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The beneficial effect of 2′-deoxycoformycin in renal ischemia-reperfusion is mediated both by preservation of tissue ATP and inhibition of lipid peroxidation

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Abstract Renal ischemia injures the renal tubular cell by disrupting the vital cellular metabolic machinery. Further cell damage is caused when the blood flow is restored by oxygen free radicals that are generated from xanthine oxidase. Oxygen radicals cause lipid peroxidation of cell and organelle membranes, disrupting the structural integrity and capacity for cell transport and energy metabolism. In the present study, the possible therapeutic usefulness of the adenosine deaminase inhibitor, 2′-deoxycoformycin (DCF), during renal ischemia and reperfusion injury was investigated. The effects of DCF on renal malondialdehyde (MDA) and ATP levels were studied after 45 min ischemia and 15 min subsequent reperfusion in rat kidneys. MDA levels remained unchanged during ischemia, but increased after the subsequent reperfusion. DCF pretreatment (2.0 mg/kg i.m.) decreased MDA and increased ATP levels during the ischemia-reperfusion period. DCF exerts a dual protective action by facilitating purine salvage for ATP synthesis and inhibiting oxygen radical-induced lipid peroxidation. These results suggest that DCF therapy could be beneficial in the treatment of ischemia-reperfusion renal injuries.

Key words. Adenosine triphosphate · 2′-Deoxycoformycin · Kidney · Ischemia/reperfusion · Malondialdehyde

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Introduction

Acute renal failure (ARF) resulting from ischemia is of great clinical importance because of its frequent occurrence and high mortality [1]. Renal ischemia results in a rapid decrease in tissue ATP and a rise in the ATP degradation products adenosine, inosine, and hypoxanthine [2]. The loss of adenosine from cells due to degradation during ischemia is believed to result in depletion of adenine nucleotides, which injure the renal tubular cell by disrupting the vital cellular metabolic machinery. Another consequence of the accumulation of hypoxanthine during renal ischemia is the generation of highly reactive oxygen free radicals during vascular reperfusion [3–5]. Molecular oxygen permits xanthine oxidase (XO) to oxidize hypoxanthine and xanthine to uric acid, leading to increased generation of hydrogen peroxide and superoxide as by products [6]. Hydrogen peroxide in the presence of Fe^{2+} can be rapidly converted to hydroxyl radicals via the Haber-Weiss reaction [7]. These free radicals can then attack a wide variety of cellular components, including DNA, proteins, and membrane lipids. The latter may result in lipid peroxidation causing damage of critical cell and organelle membrane functions, thereby exacerbating ischemic injury during the reperfusion period [8].

Research efforts designed to prevent or ameliorate ischemia-reperfusion injury have focused on the pharmocological inhibition of free radical injury. Many antioxidants with different mechanisms of action have been widely investigated [8]. Recently, our laboratory reported that an adenosine analogue and an adenosine deaminase (ADA) inhibitor may serve as protective agents in ischemia-reperfusion injury [9–11]. ADA is an ubiquitous enzyme that catalyzes the deamination of adenosine to inosine. Physiologically, the enzyme can either function in a salvage pathway or be involved in the metabolic degradation of adenosine [12]. Therefore, inhibition of ADA may result in either a decrease in the substrate flow for free radical generation or a reduction of adenosine catabolism which facilitates purine salvage for ATP synthesis. To test the hypothesis, we investigated the effect of an ADA in-

hibitor, 2′-deoxycoformycin (DCF), on the concentrations of ATP and malondialdehyde (MDA) during the ischemic and postischemic reperfusion periods in rat kidneys.

Materials and methods

The study was performed using male Sprague-Dawley rats weighting 175–250 g. After the rats were anesthetized with pentobarbital sodium (60 mg/kg i.p.), the kidneys were exposed through a midline abdominal incision. Kidney ischemia and reperfusion was performed as described previously [13]. Briefly, the left and right renal arteries were isolated and totally occluded with a smooth vascular clamp for 45 min. To minimize fluid loss during this period, all exposed tissues were moistened with Ringer's lactate solution and the abdominal incision was temporarily closed with clamps. The right kidney was then removed and the left kidney was reperfused for 15 min before removal. The left kidney was observed after unclamping, and, if visible evidence of restored blood flow was not obtained within 1 min, the animal was excluded from the study. Rats were sacrificed thereafter. Kidneys were frozen with liquid nitrogen and stored at –70°C prior to analysis. A total of 50 kidneys from 30 animals were divided into five groups as outlined below.

