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Kawashima

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[54] **METHOD FOR TREATING DIABETIC KETOACIDOSIS**

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[30] **Foreign Application Priority Data**

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[52] **U.S. Cl.** **424/178;** 424/319

[51] **Int. Cl.²** **A61K 27/00**

[58] **Field of Search** 424/319, 178

[56] **References Cited**

FOREIGN PATENTS OR APPLICATIONS

46-2247 1971 Japan 424/319

OTHER PUBLICATIONS

Canadian Journal of Biochemistry, Vol. 49, 599–605; 941–948, (1971).

The Journal of Clinical Investigation, Vol. 52, Apr. 1973, 877–884.

Primary Examiner—Frederick E. Waddell

Attorney, Agent, or Firm—Sughrue, Rothwell, Mion, Zinn & Macpeak

[57] **ABSTRACT**

A method for treating diabetic ketoacidosis comprising administering a therapeutically effective amount of a pharmaceutical composition comprising (+)-octanoylcarnitine and, optionally, insulin is disclosed.

6 Claims, 4 Drawing Figures

Fig. 1

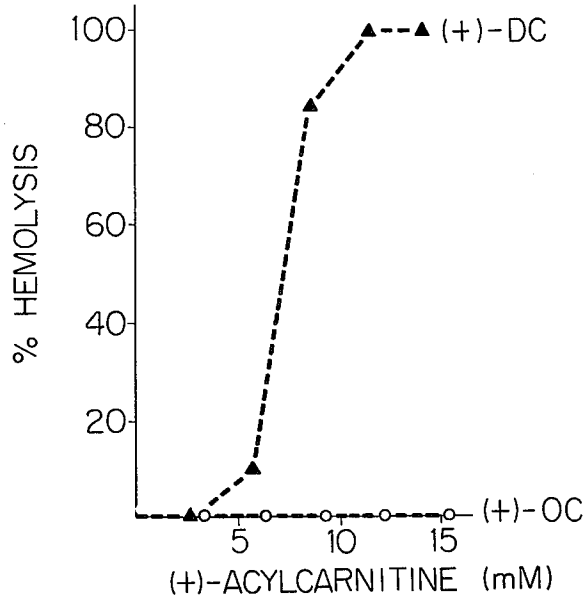

100
80
60
40
20
% HEMOLYSIS
(+)-DC
(+)-OC
5
10
15
(+)-ACYLCARNITINE (mM)

