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Research Paper

The effects of ischemia-reperfusion injury and hepatic artery ischemia on CD14 expression in canine auto-transplantation livers☆

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Abstract

Objective: To study the effect of ischemia-reperfusion injury(IRI) and hepatic artery ischemia(HAI) on CD14 expression in canine auto-transplantation livers. **Methods**:Liver orthotopic auto-transplantation models were applied with 30 healthy male Xi' an canines which were randomly divided into a control group, simultaneous reperfusion(SR) group and HAI group. CD14 protein expression, Malonaldehyde (MDA) Contents in hepatic tissues and ALT values in serum were detected, and the pathological changes of hepatic tissues was investigated under the light microscopy. **Results**:The level of CD14 protein expression in SR and HAI group tended to be time-dependent and both higher than controls with statistical significance($P < 0.01$); The peak values of these two groups both occurred at 4 h, but the level in HAI group (11.94 ± 0.43) was evidently higher than that in SR group(3.04 ± 0.34). MDA contents in liver tissue, ALT values in serum and pathological changes showed the same changing tendency as CD14 expression. **Conclusion**:① Up-regulation of CD14 expression may be the receptor-mechanism of Kupffer cells(KCs) activation in liver transplantation. ② HAI can up-regulate CD14 expression after portal vein reperfusion, improve the activity of KCs further more, increase OFRs production and cooperate with portal reperfusion, and finally aggravate the grafts injury.

Key words: liver transplantation; hepatic artery ischemia(IRI); Kupffer cells(KCs); CD14

INTRODUCTION

There are a number of causes of postoperative complications in orthotopic liver transplantation; hepatic artery ischemia(HAI) is one of these causes, relating especially to biliary complications[1]. The basal of HAI is hepatic artery delayed anastomosis after portal vein reperfusion. Among the causes of donator ischemia-reperfusion injury, the role of CD14 is increasingly studied. Recent reports[2] have suggested that lipopolysaccharide receptor CD14 plays key roles in mechanism of endotoxin mediate KCs activation in many liver diseases, but the reports of its role in liver transplantation are rare. We investigated here the effects of ischemia-reperfusion injury(IRI) and HAI on CD14 expression in canine auto-transplantation livers.

MATERIALS AND METHODS

Animals and reagents

30 healthy male Xi' an canines with an average weight of 13.5kg and average month age of 12 monthes were provided by Experimental Animal Center of Xi' an Jiaotong University(license of Shaanxi animal center was No:08-005). They were randomly divided into three groups: control group, simultaneous reperfusion(SR) group and HAI group, with 10 canines in each group.

Rabbit antidogs CD14 polyclonal antibody was provided from Boster Biotechnology Limited Company (Wuhan, china). MDA detection kit was provided from Nanjing Jiancheng Bioengineering Institute(Nanjing, china).

Model of liver auto-transplantation

Models of liver auto-transplantation were built by Yiming Li method[3]; the control group was shame-operated; portal vein and hepatic artery were opened

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simultaneously in SR group after liver cold perfusion; portal vein has been opened for two hours in HAI group before the hepatic artery was opened. Liver and blood specimens were collected at 0h, 1h, 2h and 4h; in the control group, the time of shame operation completed was recorded as 0h; in the other two groups, 0h was recorded at the time of portal vein opened.

CD14 immunohistochemical stain

Immunohistochemical staining was applied using the ABC method. Primary antibody was replaced by phosphate buffering saline solution(PBS) as negative control. Observed under light microscope, the cells with buffy granules were defined as positive cells; the result was shown as positive cells quantity(PCQ) per high power field(HP, × 400); the average of 10 fields was as the final result.

Biochemistry and chemistry examination

MDA contents were detected by chemistry method and ALT values were detected by total auto biochemistry analysis instruments(Type 7180A, HITACHI).

Pathological changes

Hematoxylin-eosin(HE) staining was given to each slide of the three groups and pathological changes were observed under the light microscope.

Statistical analysis

The results were analyzed with SPSS 12.0 commonly. Statistics comparisons between different groups were made using single factor analysis of variance. The data was expressed as $\bar{x} \pm s$($n = 10$).

