSF in patients who have no contraindication for receiving it.

KEY WORDS: Tamoxifen. Nephrogenic systemic fibrosis.

FIBROSIS SISTÉMICA NEFROGÉNICA

La fibrosis sistémica nefrogénica (FSN) es una entidad infrecuente, que se desarrolla en pacientes insuficientes renales en diálisis, vinculándose su etiopatogenia con diversos factores tales como el uso de gadolinio intravenoso, ciertos medicamentos (eritropoyetina, etc), el antecedente de cirugías vasculares, la presencia de hiperfosfatemia, la enfermedad hepática y los estados de hipercoagulabilidad y proinflamatorios.

Esta patología se caracteriza por la acumulación de tejido conectivo en la piel, por lo que esta se engrosa y clínicamente se caracteriza por progresiva induración de la piel, que se adhiere a los planos profundos, que adquiere primero una consistencia duro-elástica y luego duro-pétrea, y que van ocasionando durante su progresión la contractura en flexión de las articulaciones de los miembros. Generalmente comienza por los miembros inferiores y superiores y progresa en sentido centrípeto, comprometiendo también el tronco. El tejido celular subcutáneo, y en ocasiones hasta el músculo, se ven comprometidos. La FSN se desarrolla en un periodo de días o semanas Y puede tener afectación sistémica de otros órganos, habiéndose estimado que el 5% de los pacientes tienen una evolución rápida, progresiva y fulminante (1-10).

Con respecto al gadolinio, se sospecha que en pacientes con deterioro de la función renal, los quelatos de este elemento químico metálico sufrirían un proceso de transmetilación y este proceso conduciría a un aumento del gadolinio libre en el plasma, y a la precipitación del mismo en la dermis y otros órganos. Se ha formulado la hipótesis de que el depósito de estos compuestos causaría injuria tisular y podría actuar atrayendo fibrocitos circulantes hacia la dermis, provenientes estos de la médula ósea, y que por diversos estímulos pasarían a la sangre, y en los tejidos causarían la fibrosis.

De todas las variantes del gadolinio, la gadodiamida es la que más se ha vinculado con el desarrollo de esta entidad, La gadodiamina tiene una estructura lineal, lo cual facilitaría la liberación del gadolineo de sus sitios de unión. Los pacientes con insuficiencia renal grave (filtrado glomerular < 30 ml/min/1,73 m_2) estarían sometidos a un riesgo incrementado de FSN debido a la prolongación del tiempo de eliminación de gadolinio, que se ha estimado de 1,3 horas en voluntarios sanos frente a 34,3 horas en pacientes con insuficiencia renal terminal (11-19)

EL TAMOXIFENO

El tamoxifeno es un modulador selectivo (inhibición competitiva) de los receptores estrogénicos (MSRE o SERMs) que posee efecto estrogénico y antiestrogénico simultáneamente sobre varios tipos de tejidos. Cuando el estrógeno se une a su receptor provoca una serie de modificaciones e interacciones moleculares que culminan con la transcripción de proteínas, entre las cuales se encuentran algunas esenciales para estimular la multiplicación celular, además de que puede inhibir la transcripción de otras que modulan negativamente la progresión del ciclo celular y la división mitótica.

En cuanto al tamoxifeno, cuando éste se une al receptor de estrógeno, el complejo tamoxifeno-receptor se une al ADN, y se desencadena luego un mensaje agonista o antagonista de estrógenos según los elementos promotores presentes según el tipo celular. Así por ejemplo, el tamoxifeno bloquea la actividad del dominio de activación del receptor AF-2, por consiguiente, se manifestará como un antagonista de los estrógenos en todos los ambientes celulares sobre genes que requieran sólo al AF-2; no obstante, en los ambientes donde el AF-1 sea el activador dominante, el tamoxifeno manifestará su habilidad como agonista parcial. Debido a sus características farmacocinéticas, este fármaco puede ser administrado por vía oral, alcanzando concentraciones séricas máximas a las 4-7 horas.