Fig. 2

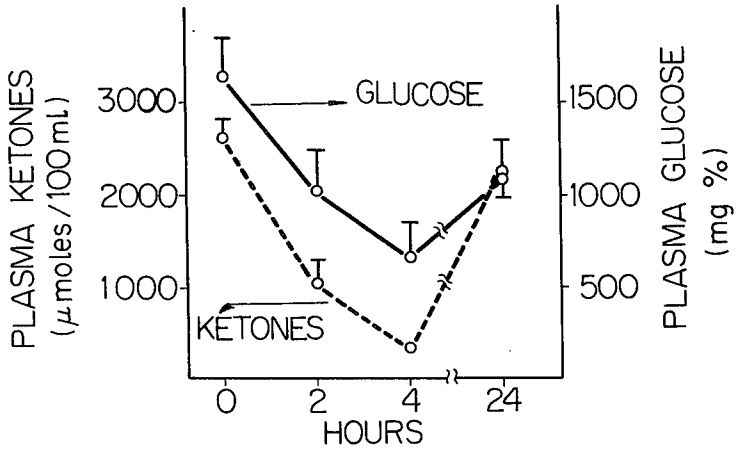

PLASMA KETONES
(μmoles/100mL)
3000
2000
1000
GLUCOSE
KETONES
1500
1000
500
PLASMA GLUCOSE
(mg %)
0
2
4
24
HOURS

Fig. 3

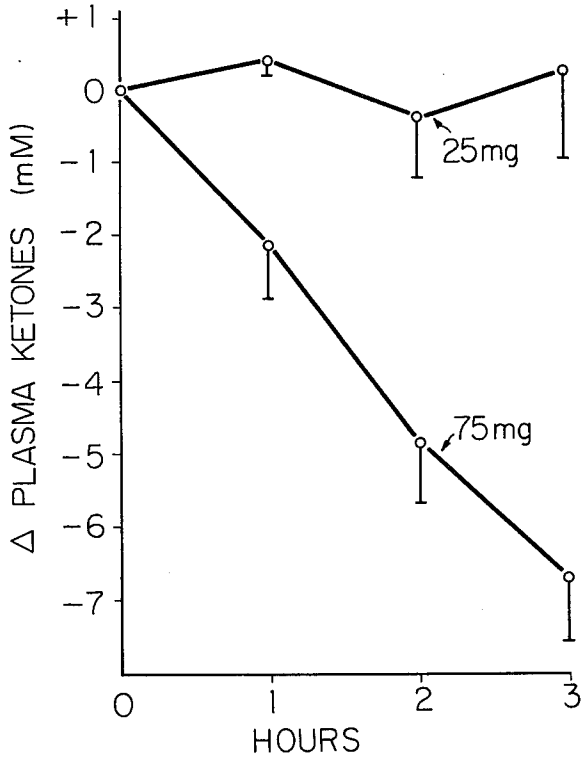

+1
0
-1
-2
-3
-4
-5
-6
-7
Δ PLASMA KETONES (mM)
25mg
75mg
0
1
2
3
HOURS

Fig. 4

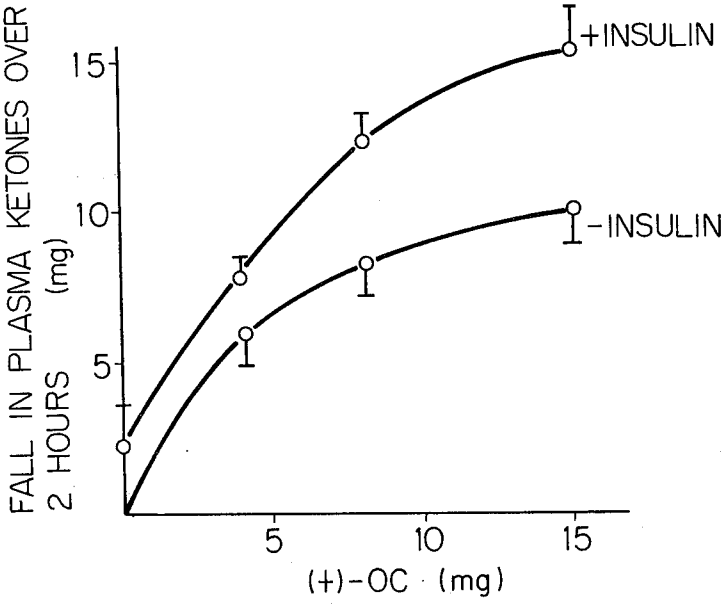
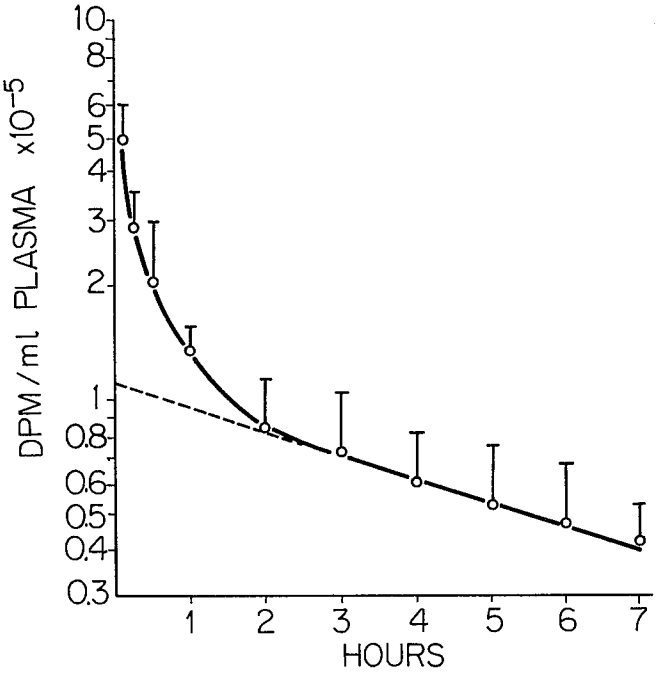