RESULTS

CD14 Protein expression

CD14 protein expressed weakly at 0 h point in all three groups and the difference was not statistically significant($P > 0.05$); The level of CD14 protein expression in SR and HAI group tended to be time-dependent and both higher than controls being statistically significant($P < 0.01$); The data showed that CD14 expression became stronger significantly in the post-reperfusion period than the pre-reperfusion period. The peak values of these two groups both occurred at 4h, but the levels in HAI group(11.94 ± 0.43) were evidently higher than that in SR group(3.04 ± 0.34), the difference was statistically significant.(Tab 1, Fig 1)

MDA and ALT

MDA contents in liver tissue and ALT values in serum showed the same changing tendency as CD14 expression; MDA contents and ALT values in SR and HAI group tended to be higher than controls in post-reperfusion period with statistical significance($P < 0.01$), the peak amplitude of HAI group(MDA 9.14 ± 0.35, ALT 145.80 ± 7.43) was evidently higher than that SR group(MDA 3.32 ± 0.20, ALT 52.60 ± 3.65). (Tab 2)

Hepatic pathological changes

Structures of liver tissue in the control group were natural with no obvious changes observed. In the SR group, pathological changes were slight and softly manifested. Severe pathological changes were found in HAI group at 2 h point, at that time hepatic artery had been closed for 2 hours; the appearances under microscope included structure disorders in hepatic lobules, hepatocyte swelling and lamellar necrosis(a great quantity of inflammatory cells infiltration).

DISCUSSION

Liver graft ischemia-reperfusion injury is an significant problem in clinical transplantation[4-6]. The canine model of liver auto-transplantation is a reasonable

Tab 1 CD14 positive cell quantity ($\bar{x} \pm s$, $n = 10$)

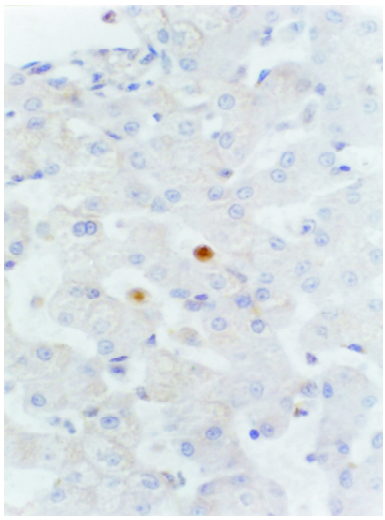
Group	CD14 positive cell quantity			
	0 h	1 h	2 h	4 h
Control	0.10 ± 0.07	0.12 ± 0.08	0.12 ± 0.08	0.14 ± 0.05
SR	0.14 ± 0.05	1.40 ± 0.12**	2.20 ± 0.20**	3.04 ± 0.34**
HAI	0.22 ± 0.13	3.18 ± 0.14**	7.04 ± 0.15**	11.94 ± 0.43**

Compared with the control group, **$P < 0.01$.

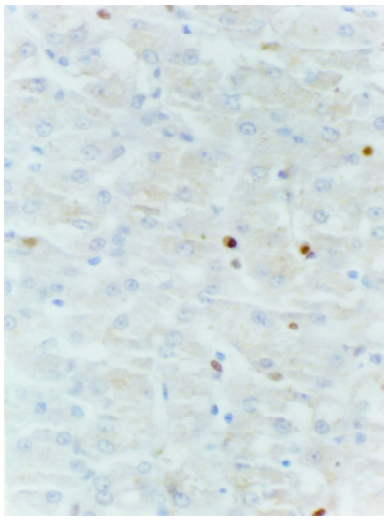
Tab 2 MDA contents and ALT levels ($\bar{x} \pm s$, $n = 10$)

Group		MDA contents and ALT levels			
		0 h	1 h	2 h	4 h
Control	MDA	0.93 ± 0.08	1.00 ± 0.15	1.03 ± 0.14	1.10 ± 0.14
	ALT	13.60 ± 1.82	14.20 ± 1.92	16.20 ± 2.56	18.60 ± 2.19
SR	MDA	1.19 ± 0.13	1.91 ± 0.17**	2.77 ± 0.11**	3.32 ± 0.20**
	ALT	23.20 ± 1.64	25.60 ± 2.07**	36.80 ± 2.77**	52.60 ± 3.65**
HAI	MDA	1.26 ± 0.07	4.64 ± 0.03**	6.76 ± 0.31**	9.14 ± 0.35**
	ALT	22.40 ± 1.14	62.60 ± 4.16**	107.20 ± 8.38**	145.80 ± 7.43**

Compared with the control group, **$P < 0.01$.



SR group(ABC method, × 400)



HAI group (ABC method, × 00)

Fig 1 CD14 positive expressed with buffy granules, PCQ in HAI group was higher than that in SR group as shown.

representation of that seen in human transplantation for two reasons. First, the cold perfusion time and HAI time can be exactly controlled. Second, the factors of immunological rejection are eliminated entirely. All animals survived these experiments.

