梨花醫大誌 : 第 19 卷 第 3 號 1996
Ewha Med J Vol. 19, No. 3, 1996

An Experimental Study in Mice for Inducing the Optimal Hepatic Ischemia Followed by Reperfusion

Kum-ja Choi

Department of Surgery, College of Medicine, Ewha Womans University

= 국 문 초 록 =

생쥐에서 임계적 간 허혈/재관류를 유도하기 위한 실험연구

이화여자대학교 의과대학 외과학교실
최 금 자

간허혈 및 재관류는 간이식술에서 뿐만아니라 복잡한 간절제술과 대형 간외상의 수술적 치료에 필연적인 과정이다. 따라서 허혈에 매우 민감하고 손상기전이 다인자성인 간의 허혈/재관류 손상에 대한 병태생리 연구는 다양한 동물을 대상으로 진행되고 있다. 그러나 생쥐는 그 크기가 매우 작아서 수술술기상 어려움이 있을 뿐만아니라 허혈 손상에도 더욱 민감하기 때문에 장기이식 면역연구에 많은 장점을 보유하고 있음에도 불구하고 간 허혈/재관류 손상에 대한 연구에 거의 이용되지 않았다.

본 연구에서는 생쥐를 대상으로 간의 임계적 허혈/재관류 손상의 실험모형을 만들고자 하였다. 상온에서 Isofulorane 흡입하에 외과용 현미경을 이용하여 문맥, 간동맥, 및 총수담관을 간문맥부에서 비외상성 미세동맥류 크립으로 함께 결찰하였을 때 결찰시간 5분, 10분, 15분, 20분, 25분 및 30분에 따른 각각의 생존율은 100%, 100%, 92%, 50%, 33% 및 0%이었다. 즉 생쥐에서 50% 사망율을 유도하기 위한 완전한 간허혈 시간은 본 실험과 같은 조건하에서 최소 20분으로 쥐에서 sodium pentobarbital 마취하의 30분보다 약 10분이 더 짧다. 이 결과는 향후 생쥐를 이용한 간 허혈/재관류 손상에 대한 병태생리 연구와 나아가 간이식 면역연구에 응용될 수 있을 것으로 사료된다.

KEY WORDS : Hepatic ischemia/reperfusion · Mouse · Model.

Introduction

Total interruption of the hepatic blood flow is often necessary during hepatic surgery. Complicated resections of liver and operation for major liver trauma may require hepatic blood in-flow control. Especially, hepatic transplantation is inevitably subjected to ischemia followed by reperfusion, and then normothermic ischemia is a major obstacle to successful liver transplantation[1].

The liver is highly susceptible to ischemia. The mechanisms of hepatic ischemia and following reperfusion injury are multifactorial, and a great deal of effort has gone into attempts to elucidate its pathophysiology in variable animals. However it is well-known in experimental pathology that complete ischemia of organs is often difficult to standardize for different reasons[2]. In particular, very little attention has been paid to the hepatic injury of mouse as-

sociated with ischemia and subsequent reperfusion since the mouse is intolerable to hepatic ischemia to venous congestion of the splanchnic and systemic systems. Moreover, the experimental surgery in the mouse is very difficult technically even under surgical microscope. There are, however, many advantages to using the mouse for immunologic research attended with transplantation

This experiment was designed to determine the optimal hepatic ischemia period of mouse under the normothermia in order to develop the standardized mouse model for the critical liver ischemia/reperfusion(I/R) injury attended with liver transplant biology.

Material and Methods

1. Experimental Protocol

All experiments were conducted with the prior approval of the IACUC(Institutional Animal Care and Use Commitee of Children's Hospital Research Foundation, Cincinnati, Ohio). Pathogen free adult male ICR mice(Harlan Teklad, Medision, WI) weighing 25-30g were used in this study after all mice were acclimated to new circumstances for 4-5 days. Mice were maintained on normal diet and tap water. The experimental animals were divided into the following six groups.

Group Ⅰ - 5 min clamp of portal triad
Group Ⅱ - 10 min clamp of portal triad
Group Ⅲ - 15 min clamp of portal triad
Group Ⅳ - 20 min clamp of portal triad
Group Ⅴ - 25 min clamp of portal triad
Group Ⅵ - 30 min clamp of portal triad

Anesthesia was induced and maintained by Isofulorane(1.5-2.0 vol%) inhalation during all surgical procedure.

2. Surgical procedure

To develop the hepatic ischemia and reperfusion the anesthesia-induced mouse was placed individually on the 35℃ warm pad of operating table. And then the mouse head was put into the anesthetic pipe with 1.5-2.0 vol% Isofulorane flow for maintaining anesthesia invariably during procedure, and the legs were fixed with plaster. After shaving and cleansing abdomen with 10% betadine solution, a 1.5 centimeters long midline abdominal incision was made, and the falciform ligament was divided, and then the portal vein, hepatic artery and common bile duct were freed from the surrounding structures and clamped together by placing atraumatic microaneurysm clip (Heifitz) during 5 min to 30 minutes of the desired period of ischemia under an operating microscope (Storz Urban, Model US-1) wth 10-12x magnification. Following declamping the clip immediately after the desired periods and observing the circulation being restored into liver, the abdominal incision was closed by running suture with 5-0 polyglycolic acid (Dexon) for musclar layer and 4-0 silk for skin. No intravenous fluid was given during surgery. But a small amount(0.2 ml) of normal saline was spreaded on top of the intraabdominal viscera to keep the organs moist.

