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(54) Title: USE OF 3-CARBOXY-N-ETHYL-N,N-DIMETHYLPROPAN-1-AMINIMIUM OR A PHARMACEUTICALLY ACCEPTABLE SALT THEREOF IN THE TREATMENT OF ATHEROSCLEROSIS

Use of 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium or a pharmaceutically acceptable salt thereof in the treatment of atherosclerosis

Technical Field

The present invention relates to 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium or a pharmaceutically acceptable salt thereof for use in the prevention and treatment of atherosclerosis. Examples of pharmaceutically acceptable salts of 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium are: 3-carboxy-A/-ethyl-/V,AA dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-A/-ethyl-/V,A/-dimethylpropan-1-aminium dihydrogen phosphate.

Background Art

Atherosclerosis is a complex chronic inflammatory process of the vascular wall that progresses over decades. It is characterized by the accumulation of oxidized low-density lipoproteins (LDL), increased cell death and hypertrophic degeneration of the arterial wall, causing narrowing of the inner diameter of the vessel and, thus, impairing blood flow. It can occur in any area of the body, but is most important when it develops in the blood vessels of the heart or brain. The narrowing is due to the formation of plaques (raised patches) in the inner lining of the arteries. These plaques consist of oxidized LDL, decaying muscle cells, fibrous tissue, clumps of blood platelets, cholesterol, macrophages, T-lymphocytes and sometimes calcium.

Atherosclerotic lesions commonly develop in regions of turbulent blood flow and are found most often in people with elevated cholesterol concentrations. The
The number and thickness of plaques increases with age, causing loss of the smooth lining of the blood vessels and encouraging the formation of thrombi (blood clots). Sometimes fragments of thrombi break off and form emboli, which travel through the bloodstream and block smaller vessels, thus, causing ischemic damage of the tissues.

Atherosclerosis and its clinical manifestations are a major cause of morbidity and mortality in the modern society. Atherosclerotic heart disease, involving the coronary arteries (coronary heart disease), is the most common cause of death, accounting for one-third of all deaths. Atherosclerotic interference with blood supply to the brain (stroke) is the third most common cause of death after cancer. Vascular insufficiency is another clinical manifestation of atherosclerosis which causes a great deal of serious illness by reducing the flow of blood in other major arteries, such as to the kidneys, legs, and intestines.

Unfortunately, atherosclerosis produces no symptoms until the damage to the arteries is severe enough to restrict blood flow. Restriction of blood flow to the heart muscle due to atherosclerosis can cause angina pectoris or a myocardial infarction (a heart attack). Restriction of blood flow to the muscles of the legs induces intermittent claudication (pain in the legs that occurs during exercise and is relieved by rest). Narrowing of the arteries supplying blood to the brain may cause transient ischemic attacks (symptoms and signs of a stroke lasting less than 24 hours) and episodes of dizziness, or ultimately, to a stroke itself.
3-Carboxy-N,N,N-trimethylpropan-1-aminium (GBB) is known mostly as a bio-
precursor of carnitine, a key molecule in the regulation of myocardial energy
metabolism, was primarily characterised as a toxic substance, which accelerates
respiration, causes salivation and lacrimation, pupil dilation, vasoconstriction and
heart stop in diastole LINNEWEH, W. Gamma-Butyrobetain, Crotonbetain und
Carnitin im tierischen Stoffwechsel. Hoppe-Seylers Zeitschrift fur physiologische
Chemie. 1929, vol.181, p.42-53. At the same time, in later papers it has been
shown that administration of 3-carboxy-/V/,V,A/-trimethylpropan-1-aminium does
not induce any toxic effects as it is extremely low toxic (LDso 7000 mg/kg, s.c.)

ROTZSCH, W. Iber die Toxizitat des Carnitins und einiger verwandter Stoffe. Acta

The combination of 3-carboxy-/V/,V,A/-trimethylpropan-1-aminium with 3-(2,2,2-
trimethylhydrazinium)propionate dihydrate in the treatment of atherosclerosis was
presented in WO 2010/149654 A (GRINDEKS JSC) 29.10.2010. Nevertheless the
effect of 3-carboxy-/V/,V,A/-trimethylpropan-1-aminium, where it is used alone, for
the treatment of atherosclerosis is not reported.

A new derivate of 3-carboxy-/V/,V,A/-trimethylpropan-1-aminium, 3-carboxy-/V-ethyl-
A/,A/-dimethylpropan-1-aminium, as a new compound with cardioprotective activity

Apolipoprotein E knockout (ApoE-) mice are frequently used experimental model
of the atherosclerosis for the assessment of anti-atherosclerotic activity of tested
substances.
Disclosure of the invention

This invention is directed to treating atherosclerosis by decreasing the total area and volume of atherosclerotic lesions.

