

Original Article

Combination of erythropoietin and alprostadil in treating acute kidney injury

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Abstract: The aim of this study was to investigate the efficacy of erythropoietin (EPO) and alprostadil (PGE1) in acute kidney injury (AKI). A total of 120 patients with AKI were enrolled from June 2009 to October 2013, and randomly divided into a control group: HD (hemodialysis), an EPO group: HD + EPO (3,000 U, 3 times/wk), a PGE1 group: HD + PGE1 (20 µg daily), and a Joint group: HD + EPO (3,000 U, 3 times/wk) + PGE1 (20 µg daily). The medication was administered for 14 days. The oliguric stages were compared between the groups; serum creatinine (Scr) and urinary neutrophil gelatinase-associated lipocalin (NGAL)/interleukin -18 (IL-18) levels were detected at D 4/8/15. The oliguric stage in the Joint group was the shortest (5±0.8 days) and with a significant difference from the other three groups (P<0.05). With sustained treatment, the Scr level of each group declined; the Joint group showed the most significant decline and the level was significantly different from that of the control group (P<0.05). The urinary NGAL in the control group was significantly higher than in the other three groups (P<0.05); the levels were similar in the EPO and PGE1 groups, but significantly higher than in the Joint group (P<0.05). Urinary IL-18 level in the control group was significantly higher than that in the other three groups (P<0.05). EPO and PGE1 can each contribute to the treatment of AKI, and the combination of these two drugs can significantly shorten the oliguric stage and improve renal function.

Keywords: Acute kidney injury, erythropoietin, alprostadil

Introduction

Acute kidney injury (AKI) is the leading cause of hospital admission and high mortality of kidney diseases [1]. The guidelines of the Acute kidney Injury Network (AKIN) in 2007 and Kidney Disease: Improving Global Outcomes (KDIGO) in 2012 both recommended serum creatinine and urine volume as diagnostic markers for AKI, but AKIN criteria, which rely on urine volume and serum creatinine (Scr) levels, are not sensitive or specific, and can only be used as markers of advanced AKI [2]. Since the etiologies of AKI vary, it is a challenge to clearly identify a novel marker of AKI [3]. However, a new biomarker is needed to further strengthen risk stratification and promote early diagnosis, in order to initiate experimental treatment and assess prognosis [4]. Previous preclinical studies using animal models showed that in the

early stages of AKI, neutrophil gelatinase-associated lipocalin (NGAL) might act as a regulatory gene and protein [5-7]. Clinical studies showed that NGAL was closely related to the severity of renal impairment, and therefore, it could be substituted as a noninvasive and sensitive biomarker for diagnosing, monitoring, and quantifying renal damage [8]; the accuracy of NGAL in early prediction of AKI would be most sensitive in patients with normal renal function while in the acute onset stage [9]. However, except for renal replacement therapies such as blood purification for AKI rescue and treatment, there has been no generally accepted treatment method. Increasing evidence has proven that erythropoietin (EPO) can generate added organ-protective effects, which might be useful in preventing or treating AKI. Alprostadil (PGE1) treatment can reduce the expression of tissue damaging factors, thus preventing ischemia-

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Table 1. Comparison of the durations of oliguria stage

Group	Oliguria stage (d)
Control	10±2.3
Alprostadil	8±2.7
EPO	8±3.2
Joint	5±0.8

Note: compared with the control group when admitted, P<0.05.

reperfusion injury [10, 11]. On a basis of hemodialysis (HD) treatment, this study combined EPO with PGE1 to investigate efficacy in treating AKI.

