

EFFECT OF D-GALACTOSAMINE ON OXIDATIVE STRESS AND APOPTOSIS IN THE LIVER OF RATS

SIÇANLARIN KARACİĞERLERİNDE D-GALAKTOZAMİNİN OKSİDATİF STRES VE APOPTOZ ÜZERİNE ETKİSİ

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ABSTRACT

Objective: This study was done to clarify the effect of high dose of D-Galactosamine (GalN) on oxidative stress and apoptosis in experimental rats.

Materials and methods: A single dose of 1 g/kg D-GalN dissolved in 0.9% NaCl was given to rats intraperitoneally. Malondialdehyde (MDA) and glutathione (GSH) levels in liver homogenates were measured spectrophotometrically. TNF- α concentration in liver was determined by enzyme linked immunosorbent assay (ELISA). DNA fragmentation which is considered to be diagnosis of apoptosis was observed by agarose gel electrophoresis.

Results: MDA level which is used as an indicator of lipid peroxidation, increased significantly, 24hr after intraperitoneal administration of D-GalN (1g/kg body weight) to rats. GSH, the endogenous antioxidant, was found decreased in D-GalN treated rats compared to control rats. Hepatic TNF- α level decreased significantly compared with that in control group. No DNA fragmentation was observed by gel electrophoresis.

Conclusion: These results suggest that high dose of D-GalN induces oxidative stress but not apoptosis in the liver of experimental rats.

Key words: D-Galactosamine, TNF- α , oxidative stress, apoptosis

ÖZET

Amaç: Çalışmamızda, yüksek doz D-galaktozamin (D-GalN) uygulamasının sıçanlarda oksidatif stres ve apoptoz üzerine etkisini inceledik.

Gereç ve yöntem: Sıçanlara intraperitoneal olarak, tek dozda 1g/kg D-GalN uygulandı. Sıçanların karaciğer homojenatlarında malondialdehit(MDA) ve glutatyon (GSH) düzeyleri spektrofotometrik yöntemle ölçüldü. Karaciğer dokusunda TNF- α düzeylerinin ölçülmesi için ELİSA yöntemi uygulandı. Apoptozun bir göstergesi olan DNA fragmentasyonu kalitatif olarak agoroz jel elektroforezi ile gözlemlendi.

Bulgular: Lipid peroksidasyonun bir göstergesi olan MDA düzeyleri 1g/kg başına D-GalN uygulamasından 24 saat sonra anlamlı olarak artış gösterirken GSH düzeylerinde azalma saptandı. Hepatik TNF- α düzeyleri ise kontrol gruba göre anlamlı olarak azaldı. DNA fragmentasyonu jel elektroforezi ile yapılan incelemede gözlemlenmedi.

Sonuç: Yüksek doz GalN uygulamasının oksidatif stresi indüklediği ama apoptoz üzerinde etkili olmadığı ileri sürülebilir.

Anahtar kelimeler: D-galaktozamin, TNF- α , oksidatif stres, apoptoz

INTRODUCTION

D-Galactosamine (D-GalN) has been shown to induce liver injury in rats. This injury closely resembles human viral hepatitis (5). In the hepatic injury induced by galactosamine, it has been suggested that reactions involving free radicals play a role based on an increase in malondial-

dehyde (MDA) levels, which had been used commonly as indicator of lipid peroxidation (6).

On the other hand, it is known that a variety of antioxidants including vitamin C, vitamin E and glutathione (GSH) scavenge free radicals to prevent oxidative damage in the cell. Some investigators reported that administ-

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ration of a hepatotoxic dose of D-GalN to rats induced a significant decrease in hepatic GSH levels (12,17).

Although D-GalN has been known as a hepatotoxin causing necrosis, it has also been reported to induce apoptosis in the liver of rats (26,27). As it is known, DNA fragmentation is an indicator of apoptotic cell death (19,28). The high incidence of apoptosis in D-GalN intoxication may be explained on the basis that toxicity of D-GalN is mediated through tumor necrosis factor (TNF- α), which causes apoptosis in liver cell (8,15, 29). TNF- α is synthesized in the Kupffer cells and may be responsible for induction of apoptotic and necrotic cell death of hepatocytes (14). While there is a lot of information on intracellular effects induced by TNF- α , its mechanism of cytotoxicity is still unknown. Several studies indicate that oxygen radicals may mediate some of the effects of TNF- α (1,3).

Taken together these observations, we aimed to study whether high dose of D-GalN could induce oxidative stress and/or apoptosis. In addition, we planned to investigate the role of TNF- α on oxidative stress and/or apoptosis in galactosamine treated rats.

