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● *Original Contribution*

EVALUATION OF ACUTE KIDNEY INJURY USING CONTRAST ULTRASONOGRAPHY IN A RABBIT MODEL OF CRUSH SYNDROME

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Abstract—This study aimed to investigate the feasibility of evaluating acute kidney injury (AKI) using contrast ultrasonography in a rabbit model of crush syndrome. Sixty-four New Zealand white rabbits were anesthetized and the left hind limb was wrapped and compressed for 4 h with a sphygmomanometer. Contrast ultrasonography of the left kidney using microbubbles as the contrast medium was performed. The serum levels of blood urea nitrogen (BUN), creatine (Cr), creatine kinase (CK) and lactate dehydrogenase (LDH) were significantly increased in comparison to before the compression. Correlation analysis showed that peak intensity was negatively correlated with the biochemical parameters and area under the curve (AUC) was positively correlated with these parameters. Contrast ultrasonography is thus sensitive to the changes in renal perfusion after limb crush injury in rabbits. The ultrasonography results correlated well with the biochemical parameters that are related to muscle injury and AKI. (E-mail: ultrasoundzc@163.com) © 2016 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Acute kidney injury, Area under the curve, Rabbits, Crush syndrome.**

INTRODUCTION

Crush injury of the limbs can be fatal despite that the major internal organs may be not affected. Acute kidney injury (AKI) is the most feared complication and often the reason of death in patients with crush injuries (Burns et al. 2010). Crush syndrome is the systemic manifestations caused by the release of cell contents into the bloodstream after traumatic rhabdomyolysis (Greaves et al. 2003). In this condition, a large amount of body fluid is accumulated in the tissue spaces, leading to hypovolemia. The myoglobin released from the myocytes is nephrotoxic and vasoconstrictive. In addition, metabolic acidosis may occur after crush injuries, precipitating the formation of myoglobin casts. All these pathogenic mechanisms contribute to the development of AKI (Gibney et al. 2014; Malinoski et al. 2004). The incidence of AKI after crush injuries ranged from 0.5% to 3.3% (Erek et al. 2002; Guner and Oncu 2014). However, once the crush syndrome manifests, the mortality can be as high as 15% to 40% (Erek et al. 2002; Guner and Oncu 2014; Oda et al. 1997), and up to 80% when complicated with multiple organ dysfunction syndrome or severe infections (Guner and Oncu 2014). Although the crush-related AKI is a fatal condition, it is preventable and reversible, given that this condition is promptly diagnosed (Sever et al. 2006). It has been shown that dialysis can significantly decrease the incidence of AKI and mortality in suspicious crush syndrome patients (Erek et al. 2002; Sever et al. 2006).

The current methods for the diagnosis of crush-related AKI, such as using creatine kinase, lack specificity and definite cut-off values (Bosch et al. 2009). A previous study showed that creatine kinase of 5000 U/L indicates a 20% probability of AKI (Huerta-Alardín et al. 2005). AKI decreases renal blood perfusion. Therefore, except blood tests, direct evaluation of renal blood perfusion using various imaging methods such as computed tomography, magnetic resonance imaging and positron emission tomography has also been used to diagnose AKI. However, the potential nephrotoxicity of iodine contrast media has restricted its application. In addition, this imaging equipment is often unavailable at the disastrous scene. It has also been shown that these imaging modalities are insensitive to early phase AKI

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(Kalantarinia 2009). Ultrasonography devices can be miniaturized for better portability and access at the scene. In the 1988 Armenia earthquake and the 1999 Turkey earthquake, ultrasonography showed its value in the evaluation of crush syndrome (Keven et al. 2001; Sarkisian et al. 1991). Microbubble contrast has been used in the ultrasonographic diagnosis of various organs, as well as in the evaluation of kidney blood perfusion and kidney functions (Quaia et al. 2009). Microbubble contrast is free of nephrotoxicity and is excreted through the lungs. The present study aimed to evaluate the feasibility of quantitative contrast ultrasonography in diagnosing early AKI after limb crush injury in rabbits.