Presenta fuerte unión a la albúmina sérica (>99%), y se metaboliza extensamente en el hígado por el citocromo P450, siendo su metabolito más importante el N-desmetiltamoxifeno, con propiedades terapéuticas similares al tamoxifeno pero del doble de vida media. Sus metabolitos se excretan sobre todo en las heces, y dado que no posee excreción renal, no requiere ajuste de dosis en

nefropatía.

Los principales usos clínicos actuales del tamoxifeno basados en sus propiedades antiestrógenas son: cáncer de mama, melanoma maligno, mastalgias, etc.; mientras que aquellos basados en sus propiedades antifibróticas son: fibrosis retroperitoneal, esclerosis peritoneal asociada a diálisis peritoneal, tumores desmoides y en algunos casos de esclerodermia.

Sus principales efectos adversos reportados en la literatura son:

- **depresión de médula ósea**
- **aparición de trombosis venosas y/o embolias pulmonares**
- **estimulación de hiperplasia endometrial y/o cáncer de endometrio . La posibilidad de un cáncer de útero es mayor en mujeres de más de 50 años y con dosis de 20-40 mg/día durante más de 2 años.**
- **inducción de cataratas o retinopatía**
- **otros: depresión, cefalea, hipercalcemia, edema, bochornos, irregularidades menstruales, flujo vaginal, constipación, movilización enzimática hepática e hipetrigliceridemia**

Estos efectos adversos antes relatados son tanto el fundamento de las contraindicaciones para su prescripción, así como la guía del tipo de monitoreos que deben realizarse durante su empleo

Esta contraindicado su combinación con warfarina, y su uso en embarazo. Las reacciones de tipo anafiláctico son extremadamente raras. Sus interacciones farmacológicas principales son de tipo inhibitoria de su metabolismo (inhibidores de la proteasa anti-retrovírica, ciclosporina, efavirenz, eritromicina, nevirapina, benzodiazepinas, nifedipina, diltizem), así como con inhibidores de la citocromo P450: ciclofosfamida, isofosfamida, etoposide, paclitaxel y los alcaloides de la vinca.

Los inductores enzimáticos, como la carbamazepina, el fenobarbital, la rifampicina, etc reducen su vida media (20-27).

HIPÓTESIS PROPUESTA:

En el presente articulo presentamos la hipótesis original de que el tamoxifeno, por su propiedades fibrinolíticas, podría ser una terapia no inmunosupresora potencialmente útil para el tratamiento de la fibrosis sistémica nefrogénica, evitándose desde ya su uso en pacientes portadores de contraindicaciones para su uso (estados protrombóticos, etc), así como implementarlo realizando los controles adecuados para su uso: hemograma, lipidograma, evaluación oftalmológica y ginecológica. Por supuesto que esta hipótesis, como cualquier otra, debe ser científicamente corroborada antes de aplicarse a la práctica clínica.

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Aunque la hipótesis es plausible, para el tratamiento de la FSN se estan utilizando ya diversosinmunosupresores y el tamoxifeno, aunque su mecanismo de acción principal sea otro, también tiene efecto inmunosupresor.Sería imposible distinguir, en caso de mostrarse útil, cual sería su efecto real. Además, para preferirlo a los inmunosupresores habría que hacer ensayos clínicos y eso va a ser imposible.

La FSN es una entidad nueva (el primer caso es de1997), solo se han descrito hasta ahora alrededor de 200 casos en todoel mundo y parece que su frecuencia comienza a disminuir debido a lostransplantes renales y el mejor tratamiento de la insuficiencia renalaguda. No está claro que tenga otra causa que el gadolinio, así que con la adecuada prevención puede que en pocos años sea una enfermedad histórica.

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El tamoxifeno está autorizado en España para el tratamiento hormonal en pacientes pre y postmenopáusicas diagnosticada de cáncer de mama y receptores hormonales positivos. Es la única indicación aprobada en nuestro pais.

Existen pocos casos publicados en la literatura sobre utilización de tamoxifeno en fibromatosis desmoide, enfermedad de Peyronie, displasia de hueso fibrosa y fibrosis retroperitoneal, con escasa evidencia.

Al tratarse de una hipótesis original, se tiene que considerar como tal, con todas las precauciones y sabiendo que se necesita aplicar el método científico de manera ética para poder contrastar dicha hipótesis.

