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SHORT COMMUNICATION

Effect of Trimetazidine on Xanthine Oxidoreductase Expression in Rat Kidney with Ischemia–Reperfusion Injury

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Ischemia/reperfusion (I/R) injury is often responsible for delayed graft function after transplantation. Trimetazidine (TMZ) is an anti-ischemic and antioxidant agent used to protect grafts from I/R injury. With the supply of molecular oxygen upon reperfusion of ischemic tissues, xanthine oxidoreductase (XOR) metabolizes xanthine and hypoxanthine to uric acid and free radicals are generated.

The aim of the study was to examine the effect of TMZ on XOR expression in rat kidney with I/R injury. The study was carried out on Wistar rats divided into two groups: animals treated with TMZ and control group receiving placebo. TMZ (10 mg/kg/day) was administered for 30 days.

There were no significant differences in XOR expression in kidneys without ischemia between rats treated with TMZ and control group, whereas the XOR expression in kidneys with ischemia was significantly decreased in rats treated with TMZ as compared with control animals. The XOR expression in ischemic kidney was significantly lower in comparison with kidney without ischemia in the group treated with TMZ.

We suggest that the decrease in xanthine oxidoreductase expression is one of the beneficial mechanisms of TMZ on I/R injury, preventing the degradation of purine nucleotides during the oxidation of hypoxanthine to xanthine and uric acid and formation of free radicals. © 2008 IMSS. Published by Elsevier Inc.

Key Words: Trimetazidine, Xanthine oxidoreductase, Rats, Kidney, Ischemia–reperfusion.

Introduction

Xanthine oxidoreductase (XOR) catalyzes the reactions of purine catabolism by oxidizing hypoxanthine to xanthine and further to uric acid. This enzyme exists in two forms that use different electron acceptors: xanthine dehydrogenase (XDH, EC 1.1.1.204), which transfers electrons to NAD^+ producing NADH, and xanthine oxidase (XO, EC 1.1.3.22), which transfers electrons to O_2 producing reactive oxygen species (ROS). The cataboism of adenine nucleotides results in an accumulation of hypoxanthine in ischemic cells. Ischemia is also associated with proteolytic conversion of XDH to XO (1,2). Along with the supply of molecular oxygen upon reperfusion of ischemic tissues, xanthine oxidase metabolizes xanthine and hypoxanthine to uric acid and free radicals are generated. Generation of ROS during reperfusion may overcome the capacity of physiological scavengers. Generated ROS are implicated in both tissue structural damage and cell signaling interference (3,4).

Trimetazidine is an anti-ischemic agent. Its anti-ischemic effects have been experimentally assessed in various models including cell cultures (5), isolated and perfused organs (6) as well as *in vivo* (7). TMZ acts mostly on mitochondria by

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restoring ATP synthesis. Moreover, TMZ inhibits oxygen consumption in mitochondria during ischemia (8) reducing the excessive release of oxygen free radicals (9), inhibits oxidation of fatty acids by inhibition of 3-ketoacyl coenzyme A thiolase (10), increases glucose metabolism, limits intracellular acidosis, protects ATP stores, reduces membrane lipid peroxidation and inhibits neutrophil infiltration after ischemia and reperfusion (6). Moreover, trimetazidine enhanced the myocardial recovery during reperfusion in diabetic and non-diabetic rat hearts (11).

The precise mechanisms of TMZ action in ischemic tissues are still not fully understood. The studies on TMZ influence on XOR expression may be helpful in better understanding of TMZ influence on cell metabolism during ischemia. Therefore, the aim of this study was to examine the effect of trimetazidine on XOR expression in rat kidney after ischemia/reperfusion (I/R) as well as the relations between XOR expression and oxypurine, nucleotide and nucleoside concentrations.

Materials and Methods

Experimental Protocol

The study was approved by the local ethics committee. Male Wistar rats weighing 300–350 g were used in this experiment and were allowed to acclimatize for a minimum of 10 days prior to the study. The rats were housed in the room maintained at 21 ± 1°C with 12 h light/dark cycle with the light cycle beginning at 6:00 A.M. Animals were divided into two groups: animals treated with TMZ (n = 11) and control group receiving placebo (n = 8). The aqueous solution of TMZ (10 mg/kg/day) was administered by gavage twice a day at 8:00 A.M. and 6:00 P.M. for 30 days. Experimental procedures were performed as described (12).

Preparation of Tissue Samples

Preparation of samples for purine determinations was done according to the modified method of Smolenski et al. (12). Analyses of 16 nucleotides, nucleosides, and oxypurines: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine (Ado), guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), guanosine (Guo), inosine monophosphate (IMP), inosine (Ino), hypoxanthine (Hyp), xanthine (Xan), uric acid (UA), uridine (Urd), nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate (NADP) were performed as described earlier (13) with Hewlett–Packard (now Agilent, Waldbronn, Germany) Series 1050/1100 chromatography system. Total adenine nucleotides (TAN = ATP + ADP + AMP) were calculated.

Xanthine oxidoreductase expression was determined using a reverse transcription-coupled competitive quantitative polymerase chain reaction assay as previously described (14).

Statistical Analysis

Mann-Whitney test was performed to compare XOR expression between groups. The significance of the differences between the parameters in the left (clamped, with ischemia) and the right (non-clamped, without ischemia) kidney was assessed using Wilcoxon test. Correlations between XOR expression and purine concentrations were assessed with Spearman rank correlation coefficient (Rs).

Results

There were no significant differences in XOR expression in kidneys without ischemia between the group of rats treated with TMZ and control group, whereas the XOR expression in kidneys with ischemia was significantly decreased in rats treated with TMZ as compared with control animals (Figure 1). The XOR expression in ischemic kidney was significantly lower in comparison with kidney without ischemia in group treated with TMZ (median 0.9 vs. 1.37, p = 0.017). There was no significant difference in XOR expression between ischemic and non-ischemic kidneys in control group.

In ischemic kidneys from rats treated with TMZ we observed statistically significant negative correlations between XOR expression and AMP (Rs = −0.74, p <0.01), TAN (Rs = −0.62, p <0.05), GMP (Rs = −0.77, p <0.01), NADP (Rs = −0.60, p <0.05) as well as Ado (Rs = −0.62, p <0.05) concentrations. Such correlations were not observed in non-ischemic kidneys, as well as in kidneys in control animals.

Discussion

In the present study we evaluated the effect of TMZ on the xanthine oxidoreductase expression in rat kidney during reperfusion. Previous reports suggest the involvement of XOR in I/R injuries. In 1981, Granger et al. hypothesized that XOR-generated ROS cause ischemic bowel injury due to ATP catabolism during hypoxia and increased electron acceptor availability on reperfusion (15). These observations have been supported by studies from other authors (16,17).

In accordance with this evidence supporting XOR involvement in I/R injury are the results of Weinbroum et al. who found that XOR activity increases >100-fold in postischemic lung lavage fluid (17). Elevated XOR activity also occurs on reperfusion of human aortic endothelial cells, with an 8-fold elevation in UA production (18). Moreover, XOR has been localized to the endothelial cells of all tissues using immunohistochemistry. In addition to this, circulating XOR has been demonstrated in human, rat and

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