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Effect of L-carnitine supplementation on xenobiotic-metabolizing hepatic enzymes exposed to methanol

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Abstract

The study aimed to evaluate the effect of L-carnitine on hepatic cytochrome P450-dependent monooxygenases exposed to methanol. Male Spraque–Dawley rats were given methanol (1/4 LD_{50} and 1/2 LD_{50}) together with L-carnitine (1 g/kg body weight). The parameters of microsome electron transport chains I and II and the levels of CYP2E1, CYP2B1/2 and CYP1A2 were measured 8, 12, 24, 48, 72 and 96 h after exposure.

L-carnitine did not affect cytochrome P450 but it significantly increased at 72 and 96 h NADPH–cytochrome P450 reductase. It stimulated cytochrome b_5 at 48 and 96 h and NADH–cytochrome b_5 reductase activity at 12, 72 and 96 h. Methanol, especially the lower dose, inhibited cytochrome P450 after 48 h, but the higher methanol dose inhibited NADH–cytochrome b_5 reductase activity in this time. L-carnitine, combined with the lower dose of methanol, stimulated NADPH–cytochrome P450 reductase after 48 h and cytochrome b_5 and NADH–cytochrome b_5 reductase over the whole period of observation.

L-carnitine stimulated CYP2B1/2 but not CYP2E1 and CYP1A2. Methanol stimulated CYP2E1 at 24 h, but CYP1A2 at 96 h in the studied doses. CYP2B1/2 was induced by the lower dose of methanol at 24 h but by the higher one at 96 h. When given together, L-carnitine and methanol (1/2 LD_{50}) significantly stimulated CYP2E1 up to 170% at 24 h and 145% at 96 h.

Keywords: L-carnitine; Methanol; P450; Liver; Rat

Introduction

Increasing environment and food pollution and widening use of drugs and pesticides have drawn the attention of toxicologists, pharmacologists and biochemists to the issue of xenobiotic transformation (Kitani, 1996; Mansuy, 1995; Spatzengger and Jaeger, 1995). The enzymes involved in this process also participate in the metabolism of such compounds as alcohols, including methanol.

Apart from physiological levels of ethanol (30 ± 20 μmml/L), human blood contains methanol (39 ± 22 μmml/L), which is formed by bacterial fermentation of fruits, juices, aspartam, honey and roasted coffee in the alimentary tract (Bouchard et al., 2001; Haffner et al., 1998). Both the alcohols are oxidized by

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enzymatic systems that metabolize exogenous alcohols. As ethanol shows higher affinity for alcohol dehydrogenase (ADH) than methanol does, the former competitively inhibits methanol biotransformation. This effect has been utilized in the treatment of acute methanol intoxication. However, ethanol also produces toxic effects. The search for more specific ADH inhibitors resulted in the discovery of pyrazole and its derivatives, which are used as analgesic and anti-inflammatory drugs. It has been shown that 4-methylpyrazole is a better and faster ADH blocker (Pietruszko, 1975). Pyrazole blocks ADH by forming an inactive ADH–NADH–pyrazole complex. However, the adverse effects of pyrazole limit its clinical use and therefore the search for non-toxic compounds that effectively block methanol oxidation continues.

Recently, some reports on the use of L-carnitine in experimental ethanol intoxication have been published. It was demonstrated that L-carnitine significantly inhibited the formation of ethyl esters of fatty acids and increased ethanol clearance (Calabrese and Rizza, 1999a). In the study it was found that L-carnitine considerably inhibited ethanol metabolism (Sachan and Berger, 1987, 1993). L-carnitine is thought to induce hepatic CYP2E1 activity that has already been increased by ethanol. It was observed that L-carnitine administration resulted in the alleviation or even complete remission of ethanol dependence syndrome (Mangano et al., 2000). Long-term L-carnitine treatment significantly reduced lipid peroxidation, which allowed normal cellular functioning (Dayanandan et al., 2001).

Those findings let us assume that L-carnitine would show a similar effect on methanol intoxication. If yes, L-carnitine might replace ethanol as a competitive inhibitor in methanol intoxication. As both the alcohols are biotransformed not only by ADH but also by the liver cytochrome P450-dependent monooxygenase system, measurement the levels and activity of P450 after exposure to methanol might provide much data on the role of the microsome enzymatic system in biotransformation (Ekstrom and Ingelman-Sundberg, 1989; Lucas et al., 1992). In the previous papers of the investigation we have reported influence of L-carnitine on methanol biotransformation (Czech et al., 2004) and effect of methanol on L-carnitine levels (Olszowy et al., 2005). The aim of this study was to determine whether the liver cytochrome P450-dependent monooxygenase system responds to a combination of methanol and L-carnitine.

Material and methods

Animals

Three-month-old Sprague–Dawley rats weighing 200–230 g each were used in this study, as there are similarities in methanol oxidation between primates and rodents.

The rats were provided by the Experimental Animal Center, the Medical University of Silesia, Katowice-Ligota, where all experiments were conducted. The study protocol was approved by the Local Ethics Committee for Animal Studies (no. 1/01).

The rats were randomized into six groups (Table 1). Prior to methanol, L-carnitine or physiological saline administration, rats were weighed in order to calculate the doses. All rats were fasted for 14 h prior to the start of each experiment. At least four rats were used for each time point in experimental groups, and three rats were included in control group.

