(54) Titre : UTILISATION DE PHYTATE COMME AGENT INHIBITEUR DE LA DISSOLUTION DE CRISTAUX DE SELS CALCIIQUES DANS LA PREVENTION DE L'OSTEOPOROSE
(54) Title: USE OF PHYTATE AS AGENT INHIBITING DISSOLUTION OF CRYSTALS OF CALCIUM SALTS FOR THE PREVENTION OF OSTEOPOROSIS

(57) Abrégé/Abstract:
The present invention relates to the utilisation of myo-inositol hexaphosphate or pharmaceutically-acceptable salts thereof for the manufacture of a medicament destined for the prevention or treatment of a disease associated with the dissolution of crystals of calcium salts, in particular osteoporosis. Said compound may be utilised in the manufacture of functional foods, dietetic complements, vitamin complements or nutritional complements or food complements or phytotherapeutic products having properties of inhibition of dissolution of crystals of calcium salts.
ABSTRACT

The present invention refers to the use of myo-inositol hexaphosphate or pharmaceutically acceptable salts thereof for the manufacture of a medicament for the prevention or treatment of an disease associated with the dissolution of crystals of calcium salts, in particular osteoporosis.

Said compound may be utilised in the manufacture of functional foods, dietetic complements, vitamin complements, nutritional complements or food complements or phytotherapeutic products having properties of inhibition of dissolution of crystals of calcium salts.
USE OF PHYTATE AS AGENT INHIBITING DISSOLUTION OF CRYSTALS OF CALCIUM SALTS FOR THE PREVENTION OF OSTEOPOROSIS

Field of the invention

This invention relates to the use of phytate (myo-inositol hexaphosphate) as an agent inhibiting the dissolution of crystals of calcium salts, particularly calcium phosphate.

In particular, in the medical field, the present invention relates to the use of phytate in the prevention of osteoporosis.

The present invention also relates to various compositions containing phytate for the inhibition of dissolution of crystals of calcium salts.

Background of the invention

Since the 1960s, when inhibitors of crystallisation were first talked about, many substances were classified as inhibitors due to their ability to prevent or reduce the formation of crystals. However, there is a shortage of substances that prevent the dissolution of already formed crystals, especially in living systems.

The dissolution of already formed salts is especially relevant in some disorders such as osteoporosis. Osteoporosis is a reduction of bone mass and mechanical strength leading to susceptibility to fractures. It is the main cause of bone fractures in post-menopausal women and in old people in general. Osteoporosis does not have a well-defined beginning and until recently, the first visible sign of the disease was often a fracture of the hip, wrist or of vertebrae that gave rise to pain or deformation. Menopause is the main cause of osteoporosis in women due to a reduction in oestrogen levels. Osteoporosis affects one out of every five women aged over 45 and four out of every ten aged over 75.

The best treatment for osteoporosis is prevention. An adequate calcium intake and physical exercise during adolescence and youth can increase the density of bone mass, which results in a reduction in bone mass loss and in less risk of fractures in later years. Adequate consumption of calcium and vitamins during maturity is essential for bone health. Hormone replacement therapy requires strict gynaecological control and careful selection of patients. In post-menopausal women with low bone mass or with established osteoporosis and
for whom hormone replacement therapy is counterindicated, bisphosphonates (alendronate or etidronate) and calcitonin are effective medicaments for preventing bone loss.


Surprisingly, the inventors of the present invention have found that the high adsorption capacity of phytate on calcium salts can be utilised to prevent the dissolution of already precipitated calcium salts, introducing a new property to phytate that has direct repercussions on certain disorders, such as osteoporosis, making it possible to utilise it to treat this disease.

The document that comes closest to the invention in the state of the art is the Chinese patent CN1295862. In summary, the patent discloses a method to treat osteoporosis based on the reaction between egg shell and acetic acid,
forming calcium acetate that is used as a calcium complement for the patient. At the same time, lysozyme and a protein of phytic acid (not phytate directly, but a different compound) were utilised to regulate the absorption of calcium, which is the agent utilised to act against osteoporosis.

In patents US 5057507, US 5015634 and WO9109601, isomers of inositol triphosphate are disclosed for the preparation of medicaments for treatment of bone disorders. As indicated in the description of this invention, the compound of the present invention, due to its structure, presents higher inhibitory potential on crystals of calcium salts and consequently provides more effective medicaments for the treatment of bone disorders, such as osteoporosis.

**Object of the invention**

The object of the present invention is to find new applications of myo-inositol hexaphosphate (hereafter referred to as phytate) related with the properties described in the background of the invention section.

The objective of the present invention is a composition that includes phytate to prevent the dissolution of crystals of calcium salts.

The applications of phytate described below have not been previously disclosed and their use can be beneficial for the treatment of certain pathologies. In particular, it has been found that the composition including phytate has an inhibitory effect on the dissolution of crystals of calcium salts, such as calcium phosphate, a fact that enables this composition to be utilised in the treatment of osteoporosis.

**Brief description of the figures**

Figure 1 is a graph showing the quantity of calcium dissolved on treating hydroxyapatite, which has been previously treated with various concentrations of phytate at pH7.4, for a period of 24 hours.

