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A new combination of sitagliptin and furosemide protects against remote myocardial injury induced by renal ischemia/reperfusion in rats

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ABSTRACT

Acute kidney injury (AKI) is associated with high mortality resulting from extra-renal organ damage, particularly the heart. The present study aimed to investigate the protective effect of sitagliptin, a dipeptidyl peptidase-4 (DPP4) inhibitor, against renal and remote cardiac damage induced by ischemia/reperfusion (IR), a leading cause of AKI. In this attempt, we compared the effects of sitagliptin to furosemide, a loop diuretic. Furosemide is commonly used clinically in AKI however, there is a lack of evidence regarding its beneficial effects in AKI. In addition, the combined administration of both drugs was also investigated. Ischemia was induced in anesthetized male Wistar rats by occluding both renal pedicles for 30 min followed by reperfusion for 24 h. Sitagliptin (5 mg kg^{-1}), furosemide (245 mg kg^{-1}) or their combination were administered orally at 5 h post-IR and 2 h before euthanasia. Administration of sitagliptin or furosemide ameliorated renal and cardiac deterioration induced by renal IR. This was manifested as significant reduction of serum creatinine, urea, cystatin c, creatine kinase-MB, cardiac troponin-I and lactate dehydrogenase ($P < 0.05$). Drug treatment significantly inhibited IR-induced elevation of TNF-α, NF-κB and caspase-3 ($P < 0.05$) in kidney and heart tissue. In addition, they significantly suppressed malondialdehyde, NO and iNOS content, whereas they increased glutathione and antioxidative enzymes activity ($P < 0.05$) in both tissues. Interestingly, a superior protection was observed with the combination compared to the individual drugs. We assume that this combination represents a promising regimen for managing AKI, particularly with the poor clinical outcome obtained with furosemide alone.

1. Introduction

Acute kidney injury (AKI) is a major clinical problem associated with high morbidity and mortality rates. It has been estimated that death in 20% of hospitalized patients and up to 50% of patients admitted to the intensive care unit is attributed to AKI [1]. In addition, AKI plays a pivotal role in the development and progression of chronic kidney disease and end-stage renal disease [2]. Moreover, it is associated with high treatment costs regarding the need for transplantation and renal replacement therapy [3].

Renal ischemia/reperfusion (IR) injury is a leading cause of AKI in native and transplanted kidneys [4,5]. It results from the reduction of renal blood flow that eventually leads to impairment of oxygen delivery to renal cells [6]. Different causes have been suggested for the reduction in renal blood flow including shock, sepsis, hepatorenal syndrome, decreased effective intravascular volume and use of some medications [7].

The mechanism of IR-induced AKI is complex involving interactions between renal tubular injury, vascular injury, and inflammation. On one hand, hypoxic damage to the renal tubular cells stimulates the release of inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and other chemotactic cytokines which aid in the recruitment of immune cells [8]. On the other hand, ischemia-induced endothelial injury plays an important role in the adhesion of leukocytes via release of adhesion molecules such as selectins and intercellular adhesion molecule-1 (ICAM-1) [9]. The interaction between endothelium

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and leukocytes results in activation of leukocytes, obstruction of capillaries, production of cytokines, and aggravation of inflammation [10]. Other possible mechanisms have been suggested including increased oxidative stress [11], impairment of nitric oxide (NO) pathway [12] and apoptosis [13].

Despite being associated with high mortality, AKI is not usually the direct cause of death [14]. However, AKI-induced systemic inflammatory response and progression of damage to remote organs have been implicated in the high patient mortality. A causal link has been suggested to exist between AKI and dysfunction of distant extra-renal organs such as the heart [15], the liver [16] and the lung [17]. It has been demonstrated that cardiac failure in AKI patients is one of the leading causes of death [18,19]. Similarly, another study of AKI patients showed that death for cardiac failure was the greatest among other AKI-induced organs failure [20]. It has been suggested that cardiac injury induced by AKI is attributed to increased systemic and cardiac TNF-α, IL-1, and ICAM-1 expression that can lead to neutrophil infiltration and myocyte apoptosis [15]. Even with increased availability and widespread application of renal replacement therapy, outcomes are not improved [21]. Therefore, identifying new therapies that can ameliorate cardiac injury during AKI is critical in limiting the high mortality and improving the outcome.

