A NEW SEMI-QUANTITATIVE METHOD FOR DETERMINING LIVER DAMAGE AFTER CONCANAVALIN A ADMINISTRATION

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NOVI SEMI-KVANTITATIVNI METOD ZA ODREĐIVANJE STEPENA OŠTEĆENJA JETRE NAKON PRIMENE KONKAVALINA A

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ABSTRACT

Concanavalin A (Con A)-mediated hepatitis is a mouse model of liver injury that resembles autoimmune and viral hepatitis in humans. Because of the similarities in pathogenesis, clinical symptoms and histological characteristics, Con A-induced liver injury is a useful model for researching hepatocellular damage in murine and human hepatitis. Although many experiments have been conducted with the aim to investigate the mechanism of Con A-induced liver injury, a precise method for determining liver damage after Con A treatment has not been established yet.

To improve the study of hepatitis, we established a new semi-quantitative method to determine liver damage after Con A injection using histological examination. Briefly, liver sections were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm-thick sections. The sections were stained with haematoxylin-eosin and examined under light microscopy (100×) to evaluate liver damage. Necrosis of hepatocytes was characterised by standard morphologic criteria (loss of architecture, vacuolisation, karyolysis), and the extent of necrosis was semi-quantitatively determined using digital camera images and the "polyline" tool of Autodesk AutoCAD 2009 software. A detailed procedure for the semi-quantitative determination of liver injury after Con A injection is presented in this paper.

Using this method, the whole tissue section can be analysed. This is a significant advantage compared to similar, previously published methods that analyse randomly chosen microscopic fields. Another big advantage of this method is its simplicity and the availability of the Autodesk AutoCAD 2009 software for public use.

Key words: Concanavalin A, hepatitis, semi-quantitative method

SAŽETAK

Konkanavalinom A izazvan hepatitis predstavlja mišji model za proučavanje autoimunskog i virusnog hepatitisa ljudi. Konkanavalinom A izazvano oštećenje jetre je relevantan model za proučavanje mehanizama i stepena oštećenja jetre, usled sličnosti sa patogenetičkim i patološkim korakima.

Iako je urađeno mnogo eksperimenata sa ciljem da se ispitaju potencijalni mehanizmi Konkanavalinom A indukovanog oštećenja jetre, i dalje ne postoji tačno opisan i precizan metod za izračunavanje stepena oštećenja jetre u ovom eksperimentalnom modelu.

Mi smo postavili novi metod za semi-kvantitativno određivanje stepena oštećenja jetre nakon aplikovanja Konkanavalina A i taj metod predstavljamo u ovom radu.

Za histološko ispitivanje, jetre se fiksiraju u 10% formalinu, nakon čega se prave preparati 4-μm. Preparati se, nakon bojenje hematoksilin-eozinom, posmatraju svetlosnim mikroskopom (uvećanje 100×), fotografisu digitalnim aparatom i korišćenjem „polyline” opcije programa Autodesk AutoCAD 2009 označavaju se polja nekroze hepatočita koje karakteriše gubitak morfologije, vakuolizacija i karioliza. Postupak semi-kvantitativnog određivanja jetre je detaljno opisan u radu.

Korišćenjem ovog metoda, određuje se stepen nekroze u celoj jetri, što je glavna prednost u odnosu na do sađa opisane metode kojima se određivaju stepen nekroze hepatočita u nasumično odabranim poljima preparata. Takođe, prednost ovog metoda je što se koristi program Autodesk AutoCAD 2009 koji je jednostavan za rad i dostupan na tržištu.

Ključne reči: Konkanavalin A, hepatitis, semi-kvantitativni metod

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INTRODUCTION

Viral hepatitis is a serious health problem worldwide, as more than two billion people have been infected by hepatitis B virus, and 170 million people have been infected by hepatitis C virus [1]. An immune-mediated mechanism is responsible for the destruction of virus-infected hepatocytes in human viral hepatitis [2-3]. Due to the highly sophisticated morphological organisation of the liver and the integrity of metabolic pathways and their specific regulation in liver cells, the development of hepatic injury has not been easily studied in cellular systems for many years. A break-through in liver injury research field was made in 1992 when a mouse model of immune-mediated liver injury, which resembles autoimmune and viral hepatitis in humans, was established by intravenous injection of Concanavalin A (Con A) [4-5]. Con A is a mitogenic plant lectin that induces polyclonal T cell activation in vitro, and causes, after intravenous injection (in dose >1.5mg/kg), severe and acute liver injury in mice, resulting in clinical and histological symptoms of acute hepatitis within 24 h [4]. Con A strongly binds to hepatocytes’ plasma membranes, and hepatocytes can be sensitised or even killed by Con A if it is injected at high concentrations [6]. Although the precise mechanisms involved in the pathogenesis of Con A-induced liver injury are not fully understood, there is direct evidence that the activation of T cells, macrophages, neutrophils and natural killer T cells (NKT) is essential for Con A-induced hepatic injury [4, 6-9]. Liver injury is associated with massive CD4+ T, NKT and macrophage activation, followed by secretion of the following pro-inflammatory cytokines: tumour necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interferon-γ (IFN-γ), interleukin 2 (IL-2), interleukin 6 (IL-6) and granulocyte macrophage-colony stimulating factor (GM-CSF) [10-11]. Marked elevation of transaminases (alanine aminotransaminase (ALT) and aspartate aminotransferase (AST)) in mouse blood occurred after Con A injection, with the maximum levels found between 6 and 8 hours after administration [4-5, 12]. In addition, intravenous injection of Con A resulted in a remarkable disruption of mouse liver tissue, manifested by widespread areas of necrosis and inflammation within the liver lobules [4-5, 13].