FALL IN PLASMA KETONES OVER 2 HOURS (mg)
15
10
5
+INSULIN
-INSULIN
5
10
15
(+)-OC (mg)

Fig. 5



METHOD FOR TREATING DIABETIC KETOACIDOSIS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method for treating diabetic ketoacidosis. More particularly, this invention relates to a method for treating diabetic ketoacidosis using a pharmaceutical composition comprising (+)-octanoylcarnitine alone or in combination with insulin.

2. Description of the Prior Art

It has been reported that about 3 percent of the population in the United States of America suffers from diabetes to a greater or lesser degree of severity. Diabetic ketoacidosis which is a lethal diabetic disorder is a common clinical emergency which is often observed. For example, at the University of Southern California, admissions of as many as 340 cases for the 3 year period from July 1, 1965 to June 30, 1968 were reported (Diabetes, 20, 490, (1972)).

For treatment of the acidosis, administrations of insulin and fluids are commonly employed but such conventional treatments require a period as long as about 7 hours to reverse the lethal disorder. Therefore, a treating agent having a rapid initial action and a higher safety has been of great interest to clinicians.

J. D. McGarry et al. have reported in *J. Clin. Invest.* 52, 877, (1973) that (+)-decanoylcarnitine which is a potent inhibitor of long-chain acylcarnitine transferase in the tests using severely ketotic alloxan diabetic rats (*Proc. Natl. Acad. Sci., U.S.A.*, 58, 790, (1967)) was found to be a substance which exhibits an extremely rapid effect in the treatment of the acidosis and that the mechanism of the action is the blockage of free fatty acids which are increased in the cytoplasm of the liver due to the insulin deficiency to transfer into the mitochondria where ketone bodies are produced.

Further, Fritz and Marquis in *Proc. Natl. Acad. Sci., U.S.A.*, 54, 1226, (1965) have reported that carnitine palmitoyltransferase is inhibited by (+)-palmitoylcarnitine; Fritz and Shulty in *J. Biol. Chem.*, 240, 2188, (1965) have reported that (+)-acetylcarnitine is the most potential competitive inhibitor for carnitine acetyltransferase; Solberg in *Biochem. Biophs. Acta.*, 280, 422, (1972) and Kopec and Fritz in *Can. J. Biochem.*, 49, 941, (1971) have reported that there will be a carnitine octanoyltransferase other than acetyl- and palmitoyltransferase; and Lee and Fritz in *Can. J. Biochem.*, 49, 599, (1971) have reported that (−)-octanoylcarnitine may be a more excellent substrate than (−)-acetylcarnitine and (−)-palmitoylcarnitine for ketone body production in rat liver. However, none of these reports suggests that (+)-octanoylcarnitine could be practically used as a therapeutic tool for diabetic ketoacidosis and, particularly, that a combination of (+)-octanoylcarnitine and insulin can markedly control or alleviate diabetic ketoacidosis.

SUMMARY OF THE INVENTION

As a result of extensive investigations on the effect of (+)-octanoylcarnitine for diabetic ketoacidosis, it has been found that when (+)-octanoylcarnitine is orally or parenterally administered, optionally in combination with insulin, for the treatment of diabetic ketoacidosis, ketone bodies in the plasma can remarkably be reduced and that the (+)-octanoylcarnitine is substantially non-toxic. Particularly, (+)-decanoylcarnitine as a control, which is an analogue of (+)-octanoylcarnitine, produces hemolysis as a deleterious side effect due to its strong surface activity when it is administered intraveneously, whereas (+)-octanoylcarnitine according to the present invention does not have such an untoward effect. Moreover, the present inventor has found that (+)-octanoylcarnitine exhibits an unexpectedly superior anti-ketogenic effect in comparison with (+)-decanoylcarnitine and (+)-hexanoylcarnitine and thereby is a promising therapeutic agent for treating ketoacidosis. It will be obvious to those skilled in the art that (+)-octanoylcarnitine of the present invention also will exhibit this excellent antiketogenic effect when administered orally and can be conveniently used in practical treatment.

