GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Free-Radical Damage to the Liver During its Transplantation Under Conditions of Oxidative Stress

V. I. Mil'chakov, I. I. Dement'eva, M. Yu. Andrianova, and M. V. Palyulina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 11, pp. 471-473, November, 1995
Original article submitted October 26, 1994

The role played by oxygenation of the recipient organism in the development of reperfusion-induced damage to the transplanted liver was evaluated in minipigs, and animals in which the transplant operations failed were found to have developed oxidative stress. The results indicate that free-radical oxidation has a role to play in the damage to cellular structures of the recipient and that it is important to correct this damage as early as possible by means of antioxidants and iron-chelating agents.

Key Words: *liver; transplantation; free-radical damage*

The prime consideration in liver transplantation is preservation of the graft. During the liverless period, substantial hemodynamic changes may occur in the patient's body [12] and promote the development of oxidative stress [11]. It has been shown in numerous studies that free oxygen radicals play an important role in the pathogenesis of damage sustained by hepatocytes during ischemia/reperfusion [3,6,8]. Under such circumstances, even a well-preserved transplant may be damaged by lipid peroxidation products in the course of reperfusion.

The purpose of the present study was to evaluate the likelihood of oxidative stress in the liver transplant recipient, the conditions under which such stress can occur, and how it might affect the outcome of the operation.

MATERIALS AND METHODS

A total of 23 male and female minipigs of the *Svetlogorskaya* breed aged 1 to 1.5 years (body weight 18-30 kg) were used. According to the results of orthotopic liver transplantation, they were divided into two groups: group 1 (n=15) consisted of animals in which the procedure was judged to be successful (they lived >24 h after it), while group 2 (n=8) comprised those which died within the first few postoperative hours.

During anesthesia and the liverless period and also after blood flow was restored through the liver, major parameters of oxygen homeostasis were measured with a BMS-3M analyzer (Radiometer); Ca^{2+} concentration, with an ICA-1 analyzer (Radiometer); and lactate levels, enzymatically with a Lactate Analyzer (Roche); the activity of free oxygen radicals was estimated in blood plasma by measuring its hydrogen peroxide-induced chemiluminescence [2]. All measurements were performed in samples of arterial and venous blood issuing from the transplant.

The results were subjected to routine statistical treatment.

RESULTS

During the liverless period, oxygen tension (Po_2) in the arterial blood remained essentially unchanged in

Research Center of Surgery, Russian Academy of Medical Sciences, Moscow (Presented by Yu. A. Vladimirov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0011-1093$12.50

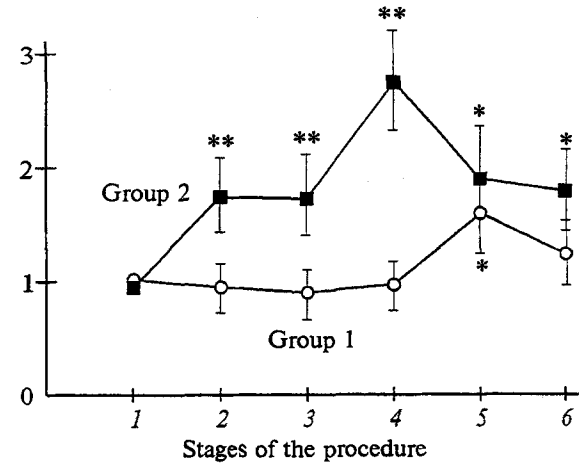

Fig. 1

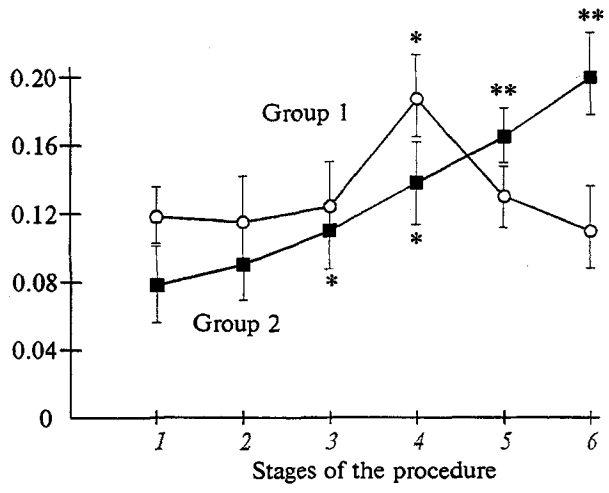

Fig. 2

Fig. 1. Temporal variation in free–radical properties of the blood. Ordinate: maximal chemiluminescence intensity, as expressed in rel. units in relation to its value at the time of anesthesia induction. Here and in Fig. 2: *1*) anesthesia induction; *2*) beginning of liverless period; *3*) end of the liverless period; *4*) resumption of blood flow; *5*) end of operation; *6*) 9 h postoperation. $p<0.05$: *in comparison with the time of anesthesia induction; **in comparison with group 1.

Fig. 2. Peroxidant activity of the blood. Ordinate: ratio of maximal chemiluminescence (in sec^{-1}) to the total chemiluminescence as measured over 5 min.

group 1 and increased by about 50% in group 2, while decreasing to a similar extent in the venous blood in both groups. After hepatic blood flow resumed, Po_2 fell slightly in the arterial blood and remained more or less stable in the venous blood in group 1, but dropped markedly in the venous blood in group 2 (Table 1).

