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(54) Title: SYNERGISTIC COMPOSITIONS CONTAINING CHOLINE BASE AND SUCCINIC ACID FOR INSULIN RESISTANCE AND DIABETES

(57) Abstract: This invention relates to compositions and methods for achieving a synergistic effect in treating insulin resistance and diabetes mellitus in a mammal. More specifically, this invention relates to synergistic composition comprising amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof, which are presented in amounts sufficient to cause synergistic effects in treating insulin resistance and diabetes mellitus in a mammal. Further, this invention relates to methods for achieving a synergistic effects in treating insulin resistance and diabetes mellitus in a mammal, which methods comprise administering to said mammal, either stepwise or simultaneously, effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof. Further, this invention relates to a novel derivative of choline. More specifically, this invention relates to bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, the novel salt of choline formed by choline base and succinic acid in the molar ratio 2:1, a process for producing the salt, and use of the salt in medicine, preferably for the manufacture of a medicament for treating insulin resistance, hyperlipidemia, dyslipidemia, or diabetes mellitus.

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SYNERGISTIC COMPOSITIONS CONTAINING CHOLINE BASE AND SUCCINIC ACID FOR INSULIN RESISTANCE AND DIABETES

TECHNICAL FIELD OF THE INVENTION

This invention relates to compositions and methods for achieving a synergistic effect in treating insulin resistance and diabetes mellitus in a mammal. More specifically, this invention relates to synergistic composition comprising amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof, which are presented in amounts sufficient to cause synergistic effects in treating insulin resistance and diabetes mellitus in a mammal. Further, this invention relates to methods for achieving a synergistic effects in treating insulin resistance and diabetes mellitus in a mammal, which methods comprise administering to said mammal, either stepwise or simultaneously, effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof. Further, this invention relates to a novel derivative of choline. More specifically, this invention relates to bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, the novel salt of choline formed by choline base and succinic acid in the molar ratio 2:1, a process for producing the salt, and use of the salt in medicine, preferably for the manufacture of a medicament for treating insulin resistance, hyperlipidemia, dyslipidemia, or diabetes mellitus.

BACKGROUND OF THE INVENTION

Insulin is the major anabolic hormone in the body and has multiple effects on a variety of tissues. Insulin stimulates the glucose uptake in muscle and fat and inhibits the glucose release from the liver. In addition, insulin regulates both plasma and tissue lipid metabolism, protein synthesis, cell growth and electrolyte balance.

Insulin resistance is defined as an impaired biological response to either exogenous or endogenous insulin. The measured biological responses could reflect metabolic processes such as changes in carbohydrate, lipid or

protein metabolism as well as mitogenic processes such as alterations in growth, differentiation, DNA synthesis, and regulation of gene transcription.

Insulin resistance has clearly emerged as an important cause of glucose intolerance leading to type 2 diabetes and a cluster of abnormalities, including hyperlipidemia and dyslipidemia, high blood pressure, hyperuricemia, and a decrease in plasma fibrinolytic activity. Reaven, G. M. Physiol-Rev. 75(3): 473-86 (1995); "Consensus development conference on insulin resistance", Diabetes Care, 21(2) 1998. Insulin resistance can be associated with a variety of disease states such as gestational diabetes mellitus, obesity, ageing, atherosclerosis, cardiovascular syndrome X, AIDS, cancer, wasting/cachexia, sepsis, trauma associated with burns, malnutrition, lupus and other autoimmune diseases, endocrine diseases, polycystic ovary syndrome, or complications arising from athletic activity.

Clinically, insulin resistance manifesting itself in pathologically elevated fasting plasma insulin levels and can be assessed by measurement of insulin concentration in plasma.

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

Clinically, diabetes mellitus manifesting itself in pathologically elevated plasma glucose levels and can be assessed by measurement of glucose concentration in blood.

Treating insulin resistance by succinic acid or salts thereof was described in the international application PCT/RU 99/00055.

