Tissue and Cell 48 (2016) 81–88

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

The role of curcumin in streptozotocin-induced hepatic damage and the trans-differentiation of hepatic stellate cells

Hesham N. Mustafa*

Anatomy Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 19 January 2016
Accepted 7 February 2016
Available online 9 February 2016

Keywords:
Curcumin
Hepatic stellate cells
Streptozotocin
Lipid peroxidation
Trans-differentiation

ABSTRACT

Diabetic patients frequently suffer from non-alcoholic steatohepatitis. The current study aimed to investigate the role of curcumin and the response of hepatic stellate cells in streptozotocin (STZ)-induced hepatic damage. Sixty male rats were divided into three groups. The normal control injected with a citrate buffer vehicle and the diabetic control group which was injected intraperitoneally (IP) with a single-dose of streptozotocin (50 mg/kg body weight) and a diabetic group was treated with an oral dose of curcumin at 80 mg/kg body weight daily for 60 days. Curcumin effectively counteracts oxidative stress-mediated hepatic damage and improves biochemical parameters. Alpha-smooth muscle actin (α-SMA) was significantly reduced, and insulin antibodies showed strong positive immunoreactivity with curcumin administration. These results optimistically demonstrate the potential use of curcumin, which is attributed to its antiradical/antioxidant activities and its potential β-cell regenerative properties. Also, it has the capability to encourage the trans-differentiation of hepatic stellate cells into insulin-producing cells for a period of time. In addition, as it is an anti-fibrotic mediator that inhibits hepatic stellate cell activation and the transition to myofibroblast-like cells, this suggests the possibility of considering curcumin's novel therapeutic effects in reducing hepatic dysfunction in diabetic patients.

1. Introduction

Diabetic patients frequently suffer from a hepatic impairment recognized as non-alcoholic steatohepatitis which is associated with severe complications such as deposition of glycogen, steatosis, cirrhosis, fibrosis and occasionally hepatic cancer (Bugianesi et al., 2007).

Streptozotocin (STZ) is an N-nitroso-N-methylurea derivative of 2-deoxy-D-glucose which is a diabetogenic agent that damages the islet β-cells in the pancreas selectively to produce insulin-dependent diabetes mellitus (IDDM) (Yang et al., 2010).

Alpha-smooth muscle actin (α-SMA) is an indicator for the recognition of myofibroblast-like cells plus hepatic stellate cells (HSCs) which are also known as Ito cells (Clement et al., 2010). In diabetes, the glucose accessibility is increased and this leads to an accelerated formation of advanced glycation end products (AGEs). AGEs interact with the receptor for AGEs (RAGEs) and consequently increase oxidative stress and cellular growth. This leads to an increased proliferation of HSCs, which is noticed during hepatic fibrogenesis that is accompanied by the up-regulation of RAGEs.

Oxidative stress plays a crucial role in the chronic complications of the diabetic liver, where it is associated with an overproduction of oxygen free radicals and lipid peroxidation (Saravanan and Ponmurugan, 2011).

The use of herbal medicine in defending against STZ-induced liver damage looks promising. Curcumin has been gaining attention because of its health benefits, such as its anti-inflammatory, antioxidant, and immune modulatory effects. Curcumin is the chief curcuminoid of *Curcuma Longa*, which is a member of the Zingiberaceae family (Kumar et al., 2015).

The polyphenol curcumin improves diabetes-induced dysfunction by decreasing the level of glucose, inhibiting protein-kinase C, and lowering superoxide production (Rungseesantivanon et al., 2010). It reverses insulin resistance, hyperglycemia, and hyperlipidemia by inhibiting the pro-inflammatory transcription factors, signal transducers and stimulating anti-inflammatory signaling pathways (Tang and Chen, 2010).

This study aimed to investigate the probable influence of hepatic stellate cells in the protective capability of curcumin in STZ-induced liver damage. It was also intended to provide an important support in understanding the mechanism of curcumin treatment for hepatic dysfunction.

* Corresponding author at: King Abdulaziz University, Faculty of Medicine, Anatomy Department, Ground Floor Building No. 8, P.O. Box: 80205, Jeddah 21589, Saudi Arabia. Tel.: +966 566 764 762.
E-mail address: hesham977@hotmail.com

http://dx.doi.org/10.1016/j.tice.2016.02.003
0040-8166/

2. Materials and methods

2.1. Ethical approval

This study was conducted after receiving the approval of the Medical Research Ethics Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

2.2. Chemicals and Reagents

Streptozotocin (STZ) and curcumin were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) kits were purchased from Randox Laboratories Ltd. (Crumlin, County Antrim, UK). Serum albumin, total protein, and total bilirubin colorimetric kits were supplied by the Bio Diagnostic Company (Cairo, Egypt).

