肝缺血後引發之細胞凋亡的病理生理變化及預防Ⅱ.致死基因的替補機轉

The pathophysiological mechanism of ischemia/reperfusion induced hepatocellular apoptosis

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一、中文摘要

本研究為探討肝臟缺血再灌流引發細胞凋亡的時間點及致死基因的表現情形和相互間的關係。實驗以大白鼠右大葉肝臟之肝動脈、門脈與膽管受阻斷不同時間及不同時段的血液灌流造成肝傷害的模式進行研究，結果發現肝臟遭受 1 小時的缺血並再灌流 6 小時後之細胞凋亡表現最強烈即肝細胞死亡數目最多，在致死基因方面 Bax、Bcl-xl、Fas 及 Fas-L 等在此時間點表現最強，而具保護作用的基因 Bcl-2 等亦在此時間點表現最強。此結果除了顯示細胞致死的原因之外，也明瞭細胞的死亡並非單一只是致死基因表現所造成的結果，同時間兼具保護作用的基因也大量表現以尋求細胞的存活，故細胞的死亡應該是兩類型基因表現不平衡所造成的結果，所以如何使保護基因充分發揮效能來抵抗致死基因作用以尋求肝細胞持續存活及具有原來的功能這將是未來研究的主題。

關鍵詞:細胞凋亡，缺血/再灌流

Abstract:

The apoptosis by ishcemia/reperfusion injury or liver transplantation induction would reduce liver function. The aims of this study are to elucidate the related gene expression and the possibly replacing or preventing mechanisms in the ischemia/reperfusion induced hepatocyte apoptosis.We used the Male Wistar rat as an experimental model to induce ischemically hepatic failure for different time course of ischemia and then we remove the clip to reperfuse the damage livers. Recently, a preliminary data in our laboratory has found that 1 hour ischemia followed by 6 hour reperfusion displays severe apoptotic expression in the damaged liver. This will provide more information for us to protect and improve the liver function .

Keywords: Apoptosis, ischemia/reperfusion

二、緣由與目的:

Liver transplantation is now an accepted therapy for end-stage liver disease. Organ preservation, transport, and subsequent transplantation all involve a period of global tissue ischemia. The pathophysioloic mechanism underlying tissue injury and cell death after ischemia remains a topic of controversy among scientists. The successful engraftment of organ transplants is dependent on multiple factors involving both major histocompatibility between donor and recipient as well as the extent of acute ischemic damage caused by reperfusion and reoxygenation of the organ. The mechanisms of acute damage following ischemia/reperfusion are thought to involve a complex interaction of immediate cellular damage caused by reactive oxygen species, and cellularresponses. Despite the vast number of experimental reports evaluating the effect of ischemia/reperfusion in the liver, the exact primary mechanism that leads to the ultimate decline in liver function and eventual organ failure remains

elusive. However, another form of cell death, apoptosis, or programmed cell death, is now widely recognized and has been implicated in various types of tissue and organ damage caused by ischemia, reperfusion, and transplantation. If the mechanism of ischemic injury can be explored, then prevention from such injuries will be possible. Apoptosis (programmed cell death) is a selective process of physiological cell deletion during embryogenesis and normal tissue turnover, but it has also been observed on some pathologic conditions. Some reports suggested that apoptosis plays an important role during ischemic renal injury especially short-term ischemia. Thus we can try some methods to prevent or reduce the liver injury during ischemia.

三、方法：

Animal and treatment：Male Wistar rat, 200~300 g, were treated as described below. The rat liver was ischemia for one hour by clamping portal vein, then reperfused for 0,1,2,4,6,10,15 and 24 hours individually.Quantitative RT-PCR：Total RNA preparation from rat liver tissues were performed as above by using trizol reagent (GIBCO/BRL). Quantitative RT-PCR to measure by using an Applied Biosystems PRISM 7700 Sequence Detector (Perkin-Elmer).

Preparation of total protein：Rat liver tissues were handily homogenized using a prechilled mortar and pestle in extraction buffer, which consisted of 10mM Tris-HCl (pH 7.6), 140 mM NaCl, 1mM PMSF, 1% NP-40, 0.5% Deoxycholicate, 2% β-mercaptoethanol, 10μg/ml Pepstain A and 10μg/ml Aprotinin. Then centrifuged at 10000×g for 20 min at 4℃. Western blotting：Proteins on the SDS-PAGE gels, each lane was loading 30μg total protein, were transferred to nitrocellulose filters. The immunoreactive bands were detected by the method of Towbin et al. (1979).

四、結果與討論

Datas have shown that the most significant frame of severe apoptosisformation after hepatic ischemia/reperfusion is 1 hour ischemia followed by 6 hour reperfusion.Biochemical method of DNA ladder displays the significant expression of apoptosis formation(Fig. 1 and 2). These data implicate some damaged signals occurring during 6 hour reperfusion. Immunocytochemical detection of bromodeoxyuridine (BrdU) incorporated into DNA has become a powerful tool for study of the cytokinetics of normal and neoplastic cells. Detection of incorporated BrdU is less laborious and time-consuming tha autoradiography of incorporated tritiated thymidine and obviates the need for radioactive DNA precursors. Use this method to address the replacing mechanism occurring in the ischemia/reperfusion hepatocytes and to determine the important time frame for this mechanism in the damaged liver. A preliminary data from our laboratory is shown in Fig. 3.

