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RAPID COMMUNICATION

Enteral Administration of Lipid Emulsions Protects Liver Cytochrome c Oxidase from Hepatotoxic Action of Thioacetamide

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Summary

During 48 hours after application sublethal doses of hepatotoxic agent thiacetamide decrease cytochrome c oxidase activity of rat liver homogenate and isolated mitochondria to 46 % and 32 % of original values, respectively. This decrease may be prevented by simultaneous application of lipid emulsion (mixture of Lipofundin and Mygliol) twice a day.

Key words

Cytochrome c oxidase – Thioacetamide – Lipids – Liver

Thioacetamide (TAA) was first reported as a hepatotoxic agent by Fitzhugh and Nelson (1948) and TAA-induced hepatic necrosis was found to be dependent on appearance of active sulphine products formed during TAA oxidative degradation (Hunter *et al.* 1977). Sublethal doses of TAA were also used for induction of regenerative processes in the liver. It was found that high activation of DNA synthesis as well as induction of cell proliferation could be detected 12–24 h after intoxication. Liver recovery from TAA-induced injury by sublethal doses was completed in about 5 days (Chanda *et al.* 1995).

The detailed mechanism of TAA toxic action has, as yet, not been fully elucidated. However, it was found that cell energy metabolism plays an important role in recovery processes because rats may be completely protected from the lethal dose of TAA by adding palmitic acid and L-carnitine to the diet (Chanda and Mehendale 1994). These findings thus indicate that lethality in this particular case is in fact due to an insufficiency of adequate nutritional substrates. The protective effect of palmitate and carnitine further indicates that the compensatory cell division targeted for tissue restoration after liver injury occurs mainly in the periportal region of the liver (Chanda and Mehendale 1994) because this oxygen-rich area obtains its energy mainly by fatty acid oxidation (Jungermann and Katz 1989). It was also found that mitochondria isolated from the intoxicated liver were uncoupled and have a lower respiratory rate (Moller and Dargel 1985). We tried therefore to evaluate the extent to which the liver oxidative capacity is affected by TAA intoxication. In our experiments, we measured the activity of cytochrome c oxidase in liver homogenates and isolated mitochondria, because this key enzyme of the respiratory chain is considered as an indicator of cell oxidative capacity. We found a decrease of cytochrome c oxidase activity after TAA and therefore, on the basis of the findings of Chanda *et al.* (1995), we also tested the extent

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to which lipid administration can protect the activity of this mitochondrial enzyme from TAA intoxication. In our experiments, we used triacylglycerols enriched by medium chain fatty acids because these fatty acids may cross the mitochondrial membrane independently of the carnitine carrier system and are thus better accessible to tissue oxidation.

The experiments were carried out on male albino Wistar rats weighing 200–230 g. Rats were housed at 23±1 °C, 55±10 % relative humidity, air exchange 12–14 times per hour with a 12 h light-dark cycle. The animals had free access to a standard laboratory diet (DOS 2B, Velaz, Prague) and tap water. All animals received care according to the guidelines set by the Institutional Animal Use and Care Committee of the Faculty of Medicine, Charles University, Hradec Králové. Thioacetamide (TAA) was administered i.p. in a single dose of 100 mg/kg. Lipid emulsion (mixture of Lipofundin 10 %, B. Braun, Melsungen and Mygliol[812], Nobel, Germany in a ratio 1:1 -LIP/MIG) was given by stomach tube in a 15 ml/kg dosage twice a day (Červinková *et al.* 1996, 1997) applied immediately after TAA administration and continued until the end of the experiment. Control rats received saline instead of the lipid emulsion in the same manner. Rats were sacrificed 24 or 48 h after TAA application by exsanguination from the abdominal aorta. The liver tissue was homogenized in 0.25 M sucrose, 10 mM tris-HCl, 1 mM EDTA medium, pH 7.4 (10 % homogenate) and mitochondria were isolated according to Schneider and Hogeboom (1950). Cytochrome c oxidase activity was determined as described before (Svátková *et al.* 1996, 1997). The enzyme activity of cytochrome c oxidase was expressed as the rate of cytochrome c oxidation, i.e as micromoles of cytochrome c oxidized per min by 1 mg of mitochondrial protein (μmol cyto c/min/mg protein). Activities of aspartate aminotransferase (AST, E.C. 2.6.1.1) and alanine aminotransferase (ALT, E.C. 2.6.1.2) in the serum were measured using Sigma kits (Bergmayer *et al.* 1978). Activities of AST and ALT were expressed as μkat/l, one microkatal being the amount of enzyme that converts 1 μmol of substrate per second under the given conditions. The values in all tables indicate arithmetic means ± S.E.M. Each group included 6 animals. The unpaired Student's t-test was used for statistical evaluation.

Table 1. Changes of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum induced by thioacetamide (TAA) intoxication and the protective effect of lipid emulsion.

