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Hepatotoxic Effect of D-Galactosamine and Protective Role of Lipid Emulsion

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Summary

D-galactosamine is a hepatotoxic agent, which induces diffuse injury of liver tissue followed by the regeneration process. Our data showed a high increase of serum aminotransferases after D-galactosamine administration, which indicates a high extent of liver injury. When lipid emulsion was applied immediately after D-galactosamine, the increase of serum aminotransferases was greatly reduced. In addition, the decrease of the cytochrome c oxidase activity induced by D-galactosamine was not observed after lipid emulsion administration and the increase of total liver oxidative capacity in the regeneration period due to activated mitochondrial biogenesis was accelerated. All these findings indicate a protective effect of lipid emulsion administration against D-galactosamine toxicity.

Key words

D-galactosamine hepatotoxicity • Liver regeneration • Cytochrome c oxidase • Lipid emulsion

Introduction

Liver regeneration is an example of tissue recovery after injury. This proliferation process can be induced in experimental conditions by partial hepatectomy or by various hepatotoxic chemical agents (tetrachlormethane, D-galactosamine, thioacetamide).

D-galactosamine (GalN) is known for inducing the features of acute hepatitis in rats. The toxic effect of GalN is connected with an insufficiency of UDP-glucose and UDP-galactose and the loss of intracellular calcium homeostasis. These changes affect cell membranes and organelles and the synthesis of proteins and nucleic acids (Keppler and Decker 1969). After GalN application, the location of proteoglycans is changed in the rat liver (Sasaki *et al.* 1996). GalN also inhibits the energy metabolism of hepatocytes (Mangeney-Andreani *et al.* 1982). This and a further study (Sire *et al.* 1983) show that GalN injures the enzymes involved in the transport of substrates to the mitochondria and modifies the phospholipid composition of membranes. The activity of mitochondrial enzymes is dependent on lipoprotein interactions and their modification by GalN may indirectly affect enzymes activities.

Damage of liver structure and function induces the regenerating process. An important role in this regeneration process is played by the cytokine IL-6. It prevents the progression of liver necrosis and thus enhances the survival of intoxicated animals and is also involved in initiating liver regeneration (Galun *et al.* 2000, Hecht *et al.* 2001).

After GalN injury, liver responds by activation of progenitor cells that proliferate and then differentiate into mature hepatocytes. Adult hepatocytes can also

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proliferate after GalN injury but these hepatocytes do not undergo dedifferentiation (Dabeva and Shafritz 1993). Other findings have demonstrated that hepatocytes can divide to restore the liver mass after GalN liver injury (Kitten and Ferry 1998).

In our study, we tested the effect of GalN administration on various parameters of liver metabolism and especially on cell energy metabolism. We also studied the effect of lipid emulsion on hepatotoxic injury because lipids are a preferred source of energy in regenerating liver tissue. We continued our previous studies (Červinková *et al.* 1995, Červinková and Drahota 1998) which showed that after partial hepatectomy and thioacetamide-induced liver injury the administration of lipid emulsion rich in medium chain fatty acids significantly attenuates the necrotic process induced by thioacetamide and accelerates the process of liver regeneration.

Methods

The experiments were made on male Wistar rats, with initial weight of 180-215 g. The rats were housed at 23±1°C, 55±10 % relative humidity, air exchange 12-14 times/h, and at 12-hour light-dark cycle periods (6:00 to 18:00). The animals had free access to standard laboratory rat chow (DOS 2B, Velaz, Prague, CR) and tap water. The animals received care according to the guidelines set by the institutional Animal Use and Care Committee of the Charles University.

D(+)-Galactosamine (Sigma) was administered i.p. in a single dose of 800 mg/kg of body weight. Lipid emulsion (Lipofundin MCT/LCT 10 % / Miglyol^{812}, in a ratio 1:1 Lipofundin / Miglyol) was given by stomach tube in a 15 ml/kg dosage twice a day (in 6-h intervals between both dosages) immediately after GalN administration and continued until the end of the experiment. Lipofundin MCT/LCT 10 % (B. Braun, Melsungen, Germany) is a sterile, non-pyrogenic fat emulsion for intravenous administration with following composition: soybean oil 50 g, medium-chain triacylglycerols (a mixture of neutral TAG of mainly caprylic acid 60 % and capric acid 40 %) 50 g, egg yolk phospholipids 12 g, glycerol 25 g, water for injections to 1000 ml. Miglyol^{812} (Dynamit Nobel, Germany) is a neutral oil containing caproic acid (max. 3 %), caprylic acid (50-65 %), capric acid (30-45 %) and lauric acid (max. 5 %). Control animals received a saline solution instead of the lipid emulsion in the same manner. Rats were sacrificed 24, 48, or 72 h after GalN 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 and mitochondria were isolated according to the previously described method (Schneider and Hogeboom 1950) and cytochrome c oxidase activity was evaluated spectrophotometrically using reduced cytochrome c (Kalous *et al.* 1989). The activity of enzyme was expressed as quantity of cytochrome c oxidized per minute per mg of protein (μmol cytochrome c/min/mg protein). Total cytochrome c oxidase activity was calculated per organ on the basis of specific activity per mg protein and total protein in the whole liver.

