Carnitine and Male Fertility

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Carnitine: The Science Behind a Conditionally Essential Nutrient
March 26, 2004
**Presentation Overview**

- Carnitine physiology
- Preclinical studies of carnitine’s effects in the testis
- Anti-apoptotic effects of carnitine
- Clinical studies of use of carnitine in male infertility
Biological Research - It’s All “Natural”…!

“People can be induced to swallow anything, provided it is sufficiently seasoned with praise.”

Jean Moliere
Carnitine-metabolic functions

- Trimethylated aminoacid-ester
- Synthesized in liver, brain, and kidney from dietary amino acids - methylation of lysine
- Most derived from diet: red meat, fish and dairy products

- Facilitates LCFA transfer into the mitochondria and their oxidation (beta-oxidation and Krebs’ cycle)
- Buffers the mitochondrial acylCoA/CoA ratio – important for carbohydrate metabolism (PDH inhibitor)
- Regulates of acetylCoA/malonylCoA ratio- important for appetite control, insulin release from pancreas and liver neoglycogenesis
- Carnitine system-enzymes involved in peroxisomal FAO
Pharmacokinetics


- Max blood concentrations are reached approximately 3.5 hrs after an oral dose, with a half-life of about 15 hrs.

- Stored in skeletal muscles, myocardium, epididymis, liver and adrenal glands


Testicular Carnitine Transport

- Free carnitine is taken up from the blood plasma, actively transported into the epididymal plasma, under the regulation of androgen.

- Carnitine is then accumulated in the spermatozoa by passive dilution.
Benefits of carnitine in sperm

- High concentrations of carnitine within the epididymis may be beneficial.

- Epididymal sperm use fatty acid oxidation as the main source of energy metabolism, carnitine is crucial to transport fatty acids into mitochondria matrix within spermatozoa for energy production.

- Carnitine contributes directly to sperm motility and sperm maturation.
Benefits of carnitine

- **Energy metabolism:** Transportation of fatty acid into mitochondria for oxidation

- **Cellular metabolism:** Anti-apoptosis
Anti-apoptotic effects

- Carnitine has been used in Alzheimer disease, congestive heart failure, chronic fatigue syndrome, end-stage renal failure, peripheral vascular disease, supplementation during exercise.

- Carnitine reduces apoptotic cell death in growth factor-deprived murine C2.8 hepatocytes, lymphocytes, teratocarcinoma cell lines, neuronal cells, cardiac myocytes after doxorubicin, skeletal muscle.
Anti-apoptotic effects

- Carnitine exerts its anti-apoptotic effects in diverse tissues
  - Does carnitine exert anti-apoptotic effects in the testis?
  - Which step(s) of apoptosis does carnitine influence?
In vivo study in testes (1)

- Effects of L-acetylcarnitine (L-ACAR) on the post-injury recovery of mouse spermatogenesis monitored by flow cytometry.
  - 1. Recovery after X-irradiation.

Carnitine in irradiated testes (1)

- First report to characterize the in vivo actions of carnitine on the testes
- The testes of mice were irradiated with a single dose of 10 Gy.
- The treatment group was given intraperitoneal L-ACAR (100mg/kg body weight) on alternate days for 4 weeks, starting from the day of irradiation.
- The effects on spermatogenesis were assessed at 28, 35, 40, 45, 50, 55, 60 days after irradiation.
- The effects were examined by flow cytometric analysis of cellular DNA content.

Carnitine in irradiated testes (1)

- In the treatment group:
  - The fraction of tetraploid cells was greater at days 28 (P<0.05) and 45 (P<0.02).
  - The round spermatid fraction was greater at 45 days (p<0.1) and the elongated spermatid fraction was higher at 50 days (P<0.1).
  - The recovery period throughout the maturation process was shortened.
- Conclusion: L-ACAR enhanced the recovery of spermatogonial cells after X ray damage
  - N.B. Despite the favorable outcome in the carnitine group, however, no significant difference was detected in the fraction of round and elongated spermatids in the control vs L-ACAR groups. It is speculative that the beneficial effect of L-ACAR would be seen throughout the maturation process of spermatogenesis.

