Mechanism of Cardioprotective Effect of Orotic Acid

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Summary. The pyrimidine base, orotic acid (OA), markedly improves the function of recently infarcted hearts subjected to global ischemia. The mechanism of cardiac action of OA is unclear, but it has been proposed that OA acts by correcting a relative deficiency of nucleotide precursors required for RNA synthesis in the stressed myocardium or by improving myocardial energy supply. The aim of this study was to investigate the mechanism of action of OA by (1) determining whether a high dose of OA can raise the concentration of pyrimidine metabolites in plasma, liver, and heart; (2) examining the effects of OA on adenine nucleotide (AN) concentrations in normal and infarcted hearts, before and after global ischemia; and (3) determining the effect of uridine, an important metabolite of OA, on myocardial energy metabolism. Three studies were performed: (1) The time course of changes in tissue and plasma concentrations of pyrimidine compounds was examined in unoperated rats after the administration of 100 mg/kg OA. (2) Rats were given OA (30 mg/kg/d) for 2 days after experimental infarction, and tissue and plasma pyrimidine concentrations were examined; the hearts were removed for perfusion in the isolated working rat heart model (37°C), subjected to 30 minutes of global ischemia, and recovery of function was assessed. AN content was assessed in the noninfarcted myocardium before and after ischemia. Isolated hearts were subjected to 30 minutes of hypoxic perfusion and the effect of adding 17 μM uridine to the perfusate was examined. Study 1 showed that OA administration produced an increase in hepatic uridine and cytidine, followed by increased plasma uridine and cytidine (cytidine, +55%, P < 0.001; uridine, +124%, P = 0.011). Myocardial uracil nucleotides increased temporarily after 4 hours (+21%, P < 0.01). In infarcted hearts after 2 days of OA administration, there were no significant changes in myocardial uracil or cytosine nucleotides or total RNA. Infarction significantly reduced functional recovery after global ischemia (sham = 62%; infarct = 26% of preischemic level; P < 0.05). OA improved the recovery of preischemic function by 133% (P < 0.05) in infarcted, but not sham-operated, hearts. Preischemic ATP and total adenine nucleotides (TAN) were decreased in the surviving myocardium of infarcted hearts (ATP reduced from 21.7 ± 0.8 to 14.7 ± 0.7 μmol/g dry wt, P < 0.001; TAN decreased from 30.3 ± 0.8 to 22.4 ± 1.1 μmol/g dry wt, P < 0.001). OA treatment prevented these reductions. Study 3 showed that uridine improved myocardial ATP and TAN levels, and decreased purine loss in hypoxic hearts. The increased AN levels were accompanied by evidence of enhancement of anaerobic glycolysis. We conclude: (1) That OA acts on the heart via the liver by increas-
nously disappears rapidly from the bloodstream and accumulates primarily in the liver [10], where it is rapidly converted to uracil nucleotides [11]. The liver is the major source of the salvageable pyrimidines in the plasma, uridine and cytidine, which are derived from liver pyrimidine nucleotides [10,12,13].

In the heart, the rate of salvage of circulating pyrimidine nucleosides is one to two orders of magnitude higher than the rate of pyrimidine de novo synthesis [14]. Therefore radiolabel from administered OA might be expected to be found in the heart, albeit diluted. If large doses of OA were administered, however, one might expect a mass action effect to raise, in turn, liver pyrimidine nucleotides, plasma cytidine, and uridine, which could increase myocardial CTP and UTP concentrations. At high doses (1% OA diet, equivalent to 500 mg/kg/d) OA increases the concentration of liver UTP eight-fold after 24 hours [15].

