"EUK-134, a synthetic superoxide dismutase and catalase mimetic, protects rat kidneys from ischemia-reperfusion-induced damage."

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ABSTRACT

The effect of a new synthetic superoxide dismutase and catalase mimetic was investigated on renal ischemia-reperfusion syndrome in rats. Synthetic salen-manganese complexes have characteristics that might facilitate their potential usefulness as therapeutic agents: (1) unlike proteinaceous antioxidant enzymes, synthetic complexes, due to their low molecular weight, have a better stability and bioavailability; (2) they have a catalytic activity enhancing their efficiency over noncatalytic reactive oxygen metabolite scavengers; and finally, (3) exhibiting combined superoxide dismutase and catalase activity, they destroy both superoxide anions and hydrogen peroxides, thereby enhancing their protective effect on ischemically injured tissues. One such compound, EUK-134, was tested in uninephrectomized rats that underwent a left renal artery clamping. After a 75-min left renal artery clamping, a single intravenous injection of EUK-134 at 0.2 mg/kg, just before unclamping, provided significantly better renal function recovery during the week after the ischemic insult compared with recovery of untreated animals. Two hours after several periods of renal ischemia (30, 45, 60, and 75 min of left renal artery clamping), EUK-134 given at a similar dose significantly improved the glomerular filtration rate after an acute ischemia of 30 and 45 min, as assessed by EDTA 51Cr. Overall, these results show that synthetic superoxide dismutase-catalase mimetics such as EUK-134 can protect ischemically injured rat kidneys from ischemia-reperfusion syndrome when administered just before reperfusion...
EUK-134, A SYNTHETIC SUPEROXIDE DISMUTASE AND CATALASE MIMETIC, PROTECTS RAT KIDNEYS FROM ISCHEMIA-REPERFUSION-INDUCED DAMAGE

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The effect of a new synthetic superoxide dismutase and catalase mimic was investigated on renal ischemia-reperfusion syndrome in rats. Synthetic salen-manganese complexes have characteristics that might facilitate their potential usefulness as therapeutic agents: (1) unlike proteinaceous antioxidant enzymes, synthetic complexes, due to their low molecular weight, have a better stability and bioavailability; (2) they have a catalytic activity enhancing their efficiency over noncatalytic reactive oxygen metabolite scavengers; and finally, (3) exhibiting combined superoxide dismutase and catalase activity, they destroy both superoxide anions and hydrogen peroxides, thereby enhancing their protective effect on ischermically injured tissues. One such compound, EUK-134, was tested in uninephrectomized rats that underwent a left renal artery clamping. After a 75-min left renal artery clamping, a single intravenous injection of EUK-134 at 0.2 mg/kg, just before unclamping, provided significantly better renal function recovery during the week after the ischemic insult compared with recovery of untreated animals. Two hours after several periods of renal ischemia (30, 45, 60, and 75 min of left renal artery clamping), EUK-134 given at a similar dose significantly improved the glomerular filtration rate after an acute ischemia of 30 and 45 min, as assessed by EDTA51 Cr. Overall, these results show that synthetic superoxide dismutase-catalase mimetics such as EUK-134 can protect ischemically injured rat kidneys from ischemia-reperfusion syndrome when administered just before reperfusion.

Major progress in organ preservation and immunosuppression has recently been made, but primary nonfunction of renal cadaveric allografts remains a critical problem. This is especially true since the scarcity of organs today favors the use of non-heart-beating donors, which significantly increases the incidence of lesions due to ischemic events (1). Therefore, there is room for research in the field of organ preservation, particularly for testing new drugs which might control ischemia-reperfusion syndrome. Although reperfusion is crucial for oxygen delivery to ischemically injured tissues, the reoxygenation is known to be detrimental because it allows the generation of reactive oxygen metabolites (ROMs) such as superoxide anions, hydroxyl radicals, and hydrogen peroxides. ROMs are able to damage cells through chemical interactions with key cellular constituents, including proteins, lipids, and DNA (2, 3). Under physiological conditions, cells contain endogenous protective agents with antioxidant properties, such as ascorbate (vitamin C) and α-tocopherol (vitamin E), or enzymatic scavengers, such as glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT), which are able to eliminate ROMs. However, under some pathological situations, such as posts ischemic injury, endogenous protective mechanisms may be overwhelmed, thereby allowing ROMs to exert their detrimental effects. The use of exogenous antioxidant enzymes, particularly SOD, has been thoroughly investigated. Studies have shown that SOD may be difficult to use because, as a protein, it has several delivery and stability shortcomings (2, 3). In addition, although SOD transforms superoxide anions into less toxic hydrogen peroxides, the latter are stable and capable of damaging cell membranes. To fully protect cells from ROM-induced damages would require the use of both SOD and CAT. There is, therefore, clear interest in developing synthetic molecules with combined SOD-CAT activity. In the present study, we have tested EUK-134, a new synthetic salen-manganese complex which does display such combined SOD-CAT activity (4). Investigating the effects of a single injection of EUK-134 just before unclamping, we found that it is able to significantly protect and improve renal function recovery after a severe kidney ischemic insult.

