Exp Toxic Pathol 2004; **55**: 367–377
http://www.elsevier-deutschland.de/etp

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The influence of L-carnitine on methanol biotransformation in rats

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With 6 figures and 7 tables

Received: January 2, 2003; Revised: August 25, 2003; Accepted: November 6, 2003

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Key words: Methanol; L-carnitine; biotransformation; rats.

Summary

There persists a need for potent and safe inhibitors of alcohol dehydrogenase (ADH), to effectively treat methanol poisoning by slowing its rate of biotransformation to there toxic products, formaldehyde and formic acid. Only a few former papers have reported on the significant effectiveness of L-carnitine in treating ethanol poisoning as well as alcohol abuse. As are no reports on the effectiveness of L-carnitine in treating methanol poisoning till now, the current studies were conducted to investigate the influence of L-carnitine on both oxydative metabolism and elimination of methanol in rats.

Male Sprague-Dawley rats, aged 3 months with the body weight of 200–230 g were divided into 6 groups at random, with two of the groups considered to be control. Rats were given drinking water (control) or methanol in two different doses of 3220 mg/kg b.m. or 6440 mg/kg b.m. intragastrically and 0.9% NaCl (control) or 6.2 mmol/kg b.m. of L-carnitine intraperitionelly.

Within 96 hours after the administration of methanol and 0.9% NaCl or L-carnitine, the urine was collected and then the animals were decapitated. To determine methanol there were taken blood samples for clot, and to determine carnitine and its derivatives blood was taken into heparinized test tubes. During the autopsy liver was also secured.

In all the experimental time points stated the methanol concentrations in blood, urine and liver homogenate were determined by a *head-space* gas chromatography.

The results of our research show that:

1. L-carnitine slows down the elimination of methanol in rats.
2. Toxicokinetic parameters indicate that L-carnitine slows down the methanol biotransformation.
3. It was observed that after the administration of L-carnitine and methanol:
- the blood methanol concentration was lower in the absorption phase but higher in the elimination phase in comparison with the methanol levels in the rats which were given only methanol
- methanoluria lasted significantly longer and was not finished within a 96-hour observation period, and the excreted urine volume was much more higher in this group
- methanol was present significantly longer in liver than blood
- the rate of methanol elimination in blood, liver and urine depended on the alcohol dose given.

4. In the case of methanol poisoning, L-carnitine reveals its protective effects. There were no deaths in the groups of the animals which were simultanously intoxicated with methanol and L-carnitine. There were 8 deaths in the group of 60 animals which were given a lower dose of methanol without L-carnitine and 27 deaths in the group of 101 animals which were given a higher dose of methanol but no L-carnitine.

Introduction

Because of its chemical and pharmacological similarities to ethanol, methanol may be a serious source of accidental poisonings, acute or fatal.

The oxydative metabolism of methanol occurs mainly in liver by both alcohol (ADH) and aldehyde (ADLH) dehydrogenases, the microsomal system (MEOS) and catalase.

Because methanol is metabolized slowly, vs. ethanol, methanol blood levels gradually rise in a person who consumes methanol. Similar to ethanol, methanol is sedating and inebriating. Methanol toxicity is associated with its metabolites formaldehyde and formic acid (Hayasaka et al. 2001; Kinoshita et al. 1998; Oyama

et al. 2002; PANDEY et al. 2000; SKRZYDLEWSKA et al. 1999; SKRZYDLEWSKA and SZYNAKA 1997; SKRZYDLEWSKA 1993; TENG et al. 2001; THEPLY 1991).

In the case of methanol intoxication, succesful medical treatment depends especially on the amount of the methanol consumed, the interval from time of its consumption to the time of treatment, as well as the extent of metabolic acidosis and extent of damage to the central nervous system (GIRAULT et al. 1999).

By competitively inhibiting alcohol dehydrogenase and thereby slowing methanol metabolism, ethanol is therapeutically useful in treating methanol. By maintaining a blood ethanol level of 100–150 mg%, ethanol effectively delays slows formation of methanol metabolites, formaldehyde and formic acid, to levels which the body can tolerate (BOGDANIK 1983).

Pyrazole is an alcohol dehydrogenase inhibitor capable of inhibiting all instances of ADH-dependent biotransformations in animal studies (JACOBSEN and MCMARTIN 1997; PIETRUSZKO 1975). However, pyrazole is not a safe antidote for methanol poisoning because pyrazole itself is toxic as is its metabolites in the presence of alcohol. Ethanol also has limitations in treating methanol poisoning, because of adverse effects of its metabolites, namely acetaldehyde, acetic acid, and fatty acid ethyl esters.

The search continues for potent, effective, and safe treatments capable of partially or totally inhibiting methanol biotransformation.

Normally, ethanol consumption in animals or man is followed by an increase in the concentration of carnitine. Several studies have been conducted to determine if ethanol-induced carnitine production is attributable to liver toxicity (ARRIGONI-MARTELLI and CASO 2001; CAMPILLO et al. 1990; FULLER and HOPPEL 1983; KRÄHENBÜHL 1996; RUDMAN et al. 1977; TSUKAMOTO and LU 2001).