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MEDICAL HYPOTHESES

USE OF TAMOXIFEN FOR THE TREATMENT OF NEPHROGENIC SYSTEMIC FIBROSIS

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SUMMARY

Nephrogenic systemic fibrosis is (NSF) a rare entity which develops in patients with renal failure undergoing dialysis, and its etiology is related to several factors such as the use of intravenous gadolinium, vascular surgeries, etc.

Tamoxifen, is a selective modulator (competitive inhibition) of estrogen receptors, and it has antifibrotic properties which are used for treating retroperitoneal fibrosis, peritoneal sclerosis associated to peritoneal dialysis, desmoids tumors and in some cases of sclerodermia.

In the present article, it is presented the hypothesis of a potential use of tamoxifen as a non immunosuppressant treatment for NSF in patients who have no contraindication for receiving it.

KEY WORDS: Tamoxifen. Nephrogenic systemic fibrosis.

RESUMEN La fibrosis sistémica nefrogénica (FSN) es una entidad infrecuente, que se desarrolla en pacientes insuficientes renales en diálisis, vinculándose su etiopatogenia con diversos factores tales como el uso de gadolinio intravenoso, el antecedente de cirugías vasculares, etc. El

Tamoxifeno, es un modulador selectivo (inhibición competitiva) de los receptores estrogénicos, que posee propiedades antifibróticas las cuales se emplean para el tratamiento de entidades tales como: fibrosis retroperitoneal, esclerosis peritoneal asociada a diálisis peritoneal, tumores desmoides y en algunos casos de esclerodermia.

En el presente articulo presentamos la hipótesis original de que el tamoxifeno, por su propiedades fibrinolíticas, podría ser una terapia no inmunosupresora potencialmente útil para el tratamiento de la fibrosis sistémica nefrogénica, evitándose desde ya su uso en pacientes portadores de contraindicaciones para su uso.

PALABRAS CLAVE: Tamoxifeno. fibrosis sistémica nefrogénica

NEPHROGENIC SYTEMIC FIBROSIS

Nephrogenic systemic fibrosis is (NSF) a rare entity which develops in patients with renal failure undergoing dialysis, and its etiology is related to several factors such as the use of intravenous gadolinium, certain types of medication (erythropoietin, etc), previous vascular surgeries, hyperphosphatemia, liver disease, hypercoagulability and proinflammatories states.

Clinically, this pathology is characterized by firm plaques in the superior/inferior limbs, which harden and thicken, and could later extend to the trunk, and cause muscle contraction when the joints of such limbs are bended, thus resulting in a progressive reduction of joint mobility. The NSF usually begins in the limbs and then progresses to the trunk, and it can involve the subcutaneous tissue, as well as the muscular one. This disease can developed in a period of days or weeks, and even having a potential systemic compromise, with a fast and severe evolution in 5% of the cases (1-10).

Regarding gadolinium, it is believed that in patients with renal failure, gadolinium chelates would suffer a process of transmetilation, this process would lead to an increase of free gadolinium in the plasma, and to its precipitation in the dermis and other organs. The hypothesis that the deposit of these compounds would cause tissue injury and could attract circulating fibrocytes to the dermis. These fibrocytes could come from the bone marrow. Of all the variants of gadolinium, gadodiamide, has been the one most related to the development of such entity.

This sort of gadolinium has a lineal structure, which would enable the release of gadolinium in its tissue binding sites. Patients suffering from severe renal insufficiency (glomerular filtration rate lower than 30 mi/min/1,73 m_2) are in a greater danger of developing FSN due to an increase in gadolinium elimination time: from 1.3 hours in healthy people to 34.3 hours in severe chronic renal failure patients (11-19).

TAMOXIFEN

Tamoxifen is a selective modulator (competitive inhibition) of estrogen receptors (MSRE or SERMs) which has a simultaneous estrogenic and antiestrogenic effect on the various types of tissues. When estrogen binds to its receptor, it causes a series of molecular changes and molecular interaction which finally produce protein transcription, among which we can mention some that are essential to stimulate cellular multiplication, besides, it can inhibit the transcription of other proteins which negatively module the progression of the cellular cycle and mitotic division.