BRIEF EXPLANATION OF ACCOMPANYING DRAWINGS

FIG. **1** shows the relative hemolytic capacity in vitro of (+)-octanoylcarnitine and (+)-decanoylcarnitine. The ordinate indicates the percent hemolysis and the abscissa indicates the concentration of (+)-octanoylcarnitine and (+)-decanoylcarnitine.

FIG. **2** shows the reversibility of the (+)-octanoylcarnitine effect in vivo. The ordinate indicates the concentrations of plasma ketones (dotted line) and plasma glucose (solid line), and the abscissa indicates the time after the administration.

FIG. **3** shows the effects of oral administration of (+)-octanoylcarnitine on plasma ketone levels. The ordinate indicates the concentration of plasma ketone bodies and the abscissa indicates the time.

FIG. **4** shows the relationship between the dosage of (+)-octanoylcarnitine and the decrease in plasma ketone levels. The ordinate indicates the amount of the decrease in plasma ketone bodies and the abscissa indicates the dosage administered.

FIG. **5** shows the disapperance of (+)-octanoylcarnitine -1-^{14}C from the blood of alloxan diabetic rats. The ordinate indicates dpm/ml plasma $\times 10^{-5}$ and the abscissa indicates the time.

DETAILED DESCRIPTION OF THE INVENTION

The (+)-octanoylcarnitine [hereinafter referred to as (+)-OC]used in the present invention is a known compound and can be easily prepared by the acylation of (+)-carnitine chloride with octanoyl chloride, these being disclosed in Japanese Patent Publication 2247/71.

The mechanism of the action of (+)-OC effective for the treatment of diabetic ketoacidosis has not yet been established. The ketosis seems to be initiated by a relative or absolute lack of insulin which in turn results in a mobilization of free acids to the liver where they are converted into acetoacetate and β-hydroxybutyrate. It is equally believed, however, that increased delivery of free fatty acids to the liver is not in itself sufficient to induce the ketosis and that the production of ketone bodies depends upon both the capacity of the tissue to generate acetyl-CoA and its ability to metabolize the free fatty acids through non-ketogenic pathways. In the normal state, the free fatty acid delivery to the liver is low and the fatty acid oxidation is inhibited at the level of the acylcarnitine transferase reaction. Fatty acids are therefore utilized primarily for triglyceride synthesis. On the other hand, in the diabetic state the free fatty acid delivery is increased and the fatty acids are

oxidized to ketone bodies through activation of the acylcarnitine transferase reaction to cause ketoacidosis.

As previously described, the (+)-OC of the present invention can be administered either orally or parenterally, e.g., subcutaneously or intravenously. For oral administration, a dose usually ranges from about 50 mg to about 100 mg, preferably 65 mg to 85 mg/kg body weight.

The compound can be used in a common preparation form, such as in the form of a powder, tablets, capsules, syrup, elixir and the like. It is preferable that 100 mg to 400 mg of the compound be contained per unit dose form. For parenteral administration, a dose usually ranges from about 1 mg to 25 mg, preferably 5 to 15 mg/Kg body weight. It is usually preferable to formulate as an aqueous solution of 100 mg to 300 mg/ml. Of course, other additives which can be commonly employed in the art can also be incorporated into these parenteral dosage forms, for example, local anesthetic agents, such as lidocain chloride and benzyl alcohol and glucose and saline etc. to render the solution isotonic, or other active ingredients. Especially when the compound is administered together with insulin as previously described, the compound is preferably given in a form of intravenous injection. In this case, the proportion of insulin to (+)-OC is usually about 1 to about 10 units, preferably 5 unit/Kg body weight.

The present invention will now be illustrated in greater detail referring to the following Examples but they are not to be construed as limiting the scope of the present invention. Unless otherwise indicated, all parts, percents, ratios and the like are by weight.

