0014-4754/93/030235-03$1.50 + 0.20

Research Articles

Lack of effect of antioxidant therapy during renal ischemia and reperfusion in dogs

L. Kónya, P. Bencsáth, G. Szénási and J. Fehér

2nd Department of Medicine, Medical School, Semmelweis University, H-1088 Budapest (Hungary)
Received 2 July 1992; accepted 26 October 1992

Abstract. Acute ischemic renal failure is of great clinical importance because of its frequent occurrence and the high mortality it causes. Recent observations indicate that reperfusion has its own dangers because of oxygen-derived free radicals. To study this problem, ischemia was evoked in dogs in one kidney, by clamping the left renal artery for 45 min. This was followed by a 90-min period of reperfusion when diuresis, GFR, PAH clearance and sodium and potassium excretion were studied. Besides a control group (n = 6), the following treatment groups were investigated. Allopurinol (n = 7): 50 mg/kg for two days p.o. and 50 mg/kg in physiological saline infusion during the experiment; a small dose of SOD (n = 6): 0.5 mg/kg in infusion, started 1 min before reperfusion and given continuously for 10 min; and a high dose of SOD (n = 7): 5 mg/kg as above.

In the first 15 min following reperfusion, the renal functions significantly worsened in all groups. Later on, the renal functions gradually improved and in the last period after reperfusion, GFR in the ischemic kidney was 64%, cPAH 59%, diuresis 60% and sodium and potassium excretion were 65% and 76%, respectively, of the basal values in the control group.

Treatment with free radical scavengers did not cause any considerable changes in the renal functions. In some respects, the worst results were observed with low-level SOD treatment (cPAH, diuresis, as well as sodium and potassium excretion).

At the end of reperfusion, there was a significant drop in sodium excretion by the right (intact circulation) kidney of the treated animals.

Key words. Antioxidant therapy; free radical; kidney reperfusion.

Ischemia lasting for a critical period of time, depending on the sensitivity of the different organs, leads to irreversible damage of the tissues. Any intervention to improve circulation, carried out soon enough, can help to preserve organ function. Recent observations indicate, however, that reperfusion has its own dangers[1]. It seems likely that the degree of tissue damage after reperfusion depends on both the preceding ischemia and the injury caused by the restarting of the circulation[2]. More and more researchers are stressing the importance of oxygen-derived free radicals in reperfusion injury under clinical and experimental conditions[3–6]. Acute ischemic renal failure is of great clinical importance because of its frequent occurrence and high mortality rate[7–9], so the role of free radicals and the protection afforded by antioxidant therapy have been widely investigated[10–16]. However, the experiments gave contradictory results[17–21], so further observations are needed. Trials of this kind with dogs have been presented relatively rarely in the literature, so we chose this species for our experiments. Oxygen-derived free radicals are formed, and exert their action, in the early phase of reperfusion. Therefore we investigated the role of these toxic agents in that period, after reversible renal ischemia was evoked by the temporary ligation of the arteria renalis.

Methods

The experiments were conducted in mongrel dogs of either sex with a body weight of 10–34 kg. The animals were narcotized with Nembutal (sodium pentobarbital, 30 mg/kg body weight i.v.), and steady narcosis was provided at 3 mg/kg body weight i.v. maintenance doses. A cuffed endotracheal tube was inserted, continuous artificial respiration was maintained (respirator RO-5, Moscow, USSR) and regular blood-gas analysis was carried out during the trial. Surgery was performed on a heatable operating table, with the rectal temperature maintained between 37 and 38 °C. For giving infusions, taking blood, and measuring blood pressure, a jugular vein and both femoral arteries and veins were catheterized. Urine was obtained through catheters from the ureters by median laparotomy. Venous blood from the kidney was obtained through the v. spermatica or v. ovarica, as appropriate, by the aid of a catheter led into the left v. renalis. The left renal artery was exposed, with the renal nerves left intact. On finishing the operation, saline infusion was administered at 20 ml/kg b.wt for 30 min, and then at 10 ml/kg b.wt/h until the end of the trial.

For measuring the glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF), inulin and para-aminohippuric acid (PAH) were infused in physiologi-

cal saline into the v. jugularis to reach a plasma concentration of 30–50 mg/100 ml and 1–3 mg/100 ml, respectively, depending on the animal's body weight (0.21–0.58 ml/min). After the operation there was a 60-min equilibration period, and then urine was collected at 15-min intervals.

