

Morphological and functional renal study in kidneys of pretreated rats with ozone and submitted to warm ischemia

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Palabras clave: isquemia caliente, reperfusión, transplante de riñón, ozonoterapia.
Key words: warm ischemia, reperfusion, kidney transplantation, ozone therapy.

RESUMEN. La isquemia caliente y fría son determinantes en la viabilidad del riñón y en el éxito del transplante. Conociendo que el ozono estimula los sistemas de defensa antioxidante, se estudió en ratas pretratadas con ozono, la morfología y función renal de riñones sometidos a un periodo de isquemia caliente. Las ratas se dividieron en: 1.-Control, animales con los riñones expuestos mediante una incisión medio abdominal; 2.-isquemia, animales sometidos a isquemia renal bilateral con posterior reperfusión; 3.-ozono+isquemia, como el grupo 2, pero con tratamiento previo con ozono por vía rectal; 4.-oxígeno+isquemia, como el grupo 3, pero empleando oxígeno. Se obtuvo una disminución significativa del flujo y de la filtración renal en los grupos 2 y 4 con respecto al 1 y al 3, sin diferencias significativas entre éstos últimos. Las alteraciones morfológicas se presentaron en el 100, 88 y 30 % de los animales en los grupos 2, 4 y 3, respectivamente. Estos resultados sugieren un procedimiento terapéutico novedoso en transplantología.

ABSTRACT. It is known that the time of warm and cold ischemia is determinant in the kidney viability and in the transplantation success. Taking into account that ozone can stimulate the cellular antioxidant enzymes, a morphofunctional renal study, applying ozone in rats before a warm ischemia, was performed. Rats were divided into: 1.-Control, a medial abdominal incision was performed for the exposure of the kidneys; 2.-ischemia, animals with a bilateral renal ischaemia, with subsequent reperfusion; 3.-ozone-ischemia, as group 2, but with previously treatment with ozone by rectal application. 4.-oxygen-ischemia, as group 3, but using oxygen. A significant decrease of the flow and renal filtration in groups 2 and 4 with regards to groups 1 and 3 was obtained, without any statistical difference between group 1 and 3. Morphological alterations were present in 100, 88 and 30 % of the animals in groups 2, 4 and 3, respectively. This finding suggests novel therapeutic approaches to tissue damage in kidney transplantation.

INTRODUCTION

During surgical revascularisation of the renal artery, but also after kidney transplantation, the kidney is temporarily rendered ischemic and subsequently reperfused. It is known that the time of warm and cold ischaemia is determinant in the kidney viability and in the transplantation success.¹ After the reimplantation, in a significant

proportion of kidneys, an acute tubular necrosis is present and the recipient must require the dialysis. In these patients the treatment with immunosuppressors is indicated, increasing the ischemic damage and in most cases this drug can not be administered because of the dysfunction of the kidney.¹ So then, the kidney preservation is of great clinical importance.

Recent advances in understanding the fundamental mechanisms of postischemic injury have suggested that most tissue injury is associated with admission of oxygen to the tissue at the time of reperfusion,² with the subsequent oxidative stress that contributes to the cellular damage.³ This reoxygenation leads to a massive production of toxic free radical species generated through several cytoplasmatic or mitochondrial mechanisms.^{2,4,5}

Several papers^{6,7} are referred to the study to increase the quality of the solutions of kidney preservation, as well as, to decrease the oxidative stress produced during the reperfusion of the transplanted organs.

In normal conditions, cells contain endogenous defenses against toxic free radicals: enzymatic defenses system such as superoxide dismutase, catalase, glutathione peroxidase, or non enzymatic components such as reduced glutathione, vitamin E, etcetera.⁸ In some pathologic situations, the defense mechanism can be overwhelmed allowing the reactive oxygen species to exert their deleterious effect without control.³

Taking into account that ozone, being a strong oxidizer, can stimulate the cellular antioxidant enzymes^{9,10} inhibiting the oxidative stress, the authors decided to study the kidney morphology and the renal function, when rectal ozone is applied before a warm ischemia.

MATERIALS AND METHODS

White Wistar rats, 250 ± 9 g weight, were divided into four groups for the study of the plasmatic renal flow and the glomerular filtration rate by means of plasmatic clearance of *p*-amino-hippurate (PAH) and inulin, respectively. PAH and inulin were determined in filtered free of proteins by cadmium sulfate,¹¹ using for PAH a photocolorimetric technique of Bratton and Marshall,¹² modified by H.W. Smith.¹³ Inulin was measured by the direct method of resorcinol without alkaline treatment.¹⁴

The first group (control) of animals were anesthetized, using sodium pentobarbital at doses of 30 mg/kg of weight, receiving 50 IU of heparin by intraperitoneal injection and after that, a medial abdominal incision was performed for the exposure of the kidneys. The second group (ischemia) were processed in the same way, but after the kidney exposition they were submitted to a bilateral renal ischemia by arterial clamping during 30 min with subsequent reperfusion before the morphofunctional renal study. The third group (ozone-ischemia) received the same procedure as group 2, but the animals were previously treated with

ozone (O₃) by rectal application, daily during 15 d, at doses of 0.05 mg/kg of weight. The fourth group (oxygen-ischemia) with a procedure similar to group 3, but using oxygen (O₂) by rectal application instead of ozone.