Time after TAA (h)	AST (μkat/l)		ALT (μkat/l)	
	Saline	+LIP/MIG	Saline	+LIP/MIG
0	1.42±0.13 (100 %)	–	0.96±0.18 (100 %)	–
24	41.50±6.86## (2922 %)	14.60±4.76**# (1028 %)	21.34±4.08## (2223 %)	5.99±2.10**## (623 %)
48	9.52±2.12## (670 %)	7.37±1.66**# (519 %)	7.83±1.69## (815 %)	4.00±1.17*# (416 %)

*Significance between the saline and TAA-treated groups and between TAA and TAA+LIP/MIG groups were calculated using Student's t-test. # $p<0.05$, ## $p<0.01$ indicate significant difference between intact and TAA treated animals. * $p<0.05$, ** $p<0.01$ indicate significant difference between groups with and without lipid emulsion. Changes induced by TAA and TAA + LIP/MIG after 24 and 48 h are also expressed in % of the control groups.*

The data presented in Table 1 show that AST and ALT activities in the serum, indicators of liver necrosis, are 20–30 times higher 24 h after TAA administration than in control animals. Fourty-eight hours after TAA administration a decline in comparison with the 24 h interval was found, however, the activities of both enzymes in TAA-treated rats were still 7–8 times higher than in control animals.

Lipid administrating depressed the 24 h peak of AST and ALT activities induced by TAA. Furthermore, the values of both enzyme activities at the 48 h interval were depressed in lipid-treated rats but were still 4–5 times higher than in control animals. This indicates that lipid administration has a significant protective effect but cannot completely eliminate TAA-induced liver injury. A similar protective effect of lipid emulsions was observed when liver injury was induced by D-galactosamine (Červinková *et al.* 1996).

As demonstrated in Table 2, 24 h after TAA administration there were no changes in the activity of cytochrome c oxidase in liver homogenate. However, a dramatic decrease was detected 48 h after TAA

administration. This demonstrates that changes of cytochrome c oxidase activity during TAA-induced liver injury begin with a certain delay and do not correlate with changes of aminotransferases. Our data presented in Table 2 further showed that, after lipid administration, the decrease of liver oxidative capacity induced by TAA administration, was completely eliminated when a lipid emulsion was applied. However, lipid substrates cannot completely prevent liver necrosis, as indicated by the increased activities of both aminotransferases after TAA+LIP/MIG administration.

We found no changes of enzyme activity in isolated mitochondria 24 h after TAA, similarly as in homogenates.

Table 2. The inhibition of cytochrome c oxidase activity by thioacetamide (TAA) in liver homogenate and protective effect of lipid emulsion

Time after TAA (h)	Cytochrome c oxidase activity (μmole cyto c/min/g wet weight) Saline	+LIP/MIG
0	145.8±6.7 (100 %)	-
24	140.9±1.8 (97 %)	174.7±11.5 (120 %)
48	66.7±3.0## (46 %)	190.2±12.5**# (130 %)

*#, ## indicate significant differences ($p<0.05$, $p<0.01$) between intact and TAA treated group. ** indicates significant difference $p<0.01$ between groups without and with lipid infusions. Changes induced by TAA and TAA + LIP/MIG after 24 and 48 h are also expressed in % of control animals.*

However, 48 h after TAA administration, cytochrome c oxidase activity was greatly depressed. This decrease was even higher than that of the homogenates, i.e. it was reduced to 30 % of the original values (see Tables 2 and 3). After lipid administration, the cytochrome c oxidase activity declined to 70 % of the values in control animals.

The difference between lipid protection of cytochrome c oxidase in homogenates (no decrease) and in mitochondria (30 % decrease) could be explained by the fact that the higher quantity with lower specific enzyme activity is formed during the TAA-induced regeneration process (Tables 2 and 3).

Table 3. The inhibition of mitochondrial cytochrome c oxidase activity by thioacetamide (TAA) and protective effect of lipid emulsion.

Time after TAA (h)	Cytochrome c oxidase activity (μmol cyto c/min/mg mito protein) Saline	+LIP/MIG
0	2.01±0.006 (100 %)	-
24	2.24±0.06 (111 %)	-
48 h	0.70±0.005## (32 %)	1.46±0.09**# (73 %)

*#, ## indicate significant differences ($p<0.05$, $p<0.01$) between control and TAA treated group. ** indicates significant difference ($p<0.01$) between groups with and without lipid emulsion. Changes induced by TAA and TAA + LIP/MIG after 24 and 48 h are also expressed in % of control animals.*

Lipid supplementation as demonstrated by our data (Table 1) cannot completely eliminate liver necrosis induced by TAA, but may eliminate the decrease of cell oxidative capacity (Table 2). Lipid supplementation may thus be an important factor supporting optimum conditions for the high rate of energy-dependent recovery processes repairing liver tissue damaged by TAA intoxication.

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Reprint requests

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