The methanol doses of 6440 and 3220 mg/kg b.w. were calculated from rat LD_{50} that is 12880 mg/kg b.w. (Williams, 1956).

Rats were sacrificed by decapitation. Liver samples were collected at necropsy in order to determine methanol, cytochrome P450 and cytochrome b_5 levels as well as NADPH–cytochrome P450 reductase and NADH–cytochrome b_5 reductase activities. The liver reductase activities were measured 8, 12, 24, 48, 72 and 96 h after exposure.

Isolation of microsomal fraction

Rats were sacrificed between 8.30 and 9.30 a.m. to avoid circadian fluctuations in the activity of the

Table 1. Treatment regimens

Group	Treatment	
	Intragastrically	Intraperitoneally
Control	Drinking water	0.9% NaCl
II	Drinking water	L-carnitine (6.2 mmol/kg b.w.)
III	Methanol (3220 mg/kg b.w.)	0.9% NaCl
IV	Methanol (3220 mg/kg b.w.)	L-carnitine (6.2 mmol/kg b.w.)
V	Methanol (6440 mg/kg b.w.)	0.9% NaCl
VI	Methanol (6440 mg/kg b.w.)	L-carnitine (6.2 mmol/kg b.w.)

cytochrome P450-dependent monooxygenase system (Czekaj et al., 1994; Plewka et al., 1992). Liver samples were placed in ice-cold physiological saline. The liver microsome fraction was isolated as described by Dallner (1974). The homogenization medium (5 cm^3/g liver tissue) consisted of 0.25 M saccharose dissolved in 10 mM Tris–HCl buffer (pH 7.4). The isolation procedure was performed at 2–4 °C.

Pure microsomal fractions were suspended in the homogenization medium containing 20% (v/v) glycerol. The microsome samples were frozen at −80 °C and stored overnight. Then they were slowly thawed and assayed biochemically.

Determination of cytochrome P450 and cytochrome b_5 levels

Cytochrome P450 and cytochrome b_5 levels were determined as described by Estabrook and Werringloer (1978). Before assay, the microsome suspension was diluted with Tris–HCl (50 mM, pH 7.4) to 0.3 mg protein/cm^3. The cytochrome levels were calculated using millimole absorption coefficients 91 and 185 $mM^{-1}\,cm^{-1}$, respectively (Omura and Sato, 1964), and were expressed in nanomoles per 1 mg microsome protein.

Determination of NADPH–cytochrome P450 reductase and NADH–cytochrome b_5 reductase activities

Both activities were determined by measuring the rate of cytochrome c reduction at 550 nm (Hodges and Leonard, 1974). The level of reduced cytochrome c was calculated with the use of the molar extinction coefficient 18.5 $mM^{-1}\,cm^{-1}$ (Hodges and Leonard, 1974). Both the reductase activities were expressed in millimoles of cytochrome c per 1 min/mg protein.

Determination of microsomal protein

Microsomal protein was determined by the method of Lowry et al. (1951) with bovine albumin as a standard.

Electrophoresis

The working medium was polyacrylamide gel (1.5 mm in thickness and 20 cm in length). The stacking gel was composed of 4% polyacrylamide dissolved in 0.125 mM Tris–HCl (pH 6.8) containing 0.1% SDS. The running gel was composed of 10% polyacrylamide dissolved in 0.375 mM Tris–HCl (pH 8.8) containing 0.1% SDS. The electrode tanks contained a 0.1% solution of SDS with Tris (6 g/dm^3) and glycine (28.8 g/dm^3). If required, the pH of the solution was adjusted to 8.3. Initially, electrophoretic separation was performed at 30 mA, and after the samples had entered the running gel, the intensity of electric current was decreased to 8 mA. Initially, the voltage was about 40 V, and at the end of separation it was increased to 70 V.

Western blot analysis of cytochrome P450 isoforms

The levels of cytochrome P450 isoforms in rat liver microsomes were determined by Western blot analysis. Microsome samples (10 μg of protein) were separated (as described above) on polyacrylamide gel (10%) in the presence of SDS according to the method of Laemmli (1970). Separated proteins were transferred onto a PVDF membrane (Millipore) and were stained immunochemically. Polyclonal anti-CYP rabbit antibodies were purchased from Chemicon Int. Inc. The antibody–CYP binding was visualized by using secondary antibodies coupled with alkaline phosphatase and by using the BCIP/NBT substrate (Sigma) as described by the manufacturer. Immunoelectrophoregrams were analyzed densitometrically with a One D-scan software (Scanalytics).

Statistical analysis

One- and two-way ANOVA followed by the Dunnett test were used for comparison amongst the multiple treated groups and the control.

Results represent the means ± SD, $n = 4$ for experimental groups and $n = 3$ for control group. The statistically significant differences at $p < 0.05$.

Results

L-carnitine had no effect on cytochrome P450 whose level remained within the range of values observed at all time points in control group (Fig. 1). NADPH–cytochrome P450 reductase activity did not change within 48 h, while afterwards it increased to reach 120–130% of the control value (Fig. 2).

L-carnitine differently affected microsome electron transport chain II. After L-carnitine administration cytochrome b_5 levels increased to 130% of the control value at 48 and 96 h (Fig. 3). NADH–cytochrome b_5 reductase activity was comparable to the control value, beside 12, 72 and 96 h time intervals when it increased (Fig. 4).

