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Insulin-like growth factor I administration in young rats with acute renal failure

Abstract The outcome of ischemic acute renal failure (IARF) is better in young than adult rats. Insulin-like growth factor I (IGF-I) treatment may increase mortality of adult rats with IARF, probably because of an exaggerated inflammatory response. We report the response to IGF-I therapy in young rats with IARF. Male rats, aged 28±1 days, with IARF were given subcutaneous IGF-I, 50 µg/100 g at 0, 8, and 16 h after reperfusion (IGF) or were untreated (ARF). Sham-operated rats were used as controls. At 2 and 7 days after ischemia, serum urea nitrogen and histological damage score, cell proliferation, apoptosis, neutrophil infiltration, and IGF-I receptor mRNA in kidneys were analyzed. The degree of renal failure, mortality rate, histological damage, cell proliferation, and neutrophil infiltration were not different between IGF-I and ARF rats. Hence, short-term IGF-I treatment did not modify the course of IARF in young rats.

Keywords Acute renal failure · Ischemia · Rat · Insulin-like growth factor-I · Neutrophil · Inflammation

Introduction

Acute renal failure (ARF) is defined as a sudden decline of renal function with resultant accumulation of potentially toxic metabolic substances. The reduction in glomerular filtration rate, together with ARF-induced exacerbation of protein catabolism [1, 2], leads to retention of nitrogenous waste products and, subsequently, to elevated serum levels of creatinine and urea nitrogen (SUN) [3].

Although the incidence of ARF in the pediatric population is about a fifth of that found in adults [3, 4], it remains a significant problem in children. It accounts for 0.2% of pediatric admissions in tertiary referral children’s hospitals [5], and in neonates and small infants the incidence is comparable to that of adults [6, 7, 8, 9]. The introduction of dialysis in the treatment of ARF in the 1950s reduced overall mortality caused by this syndrome from 80% to 25% [10]. Since then, the mortality rate has remained substantially invariable [4, 11, 12] in spite of the multiple technological and pharmacological advancements in the treatment of these patients [3, 13].

Although the etiology of ARF is frequently multifactorial, in newborns and small infants ARF is nearly always secondary to ischemia and hypoxia of kidneys caused by other organ failure [14]. Following the ischemic injury, renal function and kidney histology undergo progressive deterioration over a period ranging from 48 to 72 hours. If the individual survives, a progressive recovery process is initiated, resulting in total or partial restitution of the renal function, depending on the severity of the tissue lesion, on associated factors (malnutrition, nephrotoxic drugs, hemodynamic situation, etc.), and on the available therapeutic measures [15, 16, 17].

Over the last few years, several lines of investigation have been developed to find a suitable therapeutic agent, which should promote cellular growth and differentiation, improve renal hemodynamics, reduce inflammation, and maintain vascular integrity [18]. To this end, diuretics, dopamine, atrial natriuretic factor, calcium channel antagonists, and growth factors have been tested
in different experimental models of ARF. Insulin-like growth factor I (IGF-I) has deserved special attention in the treatment of ARF because of its theoretically useful properties, such as being mitogenic and anti-apoptotic [19], a mediator of the anabolic effects of growth hormone, a stimulator of renal plasma flow and glomerular filtration rate [20], as well as being widely expressed in kidneys [21]. Several experimental reports [1, 22, 23, 24, 25, 26, 27], although not all [28], have suggested that administration of exogenous IGF-I improves the evolution of ARF, accelerating the recovery of renal function, facilitating healing of histological lesions, and reducing the mortality rate. These results have not been confirmed by recent clinical trials in patients undergoing renal hemoperfusion [29] or with established ARF [30]. Moreover, two multicenter studies have also indicated the potentially harmful effect of growth hormone treatment in patients suffering from hypercatabolic states (ARF, sepsis, or shock) [31]. Recent experimental findings also indicate that administration of IGF-I significantly increases the mortality rate of adult rats with severe ischemic ARF [32].

Since the natural history of ischemic ARF has been shown to be more favorable in young than adult rats, as supported by earlier recovery of renal function and a better survival rate [33], and, in the clinical setting, age is also an important prognostic factor [5, 34, 35, 36, 37], it may be hypothesized that, following IGF-I treatment, the course of the disease in young rats may be different from that described in adult animals. Therefore, we designed this study to characterize in detail the response to exogenous IGF-I administration in fast-growing rats with severe ischemic ARF.

