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(54) **Titre : UTILISATION DE 3-CARBOXY-N-ETHYL-N,N-DIMETHYLPROPAN-1-AMINIUM OU D'UN SEL PHARMACEUTIQUEMENT ACCEPTABLE DE CELUI-CI DANS LA PREVENTION ET LE TRAITEMENT DU DIABETE**

(54) **Title: USE OF 3-CARBOXY-N-ETHYL-N,N-DIMETHYLPROPAN-1-AMINIUM OR A PHARMACEUTICALLY ACCEPTABLE SALT THEREOF IN THE PREVENTION AND TREATMENT OF DIABETES**

(57) **Abrégé/Abstract:**
Use of 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium and its pharmaceutically acceptable salts to decrease blood plasma levels of insulin and glucose.

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(54) Title: USE OF 3-CARBOXY-*N*-ETHYL-*N*,*N*-DIMETHYLPROPAN-1-AMINIUM OR A PHARMACEUTICALLY ACCEPTABLE SALT THEREOF IN THE PREVENTION AND TREATMENT OF DIABETES

(57) Abstract: Use of 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium and its pharmaceutically acceptable salts to decrease blood plasma levels of insulin and glucose.

Description

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

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 diabetes. Examples of pharmaceutically acceptable salts of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium are: 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate.

Background Art

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. It was primarily characterised as a toxic substance, which accelerates respiration, causes salivation and lacrimation, pupil dilation, vasoconstriction and cardiac arrest in diastole **LINNEWEH W.** Gamma- Butyrobetain, Crotonbetain und Carnitin im tierischen Stoffwechsel. *Hoppe-Seylers Zeitschrift fur physiologische Chemie.* 1929, vol.181, p.42-53. In later publications it has been shown 3-carboxy-*N,N,N*-trimethylpropan-1-aminium has extremely low toxicity (LD_{5O} 7000 mg/kg, s.c.)

ROTZSCH. W. Uber die Toxizitat des Carnitins und einiger verwandter Stoffe. *Acta biol. med. germ.* 1959, vol.3, p.28-36.

Methods for preparation of a new compound with cardioprotective activity - 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium has been disclosed in **WO 2011/048201** A (GRINDEKS, JSC) 28/04/2011 . Salts of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium as well as their use in the treatment of cardiovascular diseases has been disclosed in patent publication **WO 2012/146736** A (GRINDEKS, JSC) 27/12/2012 .

Diabetes mellitus is one of the leading causes of morbidity and mortality globally, and despite medical breakthroughs and treatment innovations, the number of adults with this condition has been increasing at a faster pace than previously expected **DANAEI G., et al.** National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet.* 2011, vol.378, p.31-40. Type 2 diabetes mellitus (T2DM) comprises about 90% of all diabetes cases throughout the world. T2DM is a metabolic disorder characterized by elevated blood glucose levels in the context of insulin resistance and relative insulin deficiency. Insulin resistance contributes to hyperinsulinaemia which is often seen in early stages of T2DM. Furthermore, hyperinsulinaemia is predictive of development of T2DM, as it is known to precede T2DM by decades. T2DM causes dysfunctions in multiple organs and tissues leading to disability and premature death of T2DM patients. Diabetes-related expenses to healthcare systems and patients are projected to increase twice during next 25 years **HUANG E.S., et al.** Projecting the future

diabetes population size and related costs for the U.S.. *Diabetes Care .* 2009, vol.32, p.2225-2229. , therefore anti-diabetes drugs are of high necessity and economical value.

Although several classes of anti-diabetic drugs are currently available, there is a significant need for novel therapies with added medical value and improved side-effect profile. Achieving and maintaining long-term glycaemic control by existing drugs in T2DM patients is often challenging, also due to insufficient insulinaemic control

MANNINO G.C., SESTI G. Individualized Therapy for Type 2 Diabetes: Clinical Implications of Pharmacogenetic Data. *Mol Diagn Ther.* 2012, vol.16, p.285-302. ,

SAFAVI M., et al. The importance of synthetic drugs for type 2 diabetes drug discovery. *Expert Opin Drug Discov. Ahead of Print (doi:10.1517/17460441.2013.837883).* 19.09.2013, p.1-25. Normalizing insulin levels is of particular preventive and therapeutic importance since uncontrolled insulin resistance and subsequent hyperinsulinaemia are major contributors in development of T2DM. Therefore, new pharmacotherapy targeting hyperglycaemia, insulin resistance and hyperinsulinaemia could be of particular interest for effective prevention and treatment of T2DM.