The lower dose of methanol (1/4 LD_{50}) had no effect on cytochrome P450 in the first 48 h of observation. At the end of observation, cytochrome P450 levels decreased to 70% of the control value. NADPH–cytochrome P450 reductase activity increased from 12 h of observation to reach 140% of the control value

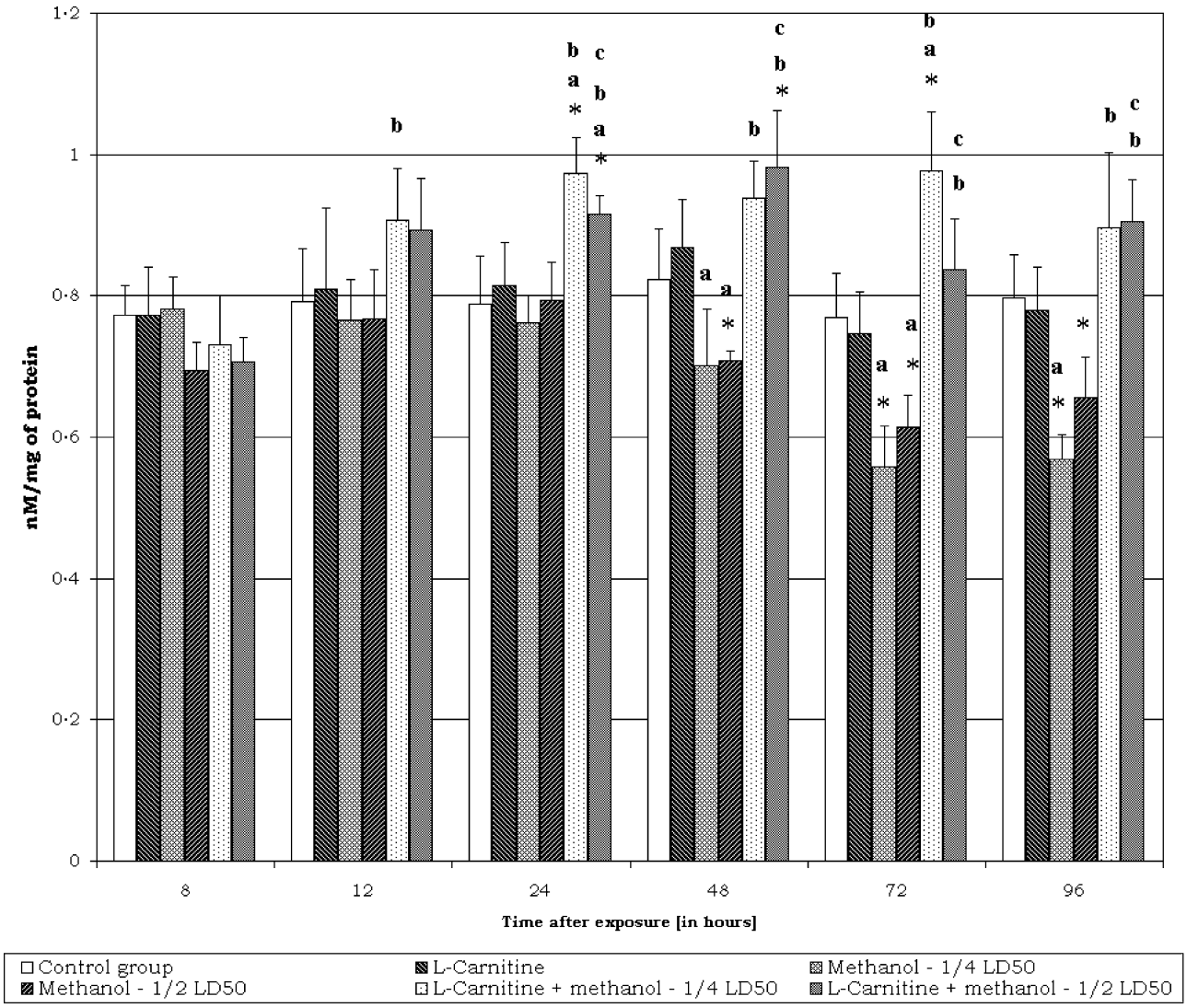

Fig. 1. Effects of methanol and/or L-carnitine on cytochrome P450 content in rat liver. Statistically significant ($p<0.05$) in comparison with: *control group; [a]L-carnitine; [b]1/4 LD_{50} methanol dose; [c]1/2 LD_{50} methanol dose.

at 96 h (not significant at 12 and 48 h). Cytochrome b_5 showed a different pattern of change compared with cytochrome P450. Cytochrome b_5 levels increased to 130% of the control value in the initial phase of observation, and then they returned to the control value. At the same time, NADH–cytochrome b_5 reductase activity did not differ from the control value.

The higher methanol dose modified all the components of the cytochrome P450-dependent monooxygenase system. In the first 24 h after methanol administration, the cytochrome P450 level did not differ from the control value, and at 48, 72, 96 h it decreased significantly to 80%. Methanol (1/2 LD_{50}) did not affect NADPH–cytochrome P450 reductase activity.