EXAMPLE 1

Determination of Hemolytic Capacity

Relative hemolytic capacities of (+)-OC and (+)-decanoylcarnitine were determined using human blood. Human erythrocytes were suspended in a solution of 5 percent bovine albumin in 0.9 percent sodium chloride (pH 7.4) containing (+)-OC or (+)-decanoylcarnitine at various concentrations as indicated in FIG. **1.** After incubation at 37°C for 20 minutes, the cells were removed by centrifuging and the amount of hemoglobin present in the supernatant was measured spectrophotometrically. The results obtained are shown in FIG. **1.**

It is apparent from the results obtained that (+)-decanoylcarnitine causes extensive hemolysis at concentrations greater than about 3mM, whereas (+)-OC is less hemolytic up to the maximum concentration, i.e., 15.4 mM.

EXAMPLE 2

Inhibition of Ketone Body Production

3 to 8 unanesthetized alloxan diabetic rats per group (Wistar; both sexes; body weight, about 120 g) received intravenously 17 mg of (+)-OC, and as controls, (+)-hexanoylcarnitine [(+)-HC] or (+)-decanoylcarnitine, [(+)-DC] with or without insulin (0.85 U) were used. Two hours after the administration, plasma glucose and ketone body concentrations were determined, and the results obtained are shown in Table 1 below. In Table 1, the values represent the means ± SEM for the number of animals in each group.

Table 1

COMPARATIVE EFFECTS OF (+)–HEXANOYLCARNITINE (+)– OCTANOYLCARNITHINE, (+)–DECANOYLCARNITINE AND INSULIN IN INTACT ALLOXAN DIABETIC RATS

Number of Animals	Treatment	Change in 2 Hours Plasma Glucose (mg%)	Change in 2 Hours Plasma Ketones (μmoles/100 ml)
8	Insulin	−423 ± 79	−248 ± 195
3	(+)–HC	+ 27 ± 48	−616 ± 100
3	Insulin; (+)–HC	−418 ± 115	−845 ± 123
6	(+)–OC	+ 23 ± 45	−1,035 ± 115
6	Insulin; (+)–OC	−604 ± 112	−1,565 ± 136
7	(+)–DC	+ 44 ± 27	−608 ± 103
8	Insulin; (+)–DC	−387 ± 51	−1,037 ± 147

From the results shown in Table 1 above, (+)-OC either alone or in combination with insulin is found to exhibit an excellent reduction of ketone bodies in blood. Further, it can be seen that (+)-OC has almost no effect on blood glucose levels despite its capacity to reverse ketosis when given to acute diabetic ketotic rats.

EXAMPLE 3

Reversibility of (+)-OC Effects

For the purpose of demonstrating the relationship between the dosage of (+)-OC and the reversal period of time of diabetic ketoacidosis in rats, 6 unanesthetized rats (body weight, about 130 g) received an infusion of 0.9 percent sodium chloride, pH 7.4, containing 1 U/ml of insulin and 20 mg/ml of (+)-OC through the venous catheter at a rate of 75 μ/min for 10 minutes. After 2 hours and 4 hours blood samples were taken, the animals were placed in cages with access to water until a 24 hour time point when the last blood sample was collected. The results obtained on plasma ketone and plasma glucose levels are shown in FIG. **2.** The values represent means ± SEM.

As is apparent from the results obtained, ketone bodies and glucose in blood which has been decreased by the infusion of (+)-OC gradually reverted to their initial values after the infusion.

EXAMPLE 4

Effects of Orally Administered (+)-OC on Plasma Ketones

Alloxan diabetic rats weighing approximately 100 g received through a stomach tube 1 ml of a neutralized aqueous solution containing 25 mg and 75 mg (+)-OC, respectively. The plasma ketone level was determined on samples taken at hourly intervals and the results obtained are shown in FIG. **3.**

It is clear from the results that no significant changes occurred in plasma ketone levels with 25 mg of the drug while a pronounced effect on reduction of plasma ketone bodies was seen with 75 mg of the drug. That is, the compound of the present invention also have an excellent effect on the rapid reduction of ketone bodies in the blood after oral administration.

