G00002

31 MAY 2002

166166

TITLE OF THE INVENTION

COMPOSITIONS AND METHODS FOR INCREASING THE CONCENTRATION AND/OR MOTILITY OF SPERMATOZOA IN HUMANS

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to compositions and methods for increasing the concentration and/or motility of spermatozoa in humans, including compositions and methods for treating humans affected by idiopathic asthenozoospermia.

Description of the Prior Art

Spermatozoa are produced in the testis and undergo post-gonadal modifications in the epididymis to acquire fertilizing ability. In epididymal plasma, high-molecular-weight proteins and such small molecules as free carnitine convert the gametes into "competent" and functional cells. Free L-carnitine is taken up from blood plasma and concentrated in the epididymal lumen. This epididymal secretion is beneficial for spermatozoa and is not merely an excretory waste. Free carnitine goes through the sperm plasma membrane by passive diffusion. Free L-carnitine is acetylated in mature spermatozoa only. The excess acetyl-CoA from the mitochondria is probably stored as acetyl-L-carnitine and modulates the reserves of free CoA essential to the function of the tricarboxylic acid cycle. This property of L-carnitine of buffering CoA in the mitochondrial matrix is known in somatic cells but is accentuated in male germinal cells. The relationship between the endogenous pool of free and acetylated L-carnitine and the percentage of progressive sperm motility indicates a more important metabolic function related to flagellar movement. Thus, the potential of initiating sperm motility which takes place in the epididymis is probably independent of the carnitine system while the energy properties of acetyl-L-carnitine is relevant in situations of "energy crisis". The uptake of cytoplasmic free L-carnitine in mature spermatozoa must be a protective form of mitochondrial metabolism useful to the survival of this isolated cell.

Idiopathic asthenozoospermia, a disorder of sperm motility, is illustrative of certain conditions in this area. It is a post-testicular cause of infertility due to various ethiology, i.e.

congenital defects of the sperm tail, maturation defects, immunological disorders or infection Several drugs for treating idiopathic asthenozoospermia, none of them completely sausfactory, are known.

Antiestrogen drugs (such as clomiphene citrate and tamoxifen) block sex hormones from inhibiting the Follicle Stimulating Hormone (FSH) and the Luteinizing Hormone (LH) in the brain. This triggers an increased release of LH and FSH, which in turn stimulates testosterone production. Increased testosterone level improves spermatogenesis, thus improving sperm density and motility. However a recent randomized, double-blind, multicenter study of 190 couples by the World Health Organization (WHO) showed no effect of clomiphene citrate. Tamoxifen was claimed to improve sperm concentration but no change in motility was usually detected. As for clomiphene, recent studies did not confirm its efficacy.

Testosterone Rebound therapy involves large doses of testosterone that suppress the activity of the patient's pituitary gland. This, in turn, reduces the intratesticular level of testosterone to systemic levels from the usual level. Then the androgen therapy is discontinued in the hope that the system will rebound and improved spermatogenesis will result.

This therapy is not recommended since a large number of treated patients continue to exhibit azoospermia after treatment.

Testolactone, an aromatase inhibitor, prevents the conversion of testosterone to estradiol. It has been tested in patients with idiopathic oligospermia but contrasting results have raised many doubts on its efficacy.

Mesterolone is a synthetic androgen widely used to treat idiopathic male infertility. A recent study sponsored by WHO failed to show any efficacy of this drug.

Human Chorionic Gonadotropin (HCG) is administered empirically to patients with defects in sperm count or motility to correct a presumed intratesticular deficiency of testosterone. Some patients actually experienced a depression of sperm count due to an increased estrogen production by the testes.

Human Menopausal Gonadotropin (HMO) has approximatively equal LH and FSH activity but its use has produced increased sperm counts in only about 50% of cases.

FSH and HCG or HCG and HMG combination therapy does not appear to improve these results any better.

Gonadotropin Releasing Hormone (GnRH) is expensive and disappointing results have been obtained.

Kallikrein can improve sperm motility with increases in sperm concentration but only in about 50% of cases.

Also L-carnitine and acetyl L-carnitine have been studied as candidate drugs for the treatment of asthenospermia.

Vitali G. et al. (Drugs Exptl. Clin. Res. XXI(4):157-159, 1995) investigated the effectiveness of L-carnitine administration in a group of patients with idiopathic asthenospermia. A favourable effect of the compound on sperm motility and rapid linear progression has been shown in 37 out of 47 patients treated. Same results were obtained by Török L. (Dermatol. Monatsschr. 169:572-575, 1983).