A number of studies have been conducted of late to demonstrate the influence of L-carnitine on the course of ethanol intoxication and consequences of alcohol abuse (CALABRESE and RIZZA 1999; CALABRESE and RIZZA 1999; CALABRESE et al. 2001; MANGANO et al. 2000; MYNATT and SACHAN 1992; SACHAN and BERGER 1987; SACHAN and BERGER 1993; SACHAN and CHA 1994; TAINAKA et al. 1993). Findings from these studies indicate that L-carnitine is useful in the treatment of poisonings by ethanol analogues such as methanol and ethylene glycol.

Carnitine, a natural metabolic product in higher organisms, is the vitamin-like nutritive factor (3-hydroxy-4-N-trimethylaminobutyrate) – discovered by Gulewitch in 1905 (ZURBRIGGEN 2000). However its significance was not appreciated until recently when its role in the oxidization of fatty acids was shown. Various L-carnitine functions in organisms (ARRIGONI-MARTELLI and CASO 2001; FELLER and RUDMAN 1988; HOPPEL 1992; LOHINGER 2000; LÖSTER 2000; ŁYSIAK-SZYDŁOWSKA 1988; RAMSAY et al. 2001; TACCONI 1997) as well as consequences of its deficiency (GUERTL et al. 2000; SPANIOL et al. 2001) have been described in recent years.

In humans a full complement of enzymes essential for carnitine biosynthesis occurs in liver, kidney and brain. Accordingly, carnitine biosynthesis occurs only in these organs (VAZ and WANDERS 2002). The participation of carnitine in the oxidization of long-chain fatty acids is the best-known function of this species in cellular metabolism. Between 60 and 70% of energy consumed by cardiac muscle is produced during the oxidation of fatty acids C_{16}–C_{18} (LOULET et al. 2001; PAULSON 1998). In the liver, carnitine is essential for the transport of long-chain fatty acids, but in the heart and skeletal muscles carnitine is used for the transport of all fatty acids regardless of the length of the carboxylic acid chains (MEYBURG et al. 2001).

Objective

The aim of the present study was to define the influence of L-carnitine on oxidative metabolism and elimination of methanol in rats, under several experimental conditions. We addressed the following questions:

1. How does methanol elimination proceed in blood, urine and liver after intraperitoneal administration of methanol only or methanol with L-carnitine?
2. Does exogenously administered L-carnitine influence methanol absorption and excretion?

Material and methods

Reagents: L-carnitine (SIGMA TAU, Roma; partial gift of the producer) was administered intraperitioneally, while methanol (Merck) was administered intragastrically.

Animals: Sprague-Dawley rats aged 3 months and with the body weight of 200–230 g each were used in this study. This strain was chosen because of the similarity of methanol oxidation in both primates and rodents.

Rats were provided by the Animal Experimental Centre at the Medical University of Silesia in Katowice-Ligota, where all experiments were conducted. The research protocol was approved by the Local Commission on Ethics for Experiments on Animals (No 1/01).

Rats were divided into 6 groups at random (table 1). All rats were fasted for 14 hours prior to the start of each study. At least 4 rats were used in the time intervals stated for experimental groups, and 3 rats were included in both control groups.

The methanol doses of 6440 mg/kg b.m. and 3220 mg/kg b.m. were calculated on the basis of the LD_{50} in rats 12880 mg/kg b.m. (Handbook of toxicology).

Urine samples were collected after 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hours from both intragastric administration of methanol or drinking water in the control group; and intraperitoneal (IP) administration of 0.9% NaCl or L-carnitine. Rats were killed by decapitation. Methanol was analyzed in blood that was collected and clotted, and in liver taken at autopsy.

Table 1. Groups of rats in this study.

Group		Number of rats	Type of the compound administered	
			intragastrically	IP
I	Control group	82	Drinking water	0.9% NaCl
II	Control group	68	Drinking water	L-carnitine (6.2 mmol/kg b.m.)
III		60	Methanol (3220 mg/kg b.m.)	0.9% NaCl
IV		60	Methanol (3220 mg/kg b.m.)	L-carnitine (6.2 mmol/kg b.m.)
V		101	Methanol (6440 mg/kg b.m.)	0.9% NaCl
VI		96	Methanol (6440 mg/kg b.m.)	L-carnitine (6.2 mmol/kg b.m.)

Methanol in blood, urine and liver was determined by a *head-space* gas chromatography (MACHATA 1967). Quantitative analysis of methanol was carried out simultaneously in duplicate by means of a gas chromatograph HRGC MEGA-5300 equipped with an integrator DP-700 and an autosampler HS 800. Two capillary columns of different polarities (DB-WAX 30 m × 0.32 mm × 0.5 μm and HP Blood Alcohol Analysis Column 7.5 m × 0.32 mm × 20 μm) were applied.

To determine carnitine and its derivatives in plasma and liver, Cederblat's method modified by Rösle (RÖSSLE et al. 1985) was used.

Statistical analysis: Illustrations were derived from the Microsoft Excel computer program, while a GraphPad Prism 2 program was used for statistical analysis (t-test, ANOVA I test).

Pharmacokinetic calculations (K_a, K_e, $t_{1/2,}$ biological $t_{1/2}$, C_{max}, t_{max}, V_d, AUC, AUC total, Cl total) were performed on the basis of the standard formulas with use of Shumaker's PKCALC programme.