Wistar male rats weighing 200–250 g were used in this study. All animals were fed standard rat chow ad libitum and

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Abbreviations: CAT, catalase; GFR, glomerular filtration rate; ROMs, reactive oxygen metabolites; SOD, superoxide dismutase.
were housed at 24°C, with an alternating cycle of 12:12-hr light/dark cycle for at least 3 days before the experiment.

Anesthesia was induced and maintained by ether inhalation. A polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was placed in the left femoral vein. Through a midline laparotomy, a right nephrectomy was performed. The left renal artery was dissected and occluded with a smooth vascular clamp for several time periods (0, 30, 45, 60, and 75 min). During the ischemic period, the body temperature was controlled and maintained between 35°C and 37°C. After unclamping of the left renal artery, the abdominal wall was sutured and the animals were placed in individual restraining cages. The femoral catheter was maintained in place for at least 2 hr after unclamping, when renal function was assessed by EDTA 51Cr. The renal catheter was excised when renal function was assessed during the week after the ischemic insult by daily creatinine.

Renal function was assessed either by daily plasmatic creatinine levels during the week after ischemia, or 2 hr after unclamping by single EDTA 51Cr injection. This allowed for the assessment of the glomerular filtration rate (GFR) using a single blood sample 1 hr after EDTA injection (5, 6). Briefly, 1 hr after unclamping, 10 μCi of EDTA 51Cr were injected in the contralateral femoral vein; then, 1 hr after injection 100 μl of blood were withdrawn through the left femoral catheter. After centrifugation, the plasma sample was counted and the GFR was estimated using the following formula:

\[ \frac{V}{t} \ln \left( \frac{P_0}{P_t} \right) \]

where \( V \) is the distribution volume, \( P_t \) is the amount of cpm/ml in the plasma sample taken at \( t \) = 60 min, and \( P_0 \) is \( I/V \) in which \( I \) is the injected amount of radioactivity (cpm) (6).

The characterization of SOD-mimetic activity of salen-manganese complexes developed by Eukarion (Bedford, MA) has been described in detail elsewhere (4, 7). Although the pharmacokinetics is not yet known, the following characteristics of EUK family complexes have been identified. First, EUK complexes exhibit superoxide-scavenging activity and inhibit malondialdehyde formation by 15% more than vitamin E (7). Second, EUK-134 exhibits powerful catalase activity at protecting human cells from toxicity by glucose and glucose oxidase, a hydrogen peroxide-generating system (4, 7). Third, EUK complexes are extremely stable in solution, even under acidic conditions. For example, it has a half-life of >15 hr at pH 1.5. Overall, synthetic EUK complexes demonstrate combined SOD and CAT activity, thereby rendering it potentially advantageous over other antioxidants (4, 7). In the present study, EUK-134 was diluted in 1 ml of glucose 5% to yield a dose of 0.2 mg/kg and injected within 5 min just before unclamping. The control groups received 1 ml of glucose 5% at the same time. The dose of 0.2 mg/kg was chosen after a dose-response study of a model of permanent coronary occlusion in rats; the dose of 0.2 mg/kg was found to be optimal and allowed uniform myocardial protection in all the treated animals (unpublished data).

All results are expressed as mean ± SD. Nonparametric Mann-Whitney \( U \) tests were used to compare control and experimental groups 2 hr after unclamping. To assess the difference between the clinical course of two groups of animals, analysis of variance for repeated measures was used. A \( P \)-value of less than 0.05 was considered to be statistically significant.