After two control periods, the left renal artery was occluded for 45 min and urine was collected from the right kidney alone. During the occlusion, the quantity of infusion was halved. Following the release of the occlusion, urine was collected from both kidneys for 6 further periods of 15 min each. Arterial blood samples were taken at the midpoint of each period.

Blood pressure, measured with a Statham P 23 db pressure transducer, was continuously recorded (Rikadenki, B 381, Tokyo, Japan).

The following groups were examined:

1) Control group (n = 6): Animals given saline alone.
2) Small-dose SOD (Peroxinorm, Grünenthal, Germany) group (n = 6): 0.5 mg/kg b.wt SOD infusion started 1 min before reperfusion and given continuously for 10 min.
3) Large-dose SOD group (n = 7): 5 mg/kg b.wt SOD administered as above.
4) Allopurinol (Milurit, EGIS, Hungary) (n = 7): Animals were pretreated with allopurinol at a dose of 50 mg/kg for two days. During the experiment, allopurinol was given i.v. at a dose of 50 mg/kg in physiological saline solution.

Inulin and PAH concentration in the plasma and the appropriately diluted urine samples were measured by the anthrone and diphenylamine methods. Sodium and potassium concentration were measured with a Flapho 4 type flame photometer (Carl Zeiss, Jena, Germany). The table contains mean values of kidney parameters expressed for 100 g kidney ± SEM. The data were analyzed by Student's t-test.

Results

In the course of statistical data processing, the parameters of the ischemic kidney were compared to the basal ones. It was observed that 15 min after the release of the renal artery occlusion, all renal parameters significantly worsened, especially GFR, which was only 21% in the control, 13% in the allopurinol, 10% in the small-dose, and 8% in the large-dose SOD group, respectively, compared to the basal level ($p < 0.001$). From that time on, the renal functions gradually improved, and 90 min after recirculation the GFR of the ischemic kidney was 64%, cPAH 59% and diuresis 60%. Sodium and potassium excretion were 65% and 76%, respectively, of the basal level in the control group. The table shows the data for two relevant periods in the experiment. Treatment with FR scavengers did not bring any improvement in the functioning of the ischemic kidney, and some functions (sodium and potassium excretion, diuresis) were even less satisfactory in the animals treated with a low dose of SOD and allopurinol. At the end of reperfusion, we observed a significant decrease of sodium excretion by the right (intact circulation) kidney. There was no difference in blood pressure between the different groups.

Discussion

To investigate the role of oxygen-derived FRs in causing damage on reperfusion, we examined whether or not antioxidant therapy could abolish tissue damage. Such an effect would provide indirect evidence for possible FR reactions during reperfusion.

Under both physiological and pathological circumstances, several processes may give rise to FR (cytochrome system, arachidonic acid metabolism, autooxidation of various compounds), but it seems that the most important during reperfusion is the role of the enzyme xanthine oxidase (XO) and of activated neu-

The effect of small (0.5 mg/kg b.wt) and large doses (5 mg/kg b.wt) of superoxide dismutase (SOD) and allopurinol on renal function and arterial blood pressure in narcotized dogs after 45 min unilateral warm renal ischemia

		Before ischemia				90 min after ischemia			
		A	B	C	D	A	B	C	D
Diuresis (ml/min)	I:	1.8 ± 0.4	1.9 ± 0.5	1.8 ± 0.3	3.1 ± 0.4	2.3 ± 0.6	0.6 ± 0.1	1.7 ± 0.4	2.8 ± 0.5
	II:	1.8 ± 0.4	2.1 ± 0.6	1.9 ± 0.3	3.4 ± 0.7	1.4 ± 0.3	1.4 ± 0.9[b]	1.3 ± 0.3	1.6 ± 0.5[b]
GFR (ml/min)	I:	65 ± 5	77 ± 6	80 ± 5	72 ± 4	66 ± 4	82 ± 6	82 ± 7	82 ± 6
	II:	63 ± 5	75 ± 6	76 ± 4	72 ± 4	39 ± 4[c]	40 ± 5[c]	32 ± 5[c]	37 ± 7[c]
cPAH (ml/min)	I:	144 ± 10	219 ± 13	237 ± 22	209 ± 15	160 ± 12	189 ± 13	213 ± 12	196 ± 19[c]
	II:	155 ± 6	204 ± 14	223 ± 17	212 ± 15	91 ± 10[a]	106 ± 14[c]	86 ± 11[c]	93 ± 19[c]
Sodium excretion (μmol/min)	I:	276 ± 57	365 ± 90	302 ± 60	455 ± 100	253 ± 61	211 ± 40[b]	205 ± 34[b]	308 ± 65[b]
	II:	319 ± 93	383 ± 95	308 ± 54	491 ± 11	165 ± 46	78 ± 10[c]	163 ± 34	167 ± 70[a]
Potassium excretion (μmol/min)	I:	67 ± 8	79 ± 12	50 ± 6	99 ± 18	76 ± 8	84 ± 17	52 ± 10	98 ± 12
	II:	70 ± 9	77 ± 11	49 ± 6	99 ± 18	58 ± 10	42 ± 6[b]	44 ± 7	55 ± 13[a]
Blood pressure (mmHg)	I:	130 ± 7	133 ± 4	123 ± 5	129 ± 4	139 ± 4	138 ± 6	131 ± 4	139 ± 4