Group 1 (n=10) included right kidneys of the rats experiencing no ischemia and no treatment and served as the control group. *Group 2* (n=10) included right kidneys of the rats experiencing 45 min ischemia but no treatment and served as the ischemia group. *Group 3* (n=10) included left kidneys of the rats experiencing 45 min ischemia and 15 min of reperfusion but no treatment and served as the reperfusion group. *Group 4* (n=10) included right kidneys of rats pretreated with DCF experiencing 45 min ischemia and served as the treated ischemic group. DCF (2.0 mg/kg) was injected i.m. 24 h before the experiments. *Group 5* (n=10) included left kidneys of rats pretreated with DCF experiencing 45 min ischemia and 15 min reperfusion and served as the treated reperfusion group. Treatment was the same as described above.

Adenosine, thiobarbituric acid, and 1,1,3,3-tetraetoxypropane were purchased from Sigma (St. Louis, Mo., USA). DCF was generously donated by Warner Lamber (Ann Arbor, Mich., USA). Glucose-6-phosphate dehydrogenase (G6P-DH), hexokinase, and NADP were purchased from Boehringer Mannheim (Germany).

MDA assay

The tissue MDA content was determined by the thiobarbituric assay as described by Mihara and Uchiyama [14]. The breakdown product of 1,1,3,3 tetraetoxypropane was used as standard and the results were expressed as nanomoles per gram of tissue.

Tissue ATP content

The amount of kidney ATP was determined by the presence of hexokinase and G6P-DH in coupled reactions [15].

$$\text{ATP} + \text{Glucose} + Mg^{2+} \xrightarrow{\text{G6P-DH}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{Hexokinase}} \text{Gluconolactone-6-phosphate} + \text{NADPH} + H^{+}$$

The end-product NADPH produced in the reaction was monitored spectrophotometrically (Milton Roy Spectronic 3000) at 340 nm. The amount of ATP in the sample was expressed as micromoles ATP per gram of tissue.

ADA enzyme activity

The ADA activity was measured using the method of Goldberg [16], and the protein content by the Lowry method. The decrease in adenosine was monitored by a decline in absorbance at 265 nm. A millimolar extinction coefficient of 12.3 was used to convert absorbance decreases to millimoles of adenosine deaminated. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the conversion of 1 nmol of adenosine to inosine in 1 min under the assay conditions. The results were expressed as nanomoles per minute per milligram of protein.

Statistical analysis

All values presented in the Fig. 1 and Table 1 are expressed as mean ±SD. Groups were compared by analysis of variance and Student's t-test with Bonferroni's modification. Differences were considered significant at $P<0.05$.

Results

Renal ATP content

Following 45 min of ischemia, total ATP fell to 29% of the control level in the ischemia group and 36% in the treated ischemia group. After 15 min reperfusion, the total ATP recovery was 74% and 110% of the control level in the reperfusion and treated reperfusion groups, respectively. The increase in the ATP level after DCF treatment was significant in the reperfusion period, but not in the ischemia period (Fig.1A).

Renal MDA content

After 45 min of renal ischemia, the MDA content was 76.6±22.9 nmol/g tissue, which was not significantly different from the value of 74.2±11.8 nmol/g tissue (n=10) obtained from normal kidneys. However, the MDA content significantly increased to 128.4±26.2 nmol/g tissue (n=10) after 15 min of reperfusion. DCF treatment decreased the MDA levels significantly, both during the ischemia and the reperfusion periods to 48.4± 16.3 nmol/g tissue (n=10) and 48.8±13.8 nmol/g tissue (n=10), respectively (Fig. 1B).

Renal ADA enzyme activity

The effect of DCF pretreatment on renal ADA activity is shown in Table 1: 24 h after DCF treatment, the reduc-

Table 1 Adenosine deaminase (*ADA*) activity in kidney tissue samples (*DCF* 2′-deoxycoformycin)

Groups (n=10)	ADA (nmol/min per mg protein) (mean±SD)
Control	9.60±1.05
Ischemia	10.98±1.39
Ischemia+reperfusion	9.86±0.90
Ischemia+DCF	4.40±0.49*
Ischemia+reperfusion+DCF	2.73±0.58**