Costa M. et al. (Andrologia, 26: 155 - 159, 1994) showed a significant improvement, both in a quantitative and qualitative manner, in spermatozoal motility after administration of L-carnitine. They speculated that in infertile patients impairment occurred either in epididymal function or in the ability of sperm to capture and utilize carnitine (Bartelloni M. et al., Acta Eur. Fertil. 18:29-31, 1987). Thus, the administration of carnitine would provide additive substrate for sperm energy metabolism and motility.

Müller-Tyl E. et al. (Fertilität 4:1-4, 1988) suggested that L-carnitine therapy can be successful in infertile patients. In fact, results demonstrated a continuous increase in the carnitine levels in sperm following carnitine treatment and a contemporary increase in motility and sperm cell count.

Loumbakis P. et al. (XIIth Congress of the European Association of Urology. Paris, September 1-4, 1996) provided preliminary data suggesting that carnitine administration may positively affect sperm quality.

Finally, Moncada M. L. et al. (Acta Eur. Fertil. 23(5):221-224, 1992) investigated the effect on sperm quality of acetyl-L-carnitine administered to patients affected by idiopathic oligoasthenospermia. Acetyl-L-carnitine had no effects on sperm density, but showed to increase progressive sperm motility.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a combination of admixed or separately packaged L-carnitine and acelyl L-carnitine capable of increasing the concentration or motility of spermatozoa in a human, said combination being in a form suitable for oral or parenteral administration, provided that said combination does not comprise propionyl L-carnitine.

Accordingly, it is an object of the invention to provide various compositions and methods for increasing the concentration of spermatozoa in humans.

It is another object of the invention to provide various composition and methods for increasing the motility of spermatozoa in humans.

It is another object of the invention to provide various compositions and methods for treating humans suffering from idiopathic asthenozoospermia.

According to another aspect of the present invention, there is provided the use of a combination of admixed or separately packaged L-carnitine and acetyl L-carnitine or the pharmacologically acceptable salts thereof for preparing a composition for increasing the concentration of spermatozoa and/or the motility of spermatozoa in humans.

The above objects and others which are apparent from the description of the invention below relate to the discovery that administration of both L-carnitine and acetyl L-carnitine, or a pharmacologically acceptable salts[1] thereof, to a human is effective for increasing the concentration and/or motility of spermatozoa, in humans, including treating idiopathic asthenozoospermia, even in individuals not responding to known, conventional aforesaid treatments.

It has been found that the compositions and methods of the present invention exhibit a marked superiority in increasing the concentration of spermatozoa and/or the motility of spermatozoa in humans, including treating humans suffering from idiopathic asthenozoospermia, over the results obtained by administering L-carnitine or acetyl L-carnitine individually, i.e. as monotherapies.

DESCRIPTION OF SPECIFIC EMBODIMENTS

The L-carnitine and acetyl L-carnitine can be in any form suitable for oral or parenteral administration to a human. The L-carnitine and acetyl L-carnitine can be formulated together, as an admixture, or they can be formulated separately (packaged separately), using known techniques. The L-carnitine and acetyl L-

carnitine can be administered in such a manner to an individual either as the admixture or separately formulated.

[1] The term "pharmacologically acceptable salts" is used herein simply to refer to those salts which are safe for use in food stuffs or in prescription products. The term is not used to indicate or suggest a product requiring a prescription.

Depending on various factors, such as concentration of active ingredient(s), the L-carnitine and acetyl L-carnitine according to the invention may be sold as food supplements, nutritional supplements, or as therapeutic products including over-the-counter and prescription products.

Various molar ratios of L-carnitine to acetyl L-carnitine, or the pharmacologically acceptable salts thereof, may be used in accordance with the invention, including molar ratios ranging from 4.0:1 to 1:1.5. Preferred ratios include molar ratios ranging from about 3.2:1 to 2.8:1 and molar ratios corresponding to about 1:1.

The combination preparations of the present invention, when in unit dosage form, comprise from 2.5 g to 0.22 g of L-carnitine inner salt and from 0.28 g to 1.3 g of acetyl L-carnitine or equimolar amounts of the pharmacologically acceptable salts thereof.

Preferred combination preparations in unit dosage form comprise 1.0 g of L-carnitine inner salt and 0.5 g of acetyl L-carnitine inner salt or equimolar amounts of the pharmacologically acceptable salts thereof.

It was, furthermore, found that although the daily dose of the aforesaid active ingredients to be administered is determined from the age, weight and condition of the patient, utilizing sound professional judgement, it is generally advisable to administer in a single dose or multiple dose administration regimen about 0.8 to 2.5 g/day of L-carnitine and about 1.0 to 1.5 g/ day of acetyl L-carnitine or equivalent molar amounts of the pharmacologically acceptable salts thereof. Larger doses can be safely administered in view of the extremely low toxicity of the aforesaid active ingredients.