Proteins were determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard. Liver protein was expressed as mg per g of wet weight or as g of total protein in the whole organ on the basis of total liver wet weight.

Table 1. Changes of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity after D-galactosamine injury.

Time after GalN	ALT (μkat/l)			AST (μkat/l)		
(h)	Control	GalN	GalN + Lipid emulsion	Control	GalN	GalN + Lipid emulsion
0	0.7±0.16	–	–	1.4±0.16	–	–
24	–	10.8±4.2	7.2±3.2	-	7.5±3.5	7.3±5.1
48	–	19.5±12	2.7±1.1*	-	15.0±10	3.1±0.7*
72	–	1.6±0.7	0.8±0.2*	–	2.2±0.7	1.4±0.2*

*$p<0.05$, ** $p<0.01$ indicate significant differences between groups with and without lipid emulsion.*

The activity of aspartate aminotransferase and alanine aminotransferase in the serum was determined using a Sigma kit (Bergmayer *et al.* 1978).

The values were expressed as means ± S.D. Each group consisted of 6 animals. The statistical differences between individual groups were calculated by Student´s t-test.

Table 2. Changes of liver protein after D-galactosamine injury

Time after GalN (h)	Liver protein (mg/g wet weight)			Total liver protein (g)		
	Control	GalN	GalN + Lipid emulsion	Control	GalN	GalN + Lipid emulsion
0	325.0±26.2	–	–	2.2±0.2	–	–
24	–	254.2±29.4^{++}	270.8±14.4	–	1.78±0.10^{++}	2.06±0.18*
48	–	231.0±17.2^{+++}	245.1±9.2	–	1.62±0.23^{++}	2.00±0.16**
72	–	193.0±9.8^{+++}	205.6±27.1	–	1.76±0.17^{++}	1.67±0.22

*$^{++}$ p<0.01, $^{+++}$ p<0.001 indicate significant differences between control and GalN group; * p<0.05, ** p<0.01 indicate significant differences between groups with and without lipid emulsion.*

Table 3. Content of liver triacylglycerols after D-galactosamine injury

Time after GalN (h)	Liver triacylglycerols (mg/g liver tissue)		
	Control	GalN	GalN + Lipid emulsion
0	4.81±1.07	–	–
24	–	7.18±1.58^{+}	29.73±7.46***
48	–	8.37±2.30^{+}	35.35±6.96***
72	–	6.75±0.91^{++}	30.44±7.99***

*$^{+}$p<0.05, $^{++}$p<0.01 indicate significant differences between control and GalN group; *** indicate significant difference (p<0.001) between groups with and without lipid emulsion.*

Results

In our experiments, we assessed the extent of liver injury 24, 48, and 72 h after D-galactosamine administration by the increase of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Table 1). We found a high increase of ALT and AST activities 24 and 48 h after GalN administration. The activities of both enzymes were normalized after 72 h, which indicated an activation of the regeneration process. The increase of both amino-transferases was significantly lower when the lipid emulsion was applied after GalN and also the recovery of normal aminotransferase activities in serum was faster.

Changes of total liver protein (Table 2) showed a significant decrease during the whole period studied. The administration of lipid emulsion reduced the decrease of total protein at 24-h and 48-h intervals. At 72-h interval, no difference between GalN and GalN plus lipid emulsion groups was found. Values of liver protein expressed per g wet weight showed a significant decrease after GalN at all tested intervals; however, the protective effect of lipid emulsion was not observed (Table 2). This could be explained by an interference of increased triglyceride content in the intoxicated liver (Table 3).

We also tested changes of liver oxidative capacity after GalN administration according to the activity of liver cytochrome c oxidase. As demonstrated in Table 4, total liver oxidase capacity was decreased 24 h after galactosamine administration. The total cytochrome c oxidase activity was again recovered 48 h after GalN administration and it was significantly higher

at 72 h compared with the control group. Data in Table 4 indicate the activation of cell oxidative capacity during the regeneration process induced by GalN intoxication. This was also confirmed by data on specific cytochrome c oxidase activity in the homogenate, which indicated a significant increase of this enzyme activity at 48-h and 72-h intervals after GalN administration. Because specific cytochrome c oxidase activity of isolated mitochondria was not changed (Table 5), the increase of homogenate activity at 72 h after GalN administration indicates that the amount of mitochondrial protein is increased.