Glucagon-like peptide-1 (GLP-1), which belongs to incretins, can reduce blood glucose through stimulation of insulin secretion from β-cells [22]. Different studies showed that GLP-1 has favorable effects in different experimental models of ischemia. For example, this peptide has been shown to exert cardioprotective effects on IR-injured hearts or cardiomyocytes [23]. In addition, exenatide, an analogue of GLP-1, has the potential to attenuate AKI induced by IR in rats [24]. The biological activity of GLP-1 can be extended via inhibiting dipeptidyl peptidase-4 (DPP4), the enzyme system responsible for its degradation. Indeed, different DPP4 inhibitors such as sitagliptin, saxagliptin and linagliptin are currently used for the treatment of type 2 diabetes based on the reduced cleavage of GLP-1 [25,26]. Previous studies showed that sitagliptin could ameliorate the deleterious effects induced by either renal IR injury [27] or cardiac IR injury [28].

The present study was conducted to investigate the effect of sitagliptin on the remote myocardial damage induced by renal IR injury in rats. In addition, we attempted to identify the underlying mechanism of action. In our attempt, we compared the effects of sitagliptin to furosemide and investigated the effect of their combination as well. Using of furosemide is based on the widespread clinical application of diuretics during AKI [29,30]. To our knowledge, this is the first report discussing not only the local effects of these drugs on renal IR injury, but also its effects on the ensuing distant cardiac complications.

2. Materials and methods

2.1. Experimental animals

Adult male Wistar rats (180–250 g) were used in the current study. Animals were obtained from the Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. Rats were acclimatized for one week prior to experiments. The animals were housed in stainless steel cages (three rats/cage) and kept at controlled temperature (23 ± 2 °C), humidity (60 ± 10%) and light/dark (12/12 h) cycle. Rats were supplied with commercially available normal chow diet and water ad libitum.

2.2. Ethical statement

Experimental design and animal handling procedures were approved by the local authorities, Ethical Committee for Animal Handling at Zagazig University (ECAHZU), at the Faculty of Pharmacy, Zagazig University, Egypt in accordance with the recommendations of the Weatherall report. Every effort was done to minimize the number of animals used and their suffering during experiments.

2.3. Drugs

Sitagliptin (Januvia®) was obtained from Merck, Sharp & Dohme (Cairo, Egypt), while furosemide was supplied from Amoun Pharmaceutical Co. S.A.E. (Obour City, Egypt). All other chemicals were of analytical grade. Drugs were dissolved in distilled water immediately before administration.

2.4. Experimental design

Rats were randomly divided into five experimental groups ($n = 6$ each). Group 1 (sham-operated), group 2 (IR injury only), group 3 (rats received sitagliptin 5 mg kg^{-1} orally at 5 h post-IR and 2 h before euthanasia), group 4 (rats received furosemide 245 mg kg^{-1} orally at 5 h post-IR and 2 h before euthanasia), and group 5 (rats received a combination of sitagliptin 5 mg kg^{-1} orally plus furosemide 245 mg kg^{-1} orally at 5 h post-IR and 2 h before euthanasia). Rats of group 1 (sham-operated) and group 2 (IR injury only) did not receive any drug treatment, but rather vehicle only.

2.5. Rationale of drug dosing

Dose of sitagliptin was chosen based on previous studies [27,31]. Dose of furosemide was chosen to simulate the oral high-dose (35 mg kg^{-1} day^{-1}) used in the clinical settings for managing AKI [32]. The equivalent rat dose was interpolated from the mentioned human dose using approximate dose conversion factors described by Freireich et al. [33]. The time points for drug administration were chosen based on the results of Williams et al. [34]. In this study, the authors demonstrated that injury might be initiated 4 h following IR procedure. Therefore, they suggested that therapeutic interventions within 6 h following IR procedure would be the most effective in ameliorating injury. In addition, their results revealed that peak renal damage occurred 24 h following IR; therefore, this time point would be helpful in monitoring the protective effects of investigated agents. Depending on these findings, the administration of drugs was started 5 h following IR procedure (i.e., within the first 6 h) in order to ensure that kidney injury has been already established. The second dose was administered 2 h before euthanasia at 24 h following IR procedure, where peak damage occurs.

2.6. Methods

2.6.1. Induction of IR injury

Rats were fasted overnight but had free access to water. At the day of surgery, animals were anaesthetized with an intraperitoneal injection of thiopental sodium (EIPICO Pharmaceuticals, 10th of Ramadan City, Egypt) at a dose of 120 mg kg^{-1} [35], and then placed on a heating pad to keep the body temperature constant at approximately 37 °C. Left and right kidneys were exposed by flank incisions on both sides, respectively. Bilateral ischemia was induced by occluding both renal pedicles using non-traumatic clamps (Dieffenbach Bulldog Clamps, Harvard Apparatus Ltd., Kent, UK) for 30 min according to the method described by Kelly et al. [36]. Complete ischemia was confirmed by blanching of kidneys. After the indicated ischemic period, clamps were released and reperfusion was started for 24 h. The kidneys were observed for 2–5 min to ensure normal blood reflow, which is indicated by