Results

During the 96-hour experiment 37 rats died. In group III 8 rats died after receiving a methanol dose of 3220 mg/kg b.m. In group V, 29 rats died (2 of the injury with a probe) with a methanol dose of 6440 mg/kg b.m. This accounts for 13.3% of 60 rats, and 26.7% of 101 rats in the groups examined. There were deaths in the first 24-hours of intoxication. Deaths did not occur in control groups I and II, nor in groups IV and VI in which methanol and L-carnitine were given concurrently.

Estimation of the half-life ($t_{1/2}$) of L-carnitine given IP

The route of administration has a major influence on drug distribution and effect. The apparent absence of pharmacokinetic data on L-carnitine (IP) was one reason to undertake this study.

Control group II was used to determine the pharmacokinetic parameters of L-carnitine (IP). It was necessary to determine carnitine levels in the 45th and 90th minutes of the observation period (table 2).

Table 2. Pharmacokinetic parameters in blood of rats which were administrated 6.2 mmol (1g)/ kg b.m. of L-carnitine (IP).

Blood pharmacokinetic parameters of L-carnitine administered in a dose of 6.2 mmol/kg b.m., IP	
Elimination constant Ke (h^{-1})	0.03
Distribution constant Ká (h^{-1})	5.17
biological half-life $t_{1/2}$ (h)	22.84
$t_{1/2}$ (h)	1.10
AUC total (mg*h/L)	19498.8
C_{max} (mmol/L)	7.31
t_{max} (h)	1
Cl (renal) (L/h)	0.051
Mean residence time (h)	24.70

Methanol elimination in blood

Methanol is rapidly absorbed from the gastrointestinal tract by diffusion. Accordingly, methanol concentrations were determined at 8 different times during the first 24-hours following its administration – every 12 hours up to the end of the experiment on the 4th day. Findings indicate that L-carnitine significantly influenced methanol levels during the intervals of this study (figs. 1, 2).

Toxicokinetic parameters, shown in table 3, indicate that L-carnitine inhibited methanol biotransformation.

Methanoluria

The level of methanol in the 24-hour urine collections shows both its inhibited elimination and increased excretion after L-carnitine administration to rats (table 4, fig. 3).

Similar correlations were observed for levels of methanol in urine of those rats treated with 6440 mg/kg b.m. of methanol (groups V and VI), (fig. 4, table 5).

Toxicokinetic parameters presented in table 6 confirm that L-carnitine can impede or accelerate rates of methanoluria. Similarly to what is observed in blood, both the total amount of methanol and its rate of elimination in urine depend on the dose of methanol and the presence (or absence) of L-carnitine.

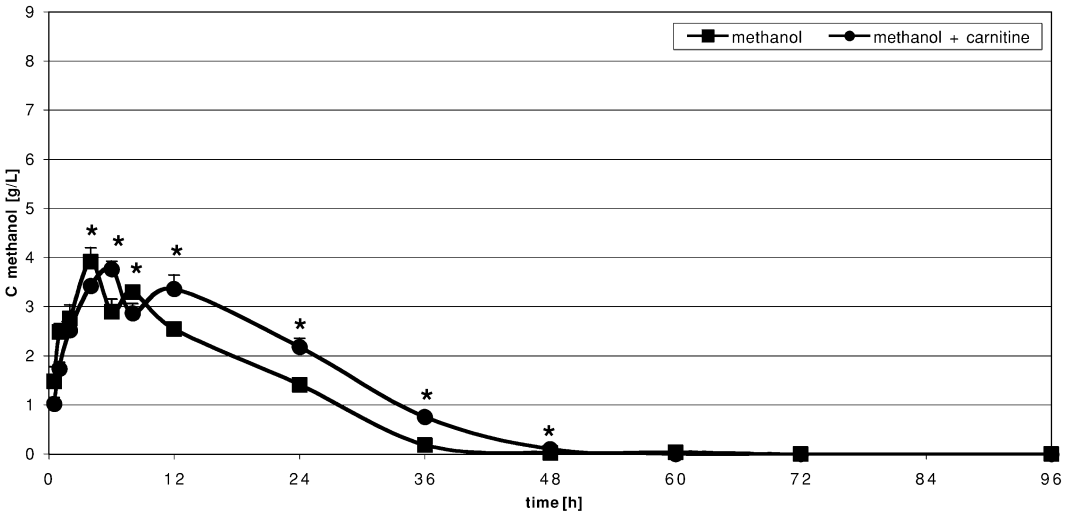

Fig. 1.