As far as tamoxifen is concerned, when it binds to its estrogen receptor, the complex tamoxifen-receptor binds to DNA, and this causes an agonist or antagonist message of estrogen according to the cellular type. So, for example, tamoxifen blocks the dominium activation activities of the receptor AF-2, thus, it will be an estrogen antagonist in all the cellular environments on genes which only need AF-2; nevertheless, in the environments where AF-1 is the dominant activator, tamoxifen will manifest its ability as a partial agonist. Due to its pharmacocynetic characteristics, this drug can be administered orally, reaching maximum serum concentrations 4-7 hours later.

It shows a strong binding to serum albumina (>99%), and it is extensively metabolized in the liver through the cytochrome P450. Its most important metabolite is N-desmetiltamoxifen, which has similar therapeutic properties to tamoxifene but half of its average life. Its metabolites are excreted mainly through faeces, and since it is not excreted through the kidney, it does not need adjustments of the doses in nephropathy.

The main clinical uses of tamoxifen based on its antiestrogen properties are: breast cancer, malign melanoma, mastalgia, etc.; while those based on antifibrotic properties are: retroperitoneal fibrosis, peritoneal sclerosis associated to peritoneal dialysis, desmoids tumors and in some cases of sclerodermia.

Its principal reported side effects are:

- **depression of the bone marrow**
- **appearance of venous thrombosis and/or pulmonary embolism**
- **stimulation of the endometrial hyperplasia and/or endometrial cancer. The possibility of having uterine cancer is higher in women older than 50 years old and with doses of 20-40 mg/day for more than 2 years.**
- **appearance of cataracts or retinopathies**
- **other: depression, cephalea, hypercalcemia, oedema, hot flashes, menstrual irregularities, vaginal flux, constipation, enzymatic mobilization in the liver and hypetriglyceridemia**

These side effects are the fundament of contraindication for its use as well as the types or monitoring which should be carried out during its use. It is not advisable to use it in combination with warfarin, or during pregnancy. Anafilactic reactions are extremely rare. Its main pharmacological interactions are of the inhibitor type of its metabolism (inhibitors of the anti-retroviral proteasa, cyclosporin, efavirenz, eritromicin, nevirapin, benzodiazepines, nifedipin, diltizem), as well as inhibitors of cytochrome P450: cyclofosfamide, isofosfamide, etoposide, paclitaxel and the alcaloids of the vinca.

Enzymatic inducers, such as carbamazepine, fenobarbital, rifampicine, etc reduce its average life.

PROPONED HYPOTHESIS:

In this article we present the original hypothesis that tamoxifen, due to its fibrinolitic properties could be a non immunosuppressant therapy, potentially useful for treating nephrogenic systemic fibrosis, but avoiding its use in patients who have contraindications to its use (prothrombotic status, etc), as well as implementing it by doing the corresponding controls: hemogram, lipidogram, oftalmological as well as gynecological evaluation. Of course, this hypothesis, as any one, must be scientifically confirmed before its clinical application.

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Comment of the reviewer Ramón Díaz-Alersi MD. Servicio de Medicina Intensiva. Hospital Puerto Real. Cádiz. España

Even though, this hypothesis makes sense, there are already several conventional immunosuppressant drugs in use for treating FSN. Since tamoxifen also has immunosuppressant effect, if this medication would be useful for treating FSN, it would be difficult to identify which of its properties, anti fibrotic or immunosuppressant, would explain its effect. Regarding if tamoxifen could replace conventional immunosuppressant drugs for treating FSN, before that it might perform a clinical trial in this sense, something that seems to be impossible.

FSN is a new entity (first case in 1997), and there are currently just 200 cases described in the world. Its incidence seems to be going down due to kidney transplant practicing and better handling of acute renal failure. Since, there is no clear cause of FSN apart from gadolinium exposition, an adequate medical preventive behaviour could become this entity a historical one in the near future.

Comment of the reviewer Blanca de la Nogal.Servicio de Farmacia. Complejo Asistencial de Burgos. España

Tamoxifen is authorized only for treating pre and post menopausal patients who suffer from breast carcinoma with positive receptors in Spain.

