Transpl Int (1995) 8: 256–261

ORIGINAL ARTICLE

Masataka Negita
Itsuo Yokoyama
Shuji Hayashi
Takaaki Kobayashi
Motohiko Yasutomi
Hiroshi Takagi

Superoxide scavenging activity in experimental liver transplantation

Received: 16 September 1994
Received after revision: 4 January 1995
Accepted: 19 January 1995

M. Negita (✉) · I. Yokoyama · S. Hayashi
T. Kobayashi · M. Yasutomi · H. Takagi
Department of Surgery II,
Nagoya University School of Medicine,
65 Tsurumai-cho, Showa-ku, Nagoya,
466 Japan,
Fax: + 81 52 741 93 18

Abstract Superoxide dismutase (SOD) activity was evaluated by measuring superoxide scavenging capability with the aid of an electron spin resonance (ESR) spin trapping method in a swine orthotopic liver transplantation (OLT) model. The animals were divided into two groups, depending on the length of the survival periods: the short survival group ($n = 8$) survived less than 6 days and the long survival group ($n = 15$) 6 days or longer. SOD activity was significantly lower in the short survival group than in the long survival group after reperfusion ($P < 0.01$). During the period of cold preservation, a minimal change in SOD activity was noted, regardless of the length of preservation. Serum aspartate aminotransferase (AST) levels after reperfusion and serum lactate dehydrogenase (LDH) levels 1 h after reperfusion were significantly higher in the short survival group than in the long survival group ($P < 0.01$ and $P < 0.05$, respectively). The difference in polymorphonuclear leukocytes (PMN) was significantly greater in the short survival group at 1 h after reperfusion ($P < 0.01$). The authors conclude that superoxide scavenging activities in the graft reflect the magnitude of reperfusion injury, which can be a reliable parameter for the estimation of graft outcome.

Key words Liver transplantation, swine, SOD · Scavenger, liver transplantation, swine · SOD, liver transplantation, swine · Spin trapping, SOD, liver transplantation

Introduction

Although the causes of graft failure after liver transplantation in the early postoperative period are many, primary nonfunction of the graft (PNF) still accounts for a significant proportion of all graft failure. Recent reports have demonstrated that oxygen free radicals are among the most important factors contributing to PNF [4, 5], and it is also known that the liver is rich in seavengers of them [1]. However, the precise mechanisms underlying the response to liver injuries caused by reactive oxygen species remain unclear. There have been a few reports on the possible involvement of radical scavengers in the mechanism of cold preservation injury of the graft [22], but direct evidence of their role in the prevention of cold preservation injury is lacking. Superoxide is thought to be promptly activated in response to reactive oxygen species [7]. Therefore, blocking the cascade process in the liberation of free radicals at an early stage of the reaction in order to maximize the scavenging ability for superoxide would appear to be justified.

In this study, the authors investigate the relationship between superoxide dismutase (SOD), which accounts for a major proportion of the superoxide scavenging activity in the liver tissue, and survival with an experimental model of swine orthotopic liver transplantation. For the measurement of SOD activity, an electron spin resonance (ESR) spin trapping method, one of the most reliable assay methods, is used together with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO).