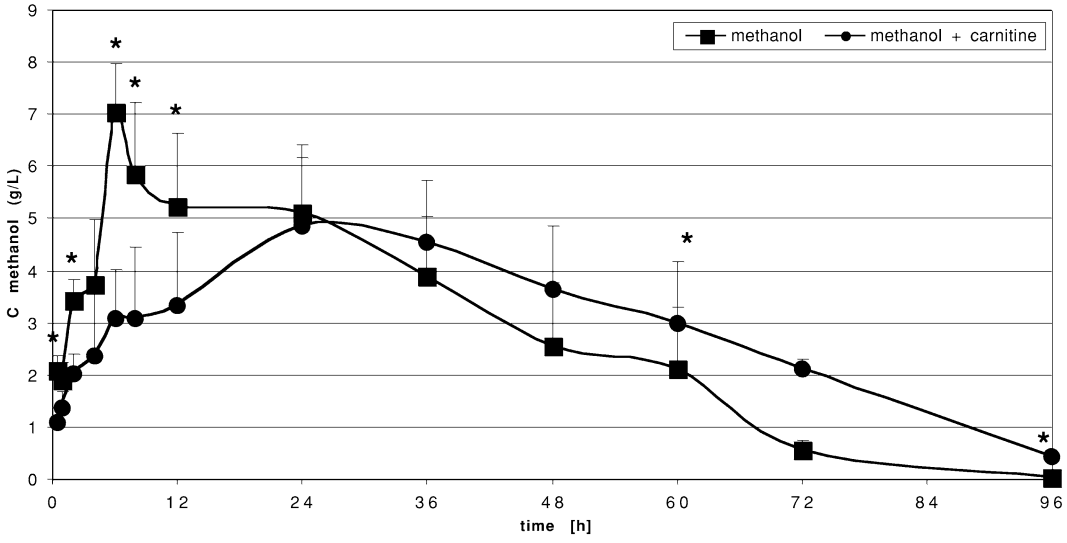

Fig. 2.

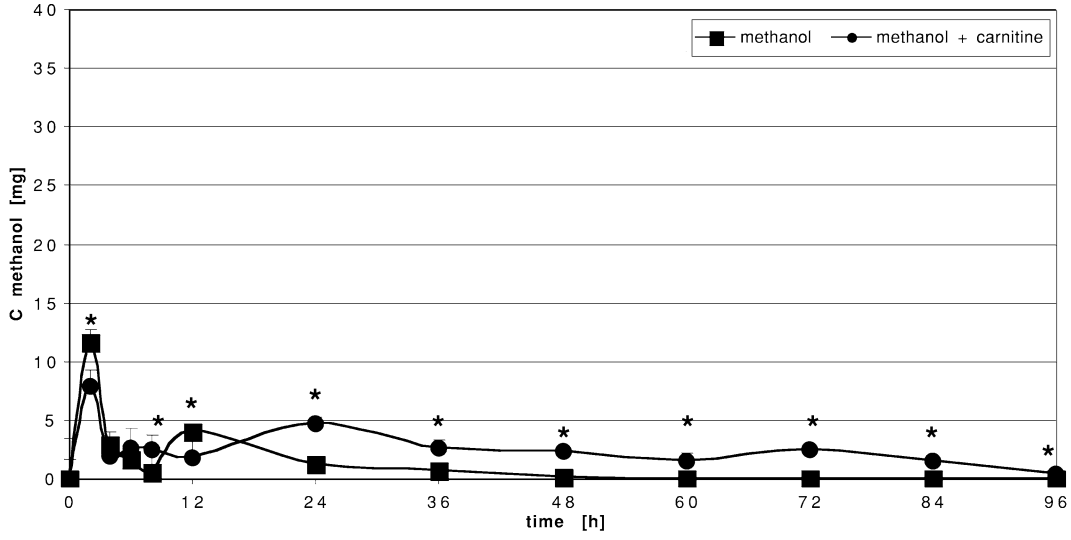

Fig. 3.

Methanol elimination in liver

The concentration of methanol in liver was lower than that in blood throughout the course of the experiment, regardless of whether rats were treated with methanol alone (groups II and V), or with methanol plus carnitine (groups IV and VI). This likely results from liver hydration but the effect of methanol metabolism cannot be excluded.

Both biological half-life ($t_{1/2}$) and appearance of methanol in liver depended on the dose of methanol as well as the presence (or absence) of exogenously administered L-carnitine. The $t_{1/2}$ was high especially in those groups of rats simultaneously treated with methanol in a dose of 6440 mg/kg b.m. and 6.2 mmol/kg b.m. of L-carnitine (table 7, figs. 5 and 6).

Table 3. Toxicokinetic parameters relating to methanol elimination in blood of rats which were administrated two different doses of methanol (3220 mg/kg b.m. or 6440 mg/kg b.m.) intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine (IP).

Parameters	Methanol 3220 mg/kg b.m. + 0.9%NaCl	Methanol 3220 mg/kg b.m. + L- carnitine	Methanol 6440 mg/kg b.m. + 0.9% NaCl	Methanol 6440 mg/kg b.m. + L- carnitine
Distribution constant Ká (h^{-1})	0.75	1.23	1.1	1.2
Elimination constant Ke (h^{-1})	0.089	0.048	0.031	0.017
Biological half-life $t_{1/2}$ (h)	7.72	14.31	22.34	40.82
C_{max} (mg/L)	3.916	3.768	7.042	4.855
t_{max} (h)	4	6	6	24
AUC (mg*h/L)	68.00 [0–36 h]	85.92 [0–36 h]	255.48 [0–72 h]	289.77 [0–96 h]
AUC_{total} (mg*h/L)	71.33	104.77	287.54	354.91
V_d (L)	503.16	634.88	722.24	1068.82
Total clearance (h)	45.14	30.73	22.40	18.14
Time of occurence in body (h)	13.85	22.26	36.51	62.33

Table 4. Total amount of methanol excreted in urine of the rats which were administrated a methanol dose of 3220 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine (IP).