Succinic acid or salts thereof were described in JP patent 61171417 as antidiabetics useful for stimulating insulin secretion. Contrary to JP 61171417, MacDonald et al. found that succinic acid and salts thereof did not stimulate insulin secretion from pancreas, and only esters of succinic acid are potent insulin secretagogues. MacDonald, M.J. and Fahien, L.A. Diabetes 37(7): 997-999 (1988). Also, succinic acid was described in JP patent 6062798 as a component of food for person with poor glucose-tolerance in combination with acetic, lactic, and gluconic acid. Disodium succinate was described as a component of the composition for decreasing blood glucose in rabbits with

alloxan diabetes in combination with citric and acetic acid. Dzvonkevich, N.D. et al., Ukr. Biokhim. Zh., 46(5):547-552(1974).

Until the invention described herein, there was no report of use of choline base or a pharmaceutically acceptable salt thereof together with succinic acid or a pharmaceutically acceptable salt thereof to achieve synergistic effects in treating insulin resistance or diabetes mellitus.

Monocholine succinate, the acid salt formed by choline base and succinic acid in the molar ratio 1:1, was described in US patent 5,124,061 as a component of plant cryoprotectant composition. However, this salt has not been obtained as the product with reproducible properties and was defined only by reference to process in which choline base and succinic acid were entered in molar ratio 1:1. Acidity of monocholine succinate and absence of reproducible properties restricts the use of the described monocholine succinate in medicine.

Until the invention described herein, there was no report of bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, the neutral salt formed by choline base and succinic acid in the molar ratio 2:1, a process for producing the salt, and use of the salt in medicine, preferably for the manufacture of a medicament for treating insulin resistance, hyperlipidemia, dyslipidemia, or diabetes mellitus. Herein and after, the chemical name "bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate" is used in the invention instead of trivial name "dicholine succinate", since the name "dicholine succinate" was frequently used in literature for description of a widely known ester of succinic acid and choline.

SUMMARY OF THE INVENTION

This invention relates to compositions and methods for achieving a synergistic effect in treating insulin resistance and diabetes mellitus in a mammal. More specifically, this invention relates to synergistic composition comprising amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof, which are presented in amounts sufficient to cause synergistic effects in treating insulin resistance and diabetes mellitus in a mammal. Further, this invention

relates to methods for achieving a synergistic effects in treating insulin resistance and diabetes mellitus in a mammal, which methods comprise administering to said mammal, either stepwise or simultaneously, effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof. Further, this invention relates to a novel derivative of choline. More specifically, this invention relates to bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, the novel salt of choline formed by choline base and succinic acid in the molar ratio 2:1, a process for producing the salt, and use of the salt in medicine, preferably for the manufacture of a medicament for treating insulin resistance, hyperlipidemia, dyslipidemia, or diabetes mellitus.

DETAILED DESCRIPTION OF THE INVENTION

The term "synergistic" as used herein means that the effect achieved with the methods and compositions of this invention is greater than the sum of the effects that result from methods and compositions comprising choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof separately and in the amounts employed in the methods and compositions hereof.

Accordingly to this invention, it is now possible to achieve a synergistic effect in treating insulin resistance and diabetes mellitus in a mammal with amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof which, if administered in said amounts singly, are not capable of achieving said effect and which effect is greater than the sum of the effects achieved for choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof separately.

The term "treating" as used herein means the management and care of a mammal for the purpose of combating the disease, condition, or disorder and includes the administration of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or

disorder. Treating insulin resistance includes increasing insulin sensitivity in a mammal and other effects, which are resulted from increasing insulin sensitivity in a mammal. Such effects include, but are not limited to, improving in carbohydrate, lipid, or protein metabolism, alterations in growth, differentiation, DNA synthesis, and regulation of gene transcription.

The present invention provides a method for achieving a synergistic effect in treating insulin resistance in a mammal in need thereof, which comprises administering to said mammal an effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof. Preferred effect achieved according to this invention in treating insulin resistance is lowering pathologically elevated plasma insulin levels. Insulin resistance in the mammal can be associated with disorders such as diabetes mellitus and its chronic complications; gestational diabetes mellitus; impaired glucose tolerance; obesity; ageing; atherosclerosis; syndrome X; cardiovascular disease; AIDS; cancer; wasting/cachexia; sepsis; trauma associated with burns; malnutrition; lupus and other autoimmune diseases; endocrine diseases; hyperuricemia; hyperlipidemia; dyslipidemia; polycystic ovary syndrome; or complications arising from athletic activity. Preferably, insulin resistance is associated with diabetes mellitus in the mammal.

