



AFRICAN REGIONAL INDUSTRIAL PROPERTY
ORGANIZATION (ARIPO)

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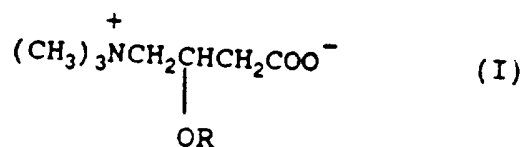
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(30) Priority Data:	
(33) Country: IT	
(31) Number: 47916 A/89	
(32) Date: 05.05.89	
(84) Designated States: BW GM KE LS MW SD SZ UG ZM ZW	

(51) International Patent Classification Int. Cl.⁵ A61K 31/205

(54) Title: PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF PROTOZOAL DISEASES PARTICULARLY OF TRYPANOSOMIASIS, COMPRISING D-CARNITINE OR AN ACYL DERIVATIVE OF D-CARNITINE.

(57) Abstract:

D-carnitine and acyl derivatives of D-carnitine of the general formula



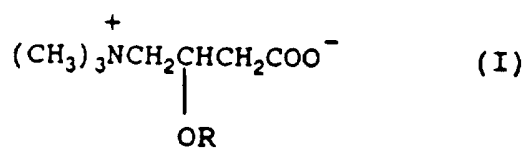
in which R is an acyl group having from 2 to 20 atoms of carbon, particularly acetyl, propionyl, butyryl, isobutyryl and isovaleryl, and their pharmacologically acceptable salts, are active in the therapeutic treatment of protozoal diseases, particularly of trypanosomiasis.

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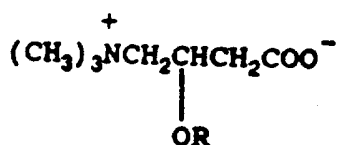


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PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF PROTOZOAL DISEASES PARTICULARLY OF TRYPANOSOMIASIS, COMPRISING D-CARNITINE OR AN ACYL DERIVATIVE OF D-CARNITINE

This invention concerns orally or parenterally administrable pharmaceutical compositions for the therapeutic treatment of protozoal diseases, containing as active ingredient D-carnitine or an acyl derivative of D-carnitine of the general formula (I)



(I)

in which R is an acyl group having 2 to 20 atoms of carbon, particularly acetyl, propionyl, butyryl, isobutyryl and isovaleryl, or their pharmacologically acceptable salts.

Carnitine (beta-hydroxy-gamma-trimethyl-aminobutyric acid) has a chiral centre and hence exists in the two stereoisomer forms, D and L. The natural and physiologically active form of carnitine is the L-(-)-carnitine which acts by transporting fatty acids through the internal mitochondrial membrane. Whereas the racemic form (D,L) of carnitine has been used in the past for some therapeutic applications, it has recently been ascertained that D-carnitine is a competitive inhibitor of fatty acyl carnitine transferases and the administration of massive doses can, therefore, cause depletion of physiological L-(-)-carnitine in the myocardium and in skeletal tissue. Consequently, in all the therapeutic applications of carnitine

known at present, for example in the cardio-vascular field, the treatment of acute and chronic myocardial ischemia, of angina pectoris, of cardiac insufficiency and cardiac arrhythmias and, in the nephrological field, the treatment of chronic uraemic patients undergoing regular haemodialytic treatment to combat muscular asthenia and the onset of muscular cramps, very pure L-(-)-carnitine is exclusively used, accurately avoiding the presence of the D-isomer.

The use of D-carnitine or of derivatives of D-carnitine has never been suggested for therapeutic purposes and it is surprising that such compounds are effective in the treatment of protozoal diseases without inducing any harmful effect in the subjects undergoing the treatment, whereas L-carnitine and the acyl derivatives of L-carnitine corresponding to the derivatives of D-carnitine of the formula (I) have not been effective in the treatment of protozoal diseases.

Henceforth, for the sake of simplicity, specific reference will be made to trypanosomiasis, considering the diffuseness and seriousness of this disease. However this invention refers to the therapeutic treatment of protozoal diseases in general, for example, leishmaniasis and toxoplasmosis.

Trypanosomiasis is a chronic disease caused by protozoa of the genus Trypanosoma. The aetiological agents of trypanosomiasis are more precisely T brucei var. Gambiense and Rhodesiense, which causes African sleeping sickness (Gambian and Rhodesian trypanosomiasis) and the T cruzi which causes Chagas disease (South American trypanosomiasis) found in Central and South America. The African forms of trypanosomiasis are transmitted by the sting of the tsetse fly (genus Glossina). Chagas disease is transmitted by contamination of the puncture wounds of Reduviid bugs, "assassin bugs" or "kissing bugs" (Triatoma

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and other related Reduviids) through the insects' infected faeces.