Day	Total excretion of methanol (mg)	
	without L-carnitine [Group III]	with L-carnitine [Group IV]
I	18.55	21.91
II	0.75	4.99
III	0.04	4.07
IV	0.00	1.95

Table 5. Total amount of methanol excreted in urine of the rats which were administrated a methanol dose of 6440 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine (IP).

Day	Total excretion of methanol (mg)	
	without L-carnitine [Group V]	with L-carnitine [Group VI]
I	41.97	44.84
II	23.52	36.59
III	4.02	11.20
IV	3.92	7.12

◀ **Fig. 1.** Methanol elimination in blood of rats which were administrated a methanol dose of 3220 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine intraperitioneally. Each point is the mean value (±SD) obtained from at least 4 animals. * $p < 0.05$.

Fig. 2. Methanol elimination in blood of rats which were administrated a methanol dose of 6440 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine intraperitioneally. Each point is the mean value (±SD) obtained from at least 4 animals. * $p < 0.05$.

Fig. 3. Methanol elimination in urine of the rats which were administrated a methanol dose of 3220 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine intraperitioneally. Each point is the mean value (±SD) obtained from at least 4 animals. * $p < 0.05$.

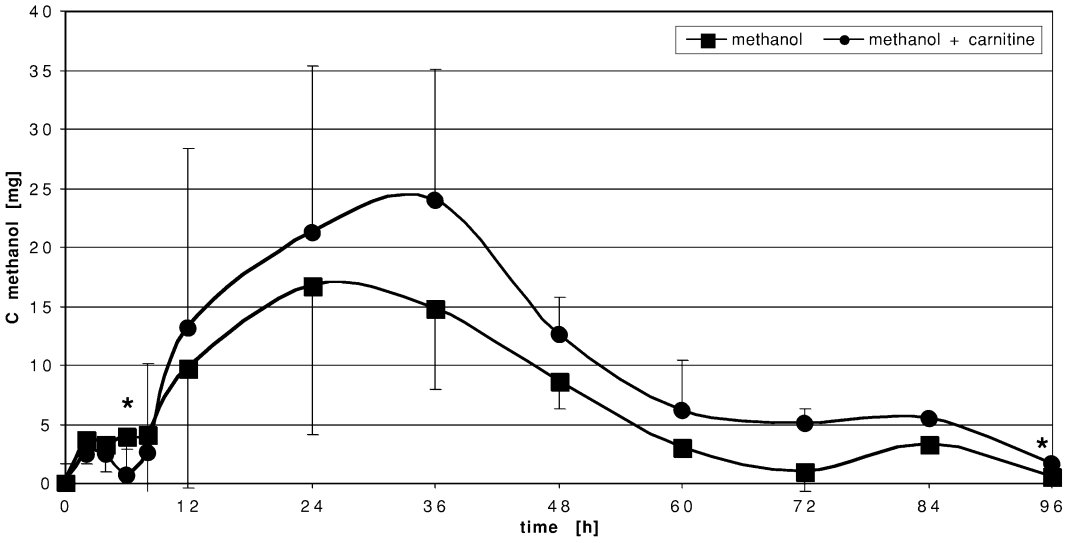

Fig. 4.

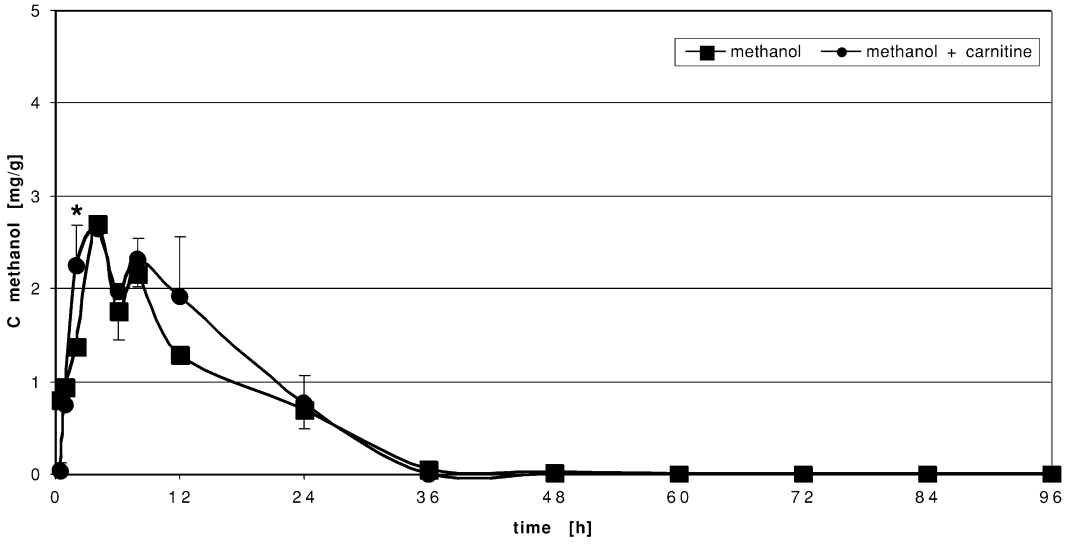

Fig. 5.

