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Calcitriol decreases pro-inflammatory cytokines and protects against severe hemorrhagic shock induced-organ damage in rats

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ABSTRACT

Introduction: Resuscitation after hemorrhagic shock (HS) could result in increased pro-inflammatory cytokines and then multiple organ dysfunctions. Calcitriol exerts pleiotropic effects in a wide variety of target tissues and has a role against anti-inflammation. The present study was aimed to investigate the modulatory effects of calcitriol on the pathophysiological and inflammatory markers following HS in rats.

Materials and methods: By withdrawing 60% of the total blood volume over 30 min via a femoral artery catheter in rats, HS was induced. Afterwards, 10 ng/kg calcitriol was injected intravenously in rats. After performing these procedures, hemodynamic status of mean arterial pressure (MAP) and heart rate (HR) were continuously monitored for 12 h. Hemoglobin, lactic dehydrogenase (LDH), creatine phosphokinase (CPK), liver and renal function were measured at 30 min before the induction of HS and 0, 1, 3, 6, 9, and 12 h after HS, while an equal volume of normal saline as replacement fluid. At 1 and 12 h after inducing HS, serum levels of tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) levels were measured, and the livers, kidneys and lungs were taken out and then examined histo-pathologically at 48 h after inducing HS.

Results: Hemoglobin and MAP were significantly decreased, liver and renal function were significantly impaired, but HR and the levels of LDH, CPK, TNF-α and IL-6 were significantly increased after HS in rats. After being treated with calcitriol following HS resulted in better survival rate, lower serum levels of TNF-α and IL-6, and lesser hepatic, renal, and pulmonary histo-pathologic scores of injury in rats.

Conclusion: Being treated with calcitriol after HS could ameliorate the pro-inflammatory reactions by modulating the effects of cytokines, which lead to prevention of subsequent major organ damages.

1. Introduction

Hemorrhagic shock (HS), which was one of the most common causes of mortality and disabilities worldwide related to traumatic injury, was responsible for up to 40% of trauma deaths [1]. When HS occurred, it could lead to dysfunction of hemodynamic status, induce tissue hypoperfusion, decrease in delivery of oxygen, and then lead to cellular hypoxia, organ injuries, and death [1]. Afterwards, nuclear factor-κB (NF-κB) was activated and provoked local and systemic liberation of both pro- and anti-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), or IL-4. In addition, IL-6 is a Th2-promoting cytokine [2]. Thus, the severe and progressing HS could cause aberrant systematic inflammatory response and ultimately resulted in multi-organ dysfunction and death [3–5]. Some agents, such as resveratrol or rosiglitazone, had been reported to exert anti-inflammatory properties against the production of TNF-α or IL-6 and reduced the adverse effects caused by HS [4,6].

Calcitriol (1,25-dihydroxyvitamin D3), was well known to have the principal physiological role of regulating homeostasis of calcium and phosphorus and bone mineralization [7]. Beyond this calcitropic effect, calcitriol was reported to have pleiotropic effects towards multiple organs and anti-inflammatory effects [7–10]. Studies had shown that dysfunction with vitamin D receptor in

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mice would develop hypertension or cardiac hypertrophy through overstimulation of renin-angiotensin system or prohypertrophic modulatory calcineurin inhibitory protein 1, which was a direct downstream target of calcineurin/nuclear factor of activated T cell signaling [9,10]. Moreover, calcitriol additionally could act as a role against anti-inflammation, such as delaying the progression of diabetic nephropathy through suppressing TNF-α and IL-6 [7,8].

The possible mechanism of organ injury caused by HS was still incompletely understood, but both experimental and clinical studies suggested that leukocytes, especially macrophages, which could release pro-inflammatory cytokines or reactive oxygen species, could be activated by the process of hypoxia/re-oxygenation and the translocated bacterial endotoxin, and then resulted in organ damages [11,12]. Although calcitriol had immune-modulatory effects beyond its calcitropic effects, there were no studies investigating its potential effects against HS. In this study, we aim to know how calcitriol could exert its potential protecting effects of major organs (liver, kidney, and lung) and examine the effects on modulating the serum levels of pro-inflammatory cytokines (TNF-α, and IL-6) by treating rats with calcitriol after HS.

2. Materials and methods

2.1. Procedures of preparations before inducing HS of rats

After purchasing from the National Animal Center (Taipei, Taiwan), these 32 male Wistar-Kyoto rats weighing 260–300 g were bred in a controlled temperature of 22 ± 1 °C with a 12-h light/dark cycle in the Animal Center at Tzu Chi University. Rats were bred with food and water *ad libitum*. The experimental protocol was approved by the Animal Care and Use Committee of Tzu Chi University.