Materials and methods

Clinical data

A total of 120 patients with AKI admitted from June 2009 to October 2013 who met the AKIN diagnostic criteria were enrolled, including 57 male and 63 female patients, aged 14 to 80. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Kunming Medical University. Written informed consent was obtained from all participants. All patients underwent HD three times a week during the entire course of treatment. The patients were randomly divided into four groups without significant differences in sex, age structure, etc. The control group had 30 patients, and was only administered conventional HD treatment. The observation group was divided into: 1) the EPO group: 30 patients, subcutaneously injected 3,000 U of EPO (Shanghai Chemo Wanbang BioPharma Co., Ltd., Shanghai, China) 3 times/wk for 14 days; 2) the alprostadil group: 30 patients, intravenously infused 20 µg of alprostadil (Harbin Pharmaceutical Group Biological Engineering Co., Ltd., Harbin, China) once/day for 14 days; 3) and the Joint group: 30 patients, subcutaneously injected 3,000 U of EPO 3 times/wk and intravenously infused 20 µg of alprostadil once/day for 14 days. All treatments were based on the control group as reference.

Observation index

The four groups were observed for 28 days. Urine amount, serum albumin, hemoglobin level, blood urea nitrogen (BUN) level, Scr level,

routine urinalysis, urinary NGAL, and urinary IL-18 levels, were evaluated on the 1st, 4th, 8th, and 15th day of admission (T1-T4).

Assay methods

An OLYMPUS AU5421 biochemistry analyzer (OLYMPUS, Tokyo, Japan) measured BUN level by the urease method, and Scr level was measured by alkaline picric acid colorimetry; a Sysmex XT-4000i hematology analyzer (Sysmex, Tokyo, Japan) measured hemoglobin by an instrument method; an ASCENT microplate reader (Thermo Fisher, USA) was used for NGAL and IL-18 by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

SPSS 19.0 software was used for analysis. The measurement data were expressed as $\bar{x} \pm s$, and the comparison among the average values used multi-factorial analysis of variance for repeated measurements, with significance level set at $\alpha=0.05$.

Results

Comparison of the durations of the oliguric stage

The Joint group exhibited a statistically significantly shorter duration of the oliguric stage than the control group (5±0.8 d vs. 10±2.3 d, P<0.05, **Table 1**).

Urinary NGAL levels

Compared with the control group, the PEG1, EPO, and Joint groups exhibited no significant differences in urinary NGAL level at T1 (P>0.05), but did show significant differences at T2-T4 (P<0.05); there was no statistically significant difference in urinary NGAL level between the PEG1 and EPO groups at T2-T4, P>0.05; however, there were statistically significant differences in urinary NGAL levels between the Joint and EPO and PEG1 groups at T2-T4 (P<0.05, **Table 2**).

Urinary IL-18 levels

Compared with the control group, the PEG1, EPO, and Joint groups exhibited no significant differences in urinary IL-18 level at T1 (P>0.05), but did show statistically significant differences

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Table 2. Urinary NGAL levels of each group at different time points (ng/ml, $\bar{x}\pm s$)

Group	T1	T2	T3	T4
Control	129.6±4.3	125.7±7.1	121.8±4.8	115.7±7.9
PEG1	84.3±25.1	106.2±130.8 ^a	81.1±15.1 ^a	82.0±19.5 ^a
EPO	78.3±2.5	77.2±7.7 ^a	77.5±7.1 ^a	101.0±13.1 ^a
Joint	64.2±29.5	64.3±32.4 ^{a,b,c}	64.3±50.9 ^{a,b,c}	60.2±19.6 ^{a,b,c}

Note: compared with the control group at T2~T4, ^aP<0.05; compared with group PEG1 at T2~T4, ^bP<0.05; compared with group EPD at T2~T4, ^cP<0.05.

Table 3. Urinary IL-18 levels of each group at different time points (ng/ml, $\bar{x}\pm s$)

Group	T1	T2	T3	T4
Control	49.7±19.7	53.4±11.5	47.6±7.8	61.9±13.3
PEG1	45.7±9.1	44.9±8.5 ^a	40.4±4.9 ^a	39.7±8.4 ^a
EPO	53.0±11.2	37.2±3.6 ^a	36.7±3.1 ^a	35.4±5.9 ^a
Joint	54.1±31.4	37.9±22.6 ^a	41.8±39.3 ^a	33.8±12.6 ^a

Note: compared with the control group at T2~T4, ^aP<0.05.