MATERIALS AND METHODS

Crush injury model

Sixty-four New Zealand white rabbits (2.5–3.3 kg, 1.0–1.5 y) were allowed to accommodate for 72 h before the experiments. The animals were provided by the Laboratory Animal Center of the PLA General Hospital, Beijing, China. The study protocol was approved by the Ethics Committee of the PLA General Hospital.

To establish the animal model of crush injury, the 64 rabbits were randomly assigned to 8 groups. The rabbits were anesthetized using ear vein injection of 3% pentobarbital sodium (Sigma-Aldrich, Shanghai, China) at a dose of 30 mg/kg, which was maintained at 10 mg/kg/h. The left hind limb was shaved and wrapped with a 5×25 gauze pad. Then a sphygmomanometer cuff was applied around the hind limb and inflated to 40 kPa. The hind limb was compressed for 4 h to establish the rabbit model of crush injury.

Contrast ultrasonography

The left side of the back of the animal was shaved for ultrasonographic examination of the kidney. A bolus of 0.2 mL microbubble contrast medium (SF6, SonoVue, Bracco, Milan, Italy) was injected *via* the ear vein followed by a flush of 5 mL normal saline (Dong et al. 2012). The ultrasonographic images were obtained using a 5–12 MHz L12-5 linear array probe (Philips IU22, Royal Philips Electronics, Amsterdam, The Netherlands) and analyzed using the QLab software (Royal Philips Electronics, Amsterdam, The Netherlands).

Blood perfusion of the left kidney was examined using ultrasonography at eight time points (immediately before compression [0 h], post compression 0.5 h, 2 h, 6 h, 24 h, 3 d, 7 d and 14 d) with animals from each randomly assigned group utilized per time point. A 3-min moving picture was recorded for each animal and analyzed off-line. An area of interest of a 5×5 square was selected in the shallow side of the renal parenchyma, avoiding large vessels. The time-intensity curve was plotted using the QLab software, and the peak intensity, area under the curve (AUC), ascending slope (AS) and descending slope (DS) were obtained. Each animal was examined three times by three different persons (C.Z., X.W. and J.T.) and the mean value was calculated.

Before injection of contrast reagent, a gray-scale ultrasound was performed of the left kidney to define a maximum longitudinal scanning plane that included the entire kidney.

Conventional color Doppler flow imaging (CDFI) demonstrated blood distribution appearance in the rabbits' left kidneys. Conventional CDFI parameters, peak systolic velocity (V_{max}), the minimal diastolic velocity (V_{min}), the mean velocity (V_{mean}) and the renal segment arterial resistance index (RI) were determined by color Doppler ultrasound using the L9-3 broadband linear array transducer.

Blood biochemistry

After the ultrasonographic examination, 2 mL blood was collected from the ear vein. Then the animal was sacrificed by injection of 50 mL air through the ear vein. The serum levels of blood urea nitrogen (BUN), creatine (Cr), creatine kinase (CK) and lactate dehydrogenase (LDH) were measured using an automated analyzer (SpotChem EZ SP-4430, Arkray, Japan).

Pathologic examination

The left kidney was harvested from each animal and fixed in 10% formalin for 48 h. The specimen was embedded in paraffin and cut into 4-μm sections. The sections were examined using hematoxylin and eosin (HE) staining by two pathologists (Y.L. and J.A. from the Department of Pathology, PLA Bethune International Peace Hospital) who were blinded to the study design.

Statistical analysis

All analyses were performed using the SPSS 13.0 software (SPSS, Chicago, IL, USA). All data are presented as mean ± standard deviation. Comparisons between groups were analyzed using one-way analysis of variance followed by Tukey's post hoc test. A p value < 0.05 was considered statistically significant.

RESULTS

Ultrasonographic results

Compression of the hind limb significantly decreased the contrast intensity of the ultrasonographic images of the kidney. The peak intensity (PI) was significantly decreased at the post compression time points except day 14 compared with that before the compression (all $p < 0.01$), and the AUC was significantly increased at the post compression time points except day 14 compared with that before the compression (all $p < 0.05$) (Fig. 1).