Monks and colleagues [16] found that infusion of ammonium chloride into isolated, perfused livers, which accelerates pyrimidine de novo synthesis, resulted in an increased concentration of liver UTP and produced a large increase in uridine release. An increased concentration of circulating pyrimidine nucleoside may augment the size of the myocardial pyrimidine nucleotide pool [14,17]. Perfusion of isolated rat hearts with high concentrations (50 μM) of cytidine or uridine substantially raised myocardial pyrimidine nucleotide levels within 30 minutes [14,17]. However, it is not known whether OA can increase pyrimidine nucleotides in the heart, nor whether OA reaches the heart directly.

We have taken three approaches to studying the mechanism of action of OA. Firstly, we investigated the metabolic fate of OA, its availability in the plasma, and how it might reach the heart. Secondly, we examined mechanical function and metabolism of OA-treated infarcted hearts before and after global ischemia [21]. Thirdly, we studied the effect of uridine, an important metabolite of OA, on myocardial energy supply [23].

The objectives of these studies were as follows: (1) To determine whether, in normal animals, administration of a high dose of OA could increase concentrations of pyrimidines in plasma, liver, and heart. (2) To determine, in animals with myocardial infarction, whether heart, liver, or plasma pyrimidine contents are increased following OA administration. (3) To examine the effect of OA treatment on function, energy production, and adenine nucleotide metabolism in normal and infarcted hearts before and after global ischemia. (4) To study the effect of uridine in hypoxic hearts.

**Materials and Methods**

**Animals**

Male Sprague-Dawley rats (8–14 weeks old, 250–350 g) were treated according to the Code of Practice for Animal Experimentation of the National Health and Medical Research Council of Australia.

**Surgery and OA administration**

In a group of unoperated animals, the time course of changes in tissue and plasma concentrations of pyrimidine compounds was examined after administering OA (100 mg/kg) by gavage. In a second group of animals with infarction, OA (30 mg/kg/d) was given by injection for 2 days after infarction, and tissue and plasma pyrimidine concentrations, postischemic function, and myocardial adenine nucleotide contents were examined.

**Study 1.** Rats were gavaged with a single dose of 100 mg/kg OA as a suspension of 30 mg/mL in 1% methylcellulose (w/v) and were anesthetized 1, 2, 4, 6, and 8 hours after OA administration for removal of the plasma, liver, and heart. A sample (1.5 mL) of whole blood was removed from the infrarenal vena cava for HPLC analysis (see later). The heart and left lateral lobe of the liver were freeze-clamped in situ in O2-ventilated animals under pentobarbital-induced anesthesia. Control samples of tissues and blood were collected without prior OA injection, representing the zero time point. Blood samples were either heparinized and centrifuged for 10 minutes at 800 × g, or immediately centrifuged for 20 seconds at 13,000 × g, and the plasma was analyzed for pyrimidine content by HPLC. Pyrimidine content did not differ between the two plasma separation methods.

**Study 2.** Rats were anesthetized with a mixture of pentobarbital (20 mg/kg), methohexitone (30 mg/kg), and atropine (0.5 mg/kg), intubated, and ventilated. A thoracotomy was performed through the fourth left intercostal space, and the left coronary artery was ligated. A sham operation was performed identically, except that the ligature was not tied. The thoracotomy was closed and the animals were allowed to recover for 2 days. They were then injected intraperitoneally with either 20 mM sodium OA 3 × daily, (equivalent to 30 mg/kg/d free OA per injection), or an equivalent volume of 20 mM saline. The operations were performed between 9 and 12 am on the first day, with two injections administered on day 1, three on the following day, and the final injection at 9 am on day 3. Six hours after the final injection the animals were anesthetized for removal of blood, heart, and liver samples as described earlier for the time-course study. Only the noninfarcted zone of the infarcted hearts was sampled. In other animals, the intact hearts were removed for perfusion.

**Perfusion**

Function in the isolated, working heart [18] was measured in four groups of rats: infarcted, infarcted OA-treated, sham-operated, and sham-operated OA-treated. The perfusate, a modified Krebs-Henseleit buffer, consisted of (in mM): NaCl, 118; KCl, 4.7; CaCl2, 1.25; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25; and glu-