In vivo study in testes (2)

- Effects of L-acetylcarnitine (L-ACAR) on the post-injury recovery of mouse spermatogenesis monitored by flow cytometry.


Carnitine in heated testis (2)

- Heat was applied by local immersion of mice in a water bath maintained at 42°C for 1 hour.
- The same dose of intra-peritoneal L-ACAR was administered in treatment group as in prior expts, starting from the day of heat treatment.
- The effects of spermatogenesis were studied at 8, 14, 21, 28, 35, 40, 45 and 60 days after heat treatment.
- The testes were excised and weighed. Their cellular DNA contents were examined by the flow cytometry.

Carnitine in heated testis (2)

- In control group, the number of primary spermatocytes was markedly reduced with complete absence of haploid cells one week after heat treatment.
- In treatment group: the haploid cell fraction was higher at day 45 in the treatment vs control group (P< 0.01).
- Histological examination of tissue sections indicated that the reorganization of the seminiferous epithelium was faster.
- Weight of the testis was higher (p< 0.05) at the above time points.
- Conclusion: There was more rapid recovery of spermatogenesis after heat treatment with L-ACAR administration.

In vivo study in testis (3)

- Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice.

Carnitine in testes after magnetic field (3)

- Testes of mice were damaged by high magnetic field exposure.
- The animals were injected either with carnitine intraperitoneally (200mg/kg) or coenzyme Q10 orally (200 mg/ kg).
- Parameters like total sperm count, motility, daily sperm production, testicular LDH- X activity as well as histopathological examinations were assessed.
- There was a significant decrease in above-mentioned parameters in the control group.
- Pre-treatment with carnitine or coenzyme Q10 1 hr before exposure to magnetic field caused a significant recovery of mice testicular damage.

Administration of L-ACAR can speed recovery of spermatogenesis in mice after various insults

- Possible mechanisms:
  1) Provision of an extra source of acetyl groups for CoA, enhancing the cellular energy metabolism with consequent favorable outcome on DNA repair, on Sertoli cells and regenerating germ cells.
  2) Anti-apoptotic effects in the testis
Possible mechanisms of carnitine’s anti-apoptotic effects

- Anti-oxidant
- Inhibition of extrinsic mitochondrial-independent Fas-triggered apoptotic signals (e.g. caspases), and of ceramide generation
- Inhibition of intrinsic mitochondrial-dependent pathways
- Others
Possible mechanisms of carnitine’s anti-apoptotic effects

- Increased reactive oxygen species (ROS) have been detected in patients with idiopathic and post-inflammatory oligoasthenospermia.

- Carnitine as an anti-oxidant
  - reduces ROS
  - increases sperm forward motility and viability in infertile patients with prostato-vesiculo-epididymitis

Possible mechanisms of carnitine’s anti-apoptotic effects

- **?? Antioxidant effect**
  - Antioxidant: ascorbate and alpha-tocopherol
  - Result: production of ROS by sperm was reduced by supplementation in vitro with ascorbate and alpha-tocopherol.
  - Supplementation of preparation media with ascorbate and alpha-tocopherol, either singly or in combination, was not beneficial to sperm motility.

Possible mechanisms of carnitine’s anti-apoptotic effects

- **Inhibit extrinsic Fas-mediated apoptosis**
- Jurkat cells were induced to undergo apoptosis with Fas ligation in the presence or absence of carnitine.
- Carnitine protected Jurkat cells against Fas-mediated apoptosis in a dose dependent fashion.
- Carnitine exerts an inhibitory effect on the activity of recombinant caspases 3, 7 and 8.
- Concentration of endogenous carnitine reduced during apoptosis.
- => endogenous carnitine might play a regulatory role in apoptosis.