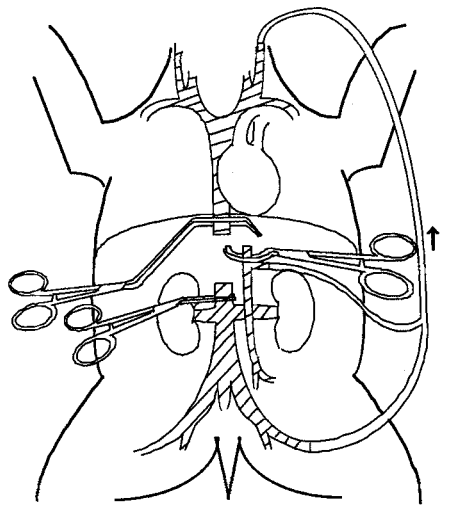

Fig. 1 Extracorporal circulation using Anthron tube during anhepatic phase

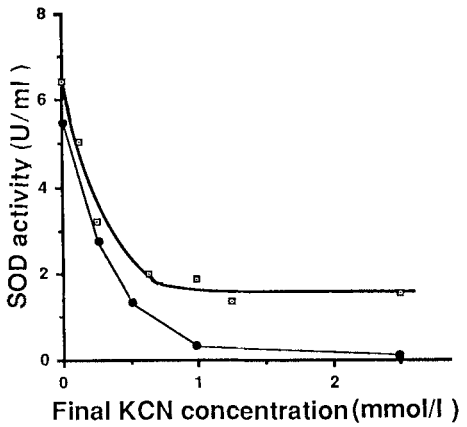

Fig. 2 Inactivation of SOD activity by KCN. A final KCN concentration greater than 0.625 mmol/l resulted in a plateau level with regard to total SOD activity (□) and almost complete inhibition of Cu, Zn-SOD activity (●) was obtained with a KCN concentration greater than 1 mmol/l. A final concentration of 1 mmol/l KCN was used to inactivate Cu, Zn-SOD

Materials and methods

Orthotopic liver transplantation (OLT)

For the animal experiments, the "Principles of laboratory animal care" (NIH Publication No. 86–23, revised 1985) were followed, as well as the regulations of the Animal Research Laboratory of Nagoya University School of Medicine. Landrace female pigs, weighing 11–13 kg, were used. All pigs received ketamine-HCl for induction and were maintained on inhalation anesthesia with halothane. For retrieval of the liver graft from the donor animal, a bolus injection of 500 U/kg body weight of heparin was administered intravenously. The splenic vein and the abdominal aorta were cannulated with 12 Fr polyethylene catheters for organ perfusion. After the thoracic aorta was clamped, infusion of cold normal saline was started rapidly via the abdominal aorta and 500 ml of 4 °C University of Wisconsin (UW) solution via the portal vein. The visceral organs were simultaneously cooled topically with iced slush. Hepatectomy was performed and the liver was immersed and stored in 4 °C UW solution. After 6 or 12 h of cold preservation, OLT was performed on the recipient animal. During the anhepatic phase (the period from termination of the native liver circulation until re-establishment of the transplanted graft circulation), passive extracorporeal circulation bypass from the portal and iliac veins to the cervical vein was established using an Anthron tube (Toray, Tokyo, Japan; Fig. 1). Postoperative maintenance immunosuppression consisted of cyclosporin A (3 mg/kg i. v., intraoperatively, and 10 mg/kg p. o., daily for 3 postoperative days) and methylprednisolone, 125 mg/body i. v., intraoperatively.

SOD measurements

Tissue samples were collected before and after preservation, immediately after reperfusion and 1 h after reperfusion. They were freeze-clamped, stored in liquid nitrogen, and frozen at –80 °C. Hypoxanthine, diethylenetriaminepenta-acetic acid (DETAPAC), which chelates several metal ions and, consequently, inhibits hydroxyl radical generation, and standard SOD from human erythrocytes were obtained from Sigma Chemical (St. Louis, Mo., US). Xanthine oxidase was purchased from Boehringer Mannheim (Mannheim, Germany) and DMPO (9.2 mol/l) and potassium cyanide (KCN) from Wako Pure Chemical Industries (Osaka, Japan); 15 µl of 9.2 mol/l DMPO was mixed with 50 µl of 2 mmol/l hypoxanthine and 35 µl of 5.5 mmol/l DETAPAC, as well as 50 µl of various concentrations of standard SOD or specimens homogenates of 20–30 mg of biopsied tissue in 5 ml of 0.1 mol/l phosphate-buffered saline (PBS) pH 7.8 in a small tube. Then, 50 µl of 0.4 U/ml xanthine oxidase was immediately added and the mixed solution was used for ESR study. Measurement of Mn-SOD activity was performed by inactivating Cu, Zn-SOD by 1 mmol/l in a final concentration of KCN using a modification of the original method described by Oberley et al. [14, 15]: KCN was mixed with the sample at a concentration of 4 mmol/l and the mixture was allowed to stand for about 30 min at room temperature. As a KCN concentration greater than 0.625 mmol/l would give a plateau level of SOD inhibition, the final concentration of KCN was adjusted to 1 mmol/l in order to inhibit Cu, Zn-SOD, as has been described by others [15] (Fig. 2). This method provides almost complete inhibition of Cu, Zn-SOD activity. Using this mixture of KCN as a sample, Mn-SOD activity was measured. Cu, Zn-SOD activity was calculated by subtracting Mn-SOD activity from total SOD activity. The protein concentration of the specimen was determined using the method of Lowry et al. [10]. SOD activity was expressed as U/mg protein. Xanthine oxidase, hypoxanthine, DETAPAC, and SOD were used by dissolving them in PBS. For the measurement of SOD activity, the ESR spin trapping method was used with a JEOL JES-RE1X spectrometer, as described by Miyagawa et al. [6, 13]. The ESR spectra recording was started 1 min after mixing at room temperature. The spectrometer was set at a microwave power of 8.0 mW, with a magnetic field of 335 ± 5 mT, sweep time of 1.5 min, field modulation width of 0.10 mT, and response time of 0.03 s.