African trypanosomiasis is characterised by irregular fever, generalised lympho-adenopathy, specially in the posterior cervical chain, cutaneous eruptions and areas of painful localised oedema. Later central nervous system symptoms predominate, such as tremors, aphasia, apathy and convulsions, progressing to coma and death. Rhodesian trypanosomiasis is more serious and more often fatal than the Gambian variety.

The extent of the phenomenon has been highlighted by the World Health Organisation (WHO) which has calculated that in 36 African countries about 50 million people risk contracting the disease. Of this large number of persons at risk, only 5 to 10 million have any possibility of protection or treatment. Moreover, it has been estimated that every year there are about 20 000 new cases, many of which remain undiagnosed.

Acute Chagas disease appears particularly in children and is characterised in the first phases by fever, lympho-adenopathy, hepatosplenomegaly and facial oedema. Meningo-encephalitis or apoplexies with convulsions may occur rarely, sometimes resulting in permanent mental or physical deficiencies or in death. Alternatively there is frequent acute myocarditis, which can also be fatal. Chronic Chagas disease can be mild or even asymptomatic, or else it can be accompanied by myocardiopathy, megaesophagus or megacolon, with a fatal outcome. These delayed symptoms are probably the result of mediated destruction by lymphocytes of the muscular tissue and of the ganglionic nerve cells during the acute form of the illness. Between the acute and the chronic form, 15 to 20 years or more can pass.

Chagas disease affects the whole Latin-American continent, particularly in the tropical and sub-tropical zones, although

there have been reports of indigenous cases in the temperate zones of North America. As far as the diffusion of the sickness is concerned, the WHO reports that it has been calculated that in non-endemic urban areas up to 20% of blood donors are infected with *T. cruzi*, to the extent that the transmission of the disease by means of transfusions has become a serious problem. About 90 million subjects are exposed to the risk of this infection while the present number of infected persons ranges from 16 to 28 million, about 20% to 30% of whom will develop chronic Chagas cardiopathy. Recent estimates support the assertion that in some areas chronic sickness caused by *Trypanosoma cruzi* is the cause of more than 10% of adult mortalities, to the point that many South American countries have defined Chagas disease as a public health problem.

Satisfactory treatments for trypanosomiasis do not exist. Suramin is the preferred medication for early Rhodesian and Gambian trypanosomiasis. Suramin has, however, a series of secondary effects which vary in intensity and frequency according to the patient's state of nutrition. Such secondary effects become very serious in debilitated subjects, a situation not uncommon in Equatorial Africa. The immediate reactions consist of nausea, vomiting, shock and loss of consciousness. More delayed reactions, which can occur up to 24 hours after the administration of the medication are, among others, cutaneous eruptions, peristalsis, photophobia, and watering eyes.

For infections brought about by *Trypanosoma brucei* var. *Gambiense*, pentamidine is also used. Cases have been reported of resistance and reactions which can be alarming, like dyspnoea, tachycardia, cephalaea, and vomiting, phenomena probably related to the fall in blood pressure which follows an



intravenous administration that is not sufficiently slow. The intramuscular method is therefore preferred as it does not produce these phenomena although it can cause sterile abscesses.

The treatment of the advanced stages of the disease is normally based on arsenical compounds, Melarsoprol being used for both trypanosomes and tryparsamide in respect of lesions by T. Gambiense. These arsenical substances are capable of passing the blood-brain barrier and therefore of curing cerebral lesions caused by the trypanosomal infection.

Nevertheless, for both these drugs, side effects have been noted in 5% to 10% of cases which can also cause death in 1% to 5% of treated subjects. A feverish reaction often manifests itself immediately after the (intravenous) injection, specially if parasitemia is high. The most serious side-effect involves the nervous system and is a reactive encephalopathy which appears 3 to 4 days after the treatment. This can be fatal.

Nifurtimox and benznidazole are the preferred drugs for curing American trypanosomiasis.

Nifurtimox, a derivative of nitrofurazone, seems to cure the treated cases of acute infection. The problem arises, however, of curing cases of chronic infection, for which administration must be prolonged for 120 days. The occurrence of nausea, loss of weight and of memory, and sleep disturbances associated with a general state of illness, means that few patients carry this treatment to its end.