Further, the present invention provides a method for achieving a synergistic effect in treating diabetes mellitus in a mammal in need thereof, which comprises administering to said mammal an effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof. Preferred effect achieved according to this invention in treating diabetes mellitus is lowering pathologically elevated blood glucose levels.

Preferably, choline base or a pharmaceutically acceptable salt thereof of the invention is choline chloride. Preferably, succinic acid or a pharmaceutically acceptable salt thereof of the invention is disodium succinate.

The administration of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof

can be stepwise in time or simultaneous with the simultaneous method being preferred.

Choline base or a pharmaceutically acceptable salt thereof can be administered orally, parenterally, topically, or rectally. Preferably, choline base or a pharmaceutically acceptable salt thereof is administered parenterally. Succinic acid or a pharmaceutically acceptable salt thereof can be administered orally, parenterally, topically, or rectally. Preferably, succinic acid or a pharmaceutically acceptable salt thereof is administered parenterally.

Preferably, the choline base or a pharmaceutically acceptable salt thereof is administered for a period of 1 day or longer, more preferably for a period of 3 to 7 days. Preferably, the succinic acid or pharmaceutically acceptable salt thereof is administered for a period of 1 day or longer, more preferably for a period of 3 to 7 days.

The present invention provides a composition for achieving a synergistic effect in treating insulin resistance in a mammal in need thereof, which comprises an effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent or carrier. Preferred effect achieved according to this invention in treating insulin resistance is lowering pathologically elevated plasma insulin levels.

Further, the present invention provides a composition for achieving a synergistic effect in treating diabetes mellitus in a mammal in need thereof, which comprises an effective amounts of choline base or a pharmaceutically acceptable salt thereof and succinic acid or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent or carrier. Preferred effect achieved according to this invention in treating diabetes mellitus is lowering pathologically elevated blood glucose levels.

The pharmaceutically acceptable salt of the choline base is prepared by known methods from nontoxic organic and inorganic acids. Such acids include, but are not limited to, hydrogen chloride, hydrogen bromide, citric acid, tartaric acid, and succinic acid. Preferably, choline base or a pharmaceutically acceptable salt thereof of the invention is choline chloride.

The pharmaceutically acceptable salt of the succinic acid is prepared by known methods from organic and inorganic bases. Such bases include, but are not limited to, nontoxic alkali metal and alkaline earth bases, for example, calcium, lithium, sodium, and potassium hydroxide; ammonium hydroxide and nontoxic organic bases, such as triethylamine, butylamine, diethanolamine, triethanolamine, and choline base. Preferably, succinic acid or a pharmaceutically acceptable salt thereof of the invention is disodium succinate.

Some examples of suitable carriers and diluents include lactose, dextrose, sorbitol, mannitol, calcium phosphate, alginates, gelatin, calcium silicate, microcrystalline cellulose, methylcellulose, polyvinylpyrrolidone, water, methyl- and propylhydroxybenzoates, talc, magnesium stearate, stearic acid, and mineral oil. The compositions of the invention can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents, or flavoring agents.

Compositions of the invention can be administered in a wide variety of different dosage forms, i.e., they may be formulated with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, aqueous solutions, elixirs, syrups and the like.

The effective amount of choline base or a pharmaceutically acceptable salt thereof for use in the methods and compositions of this invention is preferably from 3 to 300 mg per day per kg of body weight of the mammal.

The effective amount of succinic acid or a pharmaceutically acceptable salt thereof for use in the methods and compositions of this invention is preferably from 1 to 250 mg per day per kg of body weight of the mammal.

The present invention provides bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, which has the chemical formula given below:

$[(CH_3)_3N^+CH_2CH_2OH]_2\ ^-OOCCH_2CH_2COO^-$

The present invention provides a process for producing bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, which comprises reacting choline base with succinic acid in the presence of water, alcohol, or mixture thereof. Preferably, bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate is prepared by reacting the choline base and succinic acid, wherein the mole ratio of choline base to succinic acid entered in reaction is about 2:1, but no lower than 1.9:1 and no higher than 2.1:1. The reaction take place at ambient temperatures and the reaction is quantitative. The desired product is obtained as a white crystalline powder with reproducible properties.