Abbreviations. I = right kidney (intact circulation); II = left (ischemic) kidney; A = control group (n = 6); B = small dose of SOD group (n = 6); C = large dose of SOD group (n = 7); D = allopurinol group (n = 7); [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ vs basal level in the same group. The data (mean ± SEM) are expressed for 100 g wet kidney weight.

trophil granulocytes[16,19,22–25]. We investigated the effect of allopurinol, which inhibits XO, and the efficacy of SOD, which is a natural scavenger of superoxide radicals. Dog cells have been shown to contain XO[26]. The concentration of this enzyme is different from organ to organ in the same species: the level is higher in the liver and in the myocardium than in the kidney, and the XDH-XO conversion that happens during ischemia is faster in the myocardium then in the kidney[24].

The ineffectiveness of allopurinol treatment may indicate that XO does not play a critical role in reperfusion tissue damage of the kidney. Other researchers have come to the same opinion[18,27].

In our experiment, no protective effect of the SOD enzyme could be demonstrated either. It is not clear what the optimal dose of SOD is for the treatment of reperfusion injury. It depends, for example, on the route of administration.

Baker et al.[10] found in rats treated with 3.25 mg/kg b.wt of SOD following ischemia of the kidney that the serum creatinine level was lower in the first days after operation than in the untreated control group. Later on, no difference could be seen in mortality, but with a double dose (6.5 mg/kg b.wt) of the enzyme all animals survived. Koyama et al.[28] failed to find any protective effect with 0.2 2 mg of the enzyme given into the renal artery of pigs following cold-warm ischemia, while on the administration of 20 mg of the enzyme, the clearance of creatinine improved.

It was found that a small dose of SOD is not only ineffective but can cause tissue damage, because the incomplete transformation of the superoxide radical facilitates the production of even more toxic agents[29]. In our trial, some renal functions (sodium and potassium excretion) were worst in the low SOD treatment group, which may correlate with the aforementioned experience. If the major source of superoxide radicals during reperfusion is the activated polymorphonuclear neutrophils, as has been stated by some investigators[25,30], there will be continuous free radical generation, because the number of neutrophils increases after the restarting of circulation in the ischemic tissue[24,31]. Because the half-life of SOD is short (probably less than 10 min[32]), in our experiment even a large dose of the enzyme could not give total protection. Therefore, it seems reasonable to administer the enzyme continuously, or to use SOD which is conjugated with polyethylene glycol, which prolongs the half-life[33]. As the commercially available enzyme is sometimes contaminated[34], another possibility is to administer human recombinant SOD, which appears to be free of contaminants[35]. During the experiment, we also studied the function of the right kidney, in which the circulation was intact. It was observed that sodium excretion significantly decreased in all treated animals, but there was not any substantial alteration in potassium excretion or in blood pressure. It appears that the antioxidant agents have a direct effect on the regulation of sodium excretion. This observation needs further investigation.