*$P<0.001$ vs. ischemia
**$P<0.001$ vs. ischemia+reperfusion

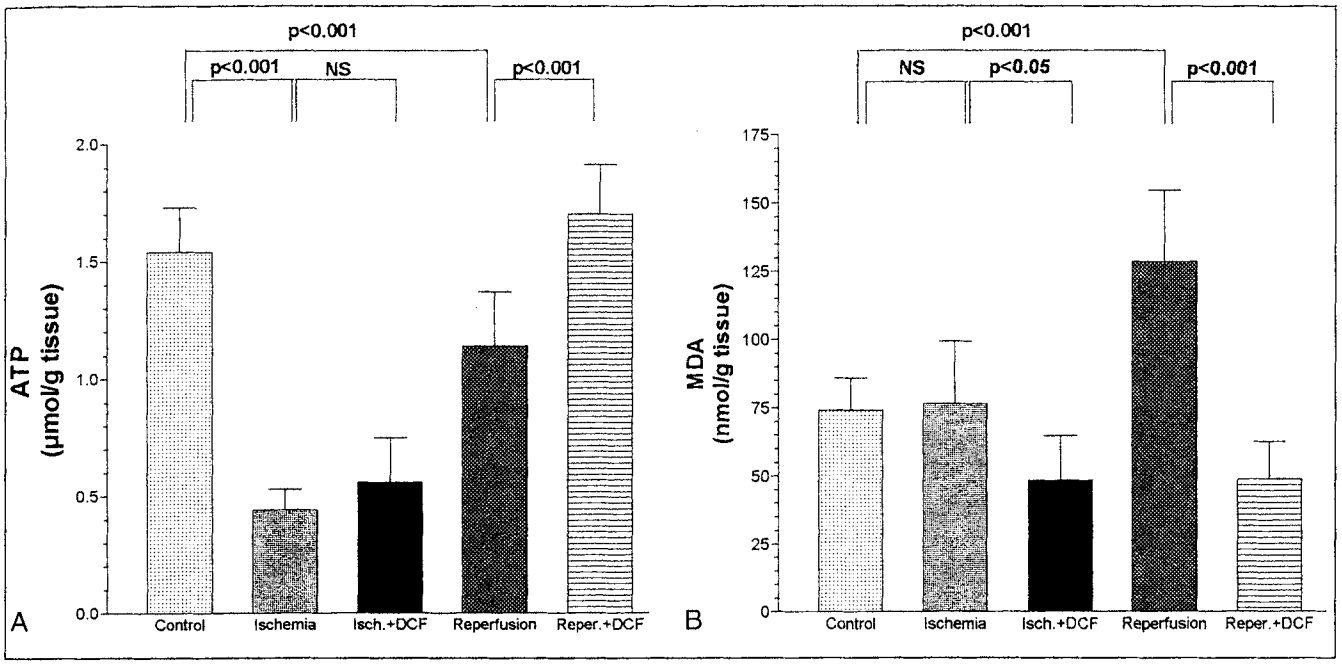

Fig. 1 Effect of 2′-deoxycoformycin (*DCF*), an inhibitor of adenosine deaminase, on renal ATP (**A**) and malondialdehyde (*MDA*) (**B**) levels during 45 min ischemia and 15 min reperfusion. Each column represents the mean ±SD of 10 kidneys

tion in ADA activity was 60% and 72% in the ischemic and reperfused kidneys, respectively.

Discussion

In addition to the lack of blood flow and oxygen delivery, the restoration of blood flow has also been reported to contribute to tubular cell damage due to the generation of free radicals [8]. Therefore, experimental approaches to the amelioration of renal ischemia-reperfusion injury have mainly focused on the prevention of free radical-induced cellular damage and the use of exogenous substrates to maintain the level of high-energy phosphometabolites and total adenine nucleotide content in the cells. Our study shows that these two therapeutic approaches can be achieved by the ADA inhibitor, DCF. We demonstrated that DCF, when used as a pretreatment, increased ATP and decreased the lipid peroxide levels during the ischemia-reperfusion period. To the best of our knowledge, this is the first report that demonstrates the inhibitory effect of DCF on cellular lipid peroxidation in renal ischemia-reperfusion injury.