The lesion progression inhibition is achieved by treatment with 3-carboxy-\(\beta\)-ethyl-\(\beta\)-dimethylpropan-1-aminium or its pharmaceutically acceptable salts: 3-carboxy-\(\beta\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium hydrogen fumarate) or 3-carboxy-\(\beta\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium dihydrogen phosphate, possibly through alteration of lipid and cholesterol metabolism.

A therapeutically effective amount of 3-carboxy-\(\alpha\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium or its pharmaceutically acceptable salt is about 0.01 to 500 mg/kg/day, preferably 0.1 to 100 mg/kg/day.

It was surprisingly and unexpectedly found that 3-carboxy-\(\beta\)-ethyl-\(\beta\),\(\beta\)-dimethylpropan-1-aminium and its pharmaceutically acceptable salts; 3-carboxy-\(\beta\)-ethyl-\(\gamma\),\(\gamma\)-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-\(\beta\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium dihydrogen phosphate posses pronounced anti-atherosclerotic effect.

The anti-atherosclerotic activity of 3-carboxy-\(\alpha\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium, 3-carboxy-\(\beta\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium-hydrogen fumarate or 3-carboxy-\(\alpha\)-ethyl-\(\alpha\),\(\alpha\)-dimethylpropan-1-aminium dihydrogen phosphate can be determined by assessing the effect of these compounds on the portion of the aortic surface covered by atherosclerotic lesions.
3-Carboxy-AAAethyl-/V,AAdimethylpropan-1-aminium can be used in pharmaceutical preparations containing the compound, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier or diluent. Suitable pharmaceutically acceptable carriers include inert solid fillers or diluents and sterile aqueous or organic solutions. Thus, for oral administration the compounds can be combined with a suitable solid or liquid carrier or diluent to form capsules, tablets, powders, syrups, solutions, suspensions and the like. The pharmaceutical compositions may, if desired, contain additional components such as flavorants, sweeteners, excipients and the like. For parenteral administration the compounds can be combined with sterile aqueous or organic media to form injectable solutions or suspensions. The injectable solutions can then be administered intravenously, intraperitoneally, subcutaneously, or intramuscularly.

Anti-atherosclerotic activity

Female ApoE^-/- mice weighing 18 - 20 g were maintained on a 12 h dark/12 h light cycle in air-conditioned rooms (22.5±0.5°C, 50±5% humidity) with unlimited access to food and water. Mice were adapted to local conditions for one week before the beginning of the study. At the age of 8 weeks, mice were randomly assigned to five equally sized groups (n = 10). To induce experimental atherosclerosis (atherosclerotic lesions in the aorta), animals of all groups were fed with WESTERN RD (P) diet (Cat 8231 6)
from Special Diets Services (Great Britain) for 4 months. During these 4 months, mice from different experimental groups received following treatment:

1. Control group - drinking water;
2. 3-Carboxy-N,N,N-trimethylpropan-1-aminium group - 3-carboxy-N,N,N-trimethylpropan-1-aminium 10mg/kg in drinking water;
3. 3-Carboxy-N-ethyl-N,N-dimethylpropan-1-aminium group - 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium 10 mg/kg in drinking water;
4. 3-Carboxy-N-ethyl-N,N-dimethylpropan-1-aminium hydrogen fumarate group - 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium hydrogen fumarate 17.5 mg/kg in drinking water;
5. 3-Carboxy-N-ethyl-N,N-dimethylpropan-1-aminium dihydrogen phosphate group - 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium dihydrogen phosphate 16.8 mg/kg in drinking water.

The doses of the tested substances were adjusted to be equimolar with 10 mg/kg dose of 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium.

The dosing of the test compounds was confirmed by measuring the consumption of drinking water every 2 days and adjusting the concentration of supplemented substances.

After 4 months, mice were injected intraperitoneally (i.p.) with 1,000 UI of heparin i.p. and sacrificed under anesthesia (sodium pentobarbital, 50 mg/kg i.p.).

The size of atherosclerotic lesions was determined in whole aorta. The aortas from arch to bifurcation were cleaned from surrounding tissues, cut out and fixed in 4%
formaldehyde. Afterwards whole aorta was longitudinally opened, pinned onto silicone plates and stained for lipids with Sudan IV. Images of the aorta were captured using a digital camera and the total area of the lesion was calculated using Image-Pro Plus 6.3 software. The extent of atherosclerosis was expressed as the percentage of the aortic surface covered by lesions compared to the total aortic surface.