1 Braunwald, E., and Kloner, R. A., J. clin. Invest. *76* (1985) 1713.
2 Bulkley, G. B., Br. J. Cancer *55* Suppl. VIII, (1987) 66.
3 Batelli, M. G., Della Corte, E., and Stirpr, F., Transplantation *29* (1980) 245.
4 Canavese, C., Stratta, P., and Vercollene, A., Nephron *49* (1988) 9.
5 Ferreira, R., Llesuy, S., Milei, J., Scordo, D., Hourquebie, H., Molteni, L., de Palma, C., and Boveris, A., Am. Heart J. *115* (1988) 307.
6 McCord, J. M., New Engl. J. Med. *312* (1985) 159.
7 Gornick, Ch. G. Jr, and Kjellstrand, C. M., Nephron *35* (1983) 145.
8 Hou, S. H., Bushinsky, D. A., Wish, I. B., Cohen, I. I., and Harrington, J. T., Am. J. Med. *74* (1983) 243.
9 Myers, B. D., Miller, D. C., Mehigan, J. T., Olcott, C. IV., Golbetz, H., Robertson, C. R., Derby, G., Spencer, R., and Friedman, S., J. clin. Invest. *73* (1984) 329.
10 Baker, G. L., Corry, R. J., and Autor, A. P., Ann. Surg. *202* (1985) 628.
11 Bayati, A., Hellberg, O., Kallskog, Ö., and Wolgast, M., Transplant. Proc., vol. XVIII, No. 1 (1986) 84.
12 Bratell, S., Polmeiz, P., Hansson, R., Jonsson, O., Lundstram, S., Petterson, S., Rippe, B., and Scherstein, T., Acta physiol. scand. *134* (1988) 35.
13 Galat, J. A., Robinson, A. V., and Rhodes, R. S., J. Surg. Res. *46* (1989) 520.
14 McCord, J. M., in: Therapeutic approaches to myocardial infarct size limitation, p. 209. Eds D. J. Hearse and D. M. Yellon. Raven Press, New York 1984.
15 Paller, M. S., and Sikora, J. J., Kidney Int. *33* (1988) 843.
16 Paller, M. S., Hoidal, J. R., and Ferris, Th. F., J. clin. Invest. *74* (1984) 1156.
17 Bird, J. E., Milhoan, K., Wilson, C. B., Young, S. G., Mundy, C. A., Parthasarathy, S., and Blantz, R. C., J. clin. Invest. *81* (1988) 1630.
18 Camelin, L. M., and Zager, R. A., Am. J. Physiol. *255* (1988) F450.
19 Hansson, R., Johansson, S., Jonsson, O., Pettersson, S., Scherstén, I., and Waldenström, J., Clin. Sci. *71* (1986) 245.
20 Ouriel, K., Smedira, N. G., and Ricotta, J. J., J. vasc. Surg. *2* (1985) 49.
21 Zager, R. A., and Gmur, D. J., Am. J. Physiol. *257* (1989) F953.
22 Hansson, R., Gustaffson, B., Jonsson, O., Lundstam, S., Petterson, S., Schersten, T., and Waldenström, J., Transplant. Proc., vol. XIV, No. 1 (1982) 51.
23 Hearse, D. J., Manning, A. S., Downey, J. M., and Yellon, D. M., Acta physiol. scand. *548* Suppl. (1986) 65.
24 Mehta, J. L., Nichols, W. W., and Mehta, P., J. Am. Coll. Cardiol. *11* (1988) 1309.
25 Romson, J. L., Hook, B. G., Kunkel, S. L., Abrams, G. D., Schork, M. A., and Lucchesi, B. R., Circulation *67* (1983) 1016.
26 Southard, J. H., Marsh, D. C., McAnulty, J. F., and Belzer, F. O., Surgery *101* (1987) 566.
27 Joannidis, M., Gstraunthaler, G., and Pfaller, W., Am. J. Physiol. *258* (1990) F232
28 Koyama, I., Bulkley, G. B., Williams, G. M., and Im, M. J., Transplantation *40* (1985) 590.
29 Kaur, A., Sethi, A. K., Ganguly, N. K., and Majumdar, S., Biochem. Int. *19* (1989) 385.
30 Linas, S. L., Whittenburg, D., Berger, E., and Repine, J. E., Kidney Int. *31* (1987) 369.
31 Tanaka, M., Brooks, S. E., Di Bernardo, L. R., Jennings, R. B., and Reimer, K. A., Circulation *82* (1990) 275.
32 Cohen, M. V., Ann. int. Med. *111* (1989) 918.
33 Tamura, Y., Chi, L., Driscoll, E. M., Hoff, P. T., Freeman, B., Gallagher, K. P., and Lucchesi, B. R., Circ. Res. *63* (1988) 944.
34 Nejima, J., Knight, D. V., Fallon, J. T., Veimura, N., Manders, T., Canfield, D. R., Cohen, M. V., and Vatner, S. F., Circulation *79* (1989) 143.
35 Ambrosio, C., Becker, L. C., Hutchins, G. M., Weisman, H. F., and Weisfeldt, M. L., Circulation *74* (1986) 1424.