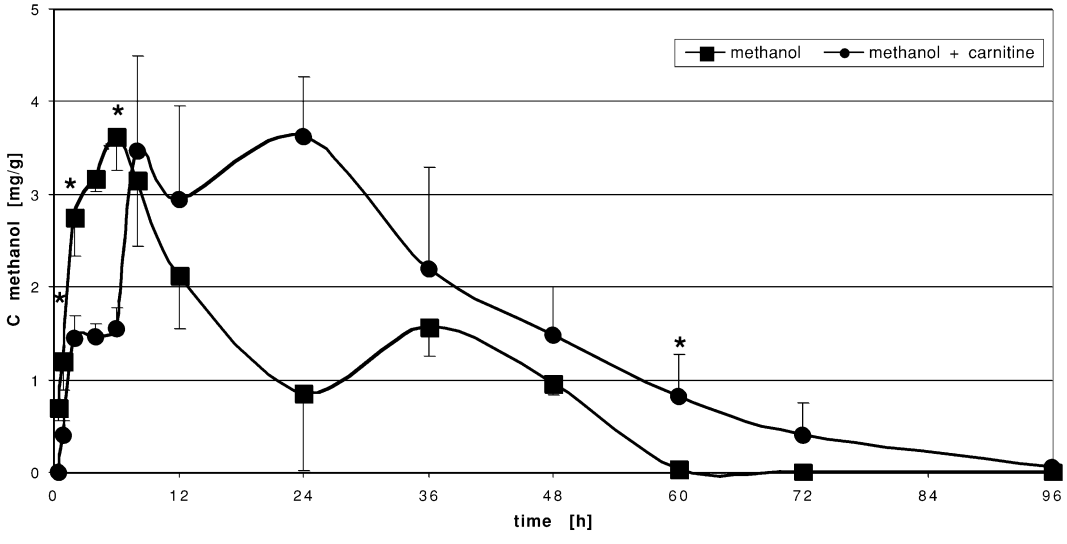

Fig. 6.

Discussion

4-Methylpyrazole (fomepizole) more potentially inhibits ADH than does ethanol, and it is therefore used in France for treating patients poisoned with methanol or ethylene glycol (BRENT et al. 2001; HAFFNER et al. 1998; SARKOLA and ERIKSSON 2001; SARKOLA et al. 2002). Fomepizole has effects similar to that of ethanol, but it has a longer active duration, which is important (BRENT et al. 2001; BURNS et al. 1997). When using ethanol to treat methanol- or ethylene glycol-poisoning, it is necessary to monitor blood ethanol concentration to ensure an adequate therapeutic level. Fomepizole causes neither inebriating nor depressive effects on the nervous system, which is important especially when treating children. No signs of hypoglycemia were observed after fomepizole

Table 6. Toxicokinetic parameters relating to methanol elimination in urine of the rats which were administrated two different doses of methanol, 3220 mg/kg b.m. or 6440 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine (IP).

Parameters	Methanol 3220 mg/kg b.m. + 0.9%NaCl	Methanol 3220 mg/kg b.m. + L- carnitine	Methanol 6440 mg/kg b.m. + 0.9% NaCl	Methanol 6440 mg/kg b.m. + L- carnitine
Elimination constant Ke (h^{-1})	0.027	0.011	0.0053	0.0007
Biological half-life $t_{1/2}$ (h)	25.35	62.04	130.53	300.2
C_{max} (mg/L)	11.615	7.967	14.853	23.928
t_{max} (h)	2	2	36	36
AUC (mg*h/L)	72.0 [0–36 h]	228.0 [0–96 h]	740.45 [0–96 h]	1026.82 [0–96 h]
AUC_{total} (mg*h/L)	100.57	325.37	1535.2	8531.56

Table 7. Toxicokinetic parameters relating to methanol elimination in liver of the rats which were administrated two different doses of methanol, 3220 mg/kg b.m. or 6440 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine (IP).

Parameters	Methanol 3220 mg/kg b.m. + 0.9% NaCl	Methanol 3220 mg/kg b.m. + L- carnitine	Methanol 6440 mg/kg b.m. + 0.9% NaCl	Methanol 6440 mg/kg b.m. + L- carnitine
Distribution constant Ká (h^{-1})	0.46	1.20	1.25	1.16
Elimination constant Ke (h^{-1})	0.0311	0.027	0.022	0.011
Biological half-life $t_{1/2}$ (h)	22.24	25.77	32.07	62.89
C_{max} (mg/L)	2.703	2.645	3.163	3.623
t_{max} (h)	4	4	4	24
AUC (mg*h/g)	32.88 [0–24]	40.54 [0–24 h]	79.73 [0–48 h]	142.28 [0–72 h]
AUC_{total} (mg*h/g)	60.80	80.10	123.47	219.4
V_d (L)	1699.72	1494.74	2413.74	2663.92
Total clearance [L/h]	52.96	40.20	52.16	29.35
Time of occurence in body (h)	31.07	35.31	45.93	75.46

◀ **Fig. 4.** Methanol elimination in urine of the rats which were administrated a methanol dose of 6440 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine intraperitoneally. Each point is the mean value (±SD) obtained from at least 4 animals. * $p < 0.05$.