Also provided according to the present invention is the use of bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate in medicine, preferably for the manufacture of a medicament for treating insulin resistance, hyperlipidemia, dyslipidemia, and diabetes mellitus in a mammal in need thereof. Preferred effect achieved according to this invention in treating insulin resistance is lowering pathologically elevated plasma insulin levels. Preferred effects achieved according to this invention in treating hyperlipidemia are lowering pathologically elevated plasma triglycerides and cholesterol levels. Preferred effects achieved according to this invention in treating dyslipidemia are lowering low-density lipoprotein cholesterol levels and increasing high-density lipoprotein cholesterol levels. Preferred effect achieved according to this invention in treating diabetes mellitus is lowering pathologically elevated blood glucose levels.

The following examples are presented to demonstrate the invention. The examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLE 1

This example shows the synergistic effects in treating insulin resistance and diabetes mellitus achieved by co-administration of effective amounts of choline chloride and disodium succinate to diabetic rats.

Assay. Plasma insulin concentrations were determined using a kit ("Dako", Dutch) with a rat insulin standard (Novo Research Institute, Bagsvard,

Denmark). Plasma glucose concentrations were determined using a kit ("Agat", Russia). Plasma triglycerides and cholesterol concentrations were determined with reagents FS, "DiaSys", Germany; HDL cholesterol with reagents "Cormay", Poland; and LDL cholesterol with reagents "Boehringer Mannheim", Germany.

Animals. Male Wistar rats 8-10 weeks of age 210-230g of body weight were used. The rats were housed at the temperature of 18 ± 21°C on a 12 hour light-dark cycle. Rats were fed on a stock laboratory diet (59 % carbohydrates; 17 % protein; 3 % fat; 21 % minerals, water, and cellulose) and allowed water *ad libitum*. Diabetes mellitus was induced in Wistar male rats by twice i.v. injection (tail vein) of alloxan (40 mg/kg body wt) with break of 48 hours. The rats were used in experiments at 6th day from the first alloxan injection. Fasting plasma glucose, insulin, total cholesterol (Ch), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides (TG) levels of these animals are presented in Table 1 as Mean±SD in comparison with healthy control. Insulin resistance, hyperinsulinemia, hyperglycemia, hyperlipidemia, and dyslipidemia characterize the rats prepared by this means.

Table 1. Fasting plasma parameters in rats at 6th day since the first alloxan injection.

Rats	Glucose,mmol/l	Insulin, μU/l	HDL, mmol/l	LDL, mmol/l	TG, mmol/l	Ch, mmol/l
Healthy rats (n=10)	4.3 ± 0.7	2.0 ± 0.5	2.0 ± 0.3	0.8 ± 0.2	0.5 ± 0.1	1.7 ± 0.3
Diabetic rats (n=10)	13.9± 2.3*	4.8 ± 0.8*	1.0 ± 0.3*	1.0 ± 0.3	1.3 ± 0.3*	2.5 ± 0.5*

*Differs significantly from healthy control ($P < 0.01$)

Treatment. The diabetic animals were divided into four groups: a diabetic control group (n=10) and three diabetic groups treated with daily i.v. injections

of choline chloride (240 mg/kg body weight, n=10), disodium succinate (70 mg/kg body weight, n=10), or the combination of choline chloride (240 mg/kg body weight) with disodium succinate (70 mg/kg body weight, n=10) for the 5 days.

Fasting plasma glucose was measured at the 6th day since treating beginning. Data are shown in Table 2. In diabetic control, the mean of glucose level was found to be 12.5 mmol/l. ΔGlucose is difference between means of fasting plasma glucose in control and treated rats calculated by equation: Δ Glucose = 12.5 – Mean (mmol/l).

Effect of the combination is 7.8 mmol/l decreasing in fasting plasma glucose level from the control. The sum of effects of choline chloride and disodium succinate administered individually is 2.8 mmol/l (–1.1 +3.9 =2.8) decreasing in fasting plasma glucose level of control. Since glucose-lowering effect of the combination is greater than the sum of glucose-lowering effects of choline and succinate administered individually, the combination is synergistically effective in lowering pathologically elevated plasma glucose levels.

Table 2. Fasting plasma glucose at 6th day since treating beginning.