Figure 2 is a graph showing the quantity of calcium dissolved on treating hydroxyapatite for a period of 24 hours in the presence of various concentrations of phytate at pH5.

**Description of the invention**

The present invention relates to the use of phytate (myo-inositol hexaphosphate) or any of the pharmaceutically acceptable salts thereof or mix-
tures of both for the manufacture of a medicament for the prevention or treatment of diseases associated with the dissolution of crystals of calcium salts, osteoporosis in particular.

The present invention also relates to the use of phytate for the manufacture of an inhibitor of dissolution of crystals of calcium salts, particularly calcium phosphate.

The present invention also relates to the manufacture of a composition, such as a functional food, dietetic complement, vitamin complement, nutritional complement, food complement or phytotherapeutic product that includes phytate, for avoiding or preventing dissolution of crystals of calcium salts.

In the present invention, it is understood that "phytate" or "myo-inositol hexaphosphate" is the molecule that corresponds to the formula:

![Phytate Structure]

and its pharmaceutically acceptable salts, which include but are not limited to salts with sodium, potassium, calcium, magnesium and calcium-magnesium.

Phytate is the most naturally abundant inositol phosphate, occurring at high concentrations in cereals, pulses, nuts and seeds in general, in the form of an insoluble salt known as phytine (mixed calcium and magnesium salt). In fact, phytate represents the greatest source of phosphorus for seeds during germination, reaching between 50% and 80% of the total phosphorus. The presence of phytates in biological fluids (blood, urine, saliva, interstitial fluid) of mammals has been clearly demonstrated. Most of the extracellular phytate (in tissues, organs and biological fluids) comes from exogenous sources (mainly dietetic although it can also be applied topically or by other administration routes) and is not a consequence of endogenous synthesis. The physiological levels needed for the molecule to exercise its biological activity depend on exogenous administration, either oral, topical or via injection and in the form of a functional food, vitamin complement or drug.
In this invention, an "inhibitor of dissolution" is understood to mean a substance that is capable of preventing or reducing the re-dissolution of already formed salts.

This composition can be administered by any known route, such as oral, parenteral, topical, subcutaneous, intravenous or intramuscular as the biological activity of phytate as an inhibitor of dissolution depends on exogenous sources.

It is well known by persons skilled in the art that the inhibitors of crystallisation, in this case phytate, exert their action by their capacity to adsorb on the surface of the crystal or crystalline nucleus in formation. The high negative electrical charge of phytate and the spatial disposition of its phosphate groups (phytate is the only inositol polyphosphate identified in eukaryotic cells that has the phosphate groups in positions 1, 2, 3 equatorial-axial-equatorial) give it much higher capacity to adsorb onto crystal surfaces than other compounds, particularly the other inositol phosphates with a smaller number of phosphate groups, such as inositol triphosphate disclosed in US patents US5057507 and US5015634.

The apparently contradictory fact that phytate is one of the inhibitors of crystallisation of calcium salts (both as regards nucleation and crystal growth) and that it is also effective in preventing their dissolution can be clearly explained by considering the mechanism of formation and destruction of a crystal. Thus, as was previously indicated, the action of phytate as inhibitor of crystallisation is attributed to its capacity to adsorb on the surface of the crystal or crystalline nucleus in formation, preventing the arrival of new atoms of material, in this way preventing the crystal from growing or the crystalline nucleus from reaching its critical size. At the same time, the adsorption of the inhibitor on the critical points of the crystal surface contribute to its stabilisation, preventing the material of the crystal from passing into solution, in this way preventing the process of crystal destruction (dissolution). The inhibitor therefore acts in both directions, preventing the process of formation but also stabilising the already formed solid, preventing both its subsequent growth and its dissolution.

Phytate can be the only active principle of the composition utilised.
However other active pharmaceutical principles may also be present, or it can be presented in the form of functional foods, dietetic complements, food complements, vitamin complements, nutritional complements or phytotherapeutic products because, as has been previously described, its bioavailability depends on exogenous supply.

In the manufacture of a phytate medicament, it can be used with a common pharmaceutically acceptable additive, an excipient or a vehicle.

**Examples of embodiment of the present invention**

The present invention is illustrated by the following examples that do not limit its scope in any way.

**Example 1**

Three homogenous suspensions of crystallised calcium phosphate in the form of hydroxyapatite in TRIS buffer at pH7.4 were prepared. These suspensions were stirred for 8 hours in the presence of 1, 3 and 12μM of phytate respectively. Later, the suspensions were filtered and the amount of phytate adsorbed on the hydroxyapatite crystals was determined by difference between the initial and final phytate in the solution at pH7.4. The results obtained indicated that 62, 66 and 56% respectively of phytate present in the solution remained fixed to the calcium phosphate structure (hydroxyapatite), showing that phytate adsorbs strongly on calcium salts and can exercise actions related to this property.