Table 4. Scr levels of each group at different time points ($\mu\text{mol/L}$, $\bar{x}\pm s$)

Group	T1	T2	T3	T4
Control	812±15.7	614±22.6	332±19.8	132±15.6
PEG1	836±16.7	634±21.3	287±15.7	154±12.3
EPO	794±26.2	642±19.6	304±14.8	148±11.9
Joint	823±17.9	562±27.8 ^a	164±18.6 ^a	78±10.2 ^a

Note: compared with the control group at T2~T4, ^aP<0.05.

at T2-T4 ($P<0.05$); there was no statistically significant difference in urinary IL-18 level between the PEG1 and EPO groups at T2-T4, $P>0.05$. The comparison of urinary IL-18 level between the Joint and EPO or PEG1 groups at T2-T4 also showed no statistically significant difference (Table 3).

Scr levels

The Joint group showed significant differences in Scr level compared to the control group at T2 ($P<0.05$) ($562\pm 27.8 \mu\text{mol/L}$ vs. $614\pm 22.6 \mu\text{mol/L}$), T3 ($P<0.05$) ($164\pm 18.6 \mu\text{mol/L}$ vs. $332\pm 19.8 \mu\text{mol/L}$), and T4 ($P<0.05$) ($78\pm 10.2 \mu\text{mol/L}$ vs. $132\pm 15.6 \mu\text{mol/L}$), respectively (Table 4).

Compared with the control group, the PEG1, EPO, and Joint groups showed no significant differences in serum albumin, hemoglobin levels, and routine urinalysis results at T2-T4 ($P>0.05$).

Discussion

AKI refers to a common critical disease with various causes that lead to a rapid decline in glomerular filtration rate; it can involve multiple disciplines, and clinically manifests with rapid decline of kidney function, progressive increase in Scr level, and decreasing urine output within 48 hr, as well as serious imbalances in the internal environment. AKI includes the entire process from a slight increase in Scr to end-stage renal failure, and renal replacement therapy is basic management for severe AKI [12]. In recent years, the mortality rate of AKI in sepsis, shock, and severe trauma-induced acute tubular necrosis has remained at more than 50%. Therefore, promoting the regeneration of renal tubular cells after necrosis and reducing mortality have become concerns in the medical field. Although the KDIGO guidelines were developed in 2012 and provided useful guidance for the diagnosis and treatment of AKI, the implementation of the guidelines in clinical practice and the investigation on whether they are suitable for Chinese patients with

AKI requires further clinical research for confirmation.

The diagnostic indicators identified thus far have good stability, with high sensitivity and specificity for the diagnosis of AKI, and their clinical application will remain useful. Among these indicators, NGAL is mainly secreted by the neutrophils, as well as by a variety of epithelial cells including the proximal renal tubular cells [13]. When epithelial cells are damaged, the expression of NGAL is significantly increased, and it is then mainly secreted and released by renal tubular epithelial cells [14]. Clinical trials found that NGAL protein could be detected in blood and urine of patients with early AKI before renal functional changes. A meta-analysis showed that urinary NGAL could not only help in the early diagnosis of AKI, but also be used to predict the short-term prognosis and could be used to determine whether a patient might need renal replacement therapy;