Fig. 5. Methanol elimination in liver of the rats which were administrated a methanol dose of 3220 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine intraperitoneally. Each point is the mean value (±SD) obtained from at least 4 animals. * $p < 0.05$.

Fig. 6. Methanol elimination in liver of the rats which were administrated a methanol dose of 6440 mg/kg b.m. intragastrically plus 0.9% NaCl or 6.2 mmol/kg b.m. of L-carnitine intraperitoneally. Each point is the mean value (±SD) obtained from at least 4 animals. * $p < 0.05$.

treatment (BURNS et al. 2001). It is a more efficient treatment for methanol poisoning compared with ethanol because of its capacity to inhibit ADH (i.e., 8000 times of ethanol) (HANTSON et al. 1999; JACOBSEN et al. 1996) and with fewer adverse effects.

Ethanol produces anaerobically fatty acid ethyl esters (FAEE). From the biological point of view, these compounds are very dangerous to human beings because of their inhibition of respiratory efficiency in both heart and liver mitochondria. Also, because of their interaction with membranes, FAEE inhibit the synthesis of various liver proteins, e.g. triacylglyceryl lipase (AUWÄRTER et al. 2001; PRAGST et al. 2001). In methanol-poisoned patients in critical condition, the outcome of fomepizole treatment was satisfactory. However, the number of the cases described is rather low. There are some limitations relative to the application of fomepizole, resulting from the lack of its registration by Food and Drug Administration (FDA) and adverse effects, such as mild rash, eosinophilia or temporary increase in the activity of liver transaminases (BRENT et al. 2001; BURNS et al. 1997).

Despites promising clinical results with pyrazole and its derivatives, ethanol infusion i.v. is still used to inhibit of methanol biotransformation. Research is ongoing in the search for non-toxic inhibitors of methyl alcohol, ethyl alcohol, and ethylene glycol (DAWIDEK-PIETRYKA et al. 2000; FACI et al. 1998; WANG et al. 1999; ZORZANO and HERRERA 1990).

In the 1990s much attention was paid to carnitine, a cofactor in the oxidation of fatty acids (SACHAN and BERGER 1993; SACHAN and MYNATT 1993; SACHAN and CHA 1994). Carnitine also plays an important role in the transport of acyl groups, in synthesis of cholesterol, and in excretion of organic acids and exogenous substances in the form of esters. Liver is the central organ of carnitine metabolism and its distribution throughout the body. Therefore, it is not surprising that in the case of hepatopathy, carnitine transformation is defective. It is known that alcohol consumption disrupts lipid homeostasis, causing an increase in the level of triacylglyceryls in blood, lipidosis, or cirrhosis (LIEBER 1994; MENON et al. 2001; NAVDER et al. 1997; SUWAKI et al. 2001; THEPLY 1991).

Sachan and colleagues have shown that carnitine inhibits ethanol biotransformation but does not influence its absorption in rats (SACHAN and BERGER 1993; SACHAN and MYNATT 1993). This leads to lower blood ethanol clearance. It was thought that carnitine competed with NAD^+ cofactor or its substrate (ethanol) for the bond point on alcohol dehydrogenase. This interaction is analogous to the reaction of ADH with the inhibitors 4-MP or trichloroethanol. Sachan and Cha (1994) have recently confirmed this hypothesis. Carnitine inhibited ethanol metabolism by virtue of its weak inhibition of the activity of ALDH (14%), catalase (5%), and MEOS (30%). Simultaneously, an increase in the level of lactate but a decrease in the level of pyruvate was observed. Moreover, this was influenced by ADH-inhibited NADH. Carnitine supplementation increased the oxidation of fatty acids, causing an increase in the endomitochondrial redox potential inhibiting the regeneration of NAD^+ with NADH. A decrease in the level of NAD^+ limits its alcohol oxidation (MYNATT and SACHAN 1992). Potential inhibition of ADH activity occurred when the ratio of NAD^+: acetylcarnitine was ≤ 1. When the ratio was 1:8 the activity of the alcohol dehydrogenase was inhibited by 45%; when 1:1, by only 7% (SACHAN and CHA 1994). The effect of acetylcarnitine was considerebly greater than that of carnitine, which inhibited ethanol oxidation by 43%. In contrast, ADH activity was inhibited 84% by acetylcarnitine, despite its concentration being 100-times lower. ADH and MEOS are the main systems involved in ethanol metabolism *in vivo*, and carnitine significantly decreases only ADH activity (SACHAN and CHA 1994).

Our experimental results in poisoning rats with methanol correlate with those of Sachan's in the case of poisoning with ethanol. When the animals were administrated an intragastric dose of methanol and an IP dose of L-carnitine, there were observed a low clearance, longer time of the alcohol existance in blood, late c_{max} and increased AUC. These parameters were dependent on the quantity of the methanol dose given (table 3).