A clinical study aimed at evaluating whether supplementation with the drug association therapy is effective in improving reduced sperm motility over L-carnitine monotherapy and acetyl L-carnitine monotherapy, respectively, is hereinbelow described.

Thirty-six patients responding to the following inclusion/exclusion criteria were enrolled.

"*Inclusion criteria:* young, infertile males with idiopathic asthenozoospermia recognized as the sole cause of infertility at least two years duration; semen parameters to be met on at least two samples: sperm concentration (M/ml) between 10-20, motility (%) > 20 <40 at 2 hours, rapid linear progression (%) < 20 at 2 hours.

Exclusion criteria. undescended testes, varicocele (grade 3), traumatic or infection related testicular atrophy, obstruction, inflammation and infection of the genital tract, any endocrine disorder affecting the hypothalamic-pituitary-gonadal axis; post-pubertal mumps, evidence of antisperm antibodies.

All patients gave their informed consent to this open study.

Semen was obtained by masturbation after at least four days of sexual abstinence. The samples were analysed within one hour after ejaculation for all the parameters by the standard methods recommended by the WHO (1987). The sperm motility was studied using a computer motility analyser on at least two specimens.

Semen analysis and motility assessment were carried out at baseline and after 4 months of L-carnitine (N=12) or acetyl-L-carnitine (N=12) or association drug (N=12) treatment.

L-carnitine was administered at the dose of 2 g/day (2 x 500 mg tablet b.i.d., after meals) for 4 months. Acetyl-L-carnitine was administered at the dose of 4 g/day (2 x 1 g sachet b.i.d., after meals) for 4 months. The association drug treatment (L-carnitine+acetyl-L-carnitine) was administered at the dose of 2 g/day (2 x 500 mg tablet composed of 220 mg L-carnitine and 280 mg acetyl-L-carnitine, b.i.d. after meals) for 4 months.

Data were analyzed using Student's "t" test for paired data.

Results

Semen analysis

Variable (mean ±SD)	Baseline	L-Carnitine	Baseline	Acetyl-L-carnitine	Baseline	LC+ALC
Motility (%)	26.8±5.4 *	33.1±4.6	24.9±4.5 #	30.9±4.5	26.3±4 5 §	40.8±6.3 a b
Concentration (M/ml)	15.7±2.0 **	26.0±2.8 b	16.7±3.6	18.1±2.0	16.2±2. 4 §	30.8±3.6 a b

Spermatozoa with rapid linear progression (%)	9.8±1.5 **	17.0±1.5	10.3±1.1 b	16.0±1.2	10.0±1.2 §	21.6±2.8 ** b

LC+ALC=L-Carnitine+Acetyl-L-carnitine

* $p \leq 0.05$ *versus* L-Carnitine
\# $p \leq 0.05$ *versus* Acetyl-L-carnitine
§ $p \leq 0.001$ *versus* L-Carnitine +Acetyl-L-carnitine
a $p \leq 0.01$ *versus* L-Carnitine
b $p \leq 0.001$ *versus* Acetyl-L-carnitine
** $p \leq$ Cool *versus* L-Carnitine

Before treatment values of seminal parameters were below those of WHO normal ranges.

The association drug treatment significantly increased the concentration and the motility of spermatozoa as well as the percentage of spermatozoa with rapid linear progression in comparison to L-carnitine and acetyl-L-carnitine monotherapy treatments.

The medicament of the present invention can be prepared by mixing -- either together or separately packaged -- the active ingredients (L-carnitine inner salt and acetyl L-carnitine inner salt or a pharmacologically acceptable salt thereof) with excipients suitable for the formulation of compositions which lend themselves to enteral administration (particularly oral administration) or to parenteral administration (particularly by the intramuscular or intravenous route). All such excipients shall be readily apparent to one having ordinary skill in this art.

Pharmaceutically acceptable salts of the aforesaid active ingredients include all pharmaceutically acceptable salts which are prepared by the addition of an acid to L-carnitine and acetyl L-carnitine inner salts and which do not give rise to undesired toxic or side effects. The formation of pharmaceutically acceptable acid addition salts is well known in pharmaceutical technology.

Non-limiting examples of suitable salts include chloride; bromide; iodide; aspartate, particularly acid aspartate; citrate, particularly acid citrate; tartrate; phosphate, particularly

acid phosphate; fumarate, particularly acid fumarate; glycerophosphate, glucose phosphate; lactate; maleate, particularly acid maleate, orotate, oxalate, particularly acid oxalate; sulphate, particularly acid sulphate; trichloroacetate; trifluoro acetate and methanesulphonate.