MATERIALS and METHODS

Male Wistar rats weighing 180-200g were used in this study. Rats were obtained from Experimental Medical Research Institute. A single dose of 1g/kg D-GalN dissolved in 0.9 % NaCl was given to rats intraperitoneally. 24 h after D-GalN administration the rats were anaesthetized with ether and the blood was drawn by cardiac puncture. The livers were removed and washed with ice-cold 0.9 % NaCl.

Serum levels of total cholesterol, HDL-cholesterol, triglyceride, AST, ALT were measured by DPP modular system (Rocche Diagnostics). All sera were kept at -80 °C until the assays were performed.

MDA levels in liver homogenate were measured according to the method described by Okhawa et al (18). Hepatic GSH content was estimated by the method of Beutler et al (2). Protein levels were assayed spectrophotometrically as described by Lowry et al.(11).

TNF- α concentration in liver was determined by enzyme linked immunosorbent assay (ELISA) using commercial kit from R&D Systems Inc., Minneapolis, USA (Quantikine M rat TNF- α immunoassay). Portions of liver were homogenized in nine parts of phosphate buffered saline, pH=7.4, containing 2 mM of phenylmethyl-sulfonyl fluoride and 1 mg/ml each of antipain, leupeptin and pepstatin A. The homogenates were centrifuged for 15 min at 15000xg at 80°C and supernatants were removed and stored at -80°C until TNF- α assay performed (21).

DNA was isolated according to the method described by Miller et al.(13). DNA samples were subjected to electrophoresis on 1.8 % agarose gel and visualized by UV fluorescence after staining with ethidium bromide (10mg/ml) in order to detect qualitative damage to genomic DNA.

All results are expressed as means \pm SD. Statistical analysis of the data obtained in each group was performed using the student's t-test and p<0.05 was considered statistically significant.

Table 1. Biochemical parameters in the serum of control and GalN treated groups (means \pm SD)

Group	Control (n=10)	D-GalN (n=10)
Triglyceride (mg/dL)	107.70 \pm 35.00	198.70 \pm 26.50**
Total Cholesterol (mg/dL)	74.00 \pm 9.80	78.00 \pm 10.30
HDL-Cholesterol (mg/dL)	39.00 \pm 8.10	4.70 \pm 1.80***
ALT (U/L)	52.20 \pm 9.80	4206.50 \pm 598.90***
AST (U/L)	51.50 \pm 12.80	4097.70 \pm 270.40***

*p<0.05, **p<0.01, ***p<0.001

Table 2. MDA, GSH and TNF- α levels in the liver of the control and GalN-treated rats (means \pm SD)

Group	Control (n=10)	D-GalN (n=10)
GSH (nmol/mg protein)	18.61 \pm 4.10	10.75 \pm 2.4*
MDA (nmol/mg protein)	2.35 \pm 0.04	18.80 \pm 4.80***
TNF- α (ng/g)	89.00 \pm 25.30	48.90 \pm 24.70**

*p<0.05, ** p<0.01, ***p<0.001

RESULTS

Although serum total cholesterol levels were found unchanged, HDL-cholesterol levels decreased in D-GalN treated group. In addition, significant elevations occurred in serum triglyceride levels after administration of this hepatotoxin. After 24 hr, it was found significant and sharp elevations of serum AST and ALT activities in rats with D-GalN (1 g/kg) administration, indicating severe liver damage (p<0.001) (Table 1). GSH content in liver homogenate decreased approximately 55 % of control value 24h following D-GalN administration. Hepatic MDA levels were found to be increased in D-GalN treated rats in comparison to control rats and the difference was statistically significant (p<0.001). Hepatic TNF- α concentration decreased by D-GalN administration as compared with the controls (Table 2).