EXAMPLE 5

Relationship between Dosage of (+)-OC and Reduction in Plasma Ketone Concentrations

4 to 6 alloxan diabetic rats per group weighing approximately 100 g received intravenously 0.5 ml of a 0.9 percent aqueous solution of sodium chloride, pH 7.4, containing 4 mg, 8 mg and 15 mg of (+)-OC, respectively, in the presence or absence of 0.75 U of insulin. The 4 and 8 mg doses were given as a single injection over a period of 1 minute and the 15 mg dose was infused over a period of 10 minutes. The decrease in plasma ketones was observed over 2 hours after the administration in each case to obtain the mean ± SEM for the 4–6 animals in each group. The results in plasma ketone levels obtained are shown in FIG. **4.**

The safety in terms of LD_{50} of the admistration of (+)-DC in the method of this invention is summarized in the following table 2.

Table 2

Animal		LD_{50} (g/Kg) Intra-venous	Intra-peritoneal	per os
Rat (Wistar)	(male)	0.198	0.53	more than 4.0
	(female)	0.213	0.54	more than 4.0
Mouse (dd)	(male)	0.175	0.51	3.84
	(female)	0.215	0.63	3.94

From the results shown in Table 2 above, it was found that the LD_{50} of (+)-OC is about 0.2 g/Kg body weight when administered intravenously, about 0.6 g/Kg body weight for intraperitoneal administration and about 4 g/Kg for oral administration.

EXAMPLE 6

Rate of Disappearance of (+)-OC from the Blood of Alloxan Diabetic Rats

Alloxan diabetic rats weighing approximately 100 g received a single intravenous injection of 10 mg of (+)-OC -1-^{14}C (sp. activity : 1 μCi/mg) in 0.5 ml of a 0.9 percent sodium chloride, pH 7.4. It was observed that the intravenously administered (+)-OC-1-^{14}C disappeared rapidly from the blood of alloxan diabetic rats. After a period of about 2 hours the disappearance curve became logarithmic with a half-life of about 4.5 hours. With one animal it was found that **41, 54** and 63 percent of the injected radioactivity (on a cumulative basis) had been excreted in the urine after 1,2 and 3 hours, respectively. Therefore, it was recognized that large quantities of (+)-OC had been excreted in the urine during a very early stage after administration.

PREPARATION EXAMPLE

Capsule:

A capsule containing 200 mg of (+)-OC was prepared using the following components.

Component	
Avicel	30 mg
Corn Starch	20 mg
Anhydrous Silicic Acid	10 mg
Magnesium Stearate	12 mg

Tablet: A tablet containing 200 mg of (+)-OC was prepared using the following components.

Component	
Avicel	25 mg
Corn Starch	25 mg
Anhydrous Silicic Acid	15 mg
Magnesium Stearate	15 mg
Methylmethacrylate-Methacrylic Acid-Copolymer (Z type)	10 mg
PEG*-6000 (sugar-coated)	0.5 mg

*Polyethylene glycol - mol.wt about 6,000.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

What is claimed is:

1. A method for treating diabetic ketoacidosis which comprises orally or parenterally administering to a subject afflicted with diabetic ketoacidosis to reduce keto bodies in plasma a therapeutically effective amount of (+)-octanoylcarnitine.

2. The method of claim **1,** wherein said administering of said (+)-octanoylcarnitine is orally at a dose level of from 50 mg to 100 mg per Kg of body weight.

3. The method of claim **1,** wherein said administering of said (+)-octanoylcarnitine is parenterally at a dose level of from 1 mg to 25 mg per Kg of body weight.

4. The method of claim **1,** wherein said administering of said (+)-octanoylcarnitine is in the form of a pharmaceutical composition containing pharmaceutically acceptable carriers or diluents.

5. The method of claim **4,** wherein said pharmaceutical composition includes insulin in such a proportion that about 1 to 10 units of insulin are administered per Kg of body weight.

6. The method of claim **5** wherein the composition is administered in an amount to provide 5 units of insulin per Kg of body weight.

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