The level of cytochrome b_5 increased in the first hours after higher methanol dose administration (115% at 8 h) but at 12 h it decreased. From then the cytochrome b_5 level did not differ significantly from the control value. Finally (at 96 h) it exceeded 125% of the control value. NADH–cytochrome b_5 reductase activity did not differ from the control value in the first hours after methanol administration. Afterwards, it decreased and showed a significant inhibition (75% of the control value) at 72 and 96 h.

After administration of the lower methanol dose given together with L-carnitine increased cytochrome P450 level at 24 and 72 h. NADPH–cytochrome P450 reductase activity increased at 72 and 96 h to reach about 140% of the control value. Cytochrome b_5 increased even up to 150% of the control value over the whole period of observation. NADH–cytochrome b_5 reductase activity also exceeded the control value throughout the experiment.

After the higher dose of methanol (1/2 LD_{50}) given together with L-carnitine, cytochrome P450 levels showed increase at 24 and 48 h, only. In this experimental

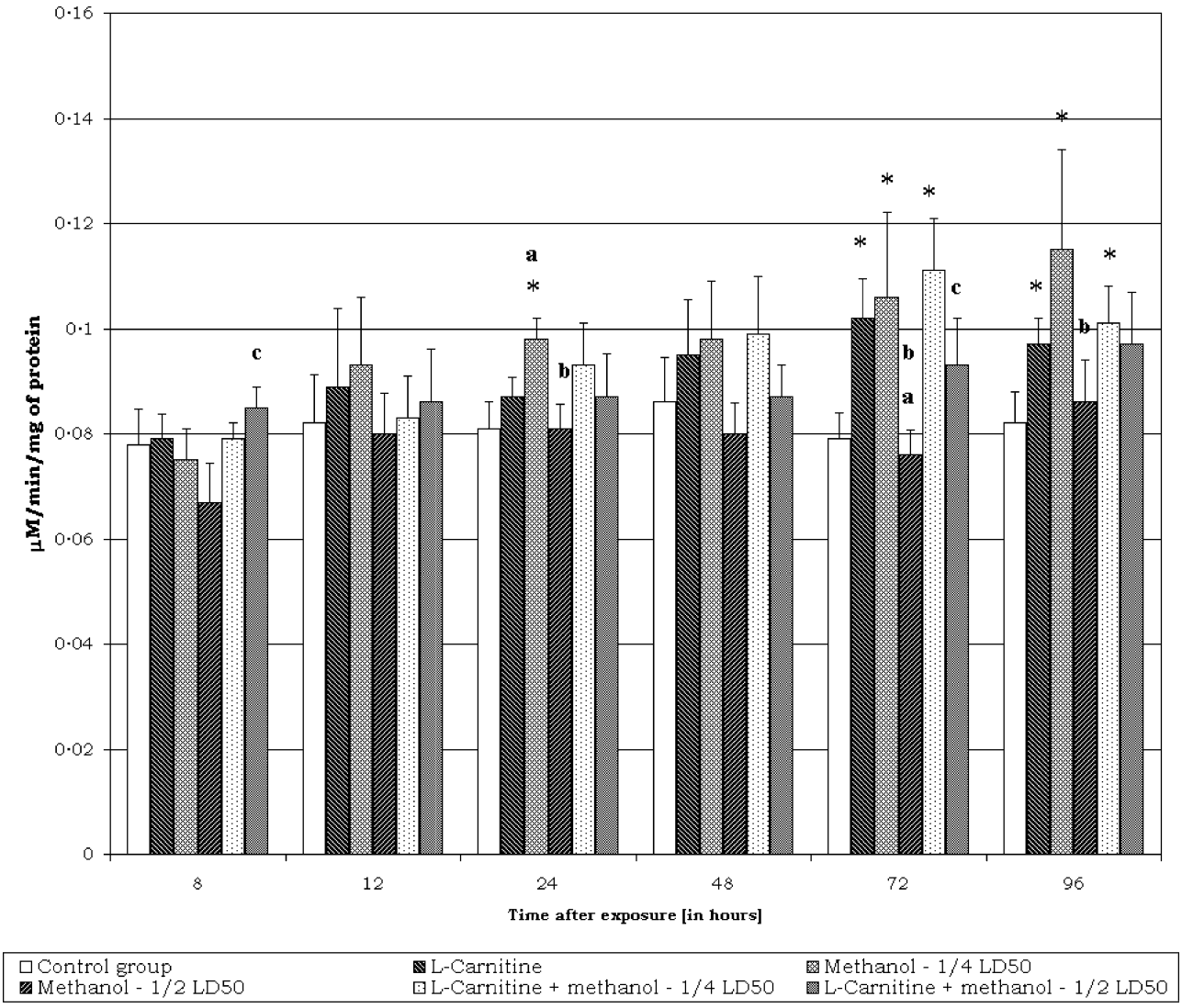

Fig. 2. Effects of methanol and/or L-carnitine on NADPH–cytochrome P450 reductase activity in rat liver. Statistically significant ($p<0.05$) in comparison with: *control group; [a]L-carnitine; [b]1/4 LD_{50} methanol dose; [c]1/2 LD_{50} methanol dose.

group, NADPH–cytochrome P450 reductase activity did not change. Cytochrome b_5 in the first 48 h increased to over 140% of the control value, and then it return to control group level. In contrast, NADH–cytochrome b_5 reductase activity showed a biphasic profile of change. In the first hours of observation it increased significantly and then it decreased to the control level until 72 h. Afterwards, it increased again to reach 130% of the control value at 96 h.

