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ABSORPTION OF *MYO*-INOSITOL HEXAKISPHOSPHATE ($InsP_6$) THROUGH THE SKIN: STUDY OF THE MATRIX EFFECTS. MECHANISM OF PHYTATE TOPICAL ABSORPTION

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1 . ABSTRACT

Myo-inositol hexakisphosphate ($InsP_6$, phytate) is a molecule to which diverse beneficial properties have been attributed. Some of these properties are related to its dermatological use as discolouring agent, on preventing calcinosis cutis or due to its important role on premature aging. Other studies also seem to demonstrate a capacity of $InsP_6$ to inhibit skin cancer. In this paper, the effect of the vehicle of topical administration of phytate is studied, using four groups of male Wistar rats (n = 6) fed with an $InsP_6$ defficient diet and treated with a hydrophyl gel or an O/W moisturizing cream with two different concentrations of $InsP_6$. Due to the correlation between $InsP_6$ absorption and its urinary excretion, these last values were used to evaluate this process. It was found that phytate was absorbed through the skin using both a gel or a cream, demonstrating that its absorption is independent on the matrix used for topical treatment. However, urinary $InsP_6$ values were slightly higher when using the gel, but in all cases values were much higher than those found with oral $InsP_6$ treatment, due to the formation of insoluble species in the gastrointestinal tract when $InsP_6$ is administered orally.

2. INTRODUCTION

Myo-inositol hexakisphosphate ($InsP_6$) is an abundant molecule in plant seeds, that ranges from 1.5 % to 6.4 % in whole grains and forms an insoluble salt with calcium and magnesium ions called phytin (1, 2). Phytate is a substance naturally present in all mammalian organs, tissues and biological fluids (3). The levels found in blood, urine and tissues clearly depend on the dietary intake (4-6).

Although many studies attributed "anti-nutritional" properties to phytate (7-11), other studies (12-17) have demonstrated that with equilibrated diets together with adequate doses of phytate non harmful effects were observed. Furthermore, many beneficial effects on human health have been demonstrated, such as its properties as an antioxidant (18, 19), its role in colon cancer prevention (20, 21) and anti-platelet activity (22). Above all these effects, the $InsP_6$ present in urine and biological fluids exerts an important role in preventing pathological calcifications as renal calculi (23-25) or calcinosis cutis (26), due to its powerful capacity to act as crystallization inhibitor of calcium salts. Finally, the latest discoveries about the properties of $InsP_6$ are related to its dermatological use, acting as an important discolouring agent of the skin or against premature aging (27). Also, some studies demonstrate a capacity of $InsP_6$ to inhibit skin cancer (28, 29) and to avoid calcinosis cutis (30).

In spite of these facts, little is known about the gastrointestinal and skin absorption of phytate. Previous studies on oral intake of phytate, demonstrated that its absorption is independent on the salt used and reaches a maximum, that corresponds to 2 % of the ingested amount, above which no further absorption ocurrs (4, 5). Also, a maximum level of absorption was observed when applying phytate topically on rats (30) and this level was attained after about 14 days of treatment. However, it is clear that there are many aspects to be clarified about phytate absorption. In this paper, a study about the effect of the matrix of phytate application on its absorption is presented.

3. MATERIALS AND METHODS

3.1. Animals, diets and experimental design

Twenty-four male Wistar rats of approximately 250 g from Harlan Iberica s.l. (Barcelona, Spain) were acclimated in the course of 7 days to our animal house. Animals were kept in Plexiglas cages (two animals per cage) at a temperature of 21 ± 1 ºC and relative humidity of 60 ± 5 % with a 12-h on-off light cycle. After this period, animals were randomly assigned into four groups of six rats respectivelly. Rats were fed with AIN-76A

Table 1. Composition of AIN-76 A diet, a purified diet in which $InsP_6$ is undetectable

Ingredient	(g/kg dry wt)
Proteins	203
Carbohydrates	650
Lipids	50
Cellulose	50
Ashes	47
Calcium	3.91
Magnesium	0.47
Zinc	0.028
Phosphorus	7.20
Phytate	undetectable
Water	58