For determination of survival rate the mice were allowed to recover from the anesthesia in the incubator with 33℃ air. And then the mice were caged individually and fed the commercial regular chow and tapwater.

3. Survival rates

Survival rate of each group was determined as counting the number of survivors over 48 hours after surgery. Data was expressed as the proportion of survivors to the experimented animals in each group. In this study, the number of the experimented animals did not include the mice died from bleeding(it is defined that 2 and more cotton sticks, Q-tips get wet with blood) or died before releasing the clamps.

Results

Normothermic ischemia was induced in 63 mice of which each 6 were used for Group Ⅰ, Ⅱ & Ⅵ, 12 for Group Ⅲ, 18 for Group Ⅳ, and 15 for Group Ⅴ respectively. The survival rates in each clamping period of protal triad are summarized in Table 1 &

Table 1. Summary of this experiment according to hepatic sichemic period

Group	Ischemia period(min)	No of animal*	No of Survival	Survival rate(%)	Death time
I	5	6	6	100	
II	10	6	6	100	
III	15	12	11	92	2hr after op
IV	20	18	9	50	2 ; not awaken
					3 ; 1hr after op
					4 ; less than 24hr after op
V	25	15	5	33	5 ; not awaken
					3 ; less than 1hr after op
					2 ; 1hr after op
VI	30	6	0	0	5 ; not awaken
					1 ; 90 min after op
Total	63	37			

*not include to mice died from bleeding(2 and more Q-tips get wet with blood) or died before releasing the clamps

Fig. 1. Within an ischemic period of 10 min, no death was observed during 48 hr after I/R inuslt of liver. Longer periods of sichemia, however, gave rise to decreasing rate of survival. The survival in group III animals clamped for 15 min was 92%. The survival for group IV animals clamped for 20 min was 50%. The survival for group V animals clamped for 25 min was 33%, and all group VI animals clamped for 30 min died within 2 hr after I/R inuslt.

Although the time of death after ischemic inuslt was variable, it occurred mostly within 2 hr after ischemia and subsequent reperfusion of liver. And twenty min may be suggested as the 50% lethal ischemic time in this experimental condition.

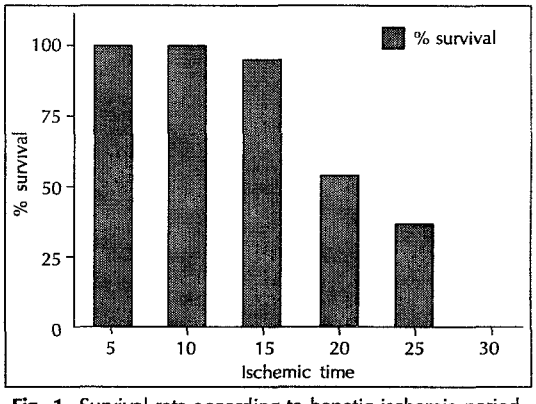

Fig. 1. Survival rate according to hepatic ischemia period.

Discussion

Portal triad occlusion results in adverse hemodyamic effects on one hand and liver ischemia on the other hand. The major cause of death with acute occlusion of the portal triad is the reduction of the circulating blood volume due to pooling of blood in the splanchnic bed[3]. However, the most critical tissue injury associated with ischemia-reperfusion is precipitated immediately after reperfsuion, and splanchnic congestion on portal occlusion and reperfusion results in disturbances of hepatic microcirculation and increased endotoxin levels in the portal vein with activation of hepatic macrophages[4]. The molecular mechanism associated with hepatic I/R injury are still unknown although the various mechanism for the damage occuring after hepatic I/R injury have been suggested[5].

Although the operation technique the author has used does not differ essentially from those used in literature[6,7], the author has described it in detail in the materials and methods section. The microsurgical clip under surgical microscopy in this experiment has been used to guarantee a complete closure of the vessels with minimal damage to the vessel wall, which should be considered important for a prompt restoration of the bloo flow after clamping for a certain period.

The anesthesia is also of importance. The author has chosen isofluorane which is known to have no direct harmful effect on the liver like nembutal or halothane[8]. In unpublished out experimental results, most of the mice died often even 5 min clamp of por-

tal triad with intramusclar injection of ketamin.

The duration of critical ischemia varies within the animals and the organs. Most laboratory animals have very few spontaneous portosystemic collateral veins. In dog, occlusion of the whole portal triad produces the same dramatic cardiovascular consequences as portal occlusion, and forty minutes of normothermic ischemia is close to a maximum tolerable duration for the liver[9]. While in rats, occlusion of the portal vein for 30 min and 50 min was lethal to about 50% and 100% of the animals respectively[3].