L-carnitine significant decreased CYP1A2 at 8 and 96 h but CYP2E1 at 8 h only (Table 2). The lower dose of methanol (1/4 LD_{50}) increased CYP2E1 levels to about 125% of the control value at 24 h, only. After the lower dose of methanol given together with L-carnitine increased CYP2E1 to over 140% at 24 h and remained at that level for the following 2 days (Table 2). The higher methanol dose only slightly stimulated CYP2E1 compared to the lower dose, for the highest CYP2E1 level was 135% of the control value. The higher dose of methanol (1/2 LD_{50}) together with L-carnitine significantly increased the level of CYP2E1. At 24 h it reached almost 170% and about 140% of the control value in the end of observation.

In the L-carnitine group significant increases in CYP 2B1/2 at 24 h and at 96 h were observed. The lower dose of methanol slightly changed the level of CYP 2B1/2 (Table 2). L-carnitine combined with the lower methanol dose did not affect CYP 2B1/2. The higher methanol dose slightly increased CYP2B1/2 to about 110% of the control value in the first phase of observation, and then even to 130%. L-carnititne combined with the higher methanol dose produced an effect similar to that caused by methanol alone.

In the L-carnitine group, CYP1A2 decreased to about 60% of the control value in the first phase of observation. At 24 h, it did not differ from the control

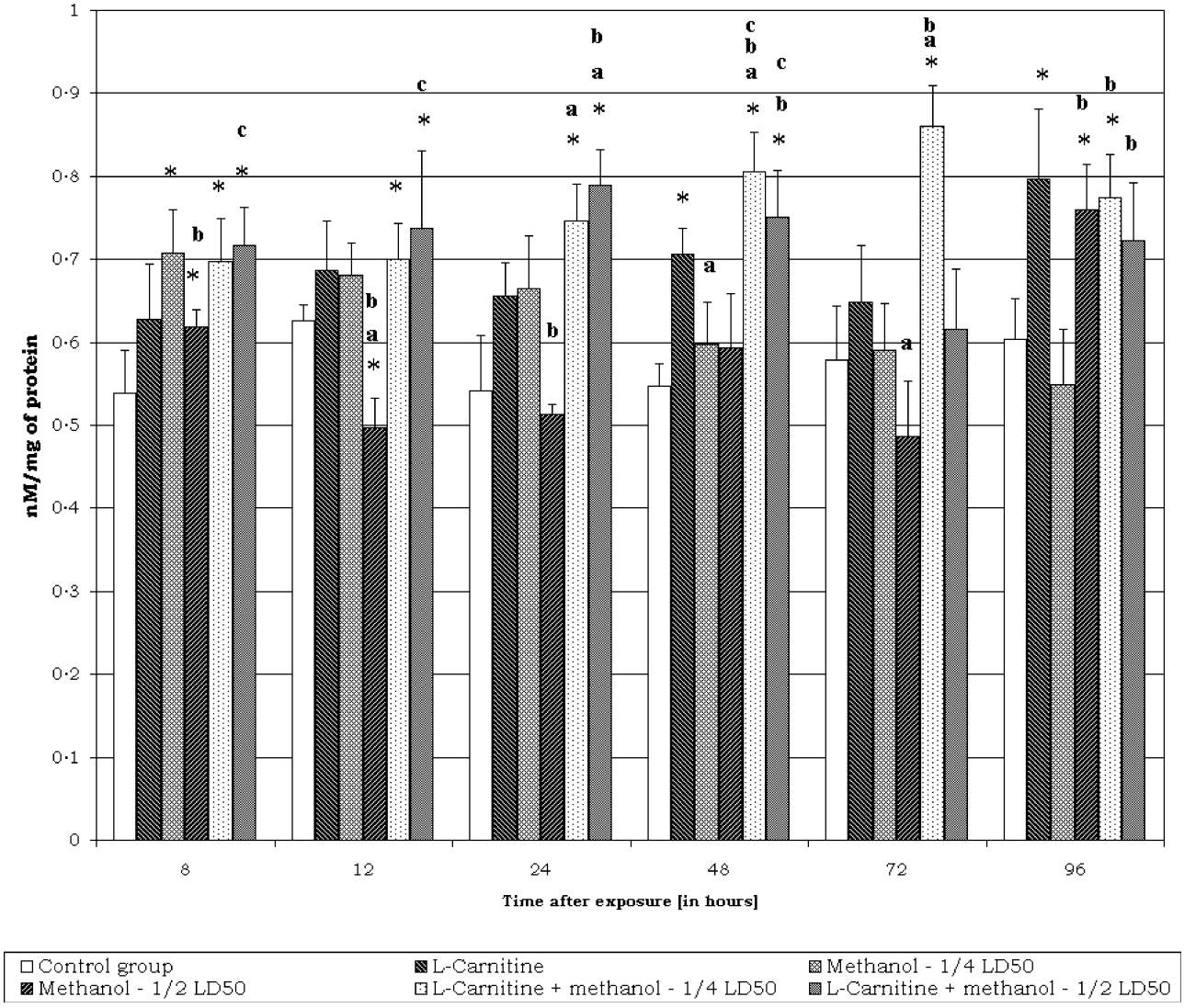

Fig. 3. Effects of methanol and/or L-carnitine on cytochrome b_5 content in rat liver. Statistically significant ($p<0.05$) in comparison with: *control group; [a]L-carnitine; [b]1/4 LD_{50} methanol dose; [c]1/2 LD_{50} methanol dose.

group, though at 96 h it decreased again to 60% of the control value (Table 2). The lower methanol dose slightly increased CYP1A2 levels (120% of the control value at 96 h). Similar changes were observed after L-carnitine combined with the lower methanol dose. When rats were treated with the higher methanol dose CYP1A2 increased to 130% of the control value at 96 h. A combination of the higher methanol dose and L-carnitine slightly increased CYP1A2 to 127% of the control value at the end of observation.