Treatment	Glucose, mmol/l (Mean ± SD)	Δ Glucose, mmol/l
Control	12.5 ± 2.4*	0
Choline chloride	13.6 ± 2.2*	–1.1
Disodium succinate	8.6 ± 1.8*t	+3.9
Combination	4.7 ± 0.8t	+7.8

* Differs significantly from the combination (P< 0.01)

t Differs significantly from control (P< 0.01)

Fasting plasma insulin levels were measured at the 6th day since treating beginning. Data are shown in Table 3. In diabetic control, the mean of insulin level was found to be 5.7 μU/l. ΔInsulin is difference between means of

fasting plasma insulin in control and treated rats calculated by equation: Δ Insulin = 5.7 – Mean (μU/l).

Effect of the combination is 4.1μU/l decreasing in fasting plasma insulin level from the control. The sum of effects of choline chloride and disodium succinate administered individually is 2.1μU/l (0.9 + 1.2 =2.1) decreasing in fasting plasma insulin level of control. Since insulin-lowering effect of the combination is greater than the sum of insulin-lowering effects of choline and succinate administered individually, the combination is synergistically effective in lowering pathologically elevated plasma insulin levels.

Table 3. Fasting plasma insulin at 6th day since treating beginning.

Treatment	Insulin, μU/l (Mean ± SD)	Δ Insulin, μU/l
Control	5.7 ± 1.1*	0
Choline chloride	4.8 ± 0.7*	0.9
Disodium succinate	4.5 ± 0.6*t	1.2
Combination	1.6 ± 0.3t	4.1

* Differs significantly from the combination (P<0.01)

t Differs significantly from control (P<0.01)

EXAMPLE 2

Bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate is effective in treating insulin resistance, hyperlipidemia, dyslipidemia, and diabetes mellitus in diabetic rats.

Animals. Diabetic rats were prepared as described in Example 1 of the invention.

Treatment. The animals were divided into two groups: a diabetic control group (n=10) and diabetic group treated with daily i.v. (tail vein) injections of bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate (120 mg/kg body weight, n=10) for the 3 days. Fasting plasma glucose, insulin, total cholesterol (Ch),

high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides (TG) levels were measured at the 7th day since treating beginning and shown in Table 4 as Mean±SD.

Table 4. Fasting plasma parameters at 7th day since treating beginning.

Blood parameters, (Mean±SD)	Treatment	
	Control rats	Treated rats
Fasting plasma glucose, mmol/l	12.5 ± 2.4	4.2 ± 0.6**
Fasting plasma insulin, μU/l	6.0 ± 1.0	1.3 ± 0.5**
Total cholesterol, mmol/l	3.0 ± 0.4	1.5 ± 0.3**
LDL cholesterol, mmol/l	1.1 ± 0.3	0.8 ± 0.2*
HDL cholesterol, mmol/l	0.8 ± 0.2	2.0 ± 0.2**
Triglycerides, mmol/l	1.4 ± 0.3	0.7 ± 0.1**

* Differs from control ($P<0.05$)

** Differs significantly from control ($P<0.01$)

EXAMPLE 3

The process for producing bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate.

To a solution of 121 g (1 mole) of choline base in methanol was added 59 g (0.5 mole) of succinic acid. The reaction mixture was stirred at ambient temperature for 20 minutes and concentrated under vacuum to dryness at a temperature of 40-50°C. The desired bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate is obtained at a yield approximating 100% as a solid white crystalline material: mp 135-137°C (from tert-butanol); NMR ^1H (D_2O):δ 2.24 (s, 4H), 3.07 (s, 18H), 3.38 (m, 4H), 3.93 (m, 4H); NMR ^{13}C (D_2O):δ 34.5, 54.2, 56.0, 67.8, 182.6; Anal. Calculated for $C_{14}H_{32}N_2O_6$: C, 51.83; H, 9.94. Found: C, 51.66; H 9.86.

WE CLAIM:

1. A method for achieving a synergistic effect in treating insulin resistance in a mammal in need thereof, which comprises administering to said mammal an effective amounts of:

(a) choline base or a pharmaceutically acceptable salt thereof; and

(b) succinic acid or a pharmaceutically acceptable salt thereof,

wherein the amount of (a) alone and the amount of (b) alone is insufficient to achieve the effect; and

wherein the effect of the sum of the amounts of (a) and (b) is greater than the sum of the effects achievable with the amounts of (a) and (b) administered individually.