For the morphological study, samples of rat kidneys of the different groups were taken and fixed in neutral 10 % formalin, processed and embedded in paraffin. The histological sections were stained with hematoxylin and eosin for their study by optical microscopy.

All data are presented as mean standard deviation. An analysis of variance of simple classification was performed, for comparison of means of different groups, for each variable and also Duncan test. For comparison of two groups, the Student's *t* test was done. Data were considered statistically different from controls at a significance level $p < 0.05$.

RESULTS AND DISCUSSION

The results demonstrated a significant decrease of the renal flow (Fig. 1) measured by means of plasmatic clearance of *p*-amino-hippurate in the groups 2: ischemia (1.66 ± 0.82) and 4: O₂-ischemia (1.16 ± 0.61) with respect to 1: control group (3.08 ± 0.73) and 3: O₃-ischemia group (3.51

± 0.90). Between the groups 2 and 4 any statistical differences were obtained, the same is observed between groups 1 and 3.

A significant decrease of the glomerular filtration is also obtained (Fig. 2) measured by means of plasmatic clearance of inulin in the groups 2: ischemia (0.32 ± 0.26) and 4: O₂-ischemia (0.44 ± 0.20) with respect to 1: control group (0.60 ± 0.20) and 3: O₃-ischemia group (0.72 ± 0.35). Between the groups 2 and 4 no statistical differences were obtained, the same is observed between groups 1 and 3.

In the same way the morphological alterations found (cortical-medullar hemorrhage, mitochondria tumefaction of the tubular epithelium, tubular cells necrosis and convoluted tubules dilatation) were present in 88 and 100 % of the animals in the groups 2 and 4, respectively, and in only 30 % of group 3.

The induction of controlled stress conditions to cardiac cells produces paradoxically positive cell responses, while prolonged ischemic stress leads to irreversible cardiac cell injury. Controlled ischemic preconditioning promotes an adaptative mechanism that results in cell protection to a subsequent sustained ischemic condition, as proved at experimental and clinical levels.¹⁵⁻¹⁷

Oxidative preconditioning with ozone could prepare the host to face physio-pathological conditions mediated by oxygen reactive species, like it is present during the ischemia-reperfusion process. It is known that in an ischemic process an activation of the antioxidant defense system can help the preservation of the organ.^{3,8,18} For that reason, the authors can consider that ozone could preserve the cellular integrity through the control of processes which generate oxygen reactive species, as has been demonstrated in several papers.^{9,10}

CONCLUSIONS

The ozone preconditioning is able to protect the renal function and the kidney cells of animals submitted to warm ischemia. The ozone protective effect on ischemia-reperfusion damage might be attributed to the upregulation of the expression of antioxidant systems. This finding suggests novel therapeutic approaches to tissue damage in kidney transplantation.

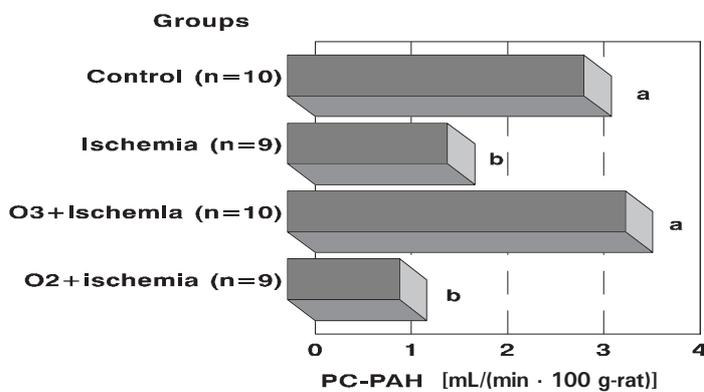


Fig. 1. Plasmatic clearances of *p*-amino-hippurate (PC-PAH) in the different groups of treatment

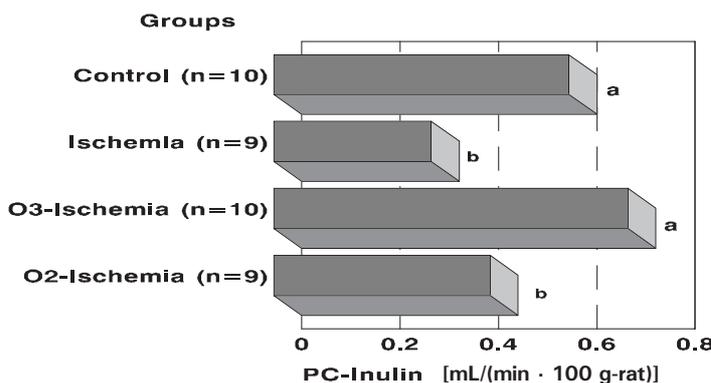


Fig. 2. Plasmatic clearances of inulin (PC-inulin) in the different groups of treatment.

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