A preferred embodiment of the invention uses L-carnitine acid fumarate and acetyl L-carnitine hydrochloride, most preferably in a molar ratio of about 3.1. This and other preferred embodiments may be illustrated by sachets containing the following composition:

	Composition 1	Composition 2	Composition 3	Composition 4
Acetyl-L-carnitine, HCl equivalent to **Acetyl-L-carnitine inner salt**	g 0.500 g 0.424 (2.1 mMoles)	g 1.000 g 0 848 (4.2 mMoles)	g 1.000 g 0.848 (4.2 mMoles)	g 1.500 g 1.272 (6.2 mMoles)
L-carnitine fumarate equivalent to **L-carnitine inner salt**	g 1.725 g 1.000 (6.2 mMoles)	g 3.450 g 2.000 (12.4 mMoles)	g 4.312 g 2.500 (15.5 mMoles)	g 1.725 g 1.000 (6.2 mMoles)
L-carnitine/acetyl-L-carnitine molar ratio	- 3	- 3	- 3.7	- 1
Fructose	g 1.000	g 2.000	g 2.000	g 2.000
Citric acid	g 0.050	g 0.050	g 0.050	g 0.050
Saccharin sodium	g 0.008	g 0.008	g 0.008	g 0.008
Tonic water flavor	g 0.050	g 0.050	g 0.050	g 0.050
D-mannitol	g 0.666	g 0.441	g 0.579	g 1.666
Colloidal silicon dioxide	g 0.001	g 0.001	g 0.001	g 0.001

The contents of one sachet should be mixed with at least 120 mL of water or other beverage.

Two sachets of the lowest dosage composition (i.e., composition 1) should be taken per day, preferably one in the morning and one at night, preferably for at least six months.

This application is a continuation-in-part of application serial no. 08/980,821, filed December 1, 1997, pending.

* * * * *

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described therein.

What is claimed is:

1. A combination of admixed or separately packaged L-carnitine and acelyl L-carnitine capable of increasing the concentration or motility of spermatozoa in a human, said combination being in a form suitable for oral or parenteral administration, provided that said combination does not comprise propionyl L-carnitine.

2. A combination of L-carnitine and acetyl L-carnitine in a form suitable for oral administration according to claim 1.

3. A combination of L-carnitine and acetyl L-carnitine in a form suitable for parenteral administration according to claim 1.

4. An admixture of L-carnitine and acetyl L-carnitine according to claim 1.

5. Separately packaged L-carnitine and acetyl L-carnitine according to claim 1.

6. The combination of claim 1, comprising a pharmacologically acceptable salt of L-carnitine or acetyl L-carnitine, said salt being selected from the group consisting of chloride, bromide, iodide, aspartate, citrate, tartrate, phosphate, fumarate glycerophosphate, glucose phosphate, lactate, maleate, orotate, oxalate, sulphate, trichloroacetate, trifluoroacetate and methanesulphonate.

7. The combination of claim 1 comprising a pharmacologically acceptable salt of L-carnitine or acetyl L-carnitine, wherein said salt is selected from the group consisting of acid aspartate, acid citrate, acid phosphate, acid furmarate, acid maleate, acid oxelate, and acid sulphate.

8. The combination of claim 6 and 7 comprising L-carnitine acid fumarate and acetyl L-carnitine hydrochloride.

9. The combination of claim 6, containing pharmacological acceptable salts of both L-carnitine and acetyl L-carnitine.

10 The combination of claim 1, capable of increasing the concentration of spermatozoa in a human.

11. The combination of claim 1, capable of increasing the motility of spermatozoa in a human.

12. The combination of claim 1, capable of treating idiopathic asthenozoospermia in a human.

13. The admixture of L-carnitine and acetyl L-carnitine of claim 4, in a form suitable for oral administration.

14. The admixture of L-carnitine and acetyl L-carnitine of Claim 4, in a form suitable for parenteral admistration.

15. The combination of claim 1, comprising L-carnitine and acetyl L-carnitine in a molar ratio of 4.0:1 to 1:1.5.

16. The combination of claim 15, comprising L-carnitine acid furmarate and acetyl L-carnitine hydrochloride.

17. The combination of claim 1, in unit dosage form, comprising from 0.22 to 2.5 g of L-carnitine inner salt and from 0.28 to 1.3 g of acetyl L-carnitine inner salt or equimolar amounts of the pharmacologically acceptable salts thereof.

18. The use of a combination of admixed or separately packaged L-carnitine and acetyl L-carnitine or the pharmacologically acceptable salts thereof for preparing a composition for increasing the concentration of spermatozoa and/or the motility of spermatozoa in humans.

19. The use of claim 18, wherein L-carnitine and acetyl L-carnitine or the pharmacologically acceptable salts thereof are in a molar ratio of 4.0:1 to 1:1.5.