Use of simvastatin in the prevention of cyclophosphamide-induced ureteral mucositis

Uso da sinvastatina na prevenção da mucosite ureteral induzida pela ciclofosfamida

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ABSTRACT

Purpose: This study was designed to investigate the pharmacological efficacy of simvastatin against ureteral mucositis cyclophosphamide-induced. **Methods:** Wistar rats weighing 287±14g were used. A single dose of cyclophosphamide (CYP) 200mg/kg IP + oral simvastatin (10mg/kg) were administered in the (CYP/SIMV) group (n=6), In the group (CYP/SAL) (n=6), saline v.o. was administered. The animals were weighed daily. After 7 days of CYP administration, blood was collected by cardiac puncture under anesthesia. After euthanasia, uterers were collected for histopathology. Serum TNF-α, IL-1α, IL-6 were determined by ELISA. **Results:** CYP-induced ureteral mucositis in rats resulted in a significant increased level of serum cytokines (TNF-α, IL-1β, IL-6). Simvastatin treated rats showed significant decreased level of inflammatory cytokines. In body weight records, CYP-treated rats showed visible significant body mass loss compared to untreated rats ($p<0.05$). Edema and inflammatory cells in ureter tissues were reduced after simvastatin treatment, as demonstrated in histological H-E staining. **Conclusion:** In conclusion, our current findings provided scientific evidence that oral simvastatin positively influenced benefits against cyclophosphamide-induced ureter mucositis, which possibly has occurred by inactivating cytokines.

Key words: Ureter. Mucositis. Cyclophosphamide. Simvastatin. Treatment. Rats.

RESUMO

Objetivo: Este estudo foi realizado para investigar a eficácia farmacológica da sinvastatina contra a mucosite ureteral induzida pela ciclofosfamida. **Métodos:** Foram utilizados ratos Wistar pesando 287±14g. Uma dose única de ciclofosfamida (CIF) 200 mg/kg i.p. + sinvastatina oral (10 mg / kg) foi administrada no grupo (CIF/SINV) (n = 6; no grupo (CIF/SAL) (n = 6) salina v.o. foi administrada. Os animais foram pesados diariamente. Após 7 dias de administração de CIF, sangue foi colhido por punção cardíaca sob anestesia. Após eutanásia, os ureteres foram coletados para exame histopatológico. Foram dosados TNF-α, IL-1α, IL-6 sérico pelo ELISA. **Resultados:** A mucosite ureteral induzida por CIF resultou num aumento significativo do nível de citocinas séricas (TNF-α, IL-1β, IL-6). Ratos tratados com sinvastatina mostraram níveis significativamente menores de citocinas pro-inflamatórias. Nos registros de peso corporal, os ratos tratados com CIF mostraram uma significativa perda de massa corporal em comparação com os ratos não tratados ($p<0,05$). Edema e células inflamatórias no tecido do ureter foram reduzidos pelo tratamento com sinvastatina, como demonstrado na coloração histológica com H-E. **Conclusão:** Em conclusão, nossos achados forneceram evidências científicas de que a sinvastatina oral influenciou positivamente o tratamento da mucosite do ureter induzida pela ciclofosfamida, o que possivelmente ocorreu pela inativação de citocinas.

Descritores: Ureter. Mucosite. Ciclofosfamida. Sinvastatina. Tratamento. Ratos

INTRODUCTION

The discovery of HMG-CoA reductase inhibitors in the 1980s and their clinical use as therapeutic agents have resulted in widely used treatments for hyperlipidemia and coronary diseases. The main mechanism of HMG-CoA reductase inhibitors, commonly referred as statins, is the inhibition of liver cholesterol synthesis by blocking the conversion of HMG-CoA to mevalonate, which is the limiting step of cholesterol biosynthesis. A potential cholesterol effect independent of statins appears to be the up-regulation of endothelial nitric oxide (eNO) synthesis[1]. It is believed that such pleiotropic effects include anti-inflammatory and antioxidant actions[2].

