THE EFFECT OF NITROPRUSSIDE ON THE RENAL ISCHAEMIA-REPERFUSION INJURY

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Abstract: ***Nitric oxide (NO) is implicated as an important mediator of, or intermediary in, inflammation, immunity and neurotransmission. In the kidney, NO is involved in haemodynamic regulation, control of vascular tone and tubular function. Formation of oxygen-derived free radicals (OFR) have been documented in renal ischaemia-reperfusion before. In this study, we investigated the significance of NO formation, by the addition of nitroprusside (0.2 mm) as an NO donor, in the ischaemia-reperfused (IR) kidneys. Induction of IR significantly reduced renal function compared to controls. In this study, nitroprusside showed beneficial effects on renal morphology although did not alter renal function over a short period of time.***

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Key Words: ***Nitric oxide, kidney, nitroprusside, ischaemia-reperfusion***

INTRODUCTION

The endothelium-derived relaxing factor, nitric oxide (NO), is a potent endogenous vasodilator which has been found to have several fundamental roles in the biology of higher organisms (1). In the kidney, NO is involved in maintenance of basal vascular tone and inhibition of contraction and proliferation of glomerular mesangial cells (2-4). The nitric oxide synthases (NOS) have been described in the kidney (1,5): constitutive NOS (cNOS) have been identified in macula densa cells and in the inner medullary collecting duct, and inducible NOS (iNOS) identified in the rat proximal tubule and renal medullary collecting duct.

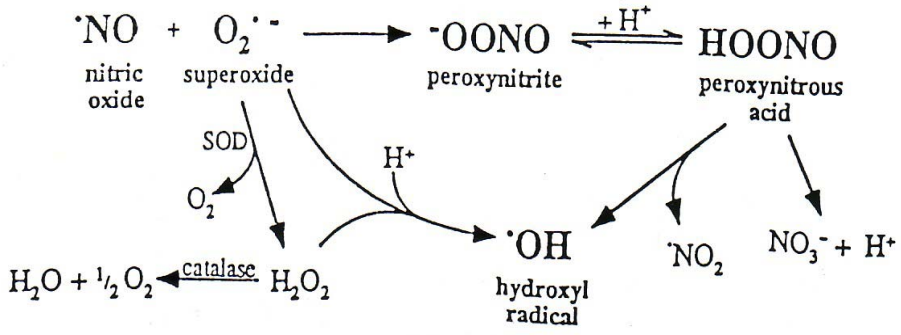

Fig.1. The production and reactions of NO/OFR/$ONOO^-$

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In chemical system, NO has high reactivity with the superoxide radical (O^-_2) and yeilds the more stable peroxynitrite anion ($ONOO^-$), an injurious oxidant molecule (6-7) which finally can decompose to $OH^·$ and NO_2 or undergoes a molecular rearrangement to yield nitrate, NO_3. Since NO and O_2 can react together, they can modulate each others activity (Fig. 1).

Reperfusion injury is believed to represent an important fact of diseases initiated by ischaemia, including organ storage and transplantation (8-9). GFR have been implicated as mediators of ischaemia-reperfusion injury (10-11).

We have previously used electron paramagnetic resonance (EPR) spectroscopy to detect and quantitate formation of the OH radicals in the isolated ischaemic-reperfused rat kidney (12).

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MATERIALS AND METHODS

Nitroprusside was from Sigma chemical company. All other chemicals used were of analytical grade. Male Sprague-Dawley rats weighing 250-350 g with free access to standard food and water were used in all experiments.

The right kidneys were isolated and perfused on bench. Ischaemia was induced by clamping off one arm of a Y piece which stopped the perfusion but allowed the perfusate to flow over the kidney. Renal function is monitored throughout in all groups by inulin clearance (GFR).

Samples of perfusate were collected at different times and rapidly frozen in liquid nitrogen.

The experimental groups were as follow:

1- Control kidneys without supplementation or intervention;

2- Ischaemia -reperfusion: 20 min ischaemia followed by 20 min reperfusion;

3- Nitroprusside (0.2 mM) with no ischaemia;

4- Nitroprusside -ischaemia-reperfusion.

After perfusion, each kidney was rapidly fixed with 4% paraformaldehyde for histological assays. Sections were prepared from kidneys using routine histological

methods. Paraffin sections (3-4 μm) were stained with haematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) for light microscopy (15).

RESULTS

In the present study, induction of ischaemia-reperfusion significantly reduced renal function compared to normoxic control kidneys. Addition of 0.2 mM nitroprusside did not significantly change renal function in either ischaemia-reperfused or normoxic control kidneys. Figure 2A is from control tissues. Sections from these kidneys had normal histology. Glomeruli were normal and tubular lumina was patent. Cellular vacuolation was seen which assumed to be as a result of perfusion.

Figure 2B and 2C demostrate the differences in damage between ischaemia-reperfusion alone (2B) and nitroprusside treatment of ischaemia-reperfused kidneys. The zones of the kidney demonstrated here are the cortico-medullary plus outer medullary zones. In the ischaemia-reperfused group, tubules show extensive tubular casts. The heavy build up of debris and cast material in the cortico-medullary and outer medullary zones in the ischaemia-reperfused tissues are greatly reduced with nitroprusside treatment.