The production of oxygen free radicals during ischemia and reperfusion of the kidney has been implicated as a major pathophysiological component of ARF [3–5, 8, 17]. Generated radicals are assumed to cause peroxidation of polyunsaturated fatty acids which may cause substantial loss in the luminal, basolateral, and mitochondrial inner membranes of renal tubular cells [18]. Evidence that this type of injury occurs in the kidney during the postischemic reperfusion rests primarily on measurement of free radical-mediated lipid peroxidation products, including MDA, ethane, and Schiff bases [3, 13, 17, 19, 20]. Similar to previous reports [3, 13, 19], the present study has also shown that a significant increase in kidney lipid peroxide, MDA, could be observed only after a 15 min postischemic reperfusion period. We have also demonstrated that 45 min renal ischemia led to a depletion of renal ATP, and the recovery of ATP was incomplete after the 15 min reperfusion period. During ischemia, high-energy phosphate supplies are depleted, and hypoxanthine derived from degradation of ATP serves as a substrate for superoxide radical generation by XO. Thus, the loss of cellular ATP and oxygen free radical production seem to be strongly related phenomena.

DCF is a competitive tight-binding inhibitor of ADA that catalyzes the deamination of adenosine to inosine [21]. Consistent with previous reports demonstrating sustained inhibition of ADA activity lasting for 72 h after 0.5–5.0 mg/kg i.m. DCF treatment [22, 23], in our study a 2.0 mg/kg dose given 24 h prior to experiments caused a 60% and 72% reduction in ADA activity in the ischemic and reperfused kidneys, respectively (Table 1). In DCF-treated rats, the tissue content of adenosine, which serves, as a precursor for ATP restoration via adenosine kinase, was reported to be increased [24, 25]. Thus, ADA inhibition not only augments the residual nucleotide pool but also increases endogenous precursors for the resynthesis of renal ATP [23–25]. It is well known that ATP is the most-important constituent of the cell and its cellular level is of critical importance for the viability of an organ. Since DCF maintains a higher concentration of ATP during ischemia-reperfusion, as was also shown in the present study, this may, at least in part, explain its efficiency in preserving the viability of tissues subjected to ischemia-reperfusion.

However, it has been speculated that DCF might have other mechanisms of action, such as the inhibition of free radical generation by decreasing the substrate flow through XO [22, 23, 26]. Our finding that ADA inhibition by DCF decreased MDA levels not only during the reperfusion but also during the ischemic period confirms the operation of this mechanism in vivo. However, it is also possible that a systemic increase in the concentration of adenosine, due to the inhibition of ADA, may also inhibit lipid peroxidation. In support, it has recently been reported from our laboratory that pretreatment with an adenosine analogue, 2-choloroadenosine, significantly suppressed cerebral MDA levels during postischemic reperfusion [9]. Furthermore, it has also been reported that adenosine inhibits neutrophil-mediated production of superoxide [27], as well as the cellular calcium influx suggested to be involved in the generation of free radicals via conversion of xanthine dehydrogenase to XO [28].

Sumpio et al. [29] have shown that the administration of ATP with magnesium chloride following ischemia results in increased tissue ATP levels and improved renal function. However, the administration of adenine nucleotides could also be harmful. If the administered ATP, like endogenous ATP, were degraded during ischemia, the increased amount of hypoxanthine could result in an increased generation of oxygen free radicals during the reperfusion period. Therefore, it has been previously suggested that ATP together with free radical scavengers could provide a more-complete protection than either alone [3]. We herein report that DCF could cover these two therapeutic approaches in ischemia-reperfusion injury. The efficiency of this combined therapy was also verified by histological examination showing less structural damage in glomerular cells than that observed in the ischemia-reperfusion group (unpublished results). Previously, DCF was found to be useful in the prevention of ischemic brain and myocardial damage via the common mechanism of preserving cellular ATP content [10, 25, 26, 30]. The protective action of DCF has been further demonstrated in renal pathological conditions. Stromski et al. [23] have shown that inhibition of ADA during a 45 min period of renal ischemia reduces the metabolic consequences and functional severity of the injury to that seen after a 30 min insult without treatment. Furthermore, puromycin aminonucleoside nephrotoxicity was totally inhibited in rat glomerular epithelial cells in vivo and in vitro using DCF [22]. Our study extends these observations and shows that the beneficial effect of DCF is not only due to ATP preservation but also to inhibition of lipid peroxidation.

In conclusion, DCF exerts a dual protective action. The reduction of adenosine catabolism facilitates purine salvage for ATP resynthesis. In addition, by decreasing the substrate flow through XO, the generation of oxygen free radicals will be limited during the reperfusion period. These results suggest that DCF therapy could be beneficial in the treatment of renal ischemia-reperfusion injuries.

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