It has been proposed that acrolein, the urotoxic metabolite of cyclophosphamide (CYP), is responsible for inducing premature changes that will lead to cyclophosphamide-induced hemorrhagic cystitis. The main effects of hemorrhagic ureteral mucositis are urothelial damage, edema, necrosis, ulceration, hemorrhage,

neovascularization and leukocyte infiltration. The involvement of eNO in urothelial damage and in the inflammatory events of this pathology has been demonstrated due to a decrease in the synthesis and release of nitric oxide, which is an endothelium-derived substance involved in the inhibition of platelet aggregation, attenuation neutrophil adhesion and microvascular permeability reduction[3]. As the inhibition of inflammation is one of the pletopenic effects of simvastatin, we outline a hypothesis to be tested: the role of simvastatin in the prevention of CYP-induced ureteral mucositis in rats.

CYP is used in cancer therapies and in non-neoplastic diseases such as nephrotic syndrome, systemic lupus erythematosus and rheumatoid arthritis. Hemorrhagic cystitis is a known adverse effect of this drug and may be a limiting factor in its use. In the absence of adequate uroprotection, the incidence of this side effect ranges from 2 to 40% in patients taking low doses of cyclophosphamide in the long term. The cause of the damage to the urinary epithelium seems to be related to acrolein, a urotoxic metabolite of CYP[4]. Although post-treatment urinary bladder involvement with CYP is well studied with good scientific evidence, the same does not occur with ureters. There are scarce and even no published studies on ureteral mucositis.

There is growing evidence suggesting that some of the clinical benefits of statin therapy can be attributed to independent mechanisms of its effect in reducing serum cholesterol levels. These are the so-called pleiotropic effects and include anti-inflammatory and antioxidant actions[5].

Urinary tract infection is a type of disease that affects bladder and ureteral function[6]. Clinically, some medications may induce nonbacterial / pathological cystitis and mucositis, such as CYP[7]. It is used in chemotherapy and is one of the most common drugs in most health systems. However, most patients experience unwanted adverse effects, including low immune cell counts, hair loss, vomiting, and bladder bleeding. In bladder and ureter impairment, CYP produces acrolein that is toxic to the urological epithelium and causes hemorrhagic cystitis and ureteral mucositis. Urinary function impairment can be linked to lower urinary tract symptoms, including dysuria,

hematuria, and hemorrhage[8,9]. Risks of hemorrhagic cystitis can further induce immune disturbances and inflammatory disorders over time[10]. Inhibition of cystitis may enhance drug effects of CYP and reduces pain in patients. Meanwhile, the potential alternative for interstitial cystitis treatment needs to be further developed and investigated.

Thus, although statins are not used in the treatment of hemorrhagic cystitis, there is scientific support for this due to its anti-inflammatory and immunomodulatory effects[1,11-13]. A pilot study performed prior to this experiment revealed ureteral changes after treatment with CYP, which is why the present study was proposed. A recent literature review has found that no published studies have tested the use of statins in experimental models involving CYP-induced ureteral mucositis.

OBJETIVE

Examine the effects of simvastatin in an experimental model of CYP-induced ureteral mucositis. Among them, we focused on: the effect of simvastatin on the survival, prevention and treatment of CYP-induced ureteral mucositis. Repercussion of the use of simvastatin in the expression of proinflammatory serum cytokines.

METHODS

Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia*) weighing 287±14g were used. They were kept in polypropylene cages and kept under temperature control in a 12h cycle overnight and permitted *ad libitum* access to water and commercially available rat feed (Prevence®). The experimental protocol was approved by the Institutional Commission on Ethics in the use of Animals (Protocol 03/2016).

Experimental design and procedures

The animals were randomly divided into two groups of six rats each and in each of them a single dose of cyclophosphamide 200mg/kg intraperitoneal (IP) was administered. In the experimental group (CYP/SIMV) (n=6), oral simvastatin (10mg/kg)

was given as oral suspension (gavage) starting one week before administration of cyclophosphamide. In the group (CYP/SAL) (n=6), saline solution was administered in the same doses and time limits. The animals were weighed daily for weight control.

