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Combination of adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-derived exosomes for protecting kidney from acute ischemia–reperfusion injury☆

Kun-Chen Lin [a,1], Hon-Kan Yip [b,c,d,e,f,1], Pei-Lin Shao [f], Shun-Cheng Wu [g], Kuan-Hung Chen [a], Yen-Ta Chen [h], Chih-Chao Yang [i], Cheuk-Kwan Sun [j], Gour-Shenq Kao [b], Sheng-Yi Chen [b], Han-Tan Chai [b], Chia-Lo Chang [k], Chih-Hung Chen [l], Mel S. Lee [m,*]

[a] Department of Anesthesiology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[b] Division of Cardiology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[c] Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[d] Center for Shockwave Medicine and Tissue Engineering, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[e] Department of Medical Research, China Medical University Hospital, China Medical University, Taichung 40402, Taiwan
[f] Department of Nursing, Asia University, Taichung 41354, Taiwan
[g] Orthopaedic Research Center, Kaohsiung Medical University, Kaohsiung, Taiwan
[h] Division of Urology, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[i] Division of Nephrology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[j] Department of Emergency Medicine, E-Da Hospital, I-Shou University School of Medicine for International Students, Kaohsiung 82445, Taiwan
[k] Division of Colorectal Surgery, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[l] Divisions of General Medicine, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[m] Department of Orthopedics, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine 83301, Kaohsiung, Taiwan

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ABSTRACT

Background: In this study, we tested the hypothesis that a combined adipose-derived mesenchymal stem cell (ADMSC) and ADMSC-derived exosome therapy protected rat kidney from acute ischemia–reperfusion (IR) injury (i.e., ligation of both renal arteries for 1 h and reperfusion for 72 h prior to euthanization).
Methods and results: Adult-male SD rats (n = 40) were equally categorized into group 1 (sham control), group 2 (IR), group 3 [IR + exosome (100 μg)], group 4 [IR + ADMSC (1.2×10^6 cells)], and group 5 (IR-exosome-ADMSC). All therapies were performed at 3 h after IR procedure from venous administration. By 72 h, the creatinine level and kidney injury score were the lowest in group 1 and the highest in group 2, significantly higher in group 3 than in groups 4 and 5, and significantly higher in group 4 than in group 5 (all $P < 0.0001$). The protein expression of inflammatory (TNF-α/NF-κB/IL-1β/MIF/PAI-1/Cox-2), oxidative-stress (NOX-1/NOX-2/oxidized protein), apoptotic (Bax/caspase-3/PARP), and fibrotic (Smad3/TGF-β) biomarkers showed an identical pattern, whereas the anti-apoptotic (Smad1/5, BMP-2) and angiogenesis (CD31/vWF/angiopoietin) biomarkers and mitochondrial cytochrome-C showed an opposite pattern of creatinine level among the five groups (all $P < 0.001$). The microscopic findings of glomerular-damage (WT-1), renal tubular-damage (KIM-1), DNA-damage (γ-H2AX), inflammation (MPO/MIF/CD68) exhibited an identical pattern, whereas the podocyte components (podocin/p-cadherin/synaptopodin) displayed a reversed pattern of creatinine level (all $P < 0.0001$).
Conclusion: Combined exosome–ADMSC therapy was superior to either one for protecting kidney from acute IR injury.

1. Introduction

Acute kidney injury (AKI) is quite often encountered in our daily clinical practice [1–6]. Despite state-of-the-art advanced pharmaceutical therapy, AKI remains one of the major causes of morbidity and mortality in hospitalized patients for any disease [2,3,6–8]. The etiology of AKI has been extensively investigated from many perspectives [1–13], including toxic substances such as chemical agents and drugs, sepsis,

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* Corresponding author.
E-mail address: mellee@cgmh.org.tw (M.S. Lee).

[1] Equally contributed.

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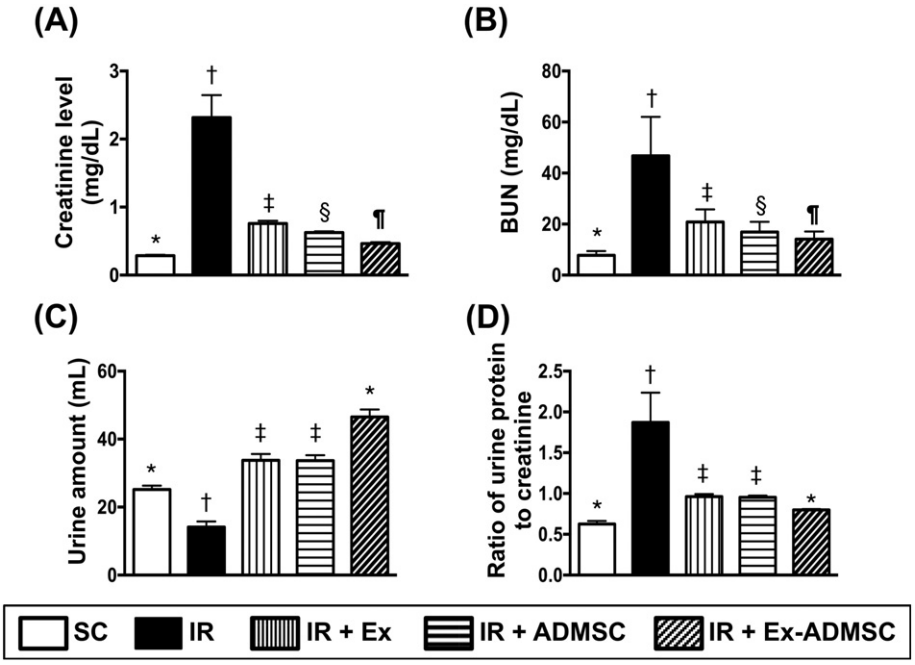