With higher magnifications proteinaceous cast material is found in tubular lumina of ischaemia-reperfused tissues, and apoptotic cells (arrows) can be identified (3A). In 3B, although proximal tubules are relatively normal (slight vacuolation), the distal tubules (arrowed) show cell swelling, intracellular vacuolation, and continuing evidence of damage.

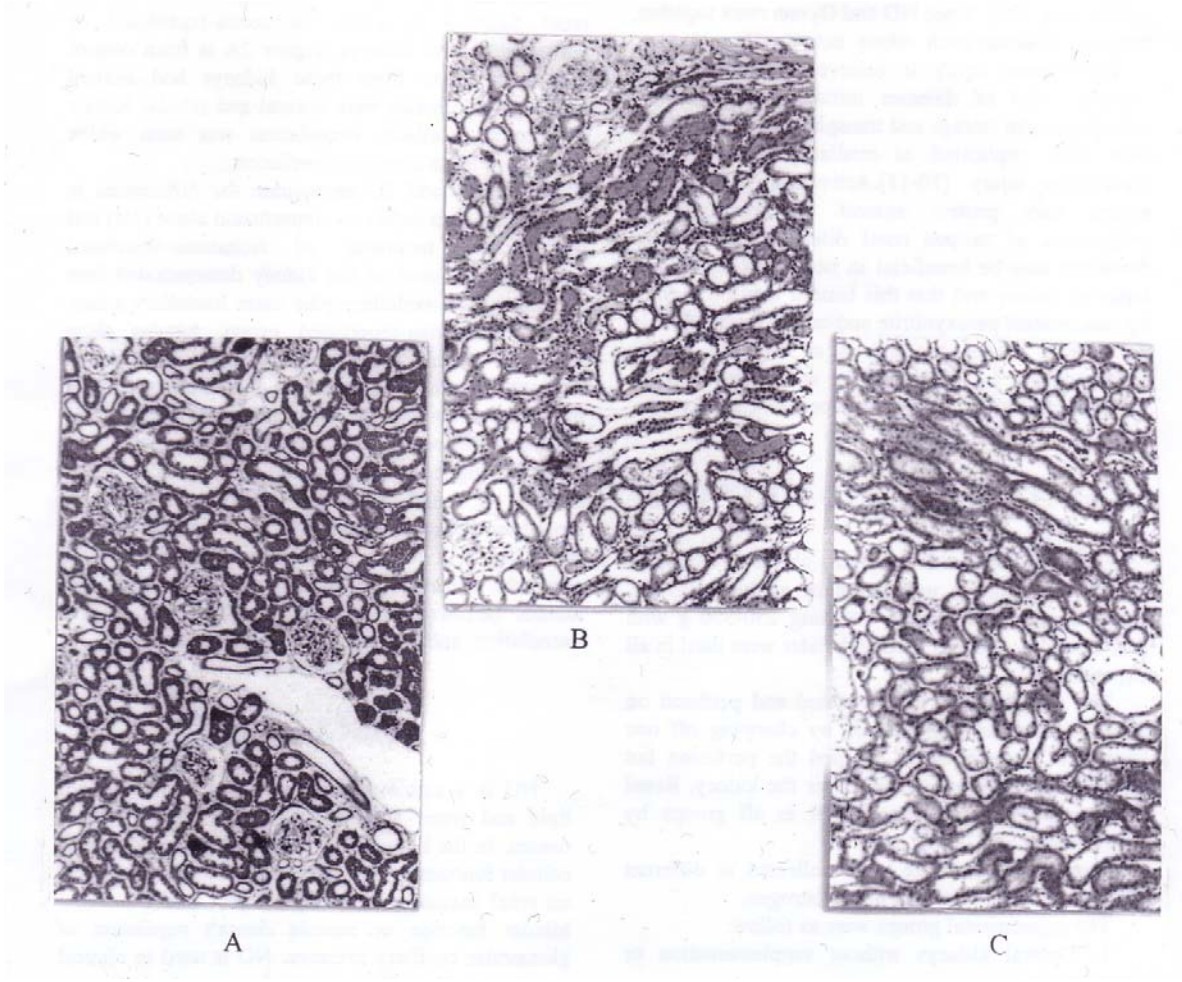
DISCUSSION

NO is a stable paramagnetic gas which is both lipid and water soluble and diffuses freely within tissues. In the body, NO is an important regulator of cellular function and in the kidney, $^\cdot$NO exerts effects on renal haemodynamics by a direct effect on renal tubular function or macula densa's regulation of glomerular capillary pressure. NO is used in clinical practice such as in severe pulmonary hypertension where it relaxes smooth muscles and improves perfusion of alveoli. Use of NO is not without problems especially in high dose. Being a free radical, NO is a highly reactive and short-lived

compound and can combine to superoxide radical to form peroxynitrite , an oxidant of higher toxicity than its precursors. Chemical studies suggest that the balance between superoxide and NO determines the extent of formation of peroxynitrite. This study aimed to explore the balance between NO and OFR on renal function and morphology in ischaemia-reperfusion injury. The relationship between renal stucture and function in free radical generated renal injury has been studied by us and others before (16-17). In the present model, we have compared alterations in functional parameters with pathology found in the nephron segments.