We investigated CD14 protein expression in dog's auto-transplantation livers under ischemia-reperfusion injury; the results showed that CD14 protein expressed weakly at 0h point in all three groups, one to two positive cells could be seen in 10 high power fields. Comparing the SR group with controls, CD14 protein expression increased after reperfusion and became stronger as time prolonged; MDA contents in liver tissues also increased evidently; MDA was lipoperoxide, its appearance indicated the production of oxygen free radicals(OFRs); KCs was the major source of OFRs in the early stage of reperfusion[7]; Serum ALT values and hepatic pathological changes in SR group were also higher and more obvious than control group. All these indicated that following CD14 expression, KCs were activated and released OFRs, which caused liver injury. CD14, a 55-kDa glycoprotein and monocyte differentiation antigen that has been identified as the principal recognition molecule for lipopolysaccharide(LPS), prominent pathogen-associated molecular patterns, presented in the outer surface of all G$^-$ bacteria[8,9]. The up-regulation of CD14 expression has been proven to be associated with many types of liver injury[10], including alcoholic liver damage[11], burns[12], and endotoxemia[13]. Tsoulfas G[14] et al reported that liver's energy metabolism was impaired due to cold preservation; ATP anabolism was depressed and catabolism increased; tissue acid environment owing to anaerobic glycolysis and lactic acid accumulation, in addition to intracellular calcium influx caused KCs to be pre-activated. On the other hand, the portal vein had to be blocked until the vascular anastomosis was completed. That caused hyperpermeability of gut walls, showing therefore that endotoxin could translocate from the guts to portal system; after reperfusion of the graft, endotoxin could flow into liver and bind with CD14. Subsequently signals delivered from extracellular to intracellular, pre-activated KCs were activated releasing a large quantity of OFRs and cytokines which contribute to liver injury[15-17].

In these experiments we also demonstrated that HAI could aggravate liver injury, (comparing the HAI group with the SR group). CD14 protein expression, MDA contents and ALT levels of dogs in HAI group were much higher than those in SR group, and hepatic pathological changes were more severe too. Moser MA[18] et al advocated that hepatic arterial anastomosis was at the late of portal vein in orthotopic liver transplantation, causing HAI to happen. After portal vein reperfusion, the liver gradually turned warm, which provoked oxygen consumption. Hepatic tissues, especially those supplied by hepatic artery, were still in the situation of warm ischemia and hypoxia, that made those tissues more likely to be damaged[19-21]. Although our experiments were a pilot study, we could respectively presume that HAI injury mechanism could be described as following: HAI maybe synergy the injury of portal vein reperfusion; By prolonging the warm ischemia in graft, HAI contributed to intracellular ATP exhaustion and calcium overload, CD14-mediated pathway was further activated subsequently, more OFRs and cytokines were released, and finally liver injury was aggravated.

What does need to be pointed out is that this experiment is a part of series research. The main problem we focused on is HAI injury in orthotopic liver transplantation, and studies concerning the expression of CD14-

mediated pathway in HAI situation are rarely reported. The canine model of liver auto-transplantation is designed by the corresponding author of this article, especially for study of HAI injury. The HAI time was exactly controlled and the factors of immunological rejection are eliminated entirely in this model. A new strategy for HAI-applying hepatic artery bridge bypass to maintain hepatic artery bloodstream flow while arterial anastomosis occurs, will be reported in our following articles.

References

[1] Li yiming, Jenkins RL, Lewis J. The primary observation and approach to effects of ischemic time of hepatic artery on the results of liver transplantation. *J Xi'an Med Univ(in Chinese)* 1993; 5: 81-3.

[2] Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14: a receptor for complex of lipopolysaccharide and LPS binding protein. *Science* 1990; 249: 1431-3.

[3] Yiming Li, Zhaoying Qin, Yaan Kang, Wenbin Yang, Jun Li, Yingbo Cheng, **et al**. The model construction of simple canine liver autotransplantation and its significance. *Chinese Journal of Experimental Surgery(in Chinese)* 1995; 12: 113-4.

[4] Gu Wenyang, Zhang Feng, Wang Xuehao, Li Guoqiang, Lu Sen. The model construction of partial orthotopic liver transplantation with hepatic arterial reconstruction in rats and its significance. *Acta Universitatis Medicinalis Nanjing*(**in Chinese**) 2005; 25: 347-9.

