Sodium butyrate decreases the activation of NF-κB reducing inflammation and oxidative damage in the kidney of rats subjected to contrast-induced nephropathy

Roberta Albino Machado¹, Larissa de Souza Constantino¹, Cristiane Damiani Tomasi¹, Hugo Alberto Rojas¹, Françieli Silva Vuolo¹, Marcelo Fontana Vitto², Patrícia Acordi Cesconetto², Cláudio Teodoro de Souza², Cristiane Ritter¹ and Felipe Dal-Pizzol¹

¹Laboratório de Fisiopatologia Experimental, Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, Brazil and ²Exercise Biochemistry and Physiology Laboratory, Postgraduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, Criciúma, Brazil

Correspondence and offprint requests to: Felipe Dal-Pizzol; E-mail: piz@unesc.net

Abstract

Background. Contrast-induced nephropathy (CIN) is associated with a combination of hypoxic and toxic renal tubular damage, renal endothelial dysfunction and altered intra-renal microcirculation. Recently, sodium butyrate (SB) has been focused on since it possesses anti-inflammatory activities. Thus, based on the lack of information on the effects of SB in acute kidney injury (AKI), we investigated the possible effects of SB after CIN in rats.

Methods. Wistar rats were divided into three groups: (1 sham) control, (2 MI) AKI treated with contrast medium and (3 MI + SB) AKI plus SB. Six days after contrast administration, blood and kidney were removed for the determination of creatinine, interleukin (IL)-6 levels, oxidative damage parameters and histologic analyses. Nuclear factor kappa B (NF-κB), pIκBα and vasodilator-stimulated phosphoprotein (VASP) protein content were determined by immunoblotting.

Results. After 6 days, the levels of creatinine increased significantly in the MI group, and this was attenuated using SB. SB treatment was associated with a decrease on the levels of lipid peroxidation, but not the protein oxidation, and IL-6 levels, as well as tubular damage. These effects are probably mediated, in part, by a decrease on the activation of NF-κB in the kidney, but not alteration in pVASP content.

Conclusions. The current experiment suggests that NF-κB induced an inflammatory response after CIN and SB could inhibit NF-κB expression protecting against CIN in rats.

Keywords: acute kidney injury; contrast-induced nephropathy; nephrotoxicity; sodium butyrate

Introduction

Acute kidney injury (AKI) is a heterogeneous disorder with multiple aetiologies, risk factors and clinical presentations [1]. AKI is commonly encountered in both hospital and outpatient settings and is associated with an increased risk of mortality. Despite improvements in our understanding of its pathogenesis, many aspects of AKI remain subject to debate [2].

Contrast-induced nephropathy (CIN) is a form of AKI following exposure to contrast media. Its pathogenesis involves renal ischaemia, particularly in the outer medulla, where oxygen delivery is already at critical levels and direct epithelial cell toxicity [3]. Thus, CIN is associated with a combination of hypoxic and toxic renal tubular damage, renal endothelial dysfunction and altered intra-renal microcirculation [4]. Various models of renal inflammation and ischaemia have shown a role of reactive oxygen species (ROS) in glomerular injury, thus the adverse effects of contrast media on renal function may involve the generation of ROS [4].

Butyric acid is a product of bacterial fermentation of carbohydrates in the rumen of multigastric animals and in the colon of omnivores, such as humans [5, 6]. The
induction of apoptosis of tumour cells by butyrate is thought to be an important mechanism in the natural protection against colorectal cancer; since one of the major actions of butyrate is to inhibit histone deacetylase (HDAC), butyrate may induce apoptosis by derepression of specific cell death genes [7]. Sodium butyrate (SB) is known to inhibit HDAC, modulate gene expression in the endothelium [8] and to induce apoptotic effects in a number of cancers [9]. Recently, SB has been focused on since it possesses anti-inflammatory activities, but its exact mechanisms of action are not well understood. Since SB inhibits HDAC resulting in the relative hyperacetylation of core histone proteins (H3 and H4), it probably regulates the expression of some inflammatory related genes [5]. In addition, some authors demonstrate the inter-relation between short chain fatty acids and the suppression of nuclear factor kappa B (NF-kB) activation, which is a well-known inflammatory mediator [10]. The acetylation of different lysines in p65 and p50 regulates different functions of NF-kB, including transcription activation, DNA-binding affinity and IkB assembly [11]. In addition, the activation of NF-kB can be linked to the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) [12] that is a defensive mechanism for counteracting cytokine-stimulated kidney damage [13] and induces vasodilatation [14].

Thus, based on the lack of information available in the literature regarding the mechanisms of contrast toxicity and the effects of SB in AKI, in this study, we aimed to investigate the possible effects of SB after CIN in rats.

Material and methods

Animals

Male Wistar rats weighing 300–350 g obtained from Central Animal House of Universidade do Extremo Sul Catarinense breeding colony were housed individually under standard conditions (12-h light/dark cycles with room temperature of 22–24°C). All experiments were performed in accordance with National Institutes of Health guidelines and with the approval of the local ethics committee.

Animal model of AKI

The rats were randomly divided into three treatment groups as follows: (1) sham group, (2) AKI induced by metaglumina diatrizoate sodium (MI group) and (3) AKI plus SB (MI + SB group), n = 10 each group, each experiment.

Twenty-four hours before contrast administration, animals had restricted access to water and after this period, animals from Groups 2 and 3 received the contrast medium (metaglumina diatrizoate sodium at a dose of 6 mL/kg through the tail vein). Sham animals received the same volume of saline. SB was administrated in the tail vein at a dose of 500 mg/kg 6 h before CIN. On the sixth day, animals were killed by decapitation and then blood was sampled to determine serum creatinine levels. Kidneys were removed for the determination of oxidative damage and inflammatory parameters, the content of NF-kB, pIkB and pVASP or histological analyses.