Materials and methods

Animals

Male Sprague-Dawley rats, weighing 60±10 g and 28±1 days of age at the start of the experiment, were used. Rats were housed in individual cages under controlled conditions of light (12-h light-darkness cycle) and temperature (21°C–23°C) and were allowed free access to standard laboratory diet (A03, Panlab, Barcelona, Spain) and tap water. After 3 days of acclimation to the experimental area, and under anesthesia with sodium thiopental (12.5 mg/kg, Tiobarbital 0.5 g, G. Braun Medical, Jaén, Spain) and ketamine hydrochloride (40 mg/kg, Ketolar 50 mg, Parke Davis, Madrid, Spain), the abdominal cavity was exposed via a midline incision. Both renal pedicles were identified and occluded with microvascular clamps (Biemer, FD 562, Aesculap, Tuttlingen, Germany) for 75 min. After this period, the clamps were removed with return of blood flow to the kidneys. If reperfusion was incomplete, as judged visually, the animal was killed and was not considered for statistical purposes. Finally, the abdominal wall was closed in two layers and animals were given an intraperitoneal injection of 1 ml/100 g of body weight of pre-warmed (37°C) saline (0.9% NaCl) to compensate for any fluid loss during surgery. Core body temperature was maintained at 37°C using a homeothermic table during surgery. Sham-operated animals underwent a similar surgical procedure but without clamping of renal pedicles. The day of surgery was considered as day 0 of the experiment. From the day of surgery onwards, animals were weighed daily and tail vein blood samples were drawn daily for determination of SUN. On day 2 or day 7, rats were sacrificed by exanguination, via the abdominal aorta. Blood and both kidneys were collected from each animal. The left kidney was processed for histopathological studies and the right one for molecular biology techniques. On day 0 the animals were distributed randomly in six experimental groups.

Long-term study

Group IGF-7d included rats with ARF treated with IGF-I, three 0.1-ml doses of 50 µg/100 g body weight of recombinant human IGF-I (Pharmacia Upjohn, Stockholm, Sweden) given subcutaneously at 0, 8, and 16 h post ischemia, and allowed to survive for 7 days after clamping. Group ARF-7d included rats with ARF given 0.1 ml of saline subcutaneously at 0, 8, and 16 h post ischemia, and allowed to survive for 7 days after clamping. Group Sham-48 h included sham-operated rats given 0.1 ml of saline subcutaneously at 0, 8, and 16 h post surgery, and allowed to survive for 7 days after operation.

Short-term study

Group IGF-48 h included rats with ARF treated with IGF-I, three 0.1-ml doses of 50 µg/100 g body weight of recombinant human IGF-I (Pharmacia Upjohn, Stockholm, Sweden) given subcutaneously at 0, 8, and 16 h post ischemia, and sacrificed 48 h after clamping. Group Sham-48 h included sham-operated rats given 0.1 ml of saline subcutaneously at 0, 8, and 16 h post surgery and sacrificed 48 h after operation. The number of initial rats per group varied so as to obtain 10 animals at the time of sacrifice in the long-term and short-term studies.

Histological examination

The left kidney was removed, stripped of its capsule, cut longitudinally into halves, fixed in 10% formaldehyde, and embedded in Paraplast. Sections of 3 µm were obtained and mounted on glass slides. Different sets of slides were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and naphthol AS-D chloroacetate esterase stains.

In order to carry out the histological studies, microscopic sections of at least 5 animals per group were analyzed. To be certain that the animals selected for the histological study were representative of the group they belonged to, their SUN values were close to the mean SUN concentration of each corresponding group.

In the sections under study, microscopic fields were chosen randomly in the corticomедullary area, where the damage produced by ischemic ARF is known to be more pronounced [38].

Scoring system of renal damage

HE and PAS samples were graded by an independent observer using a scoring system described by Miller et al. [22]. For those animals sacrificed 48 h after the induction of ARF, the scoring system assessed the following parameters related to severe tubular necrosis, prostatic hyperemia, cellular shedding towards the tubular lumen, and the appearance of hyaline deposits. In the rats killed 7 days after clamping, tubular dilatation, cellular pleomorphism, and epithelial calcification were evaluated. Grades were added together and divided by 3 to give a final score of 0–3. At least 12 microscopic fields (×40) per section were randomly chosen to be scored for each parameter.

Neutrophil infiltration

This was evaluated using naphthol AS-D chloroacetate esterase staining by counting the number of neutrophils present at the cor-