Benznidazole is capable of curing both the acute and chronic form of this disease with a course of treatment of 60 days. However, clinical experiments have shown that, with the dosage necessary to obtain results, there are serious side-effects

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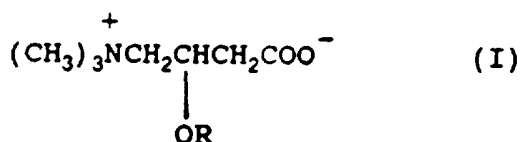
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like polyneuropathies and forms of progressive purpura of the skin. These phenomena necessitate a decrease in the doses, with correspondingly less effectiveness.

The drawbacks of such known medications are overcome by the pharmaceutical composition, administrable by the oral or the parenteral route, for the therapeutic treatment of protozoal diseases, particularly of trypanosomiasis, according to the present invention, which comprises a sufficient quantity to induce a parasitocidal effect in a patient affected by protozoal diseases of D-carnitine, or of an acyl derivative of D-carnitine, of the general formula (I)



in which R is an acyl group having 2 to 20 atoms of carbon, or an equivalent quantity of one of its pharmacologically acceptable salts, and a pharmacologically acceptable excipient. R is preferably selected from acetyl, propionyl, butyryl, isobutyryl and isovaleryl.

The efficacy of D-carnitine and its derivatives of the general formula (I) has been corroborated by extensive pharmacological experiments on parasite pathologies, both in vitro and in vivo, some of which are reported below by way of example.

- (1) Screening in vitro with parasites which are non-pathogenic to man.

Trypanosoma lewisi was used in a liquid culture. (Manganaro et al). Using various concentrations of D-carnitine inner salt (SI) inhibition of growth was obtained which varied from 50% to 70% compared to that obtained in control cultures into which were introduced a



volumetrically equivalent quantity of saline in place of the D-carnitine solution.

- (2) Screening in vivo with parasites which are non-pathogenic to man.

Male Fischer rats were used, infected with 10^7 trypanosoma by the endoperitoneal route, and selectively treated with D-carnitine. Two formulations of D-carnitine were used for the treatment: the inner salt and the chloride at a dosage of 150mg/kg body weight by mouth for five days. Monitoring of parasitemia was carried out on the 5th day of infection since it is known that in this experimental system the maximum parasitic charge occurs precisely on the 5th day.

From the various tests performed it emerged that D-carnitine, whether in the form of chloride or of inner salt, is capable of inhibiting the multiplication of Trypanosoma lewisi, and that it has an effect on this phenomenon without modifying the parasite's ability to infect.

Certain tests were moreover undertaken to establish whether the anti-parasitic activity of D-carnitine inner salt and of D-carnitine chloride and other pharmacologically acceptable salts is equivalent; whether L-carnitine and its acyl derivatives have any activity and if a dose-effect curve exists between treatment with carnitine, in various dosages by mouth, and parasitemia.

Male Fischer rats were used, sub-divided into various groups according to treatment, and infected with 10^7 trypanosoma (T lewisi).



The treatment of the different groups was carried out by mouth, once a day beginning from the day of infection until the fifth day, with L-carnitine or with D-carnitine SI or with D-carnitine chloride, in doses of 75, 150, 225 or 300 mg/kg of body weight. The parasitic counts were undertaken on the 5th day of infection and a comparison was made between the values obtained with different treatments by type of substance and dosage and those deriving from the untreated controls. (See Table 1)

The data emerging from the research, recorded in Table 1, allow one to affirm that D-carnitine (chloride and inner salt) is capable of inhibiting the multiplication of *Trypanosoma lewisi* in infected Fischer rats.

In particular an examination of the data in Table 1 leads to the following conclusions:

- (a) In going from the lowest dose (75mg) to the highest (300mg) multiplication is progressively inhibited. By normalizing these values a dose-effect curve can be obtained which is shown to be valid as much for D-carnitine chloride as for D-carnitine inner salt
- (b) It was constantly observed that the inner salt was more effective than the chloride, bearing in mind that the dose was calculated as the base substance. Moreover, it was observed that the phenomenon was far more pronounced at low dosages of the active substance, whereas it was progressively weakened when it reached a dose of 300mg/kg body weight (Table 2). In this case too the normalisation of values permitted the

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construction of a line curve whose progression was extremely enlightening, so much so that one could predict the dose at which the two effects would coincide.

- (c) The administration of L-carnitine and its acyl derivatives did not lead to significant modifications, either positive or negative, of the parasitemia in respect of the controls at any of the four dosages used.

The inhibitory activity of D-carnitine on the multiplication of *Trypanosoma lewisi* has a dose-effect progression which leads one to suppose that the phenomenon is caused by a competitive mechanism, which is therefore reversible, and not to a toxic mechanism which could be irreversible.

- (3) Tests with parasites not pathogenic to man and derivatives of D-carnitine.

