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Modulation of Mitochondrial Calcium Management Attenuates Hepatic Warm Ischemia-Reperfusion Injury

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Hepatic warm ischemia and reperfusion (IR) injury occurs in many clinical situations and has an important link to subsequent hepatic failure. The pathogenesis of this injury involves numerous pathways, including mitochondrial-associated apoptosis. We studied the effect of mitochondrial calcium uptake inhibition on hepatic IR injury using the specific mitochondrial calcium uptake inhibitor, ruthenium red (RR). Rats were subjected to 1 hour of 70% warm hepatic ischemia following RR pretreatment or vehicle injection. Sham-operated animals served as controls. Analysis was performed at 15 minutes, 1 hour, 3 hours, or 6 hours after reperfusion. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were determined. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining was performed to assess apoptosis, and hepatocellular necrosis was semiquantitated from hematoxylin and eosin–stained tissue sections. RR pretreatment significantly decreased both AST and ALT serum levels after 6 hours of reperfusion (AST: 1,556 ± 181 U/L vs. 597 ± 121 U/L, $P = 0.005$; ALT: 1,118 ± 187 U/L vs. 294 ± 39 U/L, $P = 0.005$). Apoptosis was observed within 15 minutes of reperfusion in vehicle-pretreated animals and peaked after 3 hours of reperfusion (98 ± 21 cells/high-power field [hpf]). Apoptosis was inhibited at all time points by RR pretreatment. Histologic evidence of necrosis was not observed prior to 3 hours of reperfusion (23% ± 4%), and maximal necrosis was observed after 6 hours of reperfusion (26% ± 1% percent area). RR pretreatment significantly decreased the necrotic percent area at both the 3-hour and the 6-hour time points (4.2% ± 2%; 3.7% ± 1%, respectively). Hepatic IR injury resulted in both apoptotic and necrotic cell death, which were attenuated by RR pretreatment. In conclusion, these observations implicate mitochondrial calcium uptake in the pathogenesis of hepatic IR injury. (*Liver Transpl 2005;11:663-668.*)

Hepatic warm ischemia and reperfusion (IR) injury occur in many clinical situations including transplantation, resection, trauma, shock, hemorrhage, and thermal injury. Evidence exists linking the periods of ischemia associated with these events to subsequent liver failure.[1] In addition, there is evidence that the duration of the ischemic periods during hepatic resection directly correlates with postoperative liver dysfunction.[2-5] The pathogenesis of ischemic hepatocellular injury is complex and involves numerous mediators.[6-9] Although many authors have demonstrated apoptosis following hepatic ischemia, the role of mitochondrial-associated apoptotic mechanisms in hepatic IR injury remains unclear. A better understanding of the cellular pathophysiology of IR and identification of methods to attenuate this injury has important clinical implications.

Within the last decade, a series of findings have shown that mitochondria play a central role in intracellular calcium dynamics.[10,11] Mitochondria can take up calcium rapidly via the ruthenium red (RR)-sensitive uniporter that spans the inner mitochondrial membrane. This uptake is driven by the negative potential difference across the mitochondrial membrane ($\Delta\Psi$), which is maintained by proton extrusion from the electron transport chain.[12] The mitochondrial matrix has a large calcium binding ratio, which allows mitochondria to accumulate extraordinary amounts of calcium, yet retain a negative membrane potential and avoid permeability transition pore formation. These properties allow mitochondria to function as buffers against cytosolic calcium overload; however, this ability to be an effective calcium buffer requires active electron transport.[12,13]

Hepatic ischemia increases cytosolic calcium concentrations as a result of both an increased release from the endoplasmic reticulum and other calcium stores as well as decreased calcium extrusion via membrane pumps.[14] As a result, rapid mitochondrial calcium

Abbreviations: IR, ischemia and reperfusion; $\Delta\Psi$, mitochondrial membrane potential; RR, ruthenium red; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling; hpf, high-power field; ATP, adenosine triphosphate.

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uptake occurs in an effort to buffer the cytosolic calcium incease.[15] However, as ischemia persists electron transport is inhibited, and ATP levels are depleted. The continued accumulation of calcium under these circumstances compromises $\Delta\Psi$. Ultimately if ischemia is not rectified, mitochondrial calcium overload occurs, resulting in complete collapse of $\Delta\Psi$, and mitochondrial-initiated cell death.[13,16,17]

RR is a glycoprotein stain that has a high affinity for the mitochondrial calcium uniporter and inhibits uniporter-dependent calcium uptake in a noncompetitive manner.[18] RR is a well-known and widely studied tool for modulation of mitochondrial calcium uptake. The uniporter is a gated ion channel and can be completely inhibited by micromolar concentrations of RR.[18-24] RR can prevent mitochondrial calcium uptake in isolated mitochondria, cell culture, tissue culture, and in vivo.[22,24-27] We have previously demonstrated that prevention of mitochondrial calcium uptake using RR prevents apoptosis in cultured HepG2 cells during the early rewarming period following an ischemic insult.[28] In this article, we investigate the role of pathologic mitochondrial calcium uptake on warm hepatic IR injury. We report here that prevention of mitochondrial calcium uptake using RR pretreatment attenuates reperfusion injury in a rat warm ischemia model.

Materials and Methods

Animals

All animal studies were performed in accordance with National Institutes of Health animal care procedures, and they were approved by and carried out under the guidelines of the Vanderbilt University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats weighing between 300 and 400 g were used for these experiments. Animals were housed in cages, kept on a 12:12 hour light/dark cycle, fed daily with rat chow, and given water ad libitum.

Model of Hepatic Ischemia

Following an overnight fast, animals were anesthetized using isoflurane (3%, 2 L O_2). Via a midline incision, the portal vein, hepatic artery, and bile duct were identified and isolated. Approximately 70% hepatic ischemia was created by using an atraumatic vessel clamp (S&T Microclamp B-3; Fine Science Tools, Foster City, CA) to occlude the portal vein and hepatic artery branches to the median and left hepatic lobes. This method is widely reported and avoids mesenteric congestion by allowing portal venous drainage via the right lobe and caudate lobe.

At 30 minutes prior to the initiation of hepatic ischemia, animals received RR (50 μg/kg) or vehicle (0.9% NaCl) intravenously. Ischemia was carried out for 1 hour. The total anesthesia time for the animals was 90 minutes. Sham-operated animals received laparotomy, mobilization of the liver, and underwent equivalent anesthesia.

Following vascular clamp removal, animals were allowed to reperfuse for periods of 15 minutes, 1 hour, 3 hours, or 6 hours. At the time of animal sacrifice, serum was collected, and hepatic tissue was preserved for histological analysis. A total of 40 animals were used for this experiment. A total of 10 animals were used for each time point: 4 hepatic ischemia, 4 hepatic ischemia with RR pretreatment, and 2 sham-operated animals.

Serum Transaminase Determinations

Serum collected from each animal at the time of euthanasia was analyzed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. These measurements were carried out by Mid South Vet Lab (Murfreesboro, TN).

Assessment of Apoptosis

In order to quantitate the rate of apoptosis in a sensitive manner, we performed terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay on paraffin embedded tissue sections from each animal. This assay was performed following the manufacturer's recommendations for a commercially available kit (ApopTag Plus Fluorescein *In Situ* Apoptosis Detection Kit; Chemicon International, Temecula, CA). Five randomly selected fields from each tissue section were examined at an original magnification of 200×. The number of positively staining cells per field was recorded. Only hepatocytes were included in this analysis. The average number of positively staining nuclei per field for each animal was used for analysis.

Quantitation of Hepatocellular Necrosis

Paraffin embedded tissue sections were stained with hematoxylin and eosin and examined at an original magnification of 200× (Axio-Plan 2; Zeiss, Hallbegmoos, Germany). Digital photomicrographs were made from 5 randomly selected fields of each tissue section (Axiocam HRc; Zeiss). Necrosis was defined as areas demonstrating one or more of the following characteristics: nuclear pyknosis, cytoplasmic hypereosinophilia, loss of distinct cellular borders, hemorrhage, and neutrophil infiltration. These traits correspond to grade 2 and 3 hepatic injury as defined by Camargo et al.[29] ImagePro software (ImagePro Plus v4.5; Media Cybernetics, Silver Spring, MD) was used to create specific overlays of any region of necrosis in each of the photomicrographs. Using these overlays, percent area necrosis was calculated for each photomicrograph. The average percent area necrosis for each animal was then used for analysis.

Statistical Analysis

Statistical significance between study groups was determined using a 2-tailed homoscedastic Student's *t* test. A *P* value of 0.05 or below was considered significant.

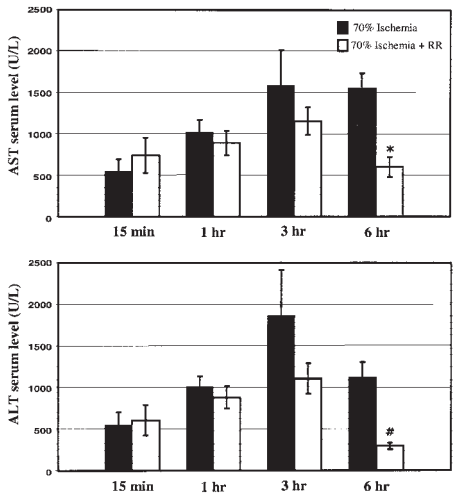

Figure 1. Serum AST and ALT levels were used as markers of reperfusion injury following hepatic ischemia. The maximal reduction of injury was observed after 6 hours of reperfusion. AST: 1,556 ± 181 U/L vs. 597 ± 121 U/L, $P = 0.005$; ALT: 1,118 ± 187 U/L vs. 294 ± 39 U/L, $P = 0.005$.

Results

RR Pretreatment Decreases Serum Transaminase Levels Following Ischemia

AST and ALT, established surrogate markers of hepatic IR injury, were measured at each reperfusion time point.[30] Peak AST and ALT levels following 1 hour of 70% hepatic ischemia occurred after 3 hours of reperfusion (Fig. 1). There was a trend for the animals pretreated with RR to have lower AST and ALT levels after 3 hours of reperfusion, and the maximal effect of RR pretreatment occurred following 6 hours of reperfusion. Inhibition of mitochondrial calcium uptake with RR resulted in a 2.5-fold reduction in AST levels and a 4-fold reduction in ALT levels at the 6-hour reperfusion time point. Pretreatment of sham-operated animals with RR did not alter AST or ALT levels when compared to sham operated animals receiving vehicle alone (data not shown).

Apoptotic Cellular Injury Begins Early in the Reperfusion Period

Recent data has shown that apoptotic mechanisms of hepatocyte death play an important role in hepatic injury following an ischemic insult.[31-33] Fragmentation of DNA strands is generally accepted as a specific marker of late apoptosis; therefore, we used TUNEL staining to evaluate the role that apoptotic cell injury played in our model of warm IR. Examples of these methods are demonstrated in Figure 2. The majority of apoptosis was noted in hepatocytes located in zone 3. The rate of apoptosis was quantified by counting the number of positively staining cells per high-power field [hpf], and 5 fields were averaged for each animal. Cellular apoptosis was observed as early as 15 minutes following reperfusion when compared to the apoptotic rates observed in the sham operated animals (6.3 ± 1.2 cells/hpf vs. 0.8 ± 0.2 cells/hpf; $P = 0.029$). The rate of TUNEL positivity increased after 1 hour and subsequently peaked at the 3-hour time point with 98 ± 21 cells/hpf. The rate of apoptosis decreased by 6 hours of reperfusion; however, the levels remain significantly higher than sham (10.6 ± 1.8 cells/hpf vs. 0.8 ± 0.2 cells/hpf; $P = 0.024$).

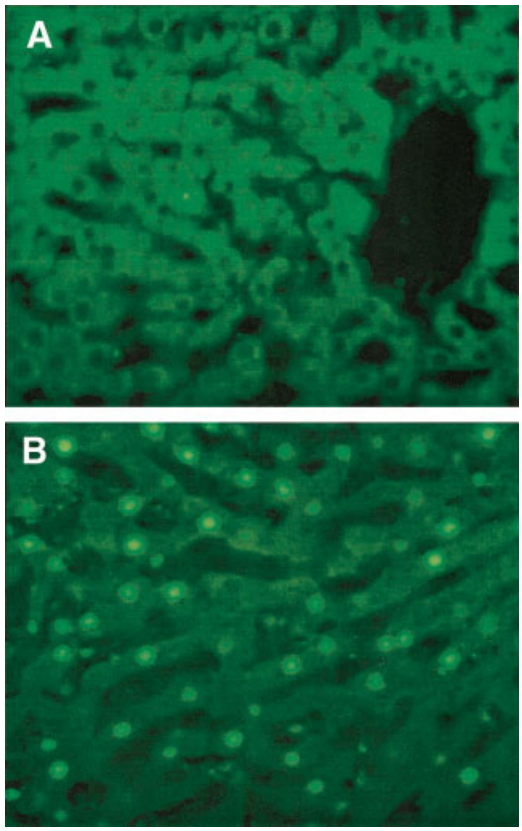

Figure 2. (A) Example of TUNEL staining of sham operated animal showing an absence of positive staining nuclei. (B) Example of a tissue section from an animal subjected to 1 hour of 70% hepatic ischemia and 3 hours of reperfusion showing multiple positively staining nuclei. Five high-power fields were examined at random for each animal, and apoptosis was recorded as the number of positively staining nuclei per high-power field.

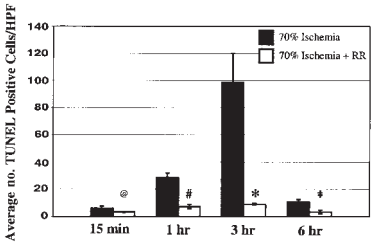

Figure 3. Summary of TUNEL data. Cellular apoptosis was first observed 15 minutes following reperfusion, and the rate of apoptosis peaked after 3 hours of reperfusion. RR significantly decreased the rate of apoptosis at all reperfusion time points measured in this study. @P = 0.029, #P = 0.003, *P = 0.014, ‡P = 0.024.

Modulation of Mitochondrial Calcium Uptake During IR Decreases the Rate of Hepatocellular Apoptosis

Our previous work has demonstrated that inhibition of mitochondrial calcium uptake using RR decreased the rate of apoptosis during the early rewarming period.[28] To determine whether the attenuation of IR injury seen in the RR pretreated animals was related to a decreased rate of apoptosis, TUNEL staining at each time point compared the RR pretreated animals to vehicle treated animals. Representative sections are illustrated in Figure 2. RR pretreated animals showed a decrease in apoptosis beginning at the 15-minute time point (6.3 ± 1.2 cells/hpf vs. 3.3 ± 0.3 cells/hpf; P = 0.011). As summarized in Figure 3, RR significantly decreased the rate of apoptosis at all reperfusion time points measured in this study. While RR pretreatment did not reduce the rate of apoptosis to sham levels at any time point, the peak rate of apoptosis in pretreated animals is 9.5 ± 0.5 cells/hpf at the 3-hour time point.

RR Pretreatment Attenuates the Percentage of Hepatic Necrosis Resulting From IR Injury

Despite the well-defined role of apoptotic cell injury in hepatic IR injury, debate remains as to whether the majority of the parenchymal injury noted during reperfusion results from apoptotic or necrotic cell death.[34,35] Many groups have reported histological evidence of both apoptosis and necrosis following hepatic IR.[34-36] We examined hematoxylin and eosin–stained tissue sections of liver taken at each time point in this study in order to determine the relationship of necrosis and apoptosis in our model and to evaluate any protection afforded by RR pretreatment.

Similar to the observation with apoptosis, necrotic cells were notably concentrated in zone 3. Necrosis was semiquantitated as described in the methods section and expressed as a percentage of the total tissue section. Histologic criteria for grade 2 or grade 3 injury[29] was not seen in tissue sections taken at 15 minutes or 1 hour following reperfusion. After 3 hours of reperfusion, the fields examined had an average of 22% ± 4% necrosis. This increased to 26% ± 8% by 6 hours of reperfusion. As summarized in Figure 4, RR pretreatment decreased the observed amount of hepatocellular necrosis to 5% ± 2% and 3% ± 1% after 3 hours and 6 hours of reperfusion (P = 0.006 and 0.026), respectively.

Discussion

This study investigated the role of mitochondrial calcium overload in warm hepatic IR injury. By using RR pretreatment to specifically inhibit uniporter dependent mitochondrial calcium uptake, we were able to globally attenuate hepatic reperfusion injury as measured by AST and ALT levels. Further analysis demonstrated a decrease in both hepatocellular necrosis and apoptosis in the pretreated rats. The decrease in apoptosis was immediately evident at the 15-minute time point and continued throughout the study period. While, RR pretreatment did not completely block the occurrence of apoptosis, it was able to reduce the peak rate of apoptosis approximately 10-fold at the 3-hour time point. Similarly, the peak appearance of necrosis in this study which occurred at the 6-hour time point was reduced approximately 10-fold by RR pretreatment.

Although debate over the roles of apoptosis versus necrosis in the etiology of hepatic IR injury remains,[34,35,37,38] many authors have demonstrated that apoptosis occurs following an ischemic injury.[31,36,39,40] In our study, we observed hepatocellular apoptosis beginning within 15 minutes of reperfusion.

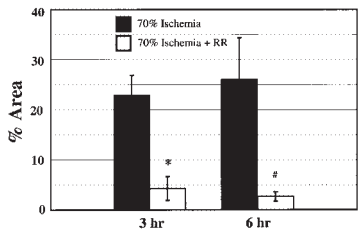

Figure 4. Necrosis was first seen after 3 hours of reperfusion, at which time the fields examined had an average of 22% ± 4% necrosis. The percent area necrosis increased to 26% ± 8% by 6 hours of reperfusion. As with apoptosis, RR pretreatment significantly decreased the observed amount of hepatocellular necrosis. *P = 0.006, #P = 0.026.

The rate of apoptosis subsequently peaked after 3 hours of reperfusion, which also corresponded with the first histological evidence of necrosis. Although apoptosis was still detected, the major histologic injury seen after 6 hours of reperfusion was hepatocellular necrosis. The coexistence of apoptosis and necrosis in hepatic IR injury has been noted by others, and some have proposed that the apoptotic mechanisms and necrotic mechanisms of cell death overlap.[37,38]

Mitochondria play a central role in apoptotic cell death.[41,42] In addition, it has become clear that intracellular calcium signaling controls a host of cellular functions including apoptosis.[12,13] Mitochondria are active participants in cellular calcium homeostasis, and they are known to act as buffers against cytosolic calcium overload provided adequate electron transport exists to preserve $\Delta\Psi$.[12,13] In fact, mitochondrial calcium uptake serves to stimulate mitochondrial ATP production as part of a poorly understood mechanism by which cytosolic adenosine diphosphate serves to drive mitochondrial ATP production.[12,43] During ischemic conditions, however, the mitochondria are unable to maintain electron transport, which results in complete collapse of $\Delta\Psi$ and mitochondrial-associated apoptotic cell death.[13,16,17] While several mechanisms of rewarming apoptosis have been demonstrated, the role of mitochondrial calcium overload in this injury remains unclear. We have previously shown that cold ischemic storage leads to mitochondrial calcium overload that can be blocked by RR.[21] In addition, storage of cells in an RR-containing solution during the period of cold ischemia prevents a caspase-independent apoptosis that occurs early in the rewarming period.[28]

The results of this study implicate mitochondrial calcium uptake and subsequent overload during the period of hepatic ischemia in the pathogenesis of hepatic IR injury. RR pretreatment led to an attenuation of early reperfusion apoptosis that remained throughout the study period. The observed decrease in hepatocellular necrosis was a surprising finding. Mitochondrial calcium overload has a clear role in some etiologies of apoptosis, but the role of mitochondrial calcium regulation in necrotic cell death is unknown. Necrotic cell death is generally believed to result from an acute metabolic disruption leading to rapid adenosine triphosphate (ATP) depletion, cellular swelling, and activation of degradative enzymes, which ends with the rupture of the cell membrane. Previous work by Qian et al.[44] demonstrated that hepatocytes experience a rapid onset of permeability transition pore formation following calcium ionophore treatment, which leads to profound ATP depletion and necrotic cell death. However, when ATP levels were maintained by various means during these experiments, permeability transition pore formation still occurred, but instead of rapid necrotic cell death, the cells underwent apoptosis.[44] From these experiments, Lemasters et al.[37,38] have proposed the necroapoptosis pathway of cell death in which a common death signal may culminate in either necrotic or apoptotic cell death.

This theory offers a plausible explanation for the observations in our study. In addition, recently RR has been demonstrated to preserve tissue ATP levels following a severe burn injury, and intracellular ATP has been demonstrated as a requirement for mitochondrial associated apoptosis.[27,45] In our study, apoptosis preceded necrosis; however, following 3 hours of reperfusion, the rate of necrosis increased as the rate of apoptosis decreased. In addition, RR decreased the rate of both apoptosis and necrosis by equivalent magnitudes, suggesting that a common cell death trigger was being affected. Given the known specificity of RR for the mitochondrial calcium uniporter, our data indicate that mitochondrial calcium overload can lead to both apoptotic and necrotic cell death.

References

1. Lemasters JJ, Thurman RG. The many facets of reperfusion injury. Gastroenterology 1995;108:1317-1320.
2. Delva E, Camus Y, Nordlinger B, Hannoun L, Parc R, Deriaz H, et al. Vascular occlusions for liver resections. Operative management and tolerance to hepatic ischemia: 142 cases. Ann Surg 1989;209:211-218.
3. Huguet C, Gavelli A, Chieco PA, Bona S, Harb J, Joseph JM, et al. Liver ischemia for hepatic resection: where is the limit? Surgery 1992;111:251-259.
4. Clavien PA, Harvey PR, Strasberg SM. Preservation and reperfusion injuries in liver allografts. An overview and synthesis of current studies. Transplantation 1992;53:957-978.
5. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, et al. Risk factors for primary dysfunction after liver transplantation—a multivariate analysis. Transplantation 1993;55:807-813.
6. Clavien PA, Emond J, Vauthey JN, Belghiti J, Chari RS, Strasberg SM. Protection of the liver during hepatic surgery. J Gastrointest Surg 2004;8:313-327.
7. Selzner N, Rudiger H, Graf R, Clavien PA. Protective strategies against ischemic injury of the liver. Gastroenterology 2003;125: 917-936.
8. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. Am J Surg 2001;181:160-166.
9. Jaeschke H. Mechanisms of reperfusion injury after warm ischemia of the liver. J Hepatobiliary Pancreat Surg 1998;5:402-408.
10. Pozzan T, Magalhaes P, Rizzuto R. The comeback of mitochondria to calcium signalling. Cell Calcium 2000;28:279-283.
11. Rizzuto R, Bernardi P, Pozzan T. Mitochondria as all-round players of the calcium game. J Physiol 2000;529(Pt 1):37-47.
12. Parekh AB. Mitochondrial regulation of intracellular Ca2+ sig-

naling: more than just simple Ca2+ buffers. News Physiol Sci 2003;18:252-256.
13. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 2003;4:552-565.
14. Farber JL. The role of calcium in lethal cell injury. Chem Res Toxicol 1990;3:503-508.
15. Amberger A, Weiss H, Haller T, Kock G, Hermann M, Widschwendter M, Margreiter R. A subpopulation of mitochondria prevents cytosolic calcium overload in endothelial cells after cold ischemia/reperfusion. Transplantation 2001;71:1821-1827.
16. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J 1999;341:233-249.
17. Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med 2000;6:513-519.
18. Ying WL, Emerson J, Clarke MJ, Sanadi DR. Inhibition of mitochondrial calcium ion transport by an oxo-bridged dinuclear ruthenium ammine complex. Biochemistry 1991;30:4949-4952.
19. Litsky ML, Pfeiffer DR. Regulation of the mitochondrial Ca2+ uniporter by external adenine nucleotides: the uniporter behaves like a gated channel which is regulated by nucleotides and divalent cations. Biochemistry 1997;36:7071-7080.
20. Belous A, Knox C, Nicoud IB, Pierce J, Anderson C, Pinson CW, Chari RS. Reversed activity of mitochondrial adenine nucleotide translocator in ischemia-reperfusion. Transplantation 2003;75:1717-1723.
21. Belous A, Knox C, Nicoud IB, Pierce J, Anderson C, Pinson CW, Chari RS. Altered ATP-dependent mitochondrial Ca2+ uptake in cold ischemia is attenuated by ruthenium red. J Surg Res 2003;111:284-289.
22. Luthra R, Olson MS. The inhibition of calcium uptake and release by rat liver mitochondria by ruthenium red. FEBS Lett 1977;81:142-146.
23. Rossi CS, Vasington FD, Carafoli E. The effect of ruthenium red on the uptake and release of Ca 2+ by mitochondria. Biochem Biophys Res Commun 1973;50:846-852.
24. Tapia R, Velasco I. Ruthenium red as a tool to study calcium channels, neuronal death and the function of neural pathways. Neurochem Int 1997;30:137-147.
25. Benzi RH, Lerch R. Dissociation between contractile function and oxidative metabolism in postischemic myocardium. Attenuation by ruthenium red administered during reperfusion. Circ Res 1992;71:567-576.
26. Miyamae M, Camacho SA, Weiner MW, Figueredo VM. Attenuation of postischemic reperfusion injury is related to prevention of [Ca2+]m overload in rat hearts. Am J Physiol 1996;271:H2145-H2153.
27. Liang WY, Tang LX, Yang ZC, Huang YS. Calcium induced the damage of myocardial mitochondrial respiratory function in the early stage after severe burns. Burns 2002;28:143-146.
28. Anderson CD, Belous A, Pierce J, Nicoud IB, Knox C, Wakata A, et al. Mitochondrial calcium uptake regulates cold preservation-induced Bax translocation and early reperfusion apoptosis. Am J Transplant 2004;4:352-362.
29. Camargo CA, Jr., Madden JF, Gao W, Selvan RS, Clavien PA. Interleukin-6 protects liver against warm ischemia/reperfusion injury and promotes hepatocyte proliferation in the rodent. Hepatology 1997;26:1513-1520.
30. Iu S, Harvey PR, Makowka L, Petrunka CN, Ilson RG, Strasberg SM. Markers of allograft viability in the rat. Relationship between transplantation viability and liver function in the isolated perfused liver. Transplantation 1987;44:562-569.
31. Kohli V, Selzner M, Madden JF, Bentley RC, Clavien PA. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. Transplantation 1999;67:1099-1105.
32. Cursio R, Gugenheim J, Ricci JE, Crenesse D, Rostagno P, Maulon L, et al. A caspase inhibitor fully protects rats against lethal normothermic liver ischemia by inhibition of liver apoptosis. FASEB J 1999;13:253-261.
33. Shimizu S, Eguchi Y, Kamiike W, Akao Y, Kosaka H, Hasegawa J, et al. Involvement of ICE family proteases in apoptosis induced by reoxygenation of hypoxic hepatocytes. Am J Physiol 1996;271:G949-G958.
34. Clavien PA, Rudiger HA, Selzner M. Mechanism of hepatocyte death after ischemia: apoptosis versus necrosis. Hepatology 2001;33:1555-1557.
35. Gujral JS, Bucci TJ, Farhood A, Jaeschke H. Mechanism of cell death during warm hepatic ischemia-reperfusion in rats: apoptosis or necrosis? Hepatology 2001;33:397-405.
36. Selzner M, Rudiger HA, Sindram D, Madden J, Clavien PA. Mechanisms of ischemic injury are different in the steatotic and normal rat liver. Hepatology 2000;32:1280-1288.
37. Lemasters JJ. V. Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. Am J Physiol 1999;276:G1-G6.
38. Lemasters JJ, Qian T, Bradham CA, Brenner DA, Cascio WE, Trost LC, et al. Mitochondrial dysfunction in the pathogenesis of necrotic and apoptotic cell death. J Bioenerg Biomembr 1999;31:305-319.
39. Rudiger HA, Clavien PA. Tumor necrosis factor alpha, but not Fas, mediates hepatocellular apoptosis in the murine ischemic liver. Gastroenterology 2002;122:202-210.
40. Selzner M, Rudiger HA, Selzner N, Thomas DW, Sindram D, Clavien PA. Transgenic mice overexpressing human Bcl-2 are resistant to hepatic ischemia and reperfusion. J Hepatol 2002;36:218-225.
41. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281:1309-1312.
42. Mayer B, Oberbauer R. Mitochondrial regulation of apoptosis. News Physiol Sci 2003;18:89-94.
43. Belous AE, Wakata A, Knox C, Nicoud IB, Pierce J, Anderson CD, Chari RS. Mitochondrial P2Y-like receptors link cytosolic adenosine nucleotides to mitochondrial calcium uptake. J Cell Biochem 2004;94:1062-1073.
44. Qian T, Herman B, Lemasters JJ. The mitochondrial permeability transition mediates both necrotic and apoptotic death of hepatocytes exposed to Br-A23187. Toxicol Appl Pharmacol 1999;154:117-125.
45. Tatsumi T, Shiraishi J, Keira N, Akashi K, Mano A, Yamanaka S, et al. Intracellular ATP is required for mitochondrial apoptotic pathways in isolated hypoxic rat cardiac myocytes. Cardiovasc Res 2003;59:428-440.