五、計劃成果自評:

本實驗根據定量PCR偵測特殊基因的表現將提供肝臟遭受缺血/再灌流產生

細胞凋亡之重要訊息與機轉。此結果有助於實際應用於臨床研究受損肝臟的肝功能與細胞凋亡之表現情形的結果則能以最直接的方式讓我們了解何種藥劑或方式處理肝缺血/再灌流傷害所引發之肝細胞凋亡與肝功能失調的實際療效;此種有意義的研究結果有助於減輕臨床上因肝臟移除後之移植肝之延遲作用或是經肝缺血後再灌流的傷害。

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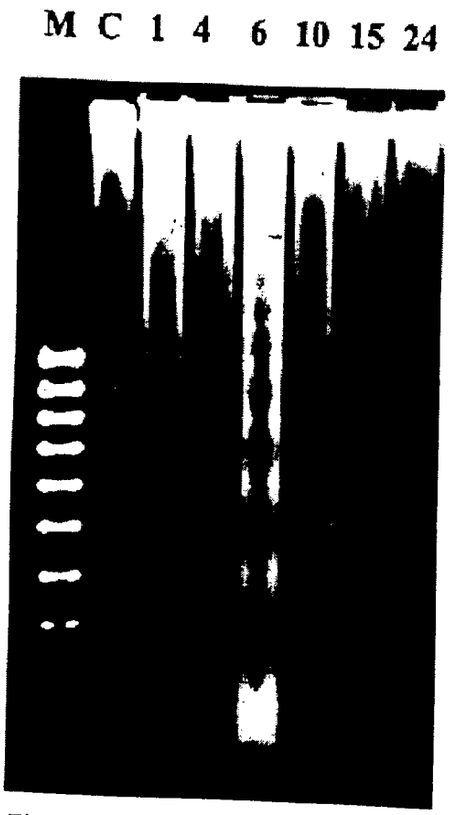

Fig 1. DNA ladder

M: DNA ladder marker C: control

1.4.6.10.15.24: reperfusion time(hours)

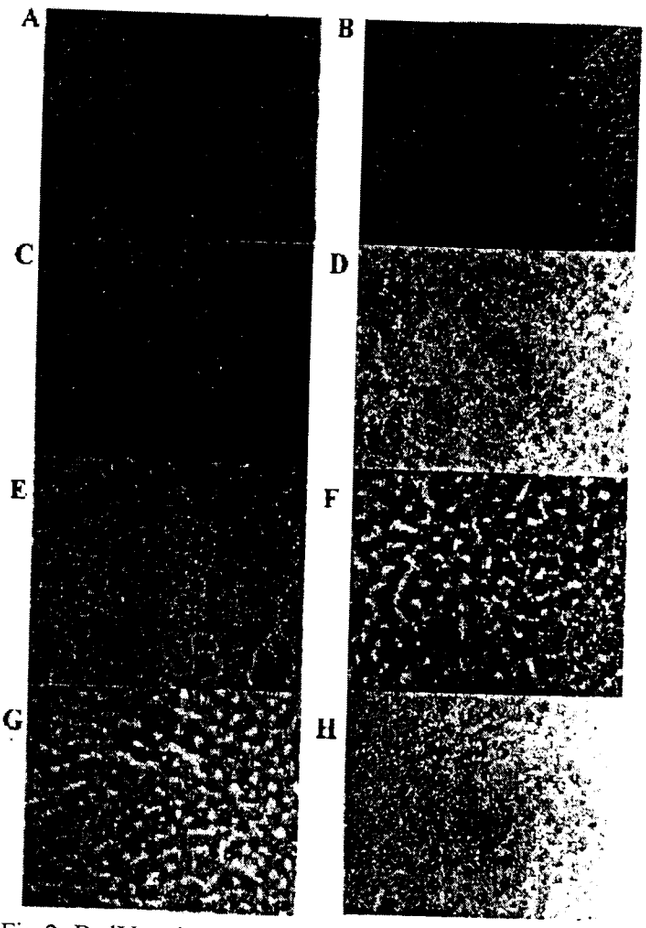

Fig 2. BrdU stained

A: postive control B: control

C~H: reperfusion times for 1.4.6.10.15 and 24 hours.

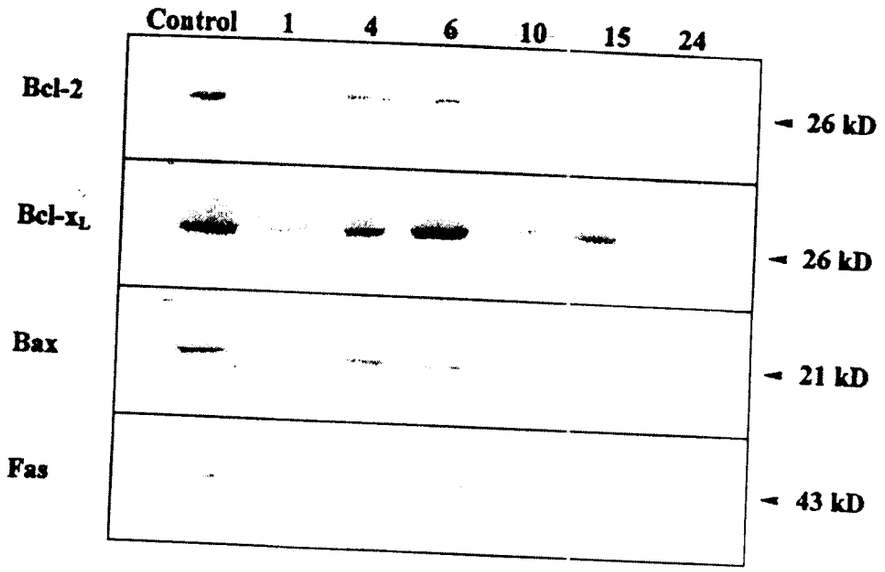

Fig 3. Western blot

1.4.6.10.15.24: reperfusion time(hours)