After 7 days of CYP administration the blood of the animals was collected by cardiac puncture under ketamine anesthesia 70 mg / kg and xylazine 10 mg/kg i.p. and then the rats were killed with superdose of the thiopental sodium anesthetic (100 mg/kg) via i.p. Blood was centrifuged and serum separated dosing TNF-α, IL-1α, IL-6 by the ELISA technique using Peprotec kits, USA, according to the manufacturer's instructions. After tricotomy and antisepsis with 70% ethyl alcohol, a median xiphobic laparotomy was performed, the ureters were removed for microscopic examination, looking for inflammation of the mucosa, congestion and hemorrhage of the organ wall.

Histopathology

The ureters were fixed in 10% formalin, embedded in paraffin, and cross sections of 5 micrometers were stained with hematoxylin and eosin. Histopathological examination was done by a pathologist without previous knowledge of the study groups, using the following criteria: normal epithelium and absence of infiltration by inflammatory cells and ulceration (score 0); moderate changes involving reduction of epithelial cells, mucosal thinning, submucosal edema, moderate bleeding and few ulcerations (score 1); intense changes including erosion of the mucosa, infiltration of inflammatory cells, fibrin deposition, hemorrhage and multiple ulcerations (score 2). For quantification of the total scores, the scores observed in 3 histological sections of the ureter per animal were added.

Statistical analysis

The results of the microscopic data were expressed as mean±standard deviation for at least 5 determinations, using Student's t-test using the BioEstat 5.0 software. The differences between groups were considered significant when $p<0.05$.

RESULTS

In body weight records, cyclophosphamide-lesioned rats showed visible body mass loss compared to untreated rats ($p< 0.05$).

Serum dosage of cytokines

Expression of TNF-α were demonstrated to a lesser extent in the serum of animals treated with simvastatin (172.3±21 pg/mL) than those receiving saline (365.4±62 pg/mL) as may be seen in table 1. There was a marked decrease in tissue reactivity with the use of simvastatin. IL-1α expression was significantly higher in the saline (CYP/SAL) group (126.9±23 pg/ml) than in the treated group simvastatin (CYP/SIMV) (47.2±7 pg/mL). IL-6 expression was also lower in the CYP/SIMV group (51.7±12 pg/mL) than in the CYP/SAL group (121.6±15 pg/mL). The data are summarized in table 1.

Table 1 - Values of the cytokines dosed in the serum of the animals submitted to treatment with CYP, saline or simvastatin.

Groups	TNF α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
CYP/SAL	365.4±62	126.9±23	121.6±15
CYP/SIMV	172.3±21*	47.2±7*	51.7±12*

* $p<0.05$ compared with the dosages of the animals of the CYP/SAL group, by Student's t-test. CYP/SAL, group of animals treated with cyclophosphamide and saline; CYP/SIMV, group of animals treated with cyclophosphamide and simvastatin.

Histopathology

Morphological characteristics of ureter cells in CYP exposed organs had a deformed cytoskeleton, hydropic degeneration in cells, increased necrotic cell counts, visible inflammatory infiltration, and a damaged ultrastructure (Figure 1). Followed by simvastatin treatment, the cytotoxicity signs of the bladder induced by CYP exposure had notable morphological improvements marked by reduced necrotic cell numbers. Comparing the scores, the differences were significant ($p<0.05$). These data are summarized in Table 2.

Table 2 - Values of ureteral histological scores of animals submitted to treatment with cyclophosphamide, saline or simvastatin.

Groups	Ureter scores
CYP/SAL	14
CYP/SIMV	7*

* p<0,05 compared with the scores of animals of the CYP/SAL group. CYP/SAL, group of animals treated with CYP and saline; CYP/SIMV, group treated with CYP and simvastatin.