Discussion

Intraperitoneal administration of L-carnitine and intragastrical administration of water to rats had no effect on the total level of cytochrome P450, probably because L-carnitine produces different effects on different CYP isoforms, and these effects cancel each other out. Out of the three cytochrome P450 isoforms studied, CYP2B1/2 significantly increased at 24 h and at 96 h but while CYP1A2 was decreased at 8 and 96 h.

One can merely speculate how L-carnitine increases the level of cytochrome b_5. Perhaps L-carnititne increases the fluidity of cytoplasmic membranes. Moreover L-carnitine enhances the β-oxidation of saturated fatty acids in mitochondria. The increasing activity of both reductases studied may prove it indirectly.

Sachan and Berger (1987), by studing ethanol metabolism in rats fed for 5 days with chow supplemented with 1% D,L-carnitine and then exposed to a single dose of ethanol, showed that L-carnitine slowed down ethanol biotransformation, but it did not affect

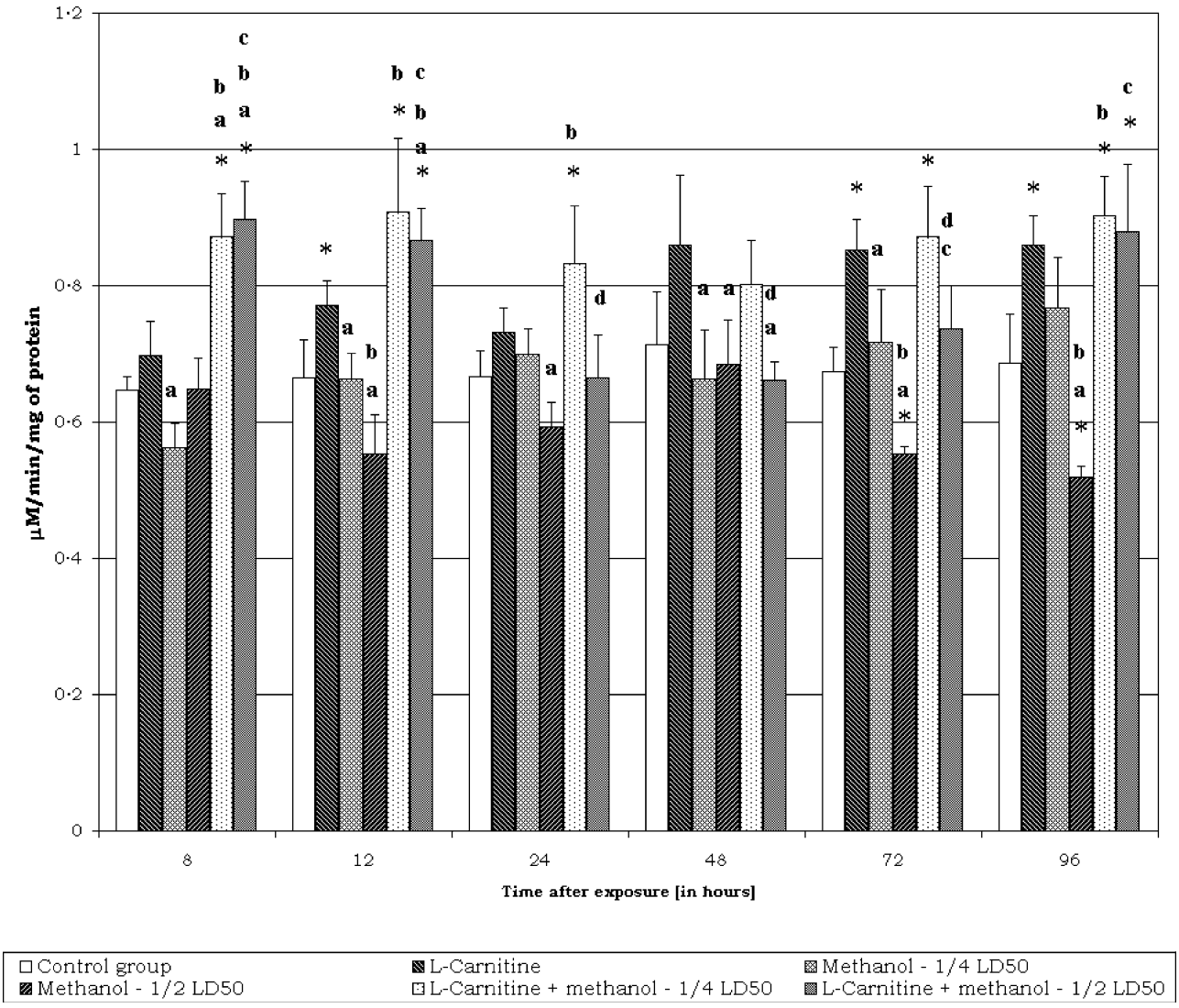

Fig. 4. Effects of methanol and/or L-carnitine on NADH–cytochrome b_5 reductase activity in rat liver. Statistically significant ($p<0.05$) in comparison with: *control group; [a]L-carnitine; [b]1/4 LD_{50} methanol dose; [c]1/2 LD_{50} methanol dose; [d]1/4 LD_{50} methanol dose plus L-carnitine.

ethanol absorption, which was reflected by a much slower ethanol clearance from blood. The authors suggested that this resulted from the competition between carnitine and the cofactor NAD^+ or ethanol for the binding site on alcohol dehydrogenase.