All analyses were performed by an observer blinded to the treatment group. After 4-month exposure to Western RD diet, the apoE-/- mice developed marked atherosclerotic lesions. Analysis of Sudan IV stained aortas showed that area of atherosclerotic lesions in the control group averaged 14-16% of the total aortic surface. Four-month administration of 3-carboxy-\(N\)-ethyl-\(N\)/V-dimethylpropan-1-aminium at the dose of 10 mg/kg, 3-carboxy-\(N\)-ethyl-\(N\)/V-dimethylpropan-1-aminium hydrogen fumarate at the dose of 17.5 mg/kg, 3-carboxy-/V-ethyl-/V,/V-dimethylpropan-1-aminium dihydrogen phosphate at the dose of 16.8 mg/kg (the latter two equimolar to 10mg/kg of 3-carboxy-/V-ethyl-/V,/V-dimethylpropan-1-aminium) induced a statistically significant reduction in the size of atherosclerotic lesions. 3-Carboxy \(-N,N,N\)-trimethylpropan-1 -aminium (10 mg/kg) had no effect on the size of atherosclerotic lesions.

dimethylpropan-1-aminium dihydrogen phosphate on the area of atherosclerotic lesions in aorta of apoE-/- mice are presented in Table 1.

Table 1

Effects of 3-carboxy-\(\text{V, V, V}\)-trimethylpropan-1-aminium, 3-carboxy-\(\text{V-ethyl-V, V}\)-dimethylpropan-1-aminium, 3-carboxy-\(\text{N-ethyl-V, V}\)-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-\(\text{N-ethyl-V, V}\)-dimethylpropan-1-aminium dihydrogen phosphate on the area of atherosclerotic lesions

<table>
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<tr>
<th>Treatment group</th>
<th>Area of atherosclerotic lesions (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>100.0±10.7</td>
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<tr>
<td>3-Carboxy-(\text{N, A, V})-trimethylpropan-1-aminium, 10 mg/kg</td>
<td>96.0±10.6</td>
</tr>
<tr>
<td>3-Carboxy-(\text{V-ethyl-V, V})-dimethylpropan-1-aminium, 10 mg/kg</td>
<td>43.6±8.6*#</td>
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<tr>
<td>3-Carboxy-(\text{V-ethyl-V, V})-dimethylpropan-1-aminium hydrogen fumarate, 17.5 mg/kg</td>
<td>47.9±5.7*#</td>
</tr>
<tr>
<td>3-Carboxy-(\text{V-ethyl-V, V})-dimethylpropan-1-aminium dihydrogen phosphate, 16.8 mg/kg</td>
<td>64.4 ±7.5*#</td>
</tr>
</tbody>
</table>
Values are given as mean ± SEM. Statistical analysis was performed by Student's t-test; *p<0.05 versus control group and #p<0.05 versus 3-carboxy-/V,/,/V-trimethylpropan-1-aminium group; n=10.
The results are presented as a percentage relative to the control group which was assigned a value of 100%.

Results presented in Table 1 show that 3-carboxy-\(N\)-ethyl-\(N,/,/V\)-dimethylpropan-1-aminium, 3-carboxy-\(N\)-ethyl-\(N,N\)-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-\(N\)-ethyl-\(N,N\)-dimethylpropan-1-aminium dihydrogen phosphate had a protective effect on formation of atherosclerotic lesions in aorta of apoE-/- mice. 3-Carboxy-\(N,N,N\)-trimethylpropan-1-aminium, in contrary, was not effective.
Claims

1. 3-Carboxy-N-ethyl-N,N-dimethylpropan-1-aminium or a pharmaceutically acceptable salt thereof for use in the prevention and treatment of atherosclerosis.

2. Use according to claim 1, wherein pharmaceutically acceptable salt is 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium hydrogen fumarate.

3. Use according to claim 1, wherein pharmaceutically acceptable salt is 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium dihydrogen phosphate.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/197 A61P9/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>A</td>
<td>WO 2012/146736 AI (GRINDEKS JSC [LV]; STONANS ILMARS [LV]; KALVINS IVARS [LV]; DAMBROVA M) 1 November 2012 (2012-11-01): claims 9-11</td>
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<td>A</td>
<td>WO 2010/151095 AI (TETRA SIA [LV]; KALVINS IVARS [LV]; BIRMANS ANATOLIJS [LV]; VEVERIS MA) 29 December 2010 (2010-12-29): claim 1</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

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Date of the actual completion of the international search: 6 March 2014
Date of mailing of the international search report: 19/03/2014

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Authorized officer: Buttner, Ulf

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