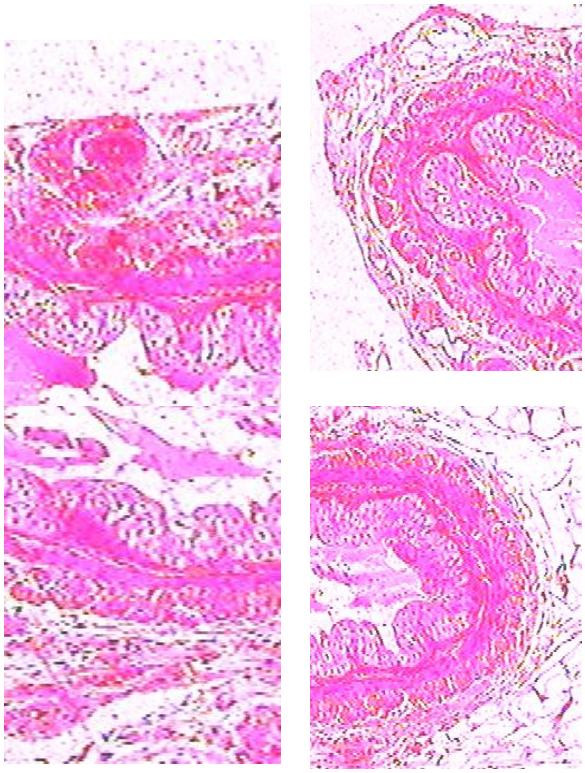

Figure 1 - Ureter: edema, inflammation, fibrin deposition in rats of group CYP/SAL. HE, 200x.

DISCUSSION

Simvastatin treatment suppressed body mass loss in treated rats, which indicates there are basic beneficial effects of simvastatin to improvements in metabolic function in the body. In this study, damaged rats showed visible body mass loss in a time-dependent manner, which suggests that the mass loss resulted from ureteral mucositis induced by CYP. Overall, our findings show that simvastatin treatment may serve as a promising alternative for combatting CYP-induced ureteral mucositis, which indicates that there are potential clinical prospects for simvastatin. Some studies have demonstrated a protective function of statins against doxorubicin-induced cytotoxicity in endothelial cells in vitro[14,15] as well as inflammatory responses

and toxicity in vivo[16]. These data point to a more general organoprotective role of statins against normal tissue damage evoked by anticancer drugs and radiotherapy.

Bearing in mind that endothelial cells are of particular relevance for inflammatory responses, protection of endothelial cells by statins together with the herein shown cytoprotective function in keratinocytes is indicative of anti-mucositis activity of statin in the context of doxorubicin-based therapeutic regimen also in vivo. This hypothesis gains support by recent data showing that atorvastatin prevents mucosal damage and inflammation in hamsters following administration of antimetabolites[17]. Thus, the underlying mechanism responsible for the beneficial effects of simvastatin-exerted cytoprotection observed in ureter of our study and this needs to be further investigated.

Inflammation represents a complex biological response of body tissue to harmful stimuli, including irritants, pathogens, and damaged cells[18]. TNF-α is a cytokine that can induce cell death (apoptosis). The cytotoxicity of TNF-α has been linked to cachexia, septic shock, and inflammatory reactions[19]. We hypothesized that reduction of cytokines may block urinary mucositis development. The present results showed that CYP-exposed rats had decreased TNF-α, IL-1β and IL-6 in the serum followed by simvastatin treatment, as a cytoprotective effect, like that of glycyrrhetinic acid liposome[20]. These improved outcomes were consistent with the our pathological examinations. This is the first time that a study has been conducted on the effect of statins on the prevention of ureteral mucositis after treatment with CYP.

We concluded that there are potential benefits of simvastatin against ureteral mucosal inflammatory stress produced by CYP, which possibly occurs by inactivating cytokines. Our data encourage new forthcoming in vivo and clinical studies addressing the usefulness of simvastatin in the prevention of ureteral mucositis.

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