Summary of invention

The present invention is directed to prevention and treatment of diabetes by decreasing blood plasma levels of insulin and glucose.

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 diabetes.

During our research to find new active substances to treat hyperglycaemia an unexpected glucose- and insulin- reducing effect of 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium salts has been discovered.

The reduction of glucose and insulin concentration in blood is achieved by treatment with 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium or its pharmaceutically acceptable salts, for example but not limited to: 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium 2-(acetyloxy)benzoate, 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium hydrogen fumarate, 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium dihydrogen phosphate, 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate, 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium 3-carboxypropanoate, preferably 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium hydrogen fumarate or 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium dihydrogen phosphate.

3-Carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-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. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium or a pharmaceutically acceptable salt therefore can also be prepared for transdermal application, for example, in a form of adhesive plaster.

A therapeutically effective amount of 3-carboxy-*N*-ethyl-*N,N*-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.

Example

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). Experimental animals of all groups were fed with WESTERN RD (P) diet (Cat 82316) from Special Diets Services (Great Britain) for 4 months. During these 4 months, mice from different experimental groups received following treatment:

Control group – drinking water;

GBB group – GBB 10mg/kg;

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;

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. GBB, 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium hydrogen fumarate or 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium dihydrogen phosphate were added to the drinking water. The doses of GBB, 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium dihydrogen phosphate were equimolar to the 10 mg/kg dose (62.5 inium d4-(ethyl(dimethyl)ammonio)butanoate. 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.

At the age of 8 and 16 weeks blood was collected into EDTA/diamide- and protease inhibitor (PMSF, pepstatin, leupeptin, aprotinin)-containing tubes. Blood glucose concentration was measured using a MediSense Optium Xceed blood glucose meter and strips. Plasma was obtained by centrifugation at 3000 x g and stored frozen (-80° C) until analysis. Plasma insulin concentrations were determined with a RIA kit (Biotrend, Germany).

Treatment-effect on plasma glucose level is presented in Table 1. Both 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium dihydrogen phosphate reduced plasma glucose levels in a statistically significant manner, while GBB lacked an effect .

Table 1

Effects of test compounds at a dose of 10mg/kg on plasma glucose concentration in fed and fasted ApoE$^{-/-}$ mice

	Week 8		Week 16	
	Fed	Fasted	Fed	Fasted
Control group	8,0 ±0,42	5,1 ±0,28	10,0 ±0,55	5,4 ±0,33
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate group	8,2 ±0,61	3,7 ±0,18*	8,2 ±0,39*	4,3 ±0,16*
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium hydrogen fumarate group	7,6 ±0,27	3,9 ±0,08*	9,2 ±0,34	4,0 ±0,22*
GBB group	8,1 ±0,25	4,4 ±0,39	8,4 ±0,29	4,5 ±0,31

Each value represents the mean ± S.E.M. of 8-10 animals. *Significantly different from the control group (Tukey's test $P < 0.05$).

Insulin levels were decreased by 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate (Table 2).

Table 2

Effects of test compounds at a dose of 10mg/kg on plasma insulin concentration in fasted ApoE$^{-/-}$ mice

	Week 8	Week 16
Control group	1,39 ±0,13	1,26 ±0,05
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate group	0,60 ±0,06*	0,97 ±0,11*
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium hydrogen fumarate group	0,62 ±0,06*	0,85 ±0,06*
GBB group	1,30 ±0,11	1,20 ±0,10

Each value represents the mean ± S.E.M. of 8-10 animals. *Significantly different from the control group (Tukey's test $P < 0.05$).

Results presented in Table 1 and Table 2 show that 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium hydrogen fumarate and 3-carboxy-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium dihydrogen phosphate have a glucose and insulin level lowering properties, thus demonstrating efficacy in prevention and treatment of diabetes.

Claims

1. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium or pharmaceutically acceptable salts thereof for use in the prevention and treatment of diabetes.

2. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium or pharmaceutically acceptable salts for use according to claim 1, wherein the pharmaceutically acceptable salt is 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium hydrogen fumarate.

3. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium or pharmaceutically acceptable salts for use according to claim 1, wherein the pharmaceutically acceptable salt is 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate.