FUNCTIONAL AND MORPHOLOGICAL CHANGES IN THE KIDNEYS AFTER ACUTE EMBOLOGENIC OCCLUSION OF THE LIMB ARTERIES

N. P. Istomin, O. D. Mishnev,
N. A. Sergeeva, T. S. Serdobintseva,
A. P. Razumnyi, and M. É. Sokolov

UDC 616.137.8/.9-005.7-07:[616.61-091+616.61-092

KEY WORDS: ischemia of the limbs; postischemic recirculation; function and morphology of the kidney.

Modern methods of surgical treatment of acute embologenic arterial occlusion of the limbs are aimed at early operative restoration of the blood flow in the main arteries [6, 7, 10]. The effect of ischemia of the limbs and, in particular, of postischemic recirculation on the body is manifested as the development of a complex syndrome of multiple failure of the vital organs, in which an important role is played by acute renal failure (ARF) [3, 11, 14]. The functional and morphological characteristics of this type of ARF have not been finally explained, and this is an obstacle to the development of effective methods of prevention and treatment [5, 8, 12].

The aim of this investigation was to study the time course of changes in the kidneys at different stages of ischemia of the limbs and restoration of the blood flow in them, on the basis of a combined functional and morphological investigation.

EXPERIMENTAL METHOD

Experiments were carried out on 45 mongrel dogs weighing 16-22 kg, in which acute occlusion of the hind limb arteries was simulated by the method of Zatevakhin et al. [1]. Premedication with trimeperidine in a dose of 1 ml of a 1% solution/kg body weight was given. Radioisotope renography was carried out by intravenous injection of ^{131}I-hippuran (12 kBq/kg body weight), with recording on a "Gamma" scintillation counter (Hungary). Curves were recorded on an N-320/3 quick-acting three-channel automatic recorder with tape winding speed of 6 mm/min. The time of appearance of maximal activity above the kidney (T_{max}), the time for maximal activity to decrease by half ($T_{1/2}$), in minutes, and the blood clearance (C) in per cent, were determined [2]. Serum K^+ and Na^+ concentrations were determined by flame photometry. The creatinine and urea nitrogen concentrations in the blood serum were determined with an Impac-400 analyzer (Gilford, USA). These parameters were recorded before occlusion, after 3, 6, and 12 h of limb ischemia, and 2 h after restoration of the blood flow in the limbs. The animals were killed by intravenous injection of hexobarbital. Activity of succinate, malate, and lactate dehydrogenases (SDH, MDH, LDH) and of alkaline phosphatase (AP) was determined by the usual methods in the proximal and distal tubules (PT and DT) in frozen sections through the kidneys. For quantitative assay of their activity, a Microvideomat television image analyzer (Opton, West Germahy) was used, with plotting on a correlation curve by Wang-720C computer (USA), using a special program. Paraffin sections through the kidneys were stained with hematoxylin and eosin, for fibrin by Mallory's method, by the PAS reaction with amylase control, and the reaction for hemoglobin and myoglobin peroxidase [9].

EXPERIMENTAL RESULTS

The experiments showed that a fixed position of the animals under the conditions of the experimental model had no significant effect on the biochemical, renographic, and morphological parameters. After 3 h of occlusion the plasma electrolyte and creatinine concentrations and the hippuran clearance of the blood remained at their initial levels (Fig. 1a, b). The

No. 3 Department of Surgical Diseases and Department of Pathological Anatomy, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Savel'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 2, pp. 169-171, February, 1987. Original article submitted March 11, 1986.