These results indicate that the cases of failed liver transplantation were associated with a marked imbalance of oxygen homeostasis involving a relative hyperoxia during the liverless period and a greatly increased oxygen consumption following the resumption of hepatic blood flow.

As shown in Table 1, lactate levels in the venous blood had risen severalfold by the end of the liverless period in both groups. The resumption of hepatic blood flow was followed by significant falls of lactate in group 1, where its level by the end of the operation was 1.6 times lower than its peak value. In group 2, by contrast, the lactate level continued to rise after the restoration of blood flow to exceed the initial value 4.4-fold by the end of the operation. Blood levels of Ca^{2+} had also risen by that time (Table 1). The impaired lactate utilization by the liver combined with the enhanced calcium release into the circulation indicates that hepatocytes were damaged as a result of reperfusion.

In group 1, as shown in Fig. 1, the maximal chemiluminescence intensity remained unchanged

TABLE 1. Po_2 Values and Lactate and Ca^{2+} Levels in the Arterial and Venous Blood of Minipigs at Different Stages of Successful (Group 1) and Unsuccessful (Group 2) Liver Transplantation ($M \pm m$)

Stage of procedure	Po_2, mm Hg				Lactate, mmol/liter		Ca^{2+}, mmol/liter	
	in arterial blood		in venous blood					
	group 1	group 2	group 1	group 2	group 1	group 2	group 1	group 2
Anesthesia induction	250.8±21.7	196.5±33.2	57.1±5.1	44.6±7.9	2.0±0.9	1.7±0.8	1.08±0.1	1.21±0.1
Beginning of liverless period	273.8±25.4	277.7±33.1*	56.6±7.9	33.6±2.6**	3.5±0.5*	3.3±0.4*	1.03±0.1	1.09±0.1
End of liverless period	257.9±7.0	283.3±22.8*	51.2±7.9	29.5±5.1**	8.7±3.3*	6.2±2.6*	1.13±0.2	1.12±0.1
Resumption of blood flow	201.9±10.1	221.9±35.9	62.3±6.2	23.1±5.3*	-	-	0.90±0.2	-
End of operation	217.1±29.6	191.0±48.7	52.0±4.6	26.7±4.5*	5.4±1.9**	7.5±1.5**	1.07±0.2	1.78±0.7**
9 h postoperation	101.5±13.3*	151.0±18.4**	39.9±2.1*	23.1±0.1*	3.8±0.6	9.15±4.2**	0.89±0.2	1.10±0.6

Note. $p<0.05$: *in comparison with the time of anesthesia induction; **in comparison with group 1.

throughout the liverless period and then increased (by 60%) for a short time after hepatic blood flow resumed. Since the maximal intensity of hydrogen peroxide-induced chemiluminescence depends on the quantity of heme compounds contained in the sample [1], the transient activation of free oxygen radicals in the blood could be attributed to surgical trauma. This hypothesis is supported by the results of measuring the rate at which the peak of chemiluminescence induction was quenched because of the presence of intracellular peroxide-degrading and antioxidant substances in the blood sample [1]. The discharge of intracellular contents from the liver in cases of successful transplantation was only observed at the stage of blood flow resumption; in cases where the procedure failed, the rate of chemiluminescence quenching was already increased (by 40%) during the liverless period and exceeded the initial value 2.4-fold by the end of the operation (Fig. 2).

An unsuccessful liver transplantation was thus characterized by a marked activation of free-radical reactions in the blood both in the liverless period and after the resumption of hepatic blood flow, which may be taken as evidence of free-radical damage to the hepatocytes.

REFERENCES

1. Yu. A. Vladimirov and A. Ya. Potapenko, in: *Physicochemical Bases of Photobiological Processes* [in Russian], Moscow (1989), p. 199.
2. Ya. I. Serkiz, E. E. Chebotarev, V. A. Baraboi, *et al.*, in: *Blood Chemiluminescence in Experimental and Clinical Oncology* [in Russian], Kiev (1984), p. 184.
3. E. Albano, G. Bellomo, M. Parola, *et al.*, *Free Radic. Biol. Med.*, **9**, Suppl. 1, 104 (1990).
4. R. C. Allen, in: Chemical and Biological Generation of Excited States (W. Adam *et al.*, eds.), New York (1982), pp. 310-344.
5. S. D. Aust, L. A. Morehouse, and C. E. Thomas, *Free Radic. Biol. Med.*, **1**, 3-25 (1985).
6. D. R. Kowalski, Tak Yee Aw, Y. Park, and D. P. Jones, *Free Radic. Biol. Med.*, **12**, 205-212 (1992).
7. K. Nakamura and K. Nomoto, *Free Radic. Biol. Med.*, **9**, Suppl. 1, 32 (1990).
8. N. Ohkohehi, M. Sakurada, H. Katoh, *et al.*, *Free Radic. Biol. Med.*, **9**, Suppl. 1, 97 (1990).
9. D. Pearson, *Perfusion*, **3**, 113-133 (1988).
10. S. I. Salaris and C. F. Babbs, *Free Radic. Biol. Med.*, **9**, Suppl. 1, 100 (1990).
11. H. Sies, in: *Oxidative Stress*, London (1985), pp. 1-7.
12. W. C. Trok, D. J. Martin, M. R. Pinski, *et al.*, *Anesthesiology*, **61**, Suppl., 8271 (1984).