ADH and MEOS are major systems that participate in the metabolism of methanol in vivo. L-carnitine inhibits their activity. The results of our rat study of methanol intoxication only partly correlated with the study by Sachan and Berger (1993). We found that single doses of ethanol and L-carnitine (i) decreased methanol clearance, (ii) prolonged alcohol blood retention time, (iii) increased c_{max}, and (iv) increased AUC (for details see Czech et al., 2004). These parameters strongly depended on a methanol dose. The c_{max} values were lower in rats treated with methanol combined with L-carnitine. The c_{max} value was 3.92 ± 0.29 mg/L in rats treated with the lower methanol dose (3220 mg/kg b.w.) and 3.70 ± 0.16 mg/L in rats treated with both the lower methanol dose and L-carnitine. After the higher methanol dose (6440 mg/kg b.w.) given alone or combined with L-carnitine c_{max} was 7.04 ± 0.93 and 4.86 ± 0.70 mg/L, respectively.

Our study has shown that L-carnitine inhibits methanol absorption in experimental methanol intoxication, which was not observed by Sachan and Berger (1987) in their studies, in which blood ethanol levels were comparable in ethanol- and ethanol with carnitine-treated rats in the first hour of the experiment. In our study, methanol- with carnitine-treated rats showed much lower levels of methanol in blood compared to those in methanol-treated rats. This phenomenon is

Table 2. CYP2E1, CYP2B1/2 and CYP1A2 contents in rat liver after pretreatment with methanol and/or L-carnitine

Treatment	[h]	CYP2E1	CYP2B1/2	CYP1A2
L-carnitine	8	74*	109	63*
	24	106	130*	96
	96	82	128*	58*
Methanol – 1/4 LD_{50}	8	97	90	85
	24	126*	119*	113
	96	115	110	120*
Methanol – 1/2 LD_{50}	8	100	92	92
	24	135*	112	125
	96	121	130*	129*
Methanol – 1/4 LD_{50} and L-carnitine	8	107	91	97
	24	143*	101	117
	96	130*	110	133*
Methanol – 1/2 LD_{50} and L-carnitine	8	112	95	104
	24	168*	109	111
	96	143*	123*	127*

In each column three subsequent values indicate percentages of the control values after 8, 24 and 96 h, respectively.
*Statistically significant ($p<0.05$) in comparison with control group.

similar to the so-called alcohol deficit observed when ethanol is consumed after a meal. Perhaps L-carnitine plays a similar role in methanol intoxication.

Lower methanol levels in blood from animals exposed to both methanol and L-carnitine accompanied by increased methanol excretion in urine indicates inhibited methanol biotransformation. One should take into account that in animals treated with methanol and L-carnitine methanol urine excretion was not finished at 96 h, which might increase the observed levels.

In a similar animal model treated with ethanol was observed a 40% decrease in ethanol levels and a significant increase in ethanol clearance (Calabrese and Rizza, 1999a, b). It is likely that this effect was caused by blockade of ethanol absorption and increased activity of the MEOS system and catalase. This may confirm the fact that L-carnitine modulates ethanol-induced CYP2El, probably via posttranslational protein stabilization, for the mRNA was not increased (Tainaka et al., 1993).

In our methanol study, we observed a slight stimulation of CYP2El. Interestingly enough, there were no differences between the lower and the higher methanol doses in their effect on CYP2El levels. In the only study available, where analogous isoforms were studied (Allis et al., 1996) there was a 2.8-fold increase in the level of CYP2E1. However, the authors studied CYP2E1 by measuring *p*-nitrophenol hydroxylase activity (PNP), which might cause such discrepancies. Allis et al. (1996) did not observed any effect of methanol on the level of CYP2B1/2 (reflected by PROD activity), while we found a 120% increase after 1/4 and 130% after 1/2 LD_{50}. The increased isoforms levels were not accompanied by an increase in the total level of cytochrome P450 because after 48 h cytochrome P450 content was decreased significantly by methanol. It is unclear why NADPH–cytochrome P450 reductase activity increased after the lower dose of methanol. Perhaps further study will clarify this issue.

What was important in our study was that in rats exposed to methanol L-carnitine supplementation increased the level of CYP2E1, a major isoenzyme induced by alcohols. This increase was proportional to the dose of methanol, which was partly true also for CYP1A2. This positive effect of carnitine was also observed for the total level of cytochrome P450 when methanol was combined with carnitine. A similar positive effect was observed for cytochrome b_5, especially after the lower dose of methanol.