In order to assess the effect of synthetic SOD-CAT mimetics after renal ischemia, we injected intravenously EUK-134 into uninephrectomized rats subjected to renal ischemia of various duration. The acute renal damage was assessed by measuring the GFR recovery 2 hr after unclamping in several different groups of animals (each containing five animals) that underwent a left renal artery clamping for 0, 30, 45, 60, and 75 min. All rats in each group received either 1 ml of glucose 5% (controls) or 1 ml of glucose 5% containing EUK-134 at 0.2 mg/kg (EUK treated), just before reperfusion. We first measured the GFR recovery 2 hr after a right nephrectomy without left renal ischemia in five animals and found that the basal GFR obtained after nephrectomy alone was 0.97 ± 0.14 ml/min or, expressed per 100 g of body weight, 0.378 ± 0.05 ml/min/100 g body weight (Fig. 1). After a 30-min left renal artery clamping, the GFR dropped significantly to 0.283 ± 0.04 ml/min/100 g body weight (P<0.015; 30% less than without ischemia) in untreated rats, whereas EUK-134-treated animals that underwent similar ischemia maintained a GFR similar to that of uninephrectomized rats without ischemia, i.e., 0.380 ± 0.02 ml/min/100 g body weight (P=0.0079 vs. untreated animals). Similarly, after 45-min left renal artery clamping, the GFR in control animals decreased significantly to 0.278 ± 0.04 ml/min/100 g body weight, whereas a single administration of EUK-134 just before unclamping allowed EUK-treated rats to maintain normal renal function (0.380 ± 0.02 ml/min/100 g body weight) (P<0.0079 vs. untreated rats; Fig. 1). EUK-134 was unable to significantly improve the GFR 2 hr after a more severe ischemic insult, i.e., 60 or 75 min of left renal clamping, but the GFR was still higher in the EUK-treated group than in control rats (Fig. 1). After 60 min of left renal artery clamping, the GFR was 0.085 ± 0.07 ml/min/100 g body weight in the control group versus 0.107 ± 0.06 ml/min/100 g body weight in the EUK-134-treated group (NS); after 75 min of ischemia, the GFR was 0.077 ± 0.08 ml/min/100 g body

![Figure 1](https://example.com/figure1.png)
weight in the control group versus 0.121±0.10 ml/min/100 g body weight in the EUK-treated group (NS).

The apparent lack of effect of EUK-134 on renal function recovery after 60 or 75 min ischemia was, however, the consequence of an inappropriate timing of renal function assessment after such a very strong ischemic insult. The recovery from the renal ischemic insult was therefore evaluated in additional animals by measuring the plasmatic creatinine levels daily for 1 week after a 75-min left renal artery clamping. We first achieved a bilateral nephrectomy in two rats in order to assess the magnitude of the increase in plasmatic creatinine, which reached 5.2±0.1 mg/dl and 8.17±0.05 mg/dl 1 and 2 days after surgery, respectively. At the end of the second day, the two anephric rats died in uremia. As shown in Figure 2, the course of the renal function recovery was significantly different between control and EUK-treated animals: after a right nephrectomy and a 75-min left renal clamping, untreated rats (n=6) had a mean creatinine value of 4.16±0.25 mg/dl compared with 3.49±0.29 mg/dl (P<0.035) for EUK-134-treated rats at 1 day after surgery, and 6.08±0.21 mg/dl compared with 4.21±0.87 mg/dl in EUK-134-treated rats (P<0.012) 2 days after surgery. Two of the untreated rats died in uremia at the end of the second day; there was no mortality in the EUK-134-treated group. Four days after surgery, the creatinine levels were still significantly lower in EUK-134-treated rats than in untreated animals (4.58±0.54 mg/dl vs. 4.68±0.25 mg/dl, respectively; P<0.0019).

Overall, these results clearly demonstrate that SOD-CAT mimetics as EUK-134 improve renal function recovery after a severe ischemic injury, probably reducing the superoxide anions, the hydroxyl radicals, and the hydrogen peroxides induced by the ischemia-reperfusion syndrome in this model. This study also demonstrates that these complexes are able to protect renal function from 75 min of warm ischemia, which is known to cause severe renal damage in uninephrectomized rats. Synthetic EUK complexes have recently been described as powerful radical scavengers in several in vivo pathological situations, and EUK-8 has been shown to significantly reduce lung tissue malondialdehyde content in endotoxemic swine, thereby significantly preventing acute respiratory distress syndrome (8). Similarly, EUK-8 protected intestinal mucosa against oxidative injury (9, 10) and also demonstrated significant protective effects after cerebral ischemic injury, even when injected up to 3 hr after the arterial occlusion (unpublished data). All these results suggest that these new synthetic and extremely stable compounds, having both SOD and CAT activity should be considered in several pathological situations of acute ischemia, i.e., organ transplantation and procurement. They might have powerful therapeutic potential.

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**Figure 2.** Renal function recovery was assessed daily by plasmatic creatinine level (mg/dl). After bilateral nephrectomy (C, n=2), rats died in uremia within 2 postoperative days with a creatinine level of up to 7.5 mg/dl. Control animals (■, n=5) demonstrated a significant degradation of renal function during the first postoperative week; in comparison, EUK-treated animals (▲, n=6) recovered a normal creatinine level within 5 days. Analysis of variance for repeated measures demonstrated a significantly lower creatinine level for EUK-treated animals on postoperative days 1 (**P<0.001), 2 (**P<0.012), and 5 (***P<0.0019) in comparison with untreated animals.