[5] Strasberg SM. Preservation injury and donor selection: it all starts here. *Liver Transpl Surg* 1997; 3: S1.

[6] Zhang Chuanyong, Qian Xiaofeng, Wang Ping, Wang Ke, Wang Xuehao. Diagnosis and treatment of postoperative biliary complications in orthotopic liver transplantation. *Acta Universitatis Medicinalis Nanjing*(**in Chinese**) 2006; 26: 1056-7.

[7] Shibuya H, Ohkohchi N,Seya K,et al. Kupffer cells generate superoxide anions and modulate reperfusion injury in rat livers after cold preservation. *Hepatology* 1997; 25:356-60.

[8] Hadley JS, Wang JE, Foster SJ, Thiemermann C, Hinds CJ. Peptidoglycan of *Staphylococcus aureus* Upregulates Monocyte Expression of CD14, Toll-Like Receptor 2 (TLR2), and TLR4 in Human Blood: Possible Implications for Priming of Lipopolysaccharide Signaling. *Infect Immun* 2005; 73: 7613-9.

[9] R Arroyo-Espliguero, P Avanzas, S Jeffery, J C Kaski. CD14 and toll-like receptor 4: a link between infection and acute coronary events? *Heart* 2004; 90: 983-8.

[10] Patrizia Carotenuto, Debby van Riel, Andr Artsen, Sven Bruijns, Fons G Uytdehaag, Jon D Laman, **et al**. Treatment with Alpha Interferon Up-Regulates CD14 on Liver Macrophages and Its Soluble Form in Patients with Chronic Hepatitis B. *Antimicrob Agents Chemother* 2005; 49: 590-9.

[11] Dai LL, Gong JP, Zuo GQ, Wu CX, Shi YJ, Li XH, **et al**. Synthesis of endotoxin receptor CD14 protein in Kupffer cells and its role in alcohol-induced liver disease. *World J Gastroenterol* 2003; 9: 622-6.

[12] Cho K, Pham TN, Crivello SD, Jeong J, Green TL, Greenhalgh DG. Involvement of CD14 and toll-like receptor 4 in the acute phase response of serum amyloid A proteins and serum amyloid P component in the liver after burn injury. *Shock* 2004; 21: 144-50.

[13] ShutoY, Kataoka M, Higuchi Y, Matsuura K, Hijiya N, Yamamoto S. Roles of CD14 in LPS-induced liver injury and lethality in mice pretreated with Propionibacterium acnes. *Immunol Lett* 2004;94: 47-55.

[14] Tsoulfas G, Takahashi Y, Ganster RW, Yagnik G, Guo Z, Fung JJ, **et al**. Activation of the lipopolysaccharide signaling pathway in hepatic transplantation preservation injury. *Transplantation* 2002; 74:7-13.

[15] Yinghua Tian, Wolfram Jochum, Panco Georgiev, Wolfgang Moritz, Rolf Graf, Pierre-Alain Clavien. Kupffer cell-dependent TNF-α signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse. *Proc Natl Acad Sci U S A* 2006; 103: 4598-603.

[16] Varsha Thakur, Michele T Pritchard, Megan R McMullen, Laura E Nagy. Adiponectin normalizes LPS-stimulated TNF-α production by rat Kupffer cells after chronic ethanol feeding. *Am J Physiol Gastrointest Liver Physiol* 2006; 290: G998-1007.

[17] Yasunobu Miyake, Kenichi Asano, Hitomi Kaise, Miho Uemura, Manabu Nakayama, Masato Tanaka. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell ssociated antigens. *J Clin Invest* 2007; 117: 2268-78.

[18] Moser MA, Wall WJ. Management of biliary problems after liver transplantation. *Liver Transplan* 2001; 7: 46-52.

[19] Su GL, Goyert SM, Fan MH, Aminlari A, Gong KQ, Klein RD, **et al**. Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14. *Am J Physiol Gastrointest Liver Physiol* 2002; 283:G640-5.

[20] Regueira FM, Espi A, Nwose P, Diez-Caballero A, Baixauli J, Rotellar F, **et al**. Comparison between two warm ischemic models in experimental liver transplantation in pigs. *Transplant Proc* 2003; 35:1591-3.

[21] van As AB, Lotz Z, Tyler M, Kahn D. Reperfusion injury associated with portal venous and hepatic arterial perfusion in liver transplantation. *Transplantation* 2002; 74:158-63.