Biochemical parameters

Serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured at the same time the biopsy specimens were taken, as well as on postoperative days 1, 3, and 7. In ten animals, tissue AST levels were also measured.

Table 1 Total SOD activity of the liver graft as measured by the ESR spin trapping method (*Pre* prepreservation, *Post* postpreservation, *Reperfusion* immediately after reperfusion, *1 h* 1 hour after reperfusion)

	Total SOD activity (U/mg protein)			
	Pre	Post	Reperfusion	1 h
Long survival[a] ($n = 15$)	34.4 ± 8.1	34.7 ± 12.8	30.7 ± 6.7[a]	33.7 ± 11.4[d]
Short survival[b] ($n = 8$)	28.1 ± 8.9[e]	27.5 ± 6.6[f]	15.4 ± 6.4[g]	15.9 ± 4.0[h]

* $P < 0.01$ for c vs g, d vs h, e vs h, and f vs h; ** $P < 0.05$ for e vs g and f vs g

[a] The group of animals with grafts preserved for 6 h
[b] The group of animals with grafts preserved for 12 h

Table 2 Activity of the SOD isozymes Mn-SOD and Cu, Zn-SOD of the liver graft as measured by the ESR spin trapping method (*Pre* prepreservation, *Post* postpreservation, *Reperfusion* immediately after reperfusion, *1 h* 1 hour after reperfusion)

	Mn-SOD activity (U/mg protein)				Cu, Zn-SOD activity (U/mg protein)			
	Pre	Post	Reperfusion	1 h	Pre	Post	Reperfusion	1 h
Long survival ($n = 6$)	12.3 ± 5.0	12.6 ± 2.2	13.2 ± 3.7	10.5 ± 2.8	19.4 ± 8.4	18.0 ± 9.0	15.9 ± 6.8[a]	16.9 ± 2.9[b]
Short survival ($n = 6$)	9.4 ± 2.0	11.1 ± 3.2	10.1 ± 3.0	8.9 ± 1.1	15.3 ± 4.3[c]	14.9 ± 7.5[d]	7.4 ± 5.4[e]	7.7 ± 3.7[f]

* $P < 0.01$ for b vs f and c vs f; ** $P < 0.05$ for a vs e and d vs f

Histology

Biopsy tissues were microscopically examined with hematoxylin-eosin (H & E) stain. The numbers of polymorphonuclear leukocytes (PMN) infiltrated in the liver tissue were counted and were expressed as the number of cells per ten high-power fields (HPF) under a magnification of × 400.

Statistics

All data were expressed as mean ± SD and statistical analysis was performed using Student's *t*-test with a one-way ANOVA. Statistical significance was defined as a *P* level below 0.05.

