Protective effects of vitamin D₃ against d-galactosamine-induced liver injury in rats

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ABSTRACT

In this study, we examined liver damage induced by d-galactosamine (d-GalN) and the protective effects of vitamin D₃ in relation to d-GalN toxicity.

Twenty Wistar albino rats were used in this study. The rats were divided into four groups. Group I rats were used as the control group. Group II rats were given a single intraperitoneal injection of d-GalN. Group III rats were given a single intraperitoneal injection of d-GalN, intramuscular vitamin D₃ for five days. Group IV rats were given intramuscular vitamin D₃ for five days. All of rats were euthanized by cervical decapitation on the fifth day of experiment. Upon completion of the experiment, a mid-sagittal incision was performed, and the livers of all rats were removed and fixed. The livers were processed to perform TUNEL technique and histochemical staining.

During the microscope examination, we observed inflammatory cell infiltration, sinusoidal dilatation, and apoptotic bodies due to d-GalN exposure. In addition, glycogen content of the group II hepatocytes was significantly decreased. Vitamin D₃ treatment provided better structural appearance of the livers in group III. TUNEL positive cells were extremely pervasive in the group II livers. The study found group III TUNEL positive cells at a reduced rate in relation to group II due to vitamin D₃ treatment.

This finding indicates that d-GalN causes inflammation in the liver. This inflammation triggers the apoptotic process gradually. Vitamin D₃ has potency to decrease the severity of d-GalN-caused structural liver damage.

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1. Introduction

Acute liver failure is a clinical syndrome characterized with high mortality (Gu et al., 2014) by the sudden loss of hepatic function in the absence of preexistent liver disease (Xie et al., 2014). Hepatic encephalopathy, coagulopathy, jaundice and hydropertoneum are life-threatening symptoms (Lee, 1994) of acute liver failure.

D-GalN/lipopolysaccharide (D-GalN/LPS) combination is often used as a hepatotoxin to perform massive hepatic inflammation, necrosis, and apoptosis. The combination has the capability to induce acute hepatic damage within a few hours after injection (Yan et al., 2013). The toxic effects of D-GalN is a result of deficiency of UDP-glucose, UDP-galactose, and lack of intracellular calcium homeostasis. These alterations affect cellular membranes and organelles and the synthesis of proteins and nucleic acids (Devaki et al., 2009). D-GalN also blocks the energy metabolism of hepatocytes (Mangeney-Andreani et al., 1982) and changes the phospholipid composition of membranes (Devaki et al., 2009). Hepatic injury induced D-GalN closely resembles human viral hepatitis (Catal and Bolkent, 2008). Devaki et al. (2009) demonstrated that administration of D-GalN decreased activities of the urea cycle enzymes, ornithine transcarbamylase, and arginase in rats.

Vitamin D₃ is an active metabolite of vitamin D, and has different biological functions. One of these biological properties is antioxidative action. Systemic-administered vitamin D₃ inhibited the elevated lipid peroxidation which was detected in vitamin D₃-deficient rats. Moreover, vitamin D₃ reduced oxidative stress by elevating the antioxidative defense system, including glutathione content, glutathione peroxidase and superoxide dismutase in astocytes and the liver (Lin et al., 2005). The liver plays a key role in metabolism of vitamin D. Epidermal and diet sourced vitamin D is hydroxylated in the liver into 25-hydroxyvitamin D [25(OH)D]. 25(OH)D, is the major circulating form of vitamin D, and is used to determine a patient’s vitamin D level. 25(OH)D is transported to the kidney via circulated blood and, subsequently undergoes a
second hydroxilation which then converts it into 1,25(OH)2D, the active form of vitamin D (Arteh et al., 2010).

In light of these observations, we planned this experimental study to induce hepatic injury and in order to improve alternative therapy and protection choices.

2. Materials and methods

2.1. Animals

Ethical approval for this study has been obtained from Firat University Faculty of Medicine Ethics Board, and all procedures conformed to the “Guide for the Care and Use of Laboratory Animals”. Twenty adult male Wistar albino rats (weighing 220–230 g) were used in this study. The rats were randomly divided into four groups. The rats were kept in Plexiglas cages (five animals per cage) and received standard food and water ad libitum in an air-conditioned room with automatically-regulated temperature (22 ± 1 °C) and light cycle (light: 07:00–19:00).

2.2. Experimental protocol

D-Galactosamine (Fluka, Italy) (D-GalN) dissolved in % 0.9 isotonic NaCl.

Group I (control) rats (n:5), received the normal diet and used as control.

Group II (D-GalN) rats (n:5), were given single intraperitoneal injection of D-GalN (500 mg/kg) and euthanized by cervical decapitation five days later.

Group III (D-GalN + vitamin D3) rats (n:5), were given single intraperitoneal injection of D-Galactosamine (D-GalN, 500 mg/kg) and intramuscular vitamin D3 for five days and euthanized by cervical decapitation on the fifth day.

Group IV (vitamin D3) rats (n:5), were given intramuscular vitamin D3 for five days and euthanized by cervical decapitation on the fifth day.

A midsagittal incision was performed at the end of the experiment, and the livers of all rats were removed and fixed in 10% formaldehyde for 24 h. After adequate fixation, the tissues were dehydrated through a graded ethanol series, cleared in xylol, and then embedded in paraffin wax. The tissue blocks were sectioned at a thickness of 5 μm to perform TUNEL technique and histochemical staining.

2.3. Histochemical staining

Tissue samples of all groups were stained by using standard Hematoxylin & Eosin (H&E), Masson’s Trichrome (MT) and Periodic acid Schiff (PAS) techniques.

2.4. TUNEL technique

Apoptotic cell deaths in liver were detected by TUNEL assay using ApopTag plus Peroxidase in situ Apoptosis Detection Kit (Chemicon, Catno: S7101,USA) based on the instructions of the producer. TUNEL method makes DNA breaks in the cells visible. Percentages of TUNEL-positive cells were quantified by counting 100 cells from ten random microscopic fields. Apoptotic index (the per-
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