0007-4888/87/0002-0189$12.50

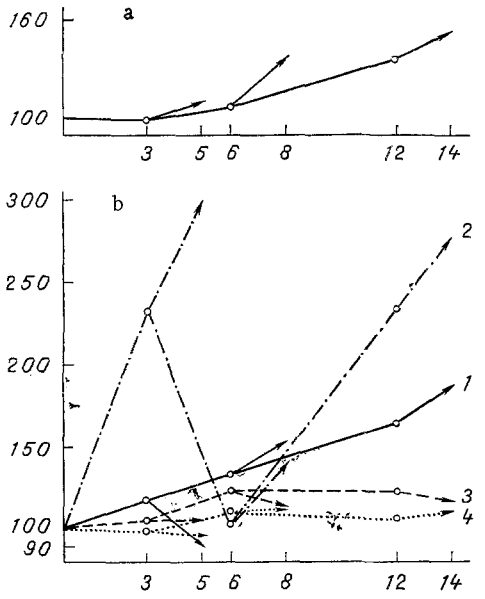

Fig. 1. Time course of blood hippuran clearance (a) and of some biochemical parameters of the blood serum (b): creatinine (1), urea nitrogen (2), potassium (3), and sodium (4) in acute arterial occlusion (in % of initial data). Arrows indicate dynamics of these parameters in the postischemic period. Abscissa, duration of experiment in hours.

values of T_{max} and $T_{1/2}$, as parameters of secretion in PT and evacuation in DT and the collecting tubules were unchanged, at 2.46 ± 0.34 and 6.57 ± 0.66 min, respectively. The epitheliocytes of these parts of the nephrons showed no significant changes but the lumen of PT and DT was constricted. The blood urea was raised by 2.3 times. Besides many other factors, a definite role in this state of affairs was evidently played by intensification of reabsorption processes in PT. Evidence of intensification of function and metabolism of the epitheliocytes of PT was given by increased AP activity in the brush border and an increase of 48% in SDH activity and 32% in MDH activity in the cytoplasm (Fig. 2a).

Prolongation of the occlusion to 6 h likewise caused no changes in the parameters of renography, but some decrease in diuresis was noted. The serum creatinine concentration remained within normal limits, and K^+ and Na^+ were increased by 23% ($P < 0.05$) and 11% ($P > 0.05$), respectively. The urea nitrogen level fell to its initial value. Congestion of the juxtamedullary zone was observed, with stasis of blood and sludging of erythrocytes. The epitheliocytes of PT were swollen, with signs of granular and vacuolar dystrophy, and the brush border was partially desquamated. The lumen of DT was considerably narrowed and the cytoplasm of the epitheliocytes was edematous, with evidence of vacuolar dystrophy (Fig. 2b). SDH activity was reduced by 67% in PT and by 43% in DT; LDH activity was raised by 60% in PT and by 40% in DT ($P < 0.05$), indicating a more important role of anaerobic metabolism in the cells. AP activity was intensified in the brush border and diffusion of the enzyme was observed into the cytoplasm of some cells in PT.

Occlusion of the arteries for 12 h led to an increase of 1.4 times in the hippuran clearance ($P < 0.05$). In three of the six animals of this group T_{max} and $T_{1/2}$ were absent from the kidney scans, and this was combined with anuria. The serum creatinine concentration was increased by 66% ($P < 0.05$), the urea nitrogen by 2.4 times ($P < 0.05$), K^+ by 22% ($P < 0.05$), and Na^+ by 9% ($P > 0.05$). The morphological changes included anemia of the cortex, congestion of the medulla, focal hemorrhages, fibrin thrombi in the venules, and cloudy-swelling and vacuolar degeneration of the epitheliocytes, progressing in some cases to partial or total necrosis of these cells (Fig. 2c). SDH activity in PT was reduced by 65% and in DT by 67%; MDH activity was reduced in PT by 50%, and in DT by 42% ($P < 0.05$). LDH activity in PT was increased by 78% and in DT by 35% ($P < 0.05$). Against the background of damage to the brush border of the epitheliocytes of PT, focal lowering of AP activity was found.

