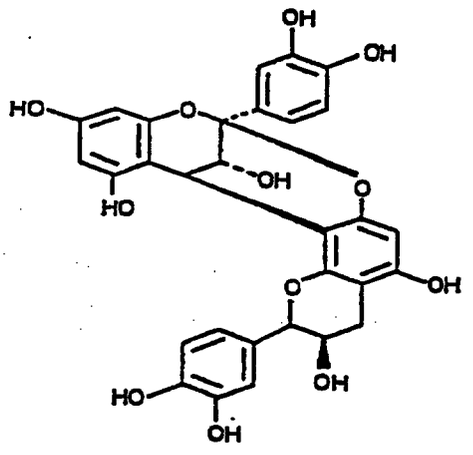
PHOSPHOLIPID COMPLEXES OF PROANTHOCYANIDIN A2 AS ANTI-ATHEROSCLEROTIC AGENTS

The present invention relates to phospholipid complexes of proanthocyanidin A2 or of extracts enriched in proanthocyanidin A2 and the use thereof for the preparation of medicaments for the prophylaxis and the therapy of atherosclerosis, and myocardial and cerebral infarction.

Proanthocyanidin A2 or 8,14-methano-2H,14H-1-benzopyrano[7,8-d][1,3]benzodioxocin-3,5,11,13,15-pentol-2,8-bis-(3,4-dihydroxyphenyl)3,4-dihydro[2R-2α,3α,8ß,14ß,15R] of formula (I)



(I)

was isolated from seed of Aesculus hippocastanum (Tetrahedron Lett., 429, 1966). The therapeutical use of proanthocyanidin A2 as cicatrising, cytoprotective, anti-ulcer, venotonic, vasoprotective and antiperoxidative agent is disclosed in EP-A-210785 (February 4, 1987).

It has now surprisingly been found that the phospholipid complexes of proanthocyanidin A2 exert a marked antiatherosclerotic activity both in animals and in humans when administered systemically, preferably through the oral route.

The complexes of the invention can consist of natural or synthetic phospholipids, such as lecithins, phosphatidylcholine, phosphatidylserine, phosphatidyl-ethanolamine. The ratio of proanthocyanidin A2 to

phospholipids ranges from 2:1 to 1:2, and preferably is about 1:1.5 w/w. A particularly preferred complex is that with soy phosphatidylcholine.

The complexes of the invention are prepared by reacting a solution of the phospholipid with a solution of proanthocyanidin A2 in suitable solvents, such as acetone, ethyl acetate, ethanol, then concentrating the reaction mixture under reduced pressure to obtain a thick residue which can be ground.

The complexes of the invention dose-dependently prevent or reduce the formation of atherosclerotic plaques. The activity was evidenced in rabbits fed with a hypercholesterolemic diet so as to induce atherosclerotic lesions similar to the human ones at the vasal level, particularly at the aortal arch, ventral aorta, carotids and cerebral vessels. In said model, the above mentioned phospholipid complexes change the macro- and microscopical vascular condition reducing, compared with untreated animals, both the number and the severity of the atheromatous plaques, with surprising vascular-tissutal benefit. In another atherosclerosis model, with the purpose of cerebral protection, wherein the vasal lumen of rabbit internal carotid had been surgically reduced while administering a hypercholesterolemic diet rich in saturated fats, a decrease in carotid obstruction, a reduction of vasal walls thickness and increased survival of the animals were observed. Atherosclerotic patients showed, after six month-treatment, a reduction of carotid obstruction due to atheromatous plaques and an improved carotid flow, evaluated by Doppler ultrasonography.

The phospholipid complexes of proanthocyanidin A2 can be used in suitable administration forms for the oral route such as tablets, soft- or hard- gelatin capsules, at dosages ranging from 50 to 500 mg two-three times a day,

depending on the severity of the disease. The preparation of the pharmaceutical formulations can be carried out according to conventional techniques and excipients.