Possible mechanisms of carnitine’s anti-apoptotic effects

- **Inhibit intrinsic mitochondrial-dependent pathway**
  - In a rat model of heart failure, an increase in apoptosis of the skeletal muscle was noted.
  - Pro-apoptotic agents, capase 3 and 9, serum TNF-alpha and its second messenger sphingosine were elevated.
  - Rats were treated with carnitine (50mg/kg) orally for 28 days.
  - In the treatment group, there were fewer TUNEL-positive nuclei and DNA break strands (ELISA ladder), which was associated with a lower expression of capases 3 and 9 and with increased expression of Bcl-2. The levels of TNF-alpha and sphingosine were also increased.

  - N.B.: Although carnitine prevents apoptosis of skeletal muscle, whether this effect is secondary to improvement of heart function or is of genuine protective role of skeletal muscle is open to question.

Possible mechanisms of carnitine’s anti-apoptotic effects

Other:

- Increase cardiolipin, enhance pyruvate transport into mitochondria and facilitates oxidation (in heart mitochondria of aging rats).
- Remove acyl CoA, a potentially toxic intermediate.
- Activate the GH/IGF-I axis.
Use of Carnitine in Male Infertility

In one study of 101 men, a positive correlation was found between carnitine content in semen and sperm motility, number and morphology (p< 0.01)

Use of Carnitine in Male Infertility

- 100 patients with idiopathic oligoasthenozoospermia all received 3g/day of oral L-CAR x 4 months
- Sperm motility determined before, during, after study
- % of motile spermatozoa increased: 26.9 ± 1.1 → 37.7 ± 1.1% (P<0.01)
- Sperm with rapid linear progression: 10.8 → 18% (P<0.01)
- Total no. of spermatozoa per ejaculate: 142 → 163 x 10^6 (P<0.01)
- N.B. uncontrolled study

Use of Carnitine in Male Infertility

- Placebo-controlled, double-blind, cross over trial
- 86 infertile men with: sperm conc 10-20 x 10^6; total motility 10-30%; forward motility < 15%; atypical forms< 70%; Velocity: 10-30u/s; Linearity <4
- L-CAR- 2g/d orally or placebo x 2 months → 2 months of washout→ 2 months placebo/L-CAR therapy.
- Significant increase in semen quality- sperm conc, total and forward sperm motility, especially in groups with lower baseline levels.
- N.B. L-CAR therapy may improve semen quality in selected cases of male infertility, but effects on fertility not examined.

Limitations of carnitine clinical studies

- Bioavailability of carnitine is small and varied from 5 to 15%.
- Intake of food related to carnitine contents was not standardized
- Dosing of carnitine may have been further complicated by the variable carnitine content in the formulation with poor dissolution properties
- Unclear whether exogenously administered carnitine can reach the epididymis or inside spermatogonia
- Action of substrates may be limited by the rate-limiting step of carnitine uptake and its metabolism
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Conclusions

- Carnitine administration improves sperm quality and/or quantity in testes of mice exposed to physical insults, and in clinical trials conducted in men with idiopathic oligoasthenospermia.

- The benefits are partly related to improvement in motility of epididymal sperm, possibly due to increased mitochondrial fatty acid oxidation.

- The anti-apoptotic properties of carnitine in the testis are likely to contribute to these beneficial effects, but require further study.
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Future Research Questions

- **Basic studies:**
  - Explore the protective mechanism(s) of carnitine’s effects in the testis
  - Enhance understanding of the pathophysiology of germ cell apoptosis
  - Develop strategies to prevent germ cell death
  - Identify specific therapy for some forms of male infertility

- **Clinical studies:**
  - Who is the optimal candidate for carnitine therapy?
  - What dose, route, duration, and formulation of carnitine therapy is best?
  - Does carnitine truly improve sperm quality and quantity, and does that result in increased fertility with normal offspring?
Acknowledgments

Jason Ng, MD
Ronalds S. Swerdloff, MD

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