Fig. 1. Circulating levels of creatinine and blood urea nitrogen (BUN) and the ratio of urine protein to creatinine at 72 h after ischemia–reperfusion (IR). A) Circulating level of creatinine by 72 h after IR procedure, *vs. other groups with different symbols (†, ‡, §, ¶), $P < 0.0001$. B) Circulating BUN level at 72 h after IR procedure, *vs. other groups with different symbols (†, ‡, §, ¶), $P < 0.0001$. C) The urine amount at 72 h, *vs. other groups with different symbols (†, ‡, §), $P < 0.0001$. D) By 72 h, the ratio of urine protein to creatinine, *vs. other groups with different symbols (†, ‡, §), $P < 0.001$. All statistical analyses were performed by one-way ANOVA, followed by Bonferroni multiple comparison post hoc test (n = 8 for each group). Symbols (*, †, ‡, §, ¶) indicate significance (at 0.05 level). SC = sham control; IR = ischemia–reperfusion; Ex = exosome; ADMSC = adipose-derived mesenchymal stem cell.

contrast medium, obstruction of the urinary tract, decompensated chronic heart failure, hepato-renal syndrome, hypoperfusion/shock, and acute ischemia–reperfusion (IR) injury. Additionally, acute kidney IR injury has been well established to be one of the major contributors of AKI [1,2,8,14], and most of the causal etiologies of AKI can also cause acute kidney IR injury [14–18]. Currently, conservative treatment and waiting for the recovery are the only management strategies recommended for acute kidney IR injury. Therefore, it is not surprising that the morbidity and mortality have not improved much in the last decade despite the guidance [19–21] for management of acute kidney IR injury being regularly updated. Accordingly, finding a new and safe therapeutic modality for patients with acute kidney IR injury is of utmost importance for clinicians.

Plentiful data show that the setting of acute IR of any tissue/organ always rapidly elicits a vigorous inflammatory reaction, inflammatory cell recruitment, cytokine production, and generation of free radicals and oxidative stress [22–25]. These cellular–molecular perturbations, in turn, directly participate in further tissue/organ damage after IR injury [24,25]. Therefore, inhibition of inflammatory reaction and oxidative stress is suggested to be pivotal for protecting the organ from acute IR injury. Abundant data have demonstrated that mesenchymal stem cells (MSCs) [25–30], especially those of adipose-derived MSCs (ADMSCs) do not only induce angiogenesis to improve ischemia-related organ dysfunction but also have capacity of anti-inflammation and immunomodulation for attenuating IR-induced organ dysfunction [25,28,30]. The exosome, one kind of micro-vesicle derived from a variety of cells, is a membrane fragment with a size around 60–120 nm [31–33]. Additionally, exosomes contain distinct subsets of microRNAs depending upon the cell type from which they are secreted [34]. Furthermore, increasing amounts of experimental data have revealed that the MSC-derived exosome has properties of (1) angiogenesis, (2) immunomodulation, and (3) paracrine effect that improve organ function following injury in preclinical studies [36–40]. Previous reports [25–38] have raised the hypothesis that combined therapy with ADMSCs and ADMSC-derived exosomes might be superior to either one for improving acute kidney IR injury.

2. Methods

2.1. Ethics

All animal experimental protocols and procedures were approved by the Institute of Animal Care and Use Committee at Kaohsiung Chang Gung Memorial Hospital (Affidavit of Approval of Animal Use Protocol No. 201406252) and performed in accordance with the Guide for the Care and Use of Laboratory Animals [The Eighth Edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011)].

Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved animal facility in our hospital with controlled temperature and light cycle (24 °C and 12/12 light cycle).

2.2. Procedure and protocol for acute kidney ischemia–reperfusion (IR) and treatment strategy

Pathogen-free, adult male Sprague-Dawley (SD) rats (n = 40) weighing 320–350 g (Charles River Technology, BioLASCO, Taiwan) were equally categorized into five groups (i.e., n = 8/each group): sham control (SC) (receiving laparotomy only), IR (receiving similar treatment as SC except for IR of both kidneys), IR + ADMSC-derived exosome (i.e., IR-Ex) (intravenous administration of 100 μg at 3 h after IR), IR + ADMISC (i.e., IR-ADMSC) intravenous administration of 1.2×10^6 cells at 3 h after IR procedure), and IR-Ex-ADMSC.