Table 2. Composition of $InsP_6$ creams used

Component	Percentage composition	
	2 % $InsP_6$ (potassium salt) cream	4 % $InsP_6$ (potassium salt) cream
Potassium $InsP_6$	2.7 (2 % $InsP_6$)	5.4 (4 % $InsP_6$)
Almond oil	4	4
Thomil ISF	3.8	3.8
Stearic acid	1	1
Lactic acid	1.6	1.6
Vitamin F 09929	2.5	2.5
Monestriol GAE	4	4
Propyl Paraben	0.1	0.1
Pumol 1618	4	4
Controx VP	0.03	0.03
Water	70.2	67.5
Triethanolamine	0.1	0.1
Laurylate S-90	0.3	0.3
Glycerine 3699 USP	4.87	4.87
Methyl Paraben	0.2	0.2
Abiol	0.3	0.3
Essence	0.3	0.3

Table 3. Composition of $InsP_6$ gels used

Component	Percentage composition	
	2 % $InsP_6$ (potassium salt) gel	4 % $InsP_6$ (potassium salt) gel
Water	89.1	86.4
Propilenglycol	6	6
PNC 400	2	2
Astro C-40	0.2	0.2
Potassium phytate	2.7 (2 % $InsP_6$)	5.4 (4 % $InsP_6$)

diet (Ssniff Especialdiäten GmbH, Soest, Germany), a purified diet (Table 1) in which $InsP_6$ is undetectable.

After a period of 16 days consuming such diet, during which the urinary $InsP_6$ became undetectable, rats were topically treated once a day with 4 g of a standard cream with a supplement 2.0 and 4.0 % of $InsP_6$ as potassium salt (Table 2) or with a gel containing 2.0 and 4.0 % of $InsP_6$, also as potassium salt (Table 3). The surface of treatment was about 50 cm^2. Samples of 24-h urine were collected at days 0, 7 and 14 of treatment to evaluate $InsP_6$ excretion by using a metabolic cage (Tecniplast, Gazzada, s.a.r.l., Italy).

Animals were sacrified at the end of the experiment. The procedures used in this experiment were carried out according to the Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes and official permission to perform this animal experiment was obtained from the Bioethical Committee of our University.

3.2. $InsP_6$ determination

The determination of $InsP_6$ levels in urine samples was performed using an analytical methodology based on column separation and following total phosphorus determination by inductively coupled plasma atomic emission spectrometry (ICP-AES) (31). This methodology allows a measurement of total $InsP_6$ with a detection limit of 60 μg/l.

3.3. Procedure

5.0 ml of urine (acidified with HCl 1:1 until pH = 3-4) was transferred to a column containing 0.2 g of anion exchange resin (the inner diameter was 4 mm). The first eluate was discarded, then the column was washed with 50 ml of HCl 50 mM. The second eluate was discarded. Then, the column was washed with 3.0 ml of HNO_3 2 M. The determination of $InsP_6$ was carried out through direct phosphorus analysis of this last eluate by ICP-AES using the corresponding calibration curve.

The ICP-AES conditions used were the following: outer argon flow 15 l/min, auxiliar argon flow 1 l/min, inner argon flow 1 l/min, nebulizer uptake rate 1 ml/min and wavelenght 213.618 nm.

3.4. Statistics

Values in the figure are expressed as mean (SE). The Student *t*-test was used to assess differences of means. Conventional Windows software was used for statistical computations. A value of $p < 0.05$ was considered to assess statistical significance.