Adopting the same experimental procedures as those in example (2) several acyl derivatives of D-carnitine were tested. Reported below are the results obtained with isovaleryl D-carnitine (IV D-carnitine), isobutyryl D-carnitine (IB) and propionyl D-carnitine (PP).

A single dose was used (300mg/kg body weight). The results are set out in Table 3.

TABLE 1

Percentage variations of parasitemia in rats infected with *Trypanosoma lewisi* and treated with various dosages of D-carnitine compared with the values applicable to infected untreated animals.



Doses used (mg)	75	150	225	300
D-Carnitine chloride	- 1.0	- 2.7***	- 10.4***	- 27.6***
D-carnitine inner salt	- 5.6***	- 9.8***	- 22.8***	- 42.9***
L-carnitine	+ 2.8	- 13.6	==	-7.3

*** = $P < 0.0005$

TABLE 2

Percentage variations of the relationship in activity D-carnitine inner salt/D-carnitine chloride (on a reference scale of 100) in relation to the different dosages used.

Dose used	Inhibitory activity inner salt/ chloride	Percentage Variation
75 mg	- 5.6 / - 1.0	+ 560
150 mg	- 9.8 / - 2.7	+ 263
225 mg	- 22.8 / - 10.4	+ 119
300 mg	- 42.9 / - 22.8	+ 55

TABLE 3

IV D-Carnitine	- 36%
IB D-Carnitine	- 38%
PP D-Carnitine	- 54%



The results confirm that even the derivatives of D-carnitine have an inhibiting effect on the multiplication of trypanosoma, with variable effectiveness according to the compounds.

(4) Tests in vivo with parasites pathogenic to man.

Tests were made on mice of the effect of D-carnitine in the treatment of infections by Rhodesian Trypanosoma brucei, pathogenic to man.

Infection by T. brucei var. Rhodesiense caused the first peak of parasitemia on the 5th day of infection and a second on approximately the 15th day. However, the treatment with D-carnitine was begun at the same time as the infection and was continued until the 15th day. Four treatment groups were formed: to the first, which acted as the control group, only saline was administered by mouth; to the other three groups 75, 150, 300mg respectively of D-carnitine per kg of body weight by mouth, every day for 15 days.

The results are set out in table 4.

TABLE 4

Variations of parasitemia compared to those of the controls.

	5th day	15th day
D-carnitine 75mg	- 37.8	- 20.6
D-carnitine 150mg	- 53.2	- 41.0
D-carnitine 300mg	- 52.3	- 50.1

Finally it was found that the most suitable dose to use in man is about a fifth of that adopted in animal experimentation,

i.e. from a minimum of 30mg to a maximum of 60mg per Kg of body weight daily. Thus an appropriate pharmaceutical composition contains, when in the form of a unitary dose, from about 500 to about 1000mg of D-carnitine, or of an acyl derivative of D-carnitine of formula (I), or an equivalent quantity of one of its pharmacologically acceptable salts.

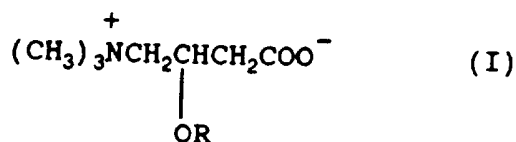
The preparation of the composition of the present invention is carried out according to the usual procedures well-known to experts of pharmaceutical technology. The nature and quantity of the excipients are generally the same as those used to prepare the known compositions with a basis of L-carnitine or acyl derivatives of L-carnitine.

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BEING NOW PARTICULARLY DESCRIBED AND ASCERTAINED
BY THE FOLLOWING CLAIMS AND IN THE MANNER IN THE CLAIMS
TO BE PERFORMED, I WE DECLARE THAT THE CLAIMS ARE

CLAIMS

1. For use in a therapeutic method of treatment of protozoal diseases, particularly trypanosomiasis, a pharmaceutical composition administrable orally or parenterally characterised in that it contains a sufficient quantity to induce a parasitocidal effect in a patient affected by protozoal diseases, of D-carnitine or of an acyl derivative of D-carnitine of the general formula (I)



in which R is an acyl group having 2 to 20 atoms of carbon, or an equivalent quantity of one of their pharmacologically acceptable salts and a pharmacologically acceptable excipient.

2. A pharmaceutical composition according to claim 1, in which R is selected from acetyl, propionyl, butyryl, isobutyryl and isovaleryl.
3. A pharmaceutical composition according to claim 1, in the form of a single dose, containing from about 500 to about 1000 mg of D-carnitine or an acyl derivative of D-carnitine of the formula (I), or an equivalent quantity of one of its pharmacologically acceptable salts.


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