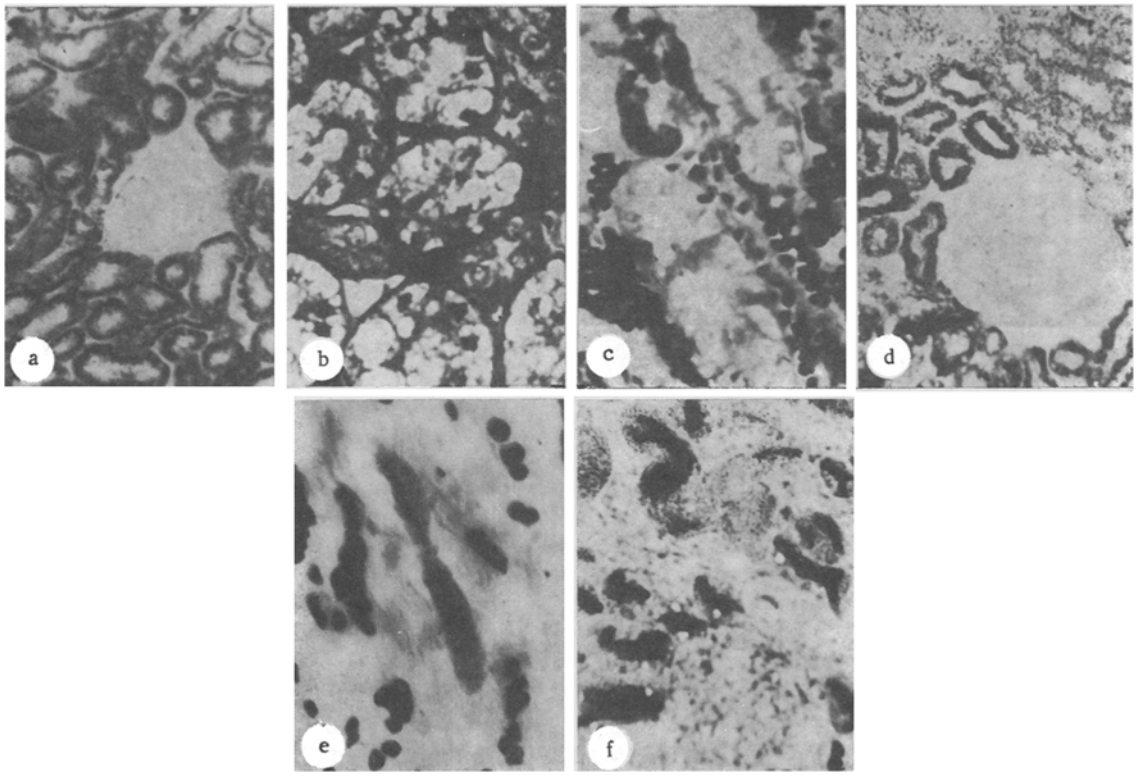

Fig. 2. Morphological changes in dogs' kidneys during acute embologenic occlusion of limb arteries. a) High SDH activity in epitheliocytes of PT, 3 h of ischemia. 100×; b) Vacuolar dystrophy of nephrocytes, 6 h of ischemia. Hematoxylin and eosin. 256×; c) Necrosis of epitheliocytes, hemorrhage, 12 h of ischemia. Hematoxylin and eosin. 256×; d) Decrease in SDH activity in tubules, recirculation after 6 h of ischemia. 100×; e) Pigment cast in tubule, Graham and Karnovsky's reaction; f) disappearance and decrease in activity of AP in brush border of PT, recirculation after 12 h of ischemia. 150×.

Restoration of the blood flow after limb ischemia for 3 h caused no changes in the blood hippuran clearance, or serum creatinine, K^+, and Na^+ concentration (Fig. 1a, b). The parameters of radioisotope renography were the same as initially: T_{max} 2.7 ± 1.12 min, $T_{1/2}$ 8.9 ± 4.19 min. Meanwhile, just as during the 3-h period of ischemia, the urea nitrogen level was raised threefold ($P < 0.001$). Morphological changes in the kidneys consisted of moderately severe disturbances of the circulation and damage to individual tubular epitheliocytes. SDH and MDH activity in PT was increased by 28 and 63%, respectively, and in DT by 57 and 50%, respectively ($P < 0.05$).