2. The method as claimed in Claim 1 wherein said effect comprises lowering plasma insulin levels.

3. A method for achieving a synergistic effect in treating diabetes mellitus in a mammal in need thereof, which comprises administering to said mammal an effective amounts of:

(a) choline base or a pharmaceutically acceptable salt thereof; and

(b) succinic acid or a pharmaceutically acceptable salt thereof,

wherein the amount of (a) alone and the amount of (b) alone is insufficient to achieve the effect; and

wherein the effect of the sum of the amounts of (a) and (b) is greater than the sum of the effects achievable with the amounts of (a) and (b) administered individually.

4. The method as claimed in Claim 3 wherein said effect comprises lowering plasma glucose levels.

5.The method as claimed in any one of Claims 1 to 4 wherein the amount of the choline base or a pharmaceutically acceptable salt thereof and the amount of the succinic acid or a pharmaceutically acceptable salt thereof is administered simultaneously.

6. The method as claimed in any one of Claims 1 to 5 wherein the pharmaceutically acceptable salt of choline is choline chloride.

7. The method as claimed in any one of Claims 1 to 6 wherein the pharmaceutically acceptable salt of succinic acid is disodium succinate.

8. The method as claimed in any one of Claims 1 to 7 wherein the effective amount of choline base or a pharmaceutically acceptable salt thereof is 3 to 300 mg per day per kg of body weight of the mammal.

9. The method as claimed in any one of Claims 1 to 8 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is 1 to 250 mg per day per kg of body weight of the mammal.

10. The method as claimed in any one of Claims 1 to 9 wherein the effective amount of choline base or a pharmaceutically acceptable salt thereof is administered orally, parenterally, topically or rectally.

11. The method as claimed in Claim 10 wherein the effective amount of choline base or a pharmaceutically acceptable salt thereof is administered parenterally.

12. The method as claimed in any one of Claims 1 to 11 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is administered orally, parenterally, topically or rectally.

13. The method as claimed in Claim 12 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is administered parenterally.

14. The method as claimed in any one of Claims 1 to 13 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is administered for a period of 1 day or longer.

15. The method as claimed in Claim 14 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is administered for a period of 3 to 7 days.

16. The method as claimed in any one of Claims 1 to 15 wherein the effective amount of choline base or a pharmaceutically acceptable salt thereof is administered for a period of 1 day or longer.

17. The method as claimed in Claim 16 wherein the effective amount of choline base or a pharmaceutically acceptable salt thereof is administered for a period of 3 to 7 days.

18. A composition for achieving a synergistic effect in treating insulin resistance in a mammal in need thereof which comprises an effective amounts of:

(a) choline base or a pharmaceutically acceptable salt thereof; and

(b) succinic acid or a pharmaceutically acceptable salt thereof,

wherein the amount of (a) alone and the amount of (b) alone is insufficient to achieve the effect; and

wherein the effect of the sum of the amounts of (a) and (b) is greater than the sum of the effects achievable with the amounts of (a) and (b) administered individually, and a pharmaceutically acceptable diluent or carrier.

19. The composition as claimed in Claim 18 wherein said effect comprises lowering plasma insulin levels.

20. A composition for achieving a synergistic effect in treating diabetes mellitus in a mammal in need thereof which comprises an effective amounts of:

(a) choline base or a pharmaceutically acceptable salt thereof; and

(b) succinic acid or a pharmaceutically acceptable salt thereof,

wherein the amount of (a) alone and the amount of (b) alone is insufficient to achieve the effect; and

wherein the effect of the sum of the amounts of (a) and (b) is greater than the sum of the effects achievable with the amounts of (a) and (b) administered individually, and a pharmaceutically acceptable diluent or carrier.

21. The composition as claimed in Claim 20 wherein said effect comprises lowering plasma glucose levels.

22. The composition as claimed in any one of Claims 18 to 21 wherein the pharmaceutically acceptable salt of choline is choline chloride.

23. The composition as claimed in any one of Claims 18 to 22 wherein the pharmaceutically acceptable salt of succinic acid is disodium succinate.

24. The composition as claimed in any one of Claims 18 to 23 wherein the effective amount of choline base or a pharmaceutically acceptable salt thereof is 3 to 300 mg per day per kilogram of body weight of the mammal.