4. RESULTS

The obtained results of $InsP_6$ urinary concentrations with four different topical treatments are shown in Figure 1. It can be seen that after 7 days of treatment, there were no statistical differences between 2 % $InsP_6$ O/W cream and gel (16.2 ± 8.7 and 14.3 ± 3.5 mg/l respectively) and between 4 % $InsP_6$ O/W cream and gel (34.8 ± 16.7 and 94.6 ± 23.4 mg/l respectively). For this period of time, the $InsP_6$ excreted urinary amount did not depend on the vehicle of $InsP_6$ administration. After fourteen days of treatment there were no significant differences between 2 % $InsP_6$ O/W cream and gel (36.2 ± 7.3 and 67.3 ± 12.9 mg/l respectively), but $InsP_6$ urinary values were higher with 4 % $InsP_6$ gel treatment (154.8 ± 19.9 mg/l) than those found with 4 % $InsP_6$ cream (47.8 ± 27.2 mg/l), for the same period of time.

A dose-response relationship was observed at days 7 and 14 for rats treated with both $InsP_6$ gels. This behaviour was not so clear in rats treated with O/W cream. However, a slight but not statistically significant increase was found when using the 4 % $InsP_6$ cream.

5. DISCUSSION

Although many $InsP_6$ based creams can be found in the market and several dermatological applications have been described, little is known about its absorption through the skin. Previous studies, demonstrated that topically administered phytate was able to inhibit the formation of subepithelial dystrophic calcifications in soft tissues (24) and that absorption of phytate depended on the salt used,

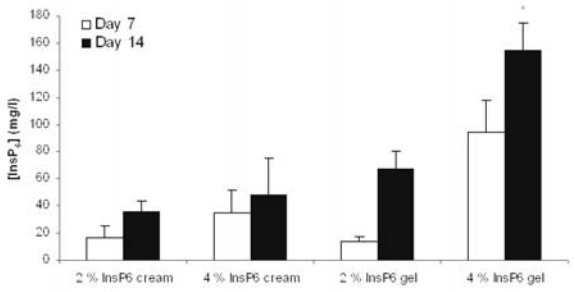

Figure 1. $InsP_6$ concentrations in urine at two different periods of time when applying different $InsP_6$ concentrations with different topical vehicles of administration (cream or gel) * $p < 0.05$ vs 4 % $InsP_6$ cream (day 14).

thus, absorption was higher when the salt was more soluble (30). From both studies it is unquestionable that phytate, in spite of being a multicharged molecule, is able to cross the epidermis and dermis, enter the blood stream and increase the urinary excretion. From the results here presented, it is clear that this absorption occurs using either a standard moisturizing cream or a gel, although they are rather different matrixes for phytate. The moisturizing cream used is an O/W cream (oil in water) where water is the major component and organic oils are forming micels. Also, in these organic micels very small aqueous drops can be found. On the other hand, a gel is a mainly aqueous matrix (approximately 90 %) with an organic polymer which gives consistency and allows the topical application. Phytate, as potassium salt, is dissolved in water when using the gel; in the case of the cream, phytate is surely dissolved in the aqueous part (due to its electric charge). The fact that phytate is in the same form and medium, independently on the matrix used, explains the fact that phytate is absorbed through the skin using both a gel and a cream.

From the obtained results, it seems that absorption of phytate using the gel is higher (despite significant differences were only found after 14 days and using a 4 % $InsP_6$ gel and cream). These could be attributed to the fact that part of the phytate in the cream is in the small water drops found in the interior of the micels and this would make its absorption more difficult.

Furthermore, comparing the obtained urinary values with those found through oral intake (24), it is deduced that with topical administration phytate reaches much higher urinary levels. It is explained if we consider that in the gastrointestinal tract phytate can form insoluble species with divalent and trivalent cations and with proteins, that come from the diet, and that can not be absorbed. The formation of these non- absorbable species is not so feasible using a topical treatment. However, in some aspects, absorption through the gastrointestinal tract and through the skin are similar and probably the mechanism of absorption is also similar. Thus, in both gastrointestinal and topical absorption, phytate enters the organism through a passive diffusion mechanism across the space between cellular unions in the gastrointestinal tract and mainly through the pores in the skin.

As a conclusion, phytate is a highly charged molecule that, dissolved in water, is absorbed through the skin, but further studies must be developed to clearly establish the real mechanism of absorption.

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