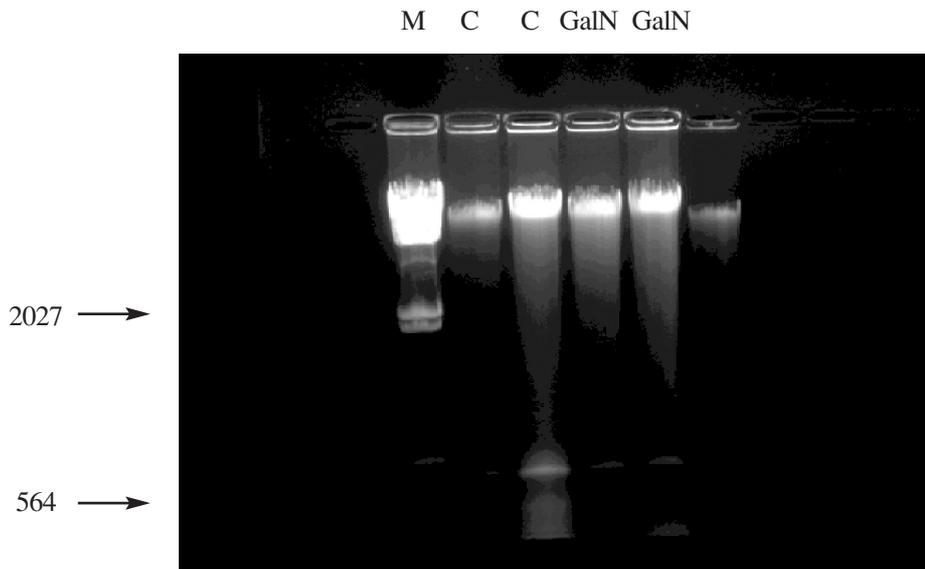


Figure 1. Qualitative evaluation of DNA fragmentation on the liver DNA of control and galactosamine treated rats. M, molecular size marker from Hind III digested I DNA; C, control; GalN, 24hr after 1g/kg GalN treatment.

In the experimental group, a ladder-like DNA fragmentation pattern (as multiples of 180-200 bp) could not be detected by agarose gel electrophoresis following 24 hr GalN administration (Figure 1).

DISCUSSION

Hepatotoxicity was estimated by measuring ALT and AST activities in serum and MDA levels in the liver tissues of D-GalN treated rats. It was found an elevation of serum ALT and AST activities after D-GalN administration. However, there are conflicting results about the relationship between lipid peroxidation and galactosamine toxicity. Some investigators have reported that lipid peroxidation may play a role in the liver damage due to galactosamine (6,26). Other investigators have failed to detect a relationship between lipid peroxidation and galactosamine toxicity (30). In this study, MDA levels as indicator of lipid peroxidation were found increased significantly in liver homogenates of D-GalN treated rats.

The mechanism of galactosamine hepatotoxicity is not clearly known. One possible mechanism is the inhibition of protein synthesis by D-GalN in hepatocytes. D-GalN causes a decrease in the level of uracil nucleotides followed by the inhibition of RNA synthesis and disturbance of the biosynthesis of glycoproteins leading to the deterioration of cellular membranes (4,10,16,22). These changes in the structure of cellular membranes may stimulate lipid peroxidation .

In addition, it is known that GSH plays a crucial role in detoxification and cellular defense and the loss in GSH content reflects oxidant defense mechanism (7,20). In this study, we also showed that GSH was depleted in the liver of rats after D-GalN administration as reported before (23). GSH content was expected to be consumed by enhanced radical reactions. On the other hand, it has been reported that some kinds of

cytotoxic chemicals such as dimethylnitrosamine, tioacetamide may induce both apoptosis and necrosis in vivo (9). However, there are a few reports of D-GalN induced apoptosis in the liver of rats (25,27). The appearance of DNA fragmentation is considered to be diagnosis of apoptotic cell death (19). While DNA fragmentations accumulate, they give the appearance of a ladder when separated by gel electrophoresis (24). In one study (27), it has been observed a ladder like DNA fragmentation at 48 hr in 1.5g/kg D-GalN treated group and at 24 hr in 3g/kg D-GalN treated group. However, in this study, no DNA fragmentation pattern was detected after 24 hr by the administration of a hepatotoxic dose of D-GalN. In the present study, undetectable ladder like DNA fragmentation could be explained by insufficient dose of D-GalN to induce apoptosis in experimental rats.

On the other hand, it has been suggested that TNF- α may also be responsible for induction of necrotic and apoptotic cell death of hepatocytes (3). In addition, it has been demonstrated that local concentrations of TNF- α in tissues rather than plasma levels would more likely reflect the biological response. This may be due to short plasma half lives of cytokines which have been measured in minutes (14). However, our results suggest that TNF- α production in rat liver was not stimulated by the administration of a hepatotoxic dose of D-GalN. This indicates that TNF- α may not be a key mediator in the induction of oxidative injury after D-GalN administration. The reduction in this cytokine may have been due to its leakage from damaged hepatic cells.

As a conclusion, our results indicate that a single dose of D-GalN (1 g/kg) may induce oxidative stress but not apoptosis in experimental rats. In addition, oxidative stress induced by D-GalN may not be related to the alterations in TNF- α levels.

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