Restoration of the blood flow after ischemia of the limbs for 6 h caused an increase in hippuran clearance by 37.5% in all the animals ($P < 0.05$). T_{max} and $T_{1/2}$ could not be determined in half of the animals, and this was combined with anuria. The serum Na^+ level was raised by 11% ($P > 0.05$). The creatinine and urea nitrogen concentration showed a tendency to rise (Fig. 1b). The K^+ level was down to its initial value. Morphological changes were characterized by more severe degeneration of the nephrocytes, or even necrosis. Hyaline and single pigment casts were seen in the dilated lumen of the tubules. AP activity also was lowered, but in individual PT it still remained high, with diffusion of enzymes into the cytoplasm.

During 2 h after the end of a 12-h period of arterial occlusion the biochemical, renographic, and morphological data (Figs. 1 and 2) indicated the development of ARF. The clearance increased by more than 50% ($P < 0.05$). T_{max} and $T_{1/2}$ were absent in four of five cases, combined with anuria. The creatinine level was 90% and the urea nitrogen level 200% higher than initially. Just as during limb ischemia for 12 h, the blood K^+ and Na^+ concentrations remained high. Morphological investigations showed that the degenerative and necrotic changes in the epitheliocytes reached their greatest severity and spread to both PT and DT. Microcirculatory disturbances were prominent and the capillaries, venules, arterioles, and lobular vessels were affected by thrombus formation. Basement membranes in PT were exposed over a wide

area and solitary albuminous pigment casts (Fig. 2e) and erythrocytes could be seen in the lumen of DT. AP activity was considerably reduced in the epitheliocytes of PT (Fig. 2f). Activity of the enzymes of aerobic and anaerobic metabolism was sharply reduced: of SDH, MDH, and LDH by 93, 62, and 90% in PT and by 63, 65, and 99% in DT, respectively ($P < 0.001$).

During acute occlusion of the limb arteries, biochemical, renographic, and morphological changes thus begin to develop actually in the ischemic period. Restoration of the blood flow in the limbs after ischemia for 6 h and, in particular, for 12 h, regularly causes impairment of the parameters of renal function and morphology which were studied, leading to the formation of ARF. The solitary pigment casts found in the lumen of the tubules are not the cause of development of ARF at the times of limb ischemia and of the postischemic period which were studied.

LITERATURE CITED

1. I. I. Zatevakhin, V. M. Koshkin, N. P. Istomin, et al., Éksp. Khir., No. 3, 16 (1976).
2. N. A. Lopatkin, Yu. Ya. Gleizer, and E. B. Mazo, Radioisotope Diagnosis in Uronephrology [in Russian], Moscow (1977), pp. 20-22.
3. O. D. Mishnev, T. S. Serdobintseva, and N. P. Istomin, Abstracts of Proceedings of the 7th All-Union Congress of Morbid Anatomists [in Russian], Tashkent (1983), pp. 391-392.
4. L. E. Nemirovskii and A. V. Zhukotskii, Tr. 2-go Mosk. Med. Inst., 86, 123 (1978).
5. N. K. Permyakov and L. N. Zimina, Acute Renal Failure [in Russian], Moscow (1982), pp. 22-32 and 185-190.
6. V. S. Savel'ev, Kariologiya, No. 8, 5 (1979).
7. V. S. Savel'ev, I. I. Zatevakhin, N. V. Stepanov, and L. I. Bogdanets, Khirurgiya, No. 5, 18 (1983).
8. G. A. Chekareva, O. D. Mishnev, T. S. Serdobintseva, and N. P. Istomin, Arkh. Patol., No. 8, 27 (1981).
9. R. Graham and M. Karnovsky, J. Histochem. Cytochem., 14, 291 (1966).
10. H. Haimovici, Surgery, 85, 461 (1979).
11. A. Lercan, P. Mathieu, J. Helmer, et al., J. Cardiovasc. Surg., 14, 609 (1973).
12. M. Mydlik, V. Spustova, R. Drurik, et al., Int. Urol. Nephrol., 1, 67 (1982).
13. A. G. E. Pearse, Histochemistry, Theoretical and Applied, Little, Brown and Company (1960).
14. D. B. Stokke, P. Anderson, K. Christensen, et al., Dan. Med. Bull., 6, 274 (1980).