Nitroprusside was used to enhance NO concentration while 20 min ischaemia plus 20 min reperfusion were used to induce oxidative stress in the kidneys. The differences in histological features between IR and nitroprusside treated kidneys suggest a beneficial role for NO formation in this model, whilst cellular blebs and casts can be seen at higher magnifications in IR kidneys, the extent of injury is lower by treatment.

In this study, although there was no significant improvement in function with nitroprusside treatment, histopathology indicates that some structural improvement is found with nitroprusside, compared with ischaemia-reperfusion alone.



A **B** **C**

Fig. 2. Histological features of control (2A), ischaemia-reperfusion (2B) and nitroprusside-treated (2C) kidneys. Magnifications: A, B and C x 150.

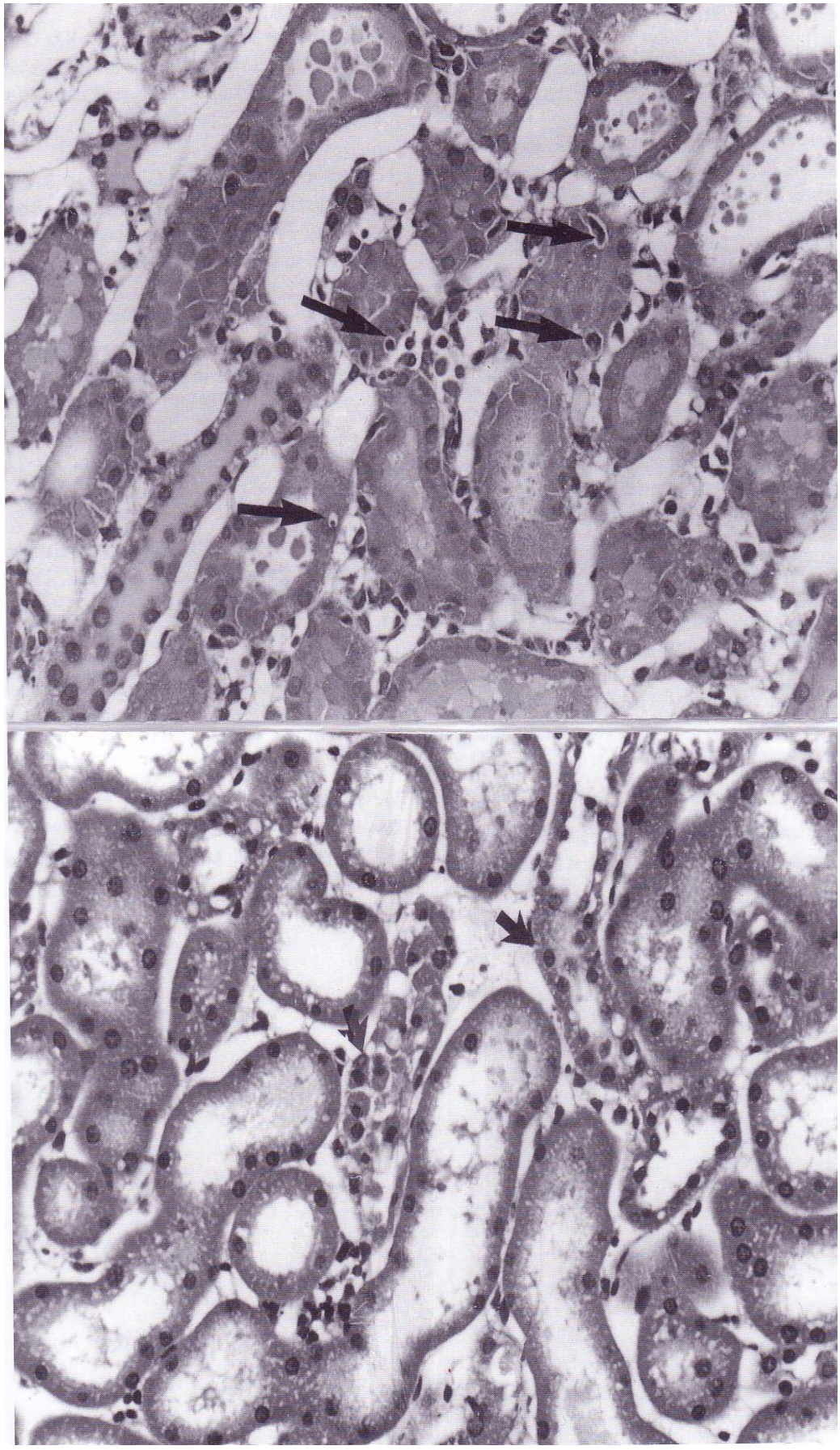


Fig. 3. High power photomicrographs from ischaemia-reperfused (3A) and nitroprusside treated (3B) tissues. Magnifications: A and B x 600

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