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urinary IL-18 secreted by tubular epithelial cells was used as an early diagnostic marker of AKI in a mouse model of acute tubular necrosis [15, 16]. Melnikov et al [17] reported that urinary IL-18 level was increased in patients with AKI, but not in patients with chronic kidney disease (CKD), urinary tract infection, nephrotic syndrome, or prerenal azotemia, indicating that the levels of IL-18 were related to disease activity [18]. Even at different levels of severity, the concentrations of NGAL and IL-18 gradually increased with disease progression. IL-18 level can be transiently increased, while NGAL level can exhibit a continuous increase or bidirectional mode, which can be explained by ongoing damage, especially in patients with sepsis [19]. KDIGO guidelines recommended etiologic treatment of AKI, strengthened nutritional support, and timely individual renal replacement therapy. Current treatments focus on maintaining renal perfusion and avoiding volume overload [20]. However, there is no guide to drug intervention. Preoperative administration of statins is used to reduce the risk of postoperative AKI [21]. Some authors tried using either recombinant human EPO or PEG1 alone, and achieved some benefits. Recombinant human erythropoietin (rHuEPO) is a type of salivary protein hormone that can stimulate the bone marrow to make blood; it is mainly secreted by the tubular para-cells near the junction of the renal cortex and medulla, and can exert its biological effects through binding with EPO receptors on target cells. Studies have found that EPO is an important cytokine, and its physiological importance has been expanded from simply promoting erythropoiesis and increasing erythrocyte volume. EPO exerts protective effects against hypoxic-ischemic injury in a variety of tissues and organs such as the brain, kidney, and heart [22, 23]. It is antioxidative, inhibits apoptosis and inflammatory responses, induces ischemic tolerance, and promotes the regeneration of renal tubular epithelial cells. EPO is therefore a multifunctional cytokine that can maintain a stable internal environment, and exhibits various protective effects in a variety of organs and tissues [24]. The gene sequence of rHuEPO is essentially the same as that of EPO, with several additional glycosylations. EPO can reduce all-cause mortality in AKI, and the incidence of AKI-induced ESRD, which depends largely on the beneficial effects of EPO after AKI [25]. The cell-protective effects of EPO are proven and

effective, but EPO can be neutralized by anti-inflammatory media in the kidney and heart, leading to chronic fibrosis after acute kidney and heart injury [26]. It was felt that vasodilation could prevent AKI, alleviate renal medullary vasoconstriction, and inhibit the activation of the renin-angiotensin-aldosterone system [27]. Alprostadil, also known as Prostaglandin E1 (PGE1), is a natural prostaglandin with potent biological activities. It can directly act on vascular smooth muscle, expand renal blood vessels, inhibit the aggregation of platelets within kidneys, inhibit the synthesis of platelet thromboxane A2, thereby preventing thrombosis, reduce the production of free radicals, stabilize cell and lysosomal membranes, prevent tissue reperfusion injury, improve microcirculation, increase renal blood flow, inhibit inflammatory cytokines, and inhibit renal natriuretic effects, thereby improving renal function. Animal studies showed that PEG1 could also reduce the expression of tissue damaging factors, thus preventing ischemia-reperfusion injury. This study found that the combination of EPO and PEG1 to treat AKI can shorten the oliguric stage and the period needed for renal function to recover, and can promote the proliferation and repair of damaged renal tubular epithelial cells compared with HD treatment alone; thus, it can be recommended in clinical practice. Compared with HD treatment, the application of either EPO or PEG1 would exhibit greater benefit than HD treatment alone for AKI. The combined effects of EPO and PEG1 were greater than EPO or PEG1 alone.

This study was limited by being a single-center small-sample report, with 28 days of observation in patients who had already developed decreased urine output and increased Scr; therefore, using urinary NGAL and IL-18 as early biomarkers of AKI was not completely valid. However, this study found that compared with existing HD treatment, use of either EPO or PEG1 alone, or EPO and PEG1 in combination, would reduce urinary NGAL and IL-18 levels to less than the control group; this suggested that urinary NGAL and IL-18 had value in predicting the prognosis of AKI. Therefore, a larger-sample, multi-center study is needed to validate the value of these markers in monitoring kidney recovery, as well as to evaluate the long-term efficacy of EPO combined with PEG1 in AKI.

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Disclosure of conflict of interest

None.

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