There were noticed some differences between Sachan's results and ours. The c_{max} values were considerably lower in the rats which were given L-carnitine**.** In the group where only a methanol dose of 3220 mg/kg b.m. was administrated, c_{max} was 3.92 ± 0.29 g/L but 3.70 ± 0.16 g/L after L-carnitine administration. In the group where a methanol dose was 6440 mg/kg b.m., c_{max} was 7.04 ± 0.93 and 4.86 ± 0.70 g/L (45% of its total value) respectively.

L-carnitine administrated to the rats which received a methanol dose, significantly slowed down the absorption. Sachan did not observe this phenomenon after ethanol administration. His results showed that within the first hour of the experiment, ethanol concentrations were similar in the blood of the animals which were administrated D,L-carnitine and those which were not. The results we obtained while investigating methanol were quite different. In the animals which were simultaneously given methanol and L-carnitine, the concentrations of methanol in blood were low just after 30 minutes. This might result from the so-called alcohol deficit observed when alcohol is drunk after the meal. In the case of methanol intoxication, L-carnitine may play a protective role, like food does after ethanol drinking. The difference between the mechanism of both methanol and ethanol diffusions may also be important. The low concentration of methanol in blood of the animals which were administrated L-carnitine corresponded with higher methanoluria.

In the fourth 24-hour urine collection of the animals which were administrated lower dose of methanol and L-carnitine, the methanol excrection in urine was 1.7 times

higher compared to the group of the rats which were not given L-carnitine. In the rats which received a larger alcohol dose and L-carnitine, the methanol excretion was 1.36 times higher (tables 4 and 5). It should be mentioned that in the group where L-carnitine was administrated, the methanol excretion was not finished even after 96 hours and the results we obtained could be higher. Compared to the rats without L-carnitine, the low concentrations of methanol in blood but significantly higher methanoluria in the animals which received this compound, suggest rather slow methanol biotransformation, as described in the case of ethanol poisoning (SACHAN and BERGER 1993).

Sachan and Berger also described a significantly decreased (30% approximately) capacity of ethanol distribution (V_d) after carnitine administration, explaining that alcohol could bind other molecules. The increased capacity of distribution was found in the experiment on methanol.

In Sachan's experimental model, Calabrese noticed a low ethanol concentration (40%) but a significantly faster clearance. (CALABRESE and RIZZA 1999; CALABRESE et al. 2001). This could result from both block of ethanol absorption and activation of adjuvant mechanisms, like the increased activity of MEOS and catalase. This finding might confirm that L-carnitine influences CYP2E1 activity in the presence of alcohol (methanol or ethanol), probably by the post-translative stabilization of proteins (TAINAKA et al. 1993), as an increase in mRNA was not noticed.

During the four-day experiment 37 rats died after a dose of methanol in the first twenty-four hours of the experiment. 2 of these rats died of the injury with a probe. All the animals survived in the groups in which methanol and L-carnitine were given simultaneously. In the group where rats received methanol only, their deaths were caused by the individual sensivity to toxic action of the xenobiotic given.

The methanol toxicity is associated mainly with products of its transformation. However, it is not possible to exclude its effect on the central nervous system. In the case of rats, this kind of toxicity is involved in the formation of formaldehyde. This compound, by inducing the formation of reactive oxygen species (ROS), evokes the oxidative stress (ALBANO 2002; BAILEY and CUNNINGHAM 2001; CEDERBAUM 2001; SKRZYDLEWSKA and FARBISZEWSKI 1997; TUMA 2002). Oxygen free radicals cause the production of hyperoxides and small aldehyde molecules (GEBICKI and GEBICKI 1993). These aldehydes with longer half-life than oxygen free radicals show their ability to diffuse to various cells, causing their destruction (SKRZYDLEWSKA and SZYNAKA 1997). The increased concentration of malondialdehyde (MDA) which is the indicator of membrane destruction, confirms the increase of peroxidation (MOTTARAN et al. 2002; TUMA 2002).

The supplementation of L-carnitine increases the level of dismutase superoxide (SOD), which alters the level of oxidative stress factors by decreasing the concentration of oxygen free radicals. L-carnitine also activates both inter- and intracellular anti-oxidative protective system by increasing the level of both glutation peroxidase and catalase (BINIENDA and ALI 2001; KAUR et al. 2001; TUMA 2002).

L-carnitine and acetyl-L-carnitine significantly reduce the level of fatty acids oxidation and regulate the function and stability of cell membrane (LÖSTER 2000; LOULET et al. 2001). The concentration of malondialdehyde decreases considerably in plasma, which confirms the decrease in lipid peroxidation (DI GIACOMO et al. 1993; KAUR et al. 2001; LÖSTER and BÖHM 2001; VESELA et al. 2001).

Conclusions

1. L-carnitine IP, decreases the elimination of methanol in rats.
2. Toxicokinetic parameters indicate that L-carnitine slows down the methanol biotransformation.
3. After L-carnitine and methanol administration:
- The concentration of methanol in blood is lower in the absorption phase but higher in the elimination phase.
- Methanol excretion in urine lasts longer and is not finished within a 96-hour observation period. The excreted amounts were significantly higher.
- The presence of methanol in the liver is considerably longer.
- The rate of the methanol elimination in blood, liver and urine depends on the alcohol dose given.

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