Application of lipid emulsion had the most pronounced effect on both total content and activity of cytochrome c oxidase 24 h after GalN administration, when the lipid emulsion completely prevented the decrease of enzyme activity induced by D-galactosamine. The protective effect of lipids was evident at 48-h and 72-h intervals after GalN administration, however, it was less pronounced in comparison with the 24-h interval (Table 4).

Table 4. Changes of cytochrome c oxidase in liver homogenate after D-galactosamine injury

Time after GalN (h)	Total content of cytochrome c oxidase (mmol cytochrome c/min/liver)			Activity of cytochrome c oxidase (µmol cytochrome c/min/mg protein)		
	Control	GalN	GalN + Lipid emulsion	Control	GalN	GalN + Lipid emulsion
0	0.93±0.10	–	–	0.43±0.07	–	–
24	–	$0.70\pm0.13^{++}$	1.01±0.09***	–	0.39±0.07	0.49±0.03*
48	–	0.95±0.18	1.17±0.09*	–	$0.58\pm0.04^{++}$	0.59±0.03
72	–	$1.25\pm0.18^{++}$	1.46±0.12*	–	$0.71\pm0.10^{+++}$	0.88±0.14*

*$^{++}p<0.01$, $^{+++}p<0.001$ indicate significant differences between control and GalN group.; *$p<0.05$, ***$p<0.001$ indicate significant differences between GalN group and group with and without lipid emulsion.*

Table 5. Activity of mitochondrial cytochrome c oxidase after D-galactosamine injury

Time after GalN (h)	Cytochrome c oxidase activity (µmol cytochrome c/min/mg mitochondrial protein)		
	Control	GalN	GalN + Lipid emulsion
0	2.33±0.39	–	–
24	–	$1.77\pm0.26^{+}$	1.45±0.26
72	–	$1.78\pm0.32^{+}$	1.82±0.23

$^{+}$ indicates significant difference ($p<0.05$) between the control and GalN group.

Discussion

In the present study, we examined the effect of G-galactosamine, a hepatotoxic agent, on the liver and the capability of specific nutritional supply to influence liver damage. We used triacylglycerols containing medium chain fatty acids (lipid emulsion Lipofundin MCT/LCT 10% / Miglyol^{812}) because these fatty acids may cross the mitochondrial membrane independently of the carnitine carrier system and are thus better available for tissue oxidation.

We verified that GalN administration induced liver injury as indicated by an increase of serum aminotransferase activities. We observed the decrease of total liver proteins evidently due to liver injury and also significant increase of the content of triacylglycerols (Tables 1 - 3). When comparing the effect of GalN alone with the effect of GalN and lipid emulsion applied for 24, 48, and 72 h, we found that lipid emulsion participates in

improving the conditions for liver reparation. The combination of GalN and lipid emulsion attenuated the decrease in the total liver protein content during the first 48 h compared with the GalN group. We cannot exactly explain why this protective effect of lipids was not evident at 72 h after GalN administration. It could be due to a negative effect of steatosis induced by lipid emulsion administration (Table 3). A similar finding was reported by Mangeney *et al.* (1985) in isolated rat hepatocytes. Their data indicated that GalN induces a decrease of triacylglycerol secretion and inhibits protein synthesis and secretion.

Lipid emulsion-treated animals have a higher total content and specific activity of cytochrome c oxidase 24, 48, and 72 h after GalN administration. In the group with GalN, we found a smaller increase of enzyme content and activity at 48-h and 72-h intervals when the regeneration process started. In the group with lipid emulsion, the increase in both content and activity of cytochrome c oxidase was already found at the 24-h interval. The decrease of total liver protein accompanying higher activity of cytochrome c oxidase suggests earlier reparation of respiratory chain enzymes compared with other cell proteins. Furthermore, cytochrome c oxidase indicates the capacity of the whole mitochondrial system of energy production. Our data suggest that mitochondrial biogenesis was activated after GalN administration because mitochondria isolated from liver of GalN and GalN plus lipid emulsion-treated groups have the same activity of cytochrome c oxidase (Table 5). A similar protective effect of the lipid emulsion on cytochrome c oxidase activity in liver homogenate was observed when liver injury was induced by thioacetamide (Červinková and Drahota 1998). In comparison with control rats the mitochondrial cytochrome c oxidase activity in GalN and GalN + lipid emulsion groups was lower (Table 5). We cannot specify from our experimental data whether this was due to the toxic effect of GalN or due to biogenesis of mitochondria with lower specific cytochrome c oxidase activity.

We may conclude from our experiments that lipid emulsion (Lipofundin MCT/LCT 10 % / Miglyol^{812}) significantly diminished the extent of liver injury induced by GalN and contributed to improvement of conditions for restoration of liver tissue and its function in the following regeneration process. Lipid supplementation may thus be an important factor supporting optimum metabolic conditions for the high rate of energy-dependent recovery processes repairing the liver tissue damaged by GalN intoxication.

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