There are few cases in the literature regarding tamoxifen use in desmoid tumors, Peyronie disease, bone fibroses dysplasia, and retroperitoneal fibrosis, with scarce evidence for its use. Since this is just a hypothesis, it must take into account that it has to be

scientifically confirmed before its clinical use.

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Letters to the Editor / Cartas al Editor

MAY A PROBLEM-BASED LEARNING CURRICULUM ENTAIL PROBLEMS?

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Sr. Editor:

Currently, there are in Argentina 26 medical schools, 10 are public and 16 are private[1]. Public education is free and public universities are autonomous although entirely dependent on the government for income. Students enter to a six-year medical curriculum right after high school (average age = 17 ± 1 years old; mean ± standard deviation) since no intermediate stages (college or similar) exist between secondary education and university. Furthermore, there exists a wide variety of admission policies resulting in a wide range of medical school admission numbers (from about 150 ± 70 to 1.500 ± 500 matriculates/year)[2].

When our public medical school at Rosario, Argentina, engaged in implementing a pure problem-based learning (PBL) curriculum, the basic requirements for successful implementation of that format, the difficulties that such curriculum could face, and the need for an unbiased and continuous monitoring and adjustment were made public, proposing simultaneously an alternative hybrid format in view of some arisen problems shortly after its first-year implementation[2,3]. However, the curriculum planners' decision was to go on with the program assuming that potential impediments for PBL curriculum implementation could be overcome.

After six years of implementation, this letter refers to the fulfillment of the aforesaid basic requirements and to some teachers' working experiences dealing with the first three years of the curriculum design intending to be helpful for curriculum planners, particularly in developing countries, in terms of being aware of potential drawbacks and ways for solving them.

Earlier reports on this subject[4-5], university database[6]and National Commission for the Evaluation and Accreditation of Universities (CONEAU)recommendations[7] were considered for comparing the aforesaid basic requirements with our present scenario. In parallel, a personal interview was carried out during 2007 to a representative and reliable group of 20 medical teachers of both sexes (48 ± 12 years old, mean ± standard deviation) selected from a population of approximately 200 teachers.

The obtained results revealed that the referred basic requirements and the improvements recommended during 2005 by CONEAU could not be fulfilled at all by internal and external reasons[8-10], despite some efforts made in this regard.

In turn, interviewed teachers identified a set of problems likely to be solved:

- **(a) a predominance of triggering enunciates instead of real medical problems,**
- **(b) a reduced background for understanding physiopathology, pharmacology and its related clinical and therapeutic contents resulting from a weak morphophysiological core,**

- **(c) poor training for correlating and integrating bio- psycho - social contents, essential for this format, The same occurred in relation with the scientific attitudes and skills despite the inclusion of a 3-month course in scientific research methodology,**
- **(d) heterogeneous evaluations resulting from different pedagogical, scientific and disciplinary teachers' expertise,**
- **(e) frequent replacement of self-learning under expert supervision, another key issue, for self-directed learning,**
- **(f) lecture lacks,**
- **(g) inadequacy in internet-based searches in many students because of their shortcomings in reading, writing and managing native and foreign languages, and**
- **(h) development of cross-disciplinary areas not suitably based on well-defined and balanced disciplines. Attention must be paid to the agreement existing between our teachers' perceptions and some students' initiatives addressed to solve the aforesaid concerns.**

Exceeding the bibliographic pros and cons [11-21] and whatever the underlying reasons for the adopted and sustained decision, an action to be faced is a quick, up-to-date and reality-grounded adjustment of the current curriculum, compatible with the CONEAU recommendations. Summing up, the best of former curricula must be retrieved, and the best of the new trends in medical education must be added, for achieving a reliable curricular hybridization.

To conclude, the lessons to take home were: (a) the greater the curriculum change the better the outcomes to be required, (b) the best ideas and purposes[22] and the most promising formats may become problematic when the contextual and operational factors are not fully considered for its implementation, and (c) an advisable flexibility must prevail whatever the curricular design being proposed[23].

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