

A NEW SE MI-QUANTITATIVE METHOD FOR DETERMINING LIVER DAMAGE AFTER CONCAVALIN A ADMINISTRATION

Vladislav Volarevic¹, Marija Milovanovic¹, Nebojsa Arsenijevic¹, Miodrag Lukic^{1,2}

¹Department of Microbiology and Immunology, Medical Faculty, University of Kragujevac, Serbia

²Department of Microbiology & Immunology, Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates

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

Vladislav Volarević¹, Marija Milovanović¹, Nebojša Arsenijević¹, Miodrag Lukić^{1,2}

¹Katedra za Mikrobiologiju i Imunologiju, Medicinski fakultet Kragujevac, Univerzitet u Kragujevcu, Srbija

²Katedra za Mikrobiologiju i Imunologiju, Medicinski fakultet Al Ain, Univerzitet Ujedinjenih Arapskih Emirata, Ujedinjeni Arapski Emirati

Received / Primljen: 8. 2. 2010.

Accepted / Prihvaćen: 1. 4. 2010.

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 animal 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 u patogenezi, kliničkoj slici i patologiji sa hepatitisom ljudi.

Iako je urađeno mnogo eksperimenata sa ciljem da se ispita patogeneza Konkanavalinom A indukovano 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 debljine 4-μm. Preparati se, nakon bojenja hematoksilin-eozinom, posmatraju svetlosnim mikroskopom (uvećanje 100×), fotografiju digitalnim aparatom i korišćenjem „polyline“ opcije programa Autodesk AutoCAD 2009 označavaju se polja nekroze hepatocita 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 sada opisane metode kojima se određivao stepen nekroze hepatocita 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.

UDK ?????? / Ser J Exp Clin Res 2009; 11 (2): 45-48