Before inducing HS, the rats were inhaled ether for about fifteen minutes for anesthetization. To collect blood specimen and monitor mean arterial pressure (MAP) and heart rate (HR), a polyethylene catheter (PE-50) was inserted into the femoral artery of these rats after being well anesthetized and was connected to a pressure transducer (Gould Instruments, Cleveland, OH, USA) on a polygraph recorder (Power Lab, AD Instruments, Mountain View, CA, USA). To administer medications or fluids intravenously, the other PE-50 catheter was inserted into the femoral vein in these rats. The whole procedure of operation was accomplished within 15 min with a small wound (less than 0.5 cm^2) left behind without giving medications towards treating these wounds. After these procedures, these rats were relocated in a conscious rat metabolic cage (Shingshieying Instruments, Hualien, Taiwan). Hemorrhagic shock would be induced 24 h later in an un-anesthetized status after these rats awoke from these procedures [3–5].

2.2. Procedures of inducing HS of rats

Of these rats, HS was generated by drawing blood from the femoral arterial catheter into a 10-ml syringe using a pump with a controlled rate of extraction to imitate a typical hemorrhagic event. During this period of 30 min, blood was extracted with the amount of 60% of total blood volume, which was calculated as 6 ml/100 gm BW + 0.77 ml and resulting the development of HS [1]. After the development of HS, samples of blood of these rats (0.5 ml) were obtained for biochemical analysis with an equal volume of 0.5 ml normal saline used as resuscitation fluid at 0, 1, 3, 6, 9, and 12 h. After the development of HS and resuscitation, these rats were monitored continuously for another 48 h and then sacrificed for histo-pathological examinations [3–5].

2.3. Grouping of rats

These rats were divided randomly into four groups. Rats in the Calcitriol group (n = 8) were not subjected to HS but were given 10 ng/kg calcitriol (Sigma Chemical, St. Louis, MO, USA) in 0.5 ml normal saline. Rats in the Control group (n = 8), were not subjected to HS and were not treated with calcitriol, but given 0.5 ml normal saline. Hemorrhagic shock was induced in rats in the HS group (n = 8) and these rats were immediately received an intravenous drip of 0.5 ml normal saline. Rats in the HS + Calcitriol group (n = 8) were induced HS and these rats were immediately received 10 ng/kg calcitriol in 0.5 ml normal saline. Normal saline and calcitriol were all infused intravenously via the PE-50 catheter in femoral vein of these rats over 10 min [13].

2.4. Biochemical analysis

Before injecting heparin (2 IU/gm BW) in 1 ml normal saline intravenously via the PE-50 catheter into rats over 20 min, samples of arterial blood were obtained (0.5 ml) to determine baseline biochemical values [3–5]. Samples were collected at 30 min before inducing HS and at 0, 1, 3, 6, 9, and 12 h after the induction of HS with infusing equal amount of normal saline as resuscitation fluid. These blood samples were sent for measuring the levels of lactic dehydrogenase (LDH), creatine phosphokinase (CPK), hepatic and renal functions, which including glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), and creatinine (Cre). Of this blood sample (about 0.5 ml), about 0.1 ml was sent for the measurement of hemoglobin (Sysmex K-1000, Sysmex American, Mundelein, IL, USA). The other 0.4 ml was immediately centrifuged at 3000*g* for 10 min, and the serum was decanted and separated into two parts. One part was stored at 4 °C within 1 h after collection of blood samples and then aimed for biochemical analysis with an autoanalyzer (COBAS C111, Roche Diagnostics, Basel, Switzerland). The other part was stored at −80 °C for measuring the levels of TNF-α and IL-6 [3–5].

2.5. Measuring serum levels of TNF-α, and IL-6 measurement

Serum levels of TNF-α and IL-6 were measured separately at 1 and 12 h after the induction of HS by antibody enzyme-linked immunosorbent assay (ELISA) using commercial antibody pairs, recombinant standards, and a biotin-streptavidin-peroxidase detection system (R&D Systems, Inc., Minneapolis, MN, USA) [3–5]. The optical density of each well was determined within 30 min, using an automated ELISA reader (Sunrise, Tecan Co., Grödingen, Austria) set to 450 nm/540 nm wavelengths. The limit of detection, calculated as the concentration of rat TNF-α and IL-6 corresponding to the blank average minus three standard deviations, was 5 pg/ml and 21 pg/ml, respectively. The inter- and intra-assay coefficients of variation for TNF-α were 8.8% and 2.1%, and for IL-6 were 8.1% and 8.8%, respectively.

2.6. Pathological examination

Rats were anesthetizedly sacrificed at 48 h after the induction of HS and the livers, kidneys, and lungs were immediately taken out. Tissue samples of livers, kidneys, and lungs were fixed, processed, and H & E stained as described previously [3–5]. One pathologist who was blinded to the grouping of these rats examined these specimens. The severity of hepatic, renal tubular and pulmonary injuries in these specimens were evaluated and scored as described previously [3–5]. Every evaluation observed on all these specimens included five sections per organ and five fields per section.