The following examples illustrate the invention in greater detail.

Example 1

Preparation of the complex of Proanthocyanidin A2 with phosphatidylcholine

A solution of 1577 g of phosphatidylcholine in 5 liters of ethyl acetate, kept at 70°C, was added with a solution of 1 kg of proanthocyanidin A2 in 5 liters of acetone.

The mixture was refluxed under stirring and evaporated to dryness under vacuum. The residue was dried under vacuum at 50°C for 24h, then ground to the desired particle size.

Example 2

32 New Zealand rabbits were divided in 4 groups of 8 animals each, and treated as follows:

Group 1): Control, normal diet

Group 2): Hypercholesterolemic diet (0.2% w/w cholesterol)

Group 3): Hypercholesterolemic diet + phospholipid complex of proanthocyanidin A2 extract (0.2% cholesterol + 2% w/w complex of Example 1).

Group 4): Hypercholesterolemic diet + proanthocyanidin A2 extract (0.2% cholesterol + amount of proanthocyanidin A2 equivalent to that present in 2% w/w of the complex of Example 1).

After 8 week-treatment, during which cholesterol, LDL/VLDL, HDL and triglycerids levels were measured, the animals were killed.

The number, size and distribution of the atherosclerotic lesions on thoracic and abdominal aorta were evaluated.

Aorta strips were fixed and stained with Sudan IV to visualize the lesions and to evaluate the vasal cholesterol and the content in oxidized cholesterol by gaschromatography.

The results reported in the following table prove that the treatment with phospholipid complexes of Proanthocyanidin A2 decreases in a statistically significant way the atherosclerotic lesions induced by hypercholesterolemic diet.

Table

Treatment	area percent of the lesion
Group 1	0.5%
Group 2	34%
Group 3	7.5%*
Group 4	30%

*$p<0.01$ compared with group 2.

Example 3

Capsules containing 500 mg of phospholipid complex of Proanthocyanidin A2.

Composition:

Complex of Proanthocyanidin A2 with soy phosphatidylcholine	150 mg
Lactose	57 mg
Modified starch	40 mg
Magnesium stearate	3.0 mg

Example 4

Gastro-resistant tablets

Complex of Proanthocyanidin A2 with soy phosphatidylcholine	200 mg
Microcrystalline cellulose	118 mg
Precipitated silica	3 mg
Magnesium stearate	4 mg
Methacrylic acid anionic polymer and esters thereof	12 mg
Talc	8 mg

Magnesium carbonate	8 mg
Maize starch	5 mg
Gum arabic	159 mg

Example 5

Soft-gelatin capsules

Complex of Proanthocyanidin A2 with soy phosphatidylcholine	216 mg
Peanut oil	209 mg
Partially hydrogenated vegetable oils	100 mg
Soy lecithin	5 mg

CLAIMS

1. Phospholipid complexes of proanthocyanidin A2 or extracts enriched in proanthocyanidin A2.

2. Complexes as claimed in claim 1, in which phospholipids are selected from lecithins, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine.

3. Complexes as claimed in claim 2, in which the phospholipid is phosphatidylcholine.

4. Complexes according to any one of claims 1 to 3, in which the weight ratio of proanthocyanidin A2 to phospholipid ranges from 2:1 to 1:2.

5. The use of the complexes of claims 1 to 4 for the preparation of medicaments for the prophylactic or therapeutical treatment of atherosclerosis, and of myocardial and cerebral infarction.

6. The use as claimed in claim 5 in which the unitary dosage of the medicament ranges from 50 to 500 mg of active ingredient.

7. The use as claimed in claim 5 or 6 in which the medicament is administrable through the oral route.

8. The use as claimed in claim 7 in which the medicament is in the form of soft- or hard- gelatin capsules or tablets.

9. Pharmaceutical compositions containing as active ingredient a complex of claims 1 - 4.

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