The mortality of mice in this experiment after 5 min and 10 min of clamping was nil or very low, whereas longer periods of ischemia entailed a linear decrease of survival rate(Fig. 1). This means that in our experimental set-up 50% mortality is around 20 min of clamping. And this result shows that most of mice with complete hepatic sichemia for less than 15 minutes can be alive under this experimental condition, and that the portal triad occlusion duration associated with 100% mortality was at least 30 minutes in mice, although the mouse is not only one-tenth the size of the rat, but is also much more intolerable to hepatic sichemia and venous congestion of the splanchnic and systemic systems.

With a solid evidence that the liver is highly susceptible to ischemia, a most important problem in liver transplantation is prevention of ischemic damage and an early diagnosis of primary graft failure based on ischemia. Hepatic transplantation is inevitably subjected to a variety of insults as a results of ischemia experienced during the organ harvest and reperfusion at the time of organ engraftment. The mechanism of hepatic I/R injury are mulifactorical and the role of cytokines, polymorphonuclear neutrophiles(PMN), cellular adhesion molecules, and oxygen free radicals have not been studied fully yet in I/R injury[10-13]. So this results that the clamping duration of portal triad less than 15 min have 92% survival rate and over 30 min of clamping, 100% of animals had mortality, implys that the ischemic period betwem these two points is the optimal range for studying the pathophysiological alterations associated with hepatic I/R injury in mice.

Now the author feels that the standardized operation technique applied in this experiment can contribute to the predictability of the results in the research of hepatic I/R injury in mice. This experimental model may be useful in the future to develop live transplantation immunology since numerous genetically defined inbred mouse strains-including congenic strains-are available. Moreover, there are many advantages to using the mouse for immunologic research associated with transplantation, because the mouse genome has been more thoroughly characterized than the rat or any other species of mammals, and the mouse H-2 system also bears a striking resemblance to human HLA system.

■ Acknowledgements

This research is supported by Children's Hospital Research Foundation in Cincinnati Ohio. The author thanks Moritz M. Ziegler MD and Gajra Aria for making the convenient arrangement in this work.

References

1) Ohhori I, Izumi R, Yabushita K, Watanabe T, Hashimoto T, Takamori M, et al : *Prevention of liver damage by using free radical scavengers and changes in plasma PG levels during liver ishcemia. Transplant Proc 1989 ; 21 : 1309-1311*
2) Trump BF, Berezeskey IK, Collan Y, Kahng MW, Mergner WJ : *Recent studies on the pathophysiology of ischemic cell injury. Beitr Path 1976 ; 158 : 363-388 cited from Frederiks WM, James J, Bosch KS, Schroeder MJR, Schuyt HC : A model for provoking ischemic necrosis in rat liver parenchyma and its quantitative analysis. Exp Path 1982 ; 22 : 245-252*
3) Meer C, Kley GA, Valkenburgh PW : *Studies on the cause of death after permanent and temporary occlusion of the portal vein in rats. Circ Shock 1976 ; 3 : 191-193*
4) Takei Y, Marzi I, Kauffman FC, Cowper KC, Lemasters JJ, Thurman RG : *Prevention of early graft failure by the calcium channel blocker nisoldipine involvement of Kupffer cells. Transplant Proc 1990 ; 22 : 2203*

5) Toledo-Pereyra LH, Suzuki S : *Neutrophils, cytokines, and adhesion molecules in hepatic ischemia and reperfusion injury. J Am College Surg 1994 ; 179 : 758-762*

6) Castillo M, Toledo-Pereyra LH, Prough D, Sharpiro E, Gordon D, Chaudhury S : *Effective timing of allopurinol administration in the ischemic liver. Transplantation 1989 ; 47 : 727-730*

7) Sark MF, Zetti GM, Hassanein TI, Hassan F, Nalesnik MA, Gavaler JS, et al : *FK 506 ameliorates the hepatic injury associated with ischemia and reperfusion in rats. Hepatology 1991 ; 13 : 947-951*

8) Wood M, Berman BL, Harbison RD, Hoyle P, Phytyon JM, Wood AJJ : *Halothane-induced hepatic necrosis in triiodotyronine-pretreated rats. Anesthesiol 1980 ; 52 : 470-476*

9) Raffucci FL : *The effects of temporary occlusion of the afferent hepatic circulation in dogs. Surgery 1953 ; 33 : 342-351*

10) Atalla SL, Toledo-Pereyra LH, MacKenzie GH, Cenderna JP : *Influence of oxygen-derinved free radical scavengers on ischemic livers. Transplantation 1985 ; 40 : 584-590*

11) Colletti LM, Remick DG, Burtch GD : *Role of tumor necrosis factor-a in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J Clin Invest 1990 ; 85 : 1936-1943*

12) Jaeschke H, Farhood A, Smith CW : *Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. FASEB J 1990 ; 4 : 3355-3359*

13) Zhou W, McCollum MO, Levine BA, Olson MS : *Inflammation and platelete-activating factor production during hepatic ischemia/reperfusion. Hepatology 1992 ; 16 : 1236-1240*