25. The composition as claimed in any one of Claims 18 to 24 wherein the effective amount of succinic acid or a pharmaceutically acceptable salt thereof is 1 to 250 mg per day per kilogram of body weight of the mammal.

26. Bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate

27. Bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate for use in medicine.

28. The use as claimed in Claim 27 wherein said use in medicine is use for the manufacture of a medicament for treating insulin resistance in a mammal in need thereof.

29. The use as claimed in Claim 27 wherein said use in medicine is use for the manufacture of a medicament for treating diabetes mellitus in a mammal in need thereof.

30. The use as claimed in Claim 27 wherein said use in medicine is use for the manufacture of a medicament for treating hyperlipidemia or dyslipidemia in a mammal in need thereof.

31. A process for producing bis(2-hydroxy-N,N,N-trimethylethanaminium) succinate, which comprises reacting choline base with succinic acid.

32. The process as claimed in Claim 32 in which the mole ratio of choline base to succinic acid is about 2:1, but no lower than 1.9:1 and no higher than 2.1:1.

INTERNATIONAL SEARCH REPORT

Internat ıl Application No
PCT/RU 00/00122

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/14 A61K31/19

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)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 010, no. 377 (C-392), 16 December 1986 (1986-12-16) & JP 61 171417 A (WAKUNAGA SEIYAKU KK), 2 August 1986 (1986-08-02) abstract ---	1-25
Y	KOREC ET AL: "Succinic acid alkyl esters-new genericoral anti-diabetic agents in alloxan and STZ diabetic rats" DIABETOLOGIA. SUPPLEMENT,DE,SPRINGER VERLAG, BERLIN, vol. 40, no. SUPPL. 01, June 1997 (1997-06), page A375 XP002123539 ISSN: 0941-5602 the whole document --- -/--	1-25

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2001	01/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gonzalez Ramon, N

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INTERNATIONAL SEARCH REPORT

Interna il Application No
PCT/RU 00/00122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MALAISSE WILLY: "Insulinotropic Nutrient Esters" DRUGS OF THE FUTURE,ES,BARCELONA, vol. 23, no. 11, 1998, pages 1205-1216, XP002123537 ISSN: 0377-8282 page 1205 -page 1210	1-25
E	WO 00 59499 A (VERTELETSKY PAVEL VASILIEVICH ;KOLESOVA OLGA EVGENIEVNA (RU); POMY) 12 October 2000 (2000-10-12) abstract page 12, line 22-25; claim 10	1-25
E	WO 00 51594 A (VERTELETSKY PAVEL VASILIEVICH ;KOLESOVA OLGA EVGENIEVNA (RU); POMY) 8 September 2000 (2000-09-08) abstract page 4, line 22-25	1-25
A	MACDONALD ET AL: "Glyceraldehyde phosphate and methyl esters of succinic acid. Two new potent insulin secretagogues" DIABETES,US,NEW YORK, NY, vol. 37, no. 7, July 1988 (1988-07), pages 997-999, XP002123535 ISSN: 0012-1797 abstract; table 1	1-25
X	GLADYCH J M Z ET AL: "SYNTHETIC NEUROMUSCULAR BLOCKING AGENTS. PART IV. COMPOUNDS RELATED TO BOTH LAUDEXIUM AND SUXAMETHONIUM" JOURNAL OF THE CHEMICAL SOCIETY,GB,CHEMICAL SOCIETY. LETCHWORTH, 1962, pages 1481-1487, XP002070649 abstract	26-32
X	FR 2 716 M (OLIN MATHIESON CHEMICAL CORPORATION) 10 August 1964 (1964-08-10) abstract; claim 3	26-32
X	ANSERMINO J M ET AL: "SUXAMETHONIUM-INDUCED MUSCLE PAINS ARE NOT RELATED TO CHOLINESTERASE ACTIVITY" ANAESTHESIA,GB,ACADEMIC PRESS, LONDON, vol. 48, no. 12, December 1993 (1993-12), pages 1097-1100, XP000901320 ISSN: 0003-2409 abstract	26-32

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna l Application No
PCT/RU 00/00122

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 61171417 A	02-08-1986	NONE	
WO 0059499 A	12-10-2000	NONE	
WO 0051594 A	08-09-2000	AU 4937499 A	21-09-2000
FR 2716 M		NONE	