**Example 2**

A homogenous suspension of crystalline calcium phosphate in the form of hydroxyapatite in TRIS buffer at pH7.4 was prepared. This suspension was stirred for 8 hours. Then the crystals obtained were filtered and dried to constant weight. Then these crystals were resuspended at pH5.0 (in acetate buffer) and the kinetics of dissolution of the salt was determined over 24 hours, continuously stirring the system. The kinetics were followed by determining the quantity of dissolved calcium and phosphorus by atomic emission spectroscopy (using inductively coupled plasma).

This experiment was repeated using concentrations of 1, 6 and 12μM of phytate during the stage at pH7.4 with the aim of fixing this compound on the
hydroxyapatite structure.

The results obtained are shown in Figure 1. They show that the phytate fixed on the calcium phosphate structure (hydroxyapatite) is capable of inhibiting the dissolution of the salt.

**Example 3**

A homogenous suspension of crystalline calcium phosphate in the form of hydroxyapatite in TRIS buffer at pH7.4 was prepared. This suspension was stirred for 8 hours. Then the crystals obtained were filtered and dried to constant weight. Then these crystals were resuspended at pH5.0 (in acetate buffer) and the kinetics of dissolution of the salt was determined over 24 hours, continuously stirring the system. The kinetics were followed by determining the quantity of dissolved calcium and phosphorus by atomic emission spectroscopy (using inductively coupled plasma).

This experiment was repeated using concentrations of 12 and 24uM of phytate in the pH5 stage with the aim of studying the inhibitory effect of dissolution by phytate present in the solution.

The results obtained are shown in Figure 2. They show that the phytate added at the stage of dissolution of hydroxyapatite (pH5) is also capable of inhibiting the dissolution of the salt.

**Example 4**

A study was carried out evaluating the effect of phytate consumption on the level of bone mass measured by axial densitometry and peripheral heel densitometry. In total, 433 axial densitometer measurements and 1473 peripheral heel densitometer measurements were made. The consumption of phytate by all subjects was evaluated by using a dietetic questionnaire. Subjects were classified into 4 groups (group 1, group 2, group 3 and group 4) according to increasing levels of phytate consumption. The results of the average T-score were as follows:

- Spinal column: group 1 (-1.48 SD 1.255), group 2 (-0.876 SD 1.135)$^a$,
- group 3 (-0.557 SD 1.349)$^a$, group 4 (-0.428 SD 1.219)$^{a,b}$.
- Femoral neck: group 1 (-0.774 SD 1.016), group 2 (-0.166 SD 1.109)$^a$,
- group 3 (-0.02 SD 1.188)$^a$, group 4 (0.168 SD 1.132)$^{a,b}$. 
Heel bone: group 1 (-0.664 SD 1.092), group 2 (-0.1411 SD 1.077)$^a$, group 3 (0.3221 SD 1.167)$^{a,b}$, group 4 (0.3283 SD 1.242)$^{a,b}$.

$^a p < 0.05 v$ group 1; $^b p < 0.05 v$ group 2

These results indicate that the consumption of a diet rich in phytate results in higher bone mass. Therefore phytate has clear potential for the prevention and treatment of osteoporosis.

**Example 5**

Composition containing 120mg phytine (calcium-magnesium phytate) and dietetic fibre as a vehicle.

Having demonstrated the positive influence of phytate on bone mass and knowing the relationship between the physiological phytate levels and its exogenous supply, this example composition (which can be used as a pharmaceutical composition or nutritional complement) can be proposed for prevention/treatment of osteoporosis.

**Example 6**

Adding cereals rich in phytate (calcium-magnesium salt) to a yogurt. Having demonstrated the positive influence of phytate on bone mass and knowing the relationship between the physiological phytate levels and its exogenous supply, this example functional food can be proposed for prevention/treatment of osteoporosis.

**Example 7**

Composition of a typical gel for topical application

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>90</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>6</td>
</tr>
<tr>
<td>PNC 400</td>
<td>2</td>
</tr>
<tr>
<td>Sodium phytate</td>
<td>2</td>
</tr>
</tbody>
</table>

Having demonstrated the positive influence of phytate on bone mass and knowing the relationship between the physiological phytate levels and its exogenous supply, this example composition for topical application can be proposed for prevention/treatment of osteoporosis.
CLAIMS

1. Use of myo-inositol hexaphosphate with formula:

![Chemical Structure](image)

or pharmacologically acceptable salts thereof or mixtures of both for the manufacture of a medicament for the prevention or treatment of a disease associated with the dissolution of crystals of calcium salts.

2. Use according to claim 1, where said disease associated with the dissolution of crystals of calcium salts is osteoporosis.

3. Use of myo-inositol hexaphosphate for the manufacture of an agent for inhibiting the dissolution of crystals of calcium salts.

4. Use according to any of the claims 1 to 3, where said salt is calcium phosphate.

5. Use according to any of the previous claims for the manufacture of a functional food.

6. Use according to any of the previous claims for the manufacture of a dietetic complement.

7. Use according to any of the previous claims for the manufacture of a vitamin complement.
8. Use according to any of the previous claims for the manufacture of a nutritional complement.
Fig 1.

![Graph showing Ca (mg/L) vs time (h) for different concentrations of IP6.](image)

Fig 2.

![Graph showing Ca (mg/L) vs time (h) for different concentrations of IP6.](image)