Results

Animals that had apparently died as a result of technical failure or from complications of general anesthesia were excluded from further analysis. Two groups of animals were then identified, depending on the length of their survival: the short survival group ($n = 8$) survived less than 6 days and the long survival group ($n = 5$) 6 days or longer. The length of cold preservation time correlated with survival; all of the animals with a preservation time of 6 h survived, whereas only one animal with a preservation time of 12 h survived longer than 6 days. For convenience sake, this latter animal was excluded from analysis. Thus, the group of animals with grafts preserved for 6 h constituted the long survival group and those with grafts preserved for 12 h the short survival group. Autopsies of the animals that died during the observation period showed that all of the grafts were diffusely ischemic with varying degrees of necrotic change. As there was no specific etiology, such as vascular or bile duct complications, the cause of death was determined to be PNF.

The changes in total SOD activity of the liver graft are shown in Table 1. SOD activity in the long survival group was maintained at a relatively stable level throughout the perioperative period, whereas that in the short survival group decreased immediately after and 1 h postreperfusion ($P < 0.01$). Both groups showed a minimal change in SOD activity during the period of cold preservation. Two types of SOD isozymes – Mn-SOD and Cu, Zn-SOD – were measured in six animals in each group. Mn-SOD activities in both of the groups were maintained within a relatively stable range throughout the study period. For Cu, Zn-SOD activity, although the change was minimal in the long survival group, it decreased significantly in the short survival group after reperfusion (Table 2).

Changes in the serum AST level are shown in Table 3 a. After reperfusion, it increased in both groups. However, in the short survival group, AST was significantly higher than in the long survival group ($P < 0.01$). Changes in the serum LDH level are shown in Table 3 b. Significantly higher LDH was noted in the short survival group at 1 h after reperfusion ($P < 0.05$).

The ratio of the activity of SOD to AST in the tissue of the liver graft was determined in five animals in each group. The ratio of SOD to AST also significantly decreased in the short survival group at 1 h after reperfusion ($P < 0.05$; Table 4). For SOD isozymes, the ratio of Mn-SOD to AST was maintained at a relatively stable level throughout the study period. On the other hand, the ratio of Cu, Zn-SOD to AST at 1 h after reperfusion was significantly lower in the short survival group than in the long survival group ($P < 0.05$; Table 5).

Table 3a Changes in serum AST level (*Pre* prepreservation, *Reperfusion* immediately after reperfusion, *1 h* 1 hour after reperfusion

	Serum AST (U/l)		
	Pre	Reperfusion	1 h
Long survival ($n = 15$)	22 ± 6^a	129 ± 77^b	191 ± 119^c
Short survival ($n = 8$)	19 ± 8^d	261 ± 90^e	375 ± 75^f

* $P < 0.01$ for a vs b, a vs c, and b vs c in the long survival group, for d vs e, d vs f, and e vs f in the short survival group, and for b vs e and c vs f between the two groups

Table 3b Changes in serum LDH level

	Serum LDH (U/l)		
	Pre	Reperfusion	1 h
Long survival ($n = 15$)	780 ± 144^a	936 ± 255^b	1072 ± 350^c
Short survival ($n = 8$)	704 ± 164^d	1225 ± 441^e	1414 ± 377^f

* $P < 0.01$ for a vs c, d vs e, and d vs f; ** $P < 0.05$ for c vs f, a vs b, b vs c, and e vs f

Table 4 Total SOD to AST ratio in the tissue (*Pre* prepreservation, *Post* postpreservation, *Reperfusion* immediately after reperfusion, *1 h* 1 hour after reperfusion

	Total SOD to AST (SOD unit/AST unit)			
	Pre	Post	Reperfusion 1 h	
Long survival ($n = 5$)	24.7 ± 6.2	28.9 ± 6.3	25.9 ± 8.2	28.0 ± 5.8^a
Short survival ($n = 5$)	26.7 ± 5.7	28.5 ± 4.5^b	22.7 ± 6.6	21.7 ± 1.3^c

* $P < 0.05$ for a vs c and b vs c

The number of PMN accumulated in the tissue in relation to survival is shown in Table 6. Although the difference in PMN infiltration was not significant between the two groups after preservation or immediately after reperfusion, it was significantly greater in the short survival group 1 h after reperfusion ($P < 0.01$; Table 6).

Discussion

Ischemia-reperfusion injury remains an important clinical consideration in liver transplantation. Severe ischemic damage leads to PNF of the graft, which accounts for approximately 10% of early graft loss. This, in turn, makes retransplantation necessary; otherwise, the patient will die [3]. Although ischemic injury can occur both prior to and during the preservation period, reperfusion injury appears to be a major factor contributing to the development of PNF. Increasing evidence has shown that oxygen free radicals, which are generated after reperfusion, play a central role in the pathogenesis of ischemia-reperfusion injury [5, 7, 22].

Though the amount of free radical production is considered of prime importance in determining the extent of tissue damage, the capacity to counteract free radicals is equally important and probably a more significant factor in preventing reperfusion injury. Of the various oxygen free radical scavengers, SOD is thought to be one of the most effective in counteracting active free radicals. Many studies on reactive oxygen species including experimental liver transplantation using small animals have reported that the liver is rich in SOD [5, 12]. However, the results obtained from these experiments are not uniformly applicable to clinical situations. One of the reasons is the scarcity of data on SOD in experimental liver transplantation using larger animals [16]. Indeed, it has been shown that the response to oxygen radicals in small animals, such as the rat, is different than that in larger animals [19]. Another reason involves a technical problem in the detection of SOD activity. The method we used in this study for the evaluation of SOD activity was ESR spin trapping, which was technically simple compared to conventional methods [13]. Moreover, this is the first report on SOD activity in experimental liver transplantation using large animals with the aid of the ESR spin trapping method. Although there are other antioxidants that can interact with superoxide anion, SOD is the major scavenger of superoxide radicals in liver tissue. Minor antioxidants include ceruloplasmin and glutathione. However, their contribution to antioxidant capacity in the liver is quite small compared to SOD. Therefore, the values of scavenging activity measured by ESR closely reflect real SOD activity. This may also be applicable to other conventional assays, such as the cytochrome c method.

The results of the present study have clearly demonstrated that SOD activity in the liver after cold preservation-reperfusion is associated with graft survival and preservation time. The fractional study of Cu, Zn-SOD and Mn-SOD, which are two isozymes of SOD detected in mammals, shows that the former isozyme is a main fraction that reflects the changes in total SOD activity after reperfusion of the liver graft. Since the Cu, Zn-SOD fraction is mostly located in the cellular cytoplasm, in contrast to Mn-SOD, a major fraction in the mitochondria of the cells, the significant reduction in SOD activity in grafts preserved for a longer period of time was most likely secondary to the change in cytoplasmic SOD [11]. This suggests that SOD in the cytoplasm of the hepatocytes is the prime source of SOD, which becomes readily available to counteract the oxygen free radicals generated upon reperfusion of the grafts.

Based on the finding that the change in the SOD value occurred immediately after reperfusion of the graft

Table 5 SOD isozymes to AST ratio in the tissue of the liver graft (*Pre* prepreservation, *Post* postpreservation, *Reperfusion* immediately after reperfusion, *1 h* 1 hour after reperfusion)

	Mn-SOD to AST (SOD unit / AST unit)				Cu, Zn-SOD to AST (SOD unit / AST unit)			
	Pre	Post	Reperfusion	1 h	Pre	Post	Reperfusion	1 h
Long survival ($n = 5$)	7.6 ± 2.9	9.5 ± 4.7	10.4 ± 5.5	8.7 ± 1.5	17.1 ± 6.0[b]	19.4 ± 2.0[c]	15.5 ± 6.1	19.2 ± 5.0[d]
Short survival ($n = 5$)	8.9 ± 1.2	10.0 ± 2.8	10.3 ± 1.6	10.5 ± 2.1	17.8 ± 5.3	18.5 ± 4.4	16.0 ± 5.7	11.7 ± 3.4[a]

* $P < 0.05$ for a vs d, b vs d, and c vs d

Table 6 Number of PMN accumulated in the tissue of the liver graft (*Post* postpreservation, *Reperfusion* immediately after reperfusion, *1 h* 1 hour after reperfusion)

	PMN (counts/10 high-powerfields)		
	Post	Reperfusion	1 h
Long survival ($n = 15$)	39 ± 8[a]	53 ± 16[b]	78 ± 27[c]
Short survival ($n = 8$)	40 ± 5[d]	65 ± 17[e]	121 ± 20[f]

* $P < 0.01$ for c vs f, a vs c, d vs e, d vs f, and e vs f; ** $P < 0.05$ for b vs c

in the animals with a poor prognosis, one may conclude that SOD activity reflects reperfusion injury of the graft more accurately than other conventional liver function tests, such as AST and LDH. In fact, none of the animals that showed a marked decrease in SOD activity survived. As it appears there is an association between a reduction in SOD activity and an increase in conventional liver enzyme levels, the question may arise of whether SOD leaks out from the damaged hepatocytes, obscuring the results. For this reason the authors measured the relative SOD activity to AST level in the liver tissue. Indeed, there was a significant correlation between SOD and AST levels in the tissue 1 h after reperfusion ($r = 0.804$, $P < 0.01$). The fact that the relative SOD activity in the long survival group is maintained at a stable level after reperfusion as compared to that in the short survival group indicates that SOD activity is adequately maintained, counteracting the oxygen free radicals produced after reperfusion. Furthermore, the fact that the Cu, Zn-SOD to AST ratio decreased significantly in the short survival group 1 h after reperfusion may also imply that inactivation of readily available SOD in the cytoplasm is the initiating event, rather than the reduction in SOD production. Although the precise mechanism of reduction in SOD activity after reperfusion is not fully understood, some explanations are plausible. A well-known fact is that when superoxide radical is dismuted by SOD, hydrogen peroxide (H_2O_2) is produced [17, 18]. Normally, H_2O_2 is catalyzed by enzymes such as catalase and glutathione peroxidase to produce water and oxygen. However, this H_2O_2 inhibits Cu, Zn-SOD activity. The other interesting aspect of this dismutation process is that the superoxide radical inactivates H_2O_2-catalyzing enzymes [2, 9]. Therefore, unless disproportionally sufficient SOD is present in the liver, Cu, Zn-SOD activity is readily influenced by the number of oxygen radicals produced. In fact, we have preliminary evidence that Cu, Zn-SOD activity did not decrease to a significant degree after oxygen free radicals had been adequately scavenged, whereas Cu, Zn-SOD activity did decrease significantly when the residual free radicals were present without been scavenged, probably due to the generation of hydroxyl radicals after the reaction of superoxide with hydrogen peroxide.

Another interesting observation in this study is that only a minimal change in SOD activity was noted during cold preservation. This further supports the concept that SOD activity is a good indicator of reperfusion injury to the graft.

Although the precise mechanism of reperfusion injury is not well understood, numerous reports by others have shown that a microcirculatory disturbance triggered by the destruction of sinusoidal endothelial cells is an initial event that causes reperfusion injury, leading to PNF [4, 7]. Although the data are not shown, from the author's preliminary study on the SOD isozyme of endothelial cells obtained from cultured bovine cells, Cu, Zn-SOD was the major SOD fraction in these cells. Therefore, it is conceivable that there is some damage not only to the hepatocytes but also to the sinusoidal endothelial cells.

The finding that there were significantly increased numbers of PMN infiltrates in the liver graft in the animals with a poor prognosis may be one of the underlying etiology of reperfusion injury. Although a significant increase in the number of PMN was not observed at the time of reperfusion of the graft, generation of these cells occurs early in the phase after reperfusion of the graft, as previously suggested by others [20, 21]. As has been shown, PMN are an important source of some, if not all, oxygen free radicals [20]. Other nonparenchymal cells of the liver that are considered to be a source of oxygen free radical production include Kupf-

fer cells, although quantification of this type of cell was not studied in this report [8].

In conclusion, the SOD level in the liver correlates well with graft outcome of liver transplantation after cold preservation, and the measurement of this enzyme may be a reliable indicator for the outcome of liver transplantation.