2.3. Animals

Sixty male adult albino Wistar rats, weighing 190 ± 20 g, that were used were obtained from the animal house. Animals were housed in a ($24\,^{\circ}C \pm 3\,^{\circ}C$) temperature-controlled room with 40–70% humidity and 12/12 h light/dark cycle. Rats were fed a standard diet and tap water *ad libitum* throughout the experiment. The experimental procedures were performed in accordance with the international guidelines for the care and the use of animals in a laboratory.

2.4. Induction of diabetes

Fasted rats (12 h) received a single intraperitoneal (IP) injection of freshly prepared STZ (50 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH 4.5). STZ-injected animals were given a 5% glucose solution for 24 h to overcome drug-induced hypoglycemia. On the third day after STZ injection, glucose levels were estimated by obtaining blood samples from the cut tip of the tail using Diagnostic Accu-Chek test strips (Roche Diagnostics, Mannheim, Germany). Blood glucose levels of 250 mg/dl or more were considered diabetic.

2.5. Experimental design

The animals were distributed into 3 groups (20/group). Group 1 (normal control) was injected IP with a citrate buffer vehicle. Group 2 (diabetic control) received a single IP injection of STZ (50 mg/kg body weight). Group 3 received a single IP injection of STZ (50 mg/kg body weight) and, on the third day after the STZ injection, curcumin was given orally with a dosage of 80 mg/kg body weight and continued daily for 60 days (Zhang et al., 2013). At the end of the experiment, blood samples were collected from the retro-orbital sinus in heparinized capillary tubes for serum analysis. Animals from all groups were sacrificed, and the livers were processed for histological studies.

2.6. Plasma glucose estimation

Plasma glucose was measured using an enzymatic colorimetric method with commercially available kits (Randox Laboratories, Ltd., Antrim, UK).

2.7. Plasma insulin estimation

Plasma insulin was determined using an insulin enzyme-linked immunosorbent assay (ELISA) kit (code no. AKRIN-010T; Shibayagi Co., Ltd., Gunma, Japan).

2.8. Serum parameters

ALT, AST, and ALP, which were increased following hepatocyte injury, were assessed according to the protocol detailed in the manuals of the diagnostic kits (Randox Laboratories Ltd., Crumlin, County Antrim, UK). Serum albumin, total protein, and total bilirubin were determined by spectrophotometer using the corresponding colorimetric kits supplied by Bio Diagnostic Company (Cairo, Egypt) (Lee et al., 2012).

2.9. Histological examination

Livers were washed with a phosphate buffer solution and then fixed in 10% neutral buffered formalin. Tissues were dehydrated through a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. Tissues were then cut into sections of 3–5 μm in thickness using a microtome and stained with hematoxylin and eosin (H&E) for histopathological evaluation and with periodic Acid-Schiff (PAS) for observation of glycogen. For each specimen, at least three to five slides were examined using an Olympus BX53 microscope equipped with a DP73 camera (Olympus, Tokyo, Japan).

2.10. Histopathological evaluation

The sections were analyzed for hydropic swelling, parenchymatous degeneration, microvesicular vacuole, macrovesicular vacuole, focal necrosis, inflammatory infiltrations, fibrosis, and sinusoids hyperemia. At the end of the analyses, the findings were presented in a table which showed the degree of degeneration (Guven et al., 2006). Score levels of 0, +1, +2, +3 were equivalent to no, mild, moderate, and severe, respectively. The scores represented values obtained from the tissue sections of six animals from each group with five fields/section (Mustafa et al., 2015).

2.11. Immunohistochemical examination

The standard peroxidase immunohistochemistry technique was applied to slides of paraffin-embedded tissue. Sections were de-waxed in xylene, rehydrated, and pretreated with 3% of hydrogen peroxide solution to block endogenous peroxidase activity. Microwave-assisted antigen retrieval was performed for 20 min. Slides were then incubated overnight at 4 °C with the primary antibody against α-SMA (a mouse monoclonal antibody [Dako, Carpinteria, California, USA] with a dilution of 1:50; cellular site was cytoplasmic) as a marker of activated HSCs with varying degrees of intensity in smooth muscles and myofibroblasts. They were similarly incubated with the primary antibody against insulin (a mouse monoclonal antibody [Dako, Carpinteria, California, USA] with a dilution of 1:100; cellular site was cytoplasmic). The sections were incubated with biotinylated IgG and then with streptavidin-peroxidase conjugate (Zymed Corp, San Francisco, CA, USA). Sections were then washed with phosphate-buffered saline (PBS) and incubated with 3, 3′-diaminobenzidine tetrachloride (DAB) substrate chromogen solution (1 drop of DAB chromogen/1 mL of substrate buffer) for 5 min to detect immunoreactivity. All sections were counter-stained with Mayer's hematoxylin. Negative control sections were prepared by omitting the primary antibody. Positive control standard laboratory slides were used for all stains to prove the success of the technique. All slides were examined under light microscopy, and the presence of labeled cells was documented. Absence of staining was recognized as a negative result (−), while the presence of brown staining was recognized as positive result (+) (Mustafa et al., 2015).