Serum creatinine

Serum creatinine was measured as a marker of CIN using a colourimetric assay. In brief, serum was exposed to 2% naphthol and 0.05% diacetyl in a final volume of 1 mL and measured spectrophotometrically after 20 min at 540 nm. Results were expressed as milligrams per deciliter.
an attenuation of creatinine increase (10 ± 5.0 mg/dL), suggesting a protection against CIN in this model (Figure 1).

**Inflammatory parameters**

Contrast medium administration was followed by an increase of IL-6 levels in the kidney (65 ± 10 versus 495 ± 55 pg/mg protein comparing sham and CIN animals), suggesting a role of inflammatory response in the genesis of CIN (Figure 2). SB administration decreased kidney IL-6 levels (195 ± 23 pg/mg protein).

**Oxidative damage**

In general, inflammatory response was associated with oxidative damage, thus we determined TBARS and protein carbonyl levels in the kidney tissue after CIN. Both oxidative markers were increased after the induction of CIN (0.2 ± 0.08 versus 0.4 ± 0.16 MDA equivalents comparing sham and CIN animals) and (1.5 ± 0.3 versus 6.1 ± 2.0 protein carbonyls comparing sham and CIN animals), but only to TBARS, it demonstrated a protective effect of SB administration (0.25 ± 0.09 MDA equivalents) and (5.8 ± 2.1 protein carbonyls) (Figures 3 and 4).

**Histology**

At 6 days after CIN, there were only minor changes in the histology scores. Compared with sham animals, the mean score for the degree of tubular necrosis was significantly higher in the CIN group (0.41 ± 0.16 versus 1.33 ± 0.4) and this was attenuated with the use of SB (0.85 ± 0.2, P = 0.03). There were no differences in the medullary congestion scores (data not shown).

**NF-κB activation**

NF-κB activation was also investigated by determining its nuclear content and by studying the phosphorylation of its
inhibitory protein. CIN leads to an increase in the phosphorylation of IκBα and a consequent increase translocation of NF-κB to the nucleus (Figure 5). The use of SB could decrease NF-κB nuclear translocation at least in part by inhibiting the phosphorylation of its inhibitor (Figure 5).

**VASP phosphorylation**

In CIN group, VASP phosphorylation increased 1.26 times when compared with control group, but we cannot demonstrate any significant effect of SB in this model (Figure 6).

**Discussion**

We here demonstrated that SB prevents the translocation of NF-κB into the nucleus, probably by reducing the phosphorylation of its inhibitor, decreasing oxidative damage, inflammatory response and tubular damage and attenuating the AKI associated with contrast medium administration in rats.

A common signalling molecule involved in several inflammatory pathways is the NF-κB. IkBα plays a pivotal role in the NF-κB signalling pathway by regulating NF-κB activation. The primary level of regulation of NF-κB activity is through its retention in the cytoplasm via interactions with IkBα. The secondary levels of regulation are following stimulation with pro-inflammatory cytokines leading to the re-synthesis of IkBα and the post-induction nuclear accumulation of IkBα inducing nuclear export of NF-κB [11]. Our results demonstrate that SB inhibits NF-κB translocation to the nucleus in the kidney, probably by the phosphorylation of IkBα, preventing the activity of the inhibitory protein IkBα in the cytoplasm. This was associated with a decrease of kidney IL-6 levels similar to sham levels, suggesting a pivotal role of HDAC inhibitors as therapeutic tools. In a permanent middle cerebral artery occlusion, it was shown that the administration of HDAC inhibitors decreased brain infarct volume, suppressed microglial activation and inhibited inflammatory markers in the ischaemic brain [21]. In a recent study, the administration of trichostatin A or SB alleviated sepsis-induced lung injury. This was accompanied by reduced neutrophil infiltration, decreased intercellular adhesion molecule-1 and E-selectin expression in lung tissue and lower IL-6 level in plasma [22]. It has been shown that SB can inhibit the lethality of severe sepsis in rats and presented protective effects on multiple organ damage associated with severe sepsis [23].

Despite the complete inhibition of NF-κB activation after administration of SB, we could not demonstrate a complete inhibition of oxidative damage, a complete recuperation of plasma creatinine levels nor a complete prevention of tubular necrosis suggesting that several other aspects of AKI are not mediated by NF-κB-induced genes. AKI induces an inflammatory response, which results in the formation of ROS that augments local tissue damage [24], and this is probably mediated by the activation of NF-κB [25]. In addition, ROS could be generated in the reperfusion injury that also follows CIN, thus, it would seem that a multicomponent strategy must be used to completely prevent AKI in the setting of CIN [26]. Since it seems that other mechanism could account...
to the observed protective effects of SB, we determined the phosphorylation of VASP in our model. The degradation of IκB is associated with the phosphorylation of VASP [12] and this protein promotes vasodilatation [14] and decrease calcium influx in mesangial cells [13], therefore it may have a protective effect on kidney function. At 6 days after CIN, there was an increase in the kidney content of pVASP, suggesting that intrinsic mechanisms are activated trying to improve blood flow and to regenerate damaged tubular cells. SB protective mechanisms seemed to be independent on blood flow or regulation of calcium influx since we cannot demonstrate any significant effect of SB in pVASP content.

In summary, the current experiment suggests that NF-κB is related to kidney inflammatory response after CIN and that SB could inhibit NF-κB nuclear translocation protecting kidneys from CIN mainly inhibiting inflammatory and oxidative tubular damage, but not controlling blood flow.

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Conflict of interest statement. None declared.

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