References

1. Belzer FO, Southard JH (1988) Principles of solid-organ preservation by cold storage. Transplantation 45: 673–676
2. Blum J, Fridovich I (1985) Inactivation of glutathione peroxidase by superoxide radical. Arch Biochem Biophys 240: 500–508
3. Boudjema K, Lindell SL, Southard JH, Belzer FO (1990) The effect of fasting on the quality of liver preservation by simple cold storage. Transplantation 50: 943–948
4. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ (1991) Kupffer cell activation and endothelial cell damage after storage or rat livers: effects of reperfusion. Hepatology 13: 83–95
5. Connor HD, Gao W, Nukina S, Lemasters JJ, Mason RP, Thurman RG (1992) Evidence that free radicals are involved in graft failure following orthotopic liver transplantation in the rat – an electron paramagnetic resonance spin trapping study. Transplantation 54: 199–204
6. Hiramatsu M, Kohno M, Edamatsu R, Mitsuta K, Mori A (1992) Increased superoxide dismutase activity in aged human cerebrospinal fluid and rat brain determined by electron spin resonance spectrometry using the spin trap method. J Neurochem 58: 1160–1164
7. Jaeschke H (1991) Reactive oxygen and ischemia reperfusion injury of the liver. Chem Biol Interact 79: 115–136
8. Jaeschke H, Farhood A (1991) Neutrophil and Kupffer cell-induced oxidant stress and ischemia-repefusion injury in rat liver. Am J Physiol 260: G355–G362
9. Kono Y, Fridovich I (1982) Superoxide radical inhibits catalase. J Biol Chem 257: 5751–5754
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275
11. Marklund S (1980) Distribution of CuZn superoxide dismutase and Mn superoxide dismutase in human tissues and extracellular fluids. Acta Physiol Scand 492 [Suppl]: 19–23
12. Marzi I, Zhong Z, Zimmermann FA, Lemasters JJ, Thurman RG (1989) Xanthine and hypoxanthine accumulation during storage may contribute to reperfusion injury following liver transplantation in the rat. Transplant Proc 21: 1319–1320
13. Miyagawa H, Yoshikawa T, Tanigawa T, Yoshida N, Sugino S, Kondo M, Nishikawa H, Kohno M (1988) Measurement of serum superoxide dismutase activity by electron spin resonance. J Clin Biochem Nutr 5: 1–7
14. Oberley LW, Spitz DR (1984) Assay of superoxide dismutase activity in tumor tissue. In: Colowick SP, Kaplan NO (eds) Methods in enzymology vd. 105. Academic Press, New York, pp 457–464
15. Oyanagui Y (1984) Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. Anal Biochem 42: 290–296
16. Sakurada M, Ohkohchi N, Kato H, Koizumi M, Fujimori K, Satomi S, Sasaki T, Taguchi Y, Mori S (1989) Mitochondrial respiratory function, adenine nucleotides and antioxygenic enzymes in pig liver transplantation. Transplant Proc 21: 1321–1322
17. Sato K, Akaike T, Kohno M, Ando M, Maeda H (1992) Hydroxyl radical production by H_2O_2 plus Cu, Zn-superoxide dismutase reflects the activity of free copper released from the oxidatively damaged enzyme. J Biol Chem 267: 25371–25377
18. Sinet PM, Garber P, Jérome H (1981) Inactivation of human CuZn superoxide dismutase during exposure to superoxide radical and hydrogen peroxide. Clin Respir Physiol 17 [Suppl]: 91–97
19. Southard JM, Marsh DC, McAnulty JF, Belzer FO (1987) Importance of O_2-derived free radical injury organ preservation and transplantation. Transplant Proc 19: 1380–1381
20. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D (1993) Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Transplantation 55: 1265–1272
21. Takei Y, Marzi I, Gao W, Gores GJ, Lemasters JJ, Thurman RG (1991) Leukocyte adhesion and cell death following orthotopic liver transplantation in the rat. Transplantation 51: 959–965
22. Weisiger RA (1986) Oxygen radicals and ischemic tissue injury. Gastroenterology 90: 494–496