Literature data indicate that L-carnitine directly affects the turnover and metabolism of lipids (Calabrese and Rizza, 1999b) and it decreased the accumulation of esters of long-chain fatty acids (Calabrese et al., 2001), thus modulating the activity of cellular enzymes involved in such processes as peroxidation. This may suggest that carnitine acts as a factor that protects cells against alcohol-induced injury.

Conclusions

1. L-carnitine has no effect on microsome electron transport chain I, while it has a mild, stimulating effect on cytochrome b_5 and NADH–cytochrome b_5 reductase.

2. L-carnitine and methanol (especially 1/4 LD_{50} dose) increased all the parameters studied, but effect is time related (cytochromes P450 and b_5 and their reductases).
3. L-carnitine decreased the levels of CYP1A2, but increased CYP2B1/2. L-carnitine and methanol (especially 1/2 LD_{50} dose) increased the levels of P450 isoforms.

References

Allis JW, Brown BL, Simmons JE, et al. Methanol potentiation of carbon tetrachloride hepatotoxicity: the central role of cytochrome P450. Toxicology 1996;112:131–40.

Bouchard M, Brunet R, Droz O, et al. A biologically based dynamic model for predicting the disposition of methanol and its metabolites in animals and humans. Toxicol Sci 2001;64:169–84.

Calabrese V, Rizza V. Effects of L-carnitine on the formation of fatty acid ethyl esters in brain and peripheral organs after short-term ethanol administration in rat. Neurochem Res 1999a;24:79–84.

Calabrese V, Rizza V. Formation of propionate after short-term ethanol treatment and its interaction with carnitine pool in rat. Alcohol 1999b;19:169–75.

Calabrese V, Scapagnini G, Catalano C, et al. Effects of acetyl-L-carnitine on the formation of fatty acid ethyl esters in brain and peripheral organs after short-term ethanol administration in rat. Neurochem Res 2001; 26:167–74.

Czech E, Olszowy Z, Nowicka J. The influence of L-carnitine on methanol biotransformation in rats. Exp Toxicol Pathol 2004;55:367–77.

Czekaj P, Plewka A, Kamiński M. Daily and circadian rhythms in the activity of mixed function oxidases system in rats of different age. Biol Rhythm Res 1994;25:67–75.

Dallner G. Isolation of rough and smooth microsomes – general. Methods Enzymol 1974;32:191–215.

Dayanandan A, Kumar P, Panneerselvam C. Protective role of L-carnitine on liver and heart lipid peroxidation in atherosclerotic rats. J Nutr Biochem 2001;12:254–7.

Ekstrom G, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). Biochem Pharmacol 1989;38:1313–9.

Estabrook RW, Werringloer J. The measurement of difference spectra: application to the cytochromes of microsomes. Methods Enzymol 1978;52:212–20.

Haffner H, Graw M, Besserer K, et al. Curvilinear increase in methanol concentration after inhibition of oxidation ethanol: significance for the investigation of endogenous methanol concentration and formation. Int J Legal Med 1998;111:27–31.

Hodges TK, Leonard RT. Purification of a plasma membrane-bound adenosine triphosphatase from plant roots. Methods Enzymol 1974;32:392–406.

Kitani K. Drug hepatotoxicity in the elderly. Drug-Induced Hepatotoxicity 1996;121:341–66.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.

Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

Lucas D, Menez JF, Berthou F, et al. Differences in hepatic microsomal cytochrome P-450 isoenzyme induction by pyrazole, chronic ethanol, 3-methylcholanthrene, and phenobarbital in high alcohol sensitivity (HAS) and low alcohol sensitivity (LAS) rats. Alcoholism: Clin Exp Res 1992;16:916–21.

Mangano NG, Clementi G, Costantino G, et al. Effect of acetyl-L-carnitine on ethanol consumption and alcohol abstinence syndrome in rats. Drug Exp Clin Res 2000; 26:7–12.

Mansuy D. Cytochromes P450 and drug toxicity. Clin Rev Allergy Immunol 1995;13:201–9.

Olszowy Z, Czech E, Nowicka J. Effect of methanol on endogenous and exogenous carnitine levels in rat plasma. Arch Toxicol 2005 [in press].

Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 1964;239:2370–8.

Pietruszko R. Human liver alcohol dehydrogenase – inhibition of methanol activity by pyrazole, 4-methylpyrazole, 4-hydroxymethylpyrazole and 4-carboxy-pyrazole. Biochem Pharmacol 1975;24:1603–7.

Plewka A, Czekaj P, Kamiński M, et al. Circadian changes of cytochrome P-450-dependent monooxygenase system in the rat liver. Pol J Pharmacol Pharm 1992;44:655–61.

Sachan DS, Berger R. Attenuation of ethanol metabolism by supplementary carnitine in rats. Alcohol 1987;4:31–5.

Sachan DS, Berger R. Specificity of carnitine in attenuation of ethanol metabolism in rat. Biochem Arch 1993;9:141–6.

Spatzengger M, Jaeger W. Clinical importance of hepatic cytochrome P450 in drug metabolism. Drug Metabol Rev 1995;27:397–417.

Tainaka H, Naito T, Murayama N, et al. Modulation of ethanol-mediated CYP2E1 induction by clofibrate and L-carnitine in rat liver. Biol Pharmacol Bull 1993;16:1240–3.

Williams S. Acute toxicities. Spector WS, editor. Handbook of toxicology, vol. I. Philadelphia, PA: WB Saunders Company; 1956. p. 188.