Because of the similarities in pathogenesis, clinical symptoms and histological characteristics, Con A-induced liver injury is a readily available and useful animal model relevant for the research of hepatocellular damage in murine and human hepatitis [4-5].

MATERIALS AND METHODS

Animals

To determine Con A-induced liver injury, we used 6-8 weeks old male BALB/c mice. Mice received standard laboratory chow and water ad libitum and were kept in 12 h light/dark cycles.

Con A-induced liver injury

Con A was purchased from Sigma Chemical Co. (St. Louis, MO).

Mouse liver damage was induced by injection of Con A (12 mg/kg), dissolved in 250μL of saline, through the tail vein.

Determination of liver injury

Transaminase (ALT and AST) measurement and liver histology are the standard methods for determining liver damage. To measure the levels of AST and ALT, plasma samples or sera were collected from mice at indicated time points after Con A injection. AST and ALT levels were determined by a biochemical kit (Olympus medical kit) according to the manufacturer’s instruction.

Compared with transaminase measurement, which is a standardised procedure, there is no standardised and generally accepted semi-quantitative method for determining hepatocellular damage. Because of that, we present here, a new method for quantifying hepatic injury after intravenous administration of Con A using histological examination.

For histological examinations, livers were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm-thick sections. The sections were stained with haematoxylin-eosin and examined under light microscopy to evaluate liver damage.

Necrosis was examined using low-power (100×) light microscopy, and images were obtained using a digital camera. The area of necrosis was quantified using the Autodesk AutoCAD 2009 software application for design and drafting.

Liver tissue sections were photographed using (100×) light microscopy and a digital camera. Each photo of the tissue sample was imported into a newly-created Autodesk AutoCAD 2009.dwg file. Using the “polyline” tool, we drew “polyline” regions around the whole sample (marked with A) and around each of the necrotic areas in the photo (marked with B). We then determined the surface area of the drawn regions. First, we determined the surface area of the A region, and then we determined the surface areas of each of the B regions (one by one). The surface area of each drawn region is presented as a unitless number in the Autodesk AutoCAD program. After we examined all of the photos from a whole liver tissue section, we calculated the percentage of necrotic area using the formula:

\[ N = \frac{Bt \times 100}{At} \]

where:

- \( N \) is the percentage (%) of necrotic area in the whole tissue section,
- \( At \) is the sum of the sample surface areas in the whole tissue section (\( At = A1 + A2 + \ldots n \), where \( n \) is the number of photos),
- \( Bt \) is the sum of the necrotic surface areas in the whole tissue section (\( Bt = B1 + B2 + \ldots Bm \), where \( m \) is the number of marked necrotic fields).
RESULTS

Histological analysis of the liver sections of Con A-treated mice showed widespread areas of necrosis and inflammation within liver lobules and around central veins and portal tracts. Extensive lesions are characterised by massive hepatocytes, coagulative necrosis, and cytoplasmic swelling of most living hepatocytes (Figures 1 and 2). Nuclear chromatin condensation was found frequently, which indicates hepatocyte apoptosis. Moderate infiltration of lymphocytes and mononuclear cells in portal areas and around central veins was also observed.

The necrosis of hepatocytes was characterised by standard morphologic criteria (loss of architecture, vacuolisation, karyolysis, increased eosinophilia), and the extent of necrosis was semi-quantitatively estimated using the method we presented here (Figures 3 and 4).

Using this semi-quantitative method, hepatic necrosis was assessed in each section as the percentage of the liver parenchyma with necrotic damage.

Further, the extent of liver damage was scored with a grade from 0 to 4 as follows:

Grade 0: normal histology where necrotic area is 0%;
Grade 1: minor necrosis, necrotic area covered < 10% of the whole tissue section;
Grade 2: necrotic area covered 10–25% of the whole tissue section;
Grade 3: necrotic area covered 25–50% of the whole tissue section;
Grade 4: necrotic area covered >50% of the whole tissue section.

DISCUSSION

This method is a newly designed, reliable semi-quantitative way to determine liver damage in Con A-induced hepatitis.

Using this method, the whole tissue section is analysed. This is a significant advantage compared with similar, previously published methods that analysed randomly chosen microscopic fields [11, 14].

In addition, the big advantage of this method is its simplicity and the availability of Autodesk AutoCAD 2009 software for public use.

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REFERENCES