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p-Cresyl sulfate affects the oxidative burst, phagocytosis process, and antigen presentation of monocyte-derived macrophages

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HIGHLIGHTS

- Lowest pCS concentrations induce ROS production and increases phagocytosis.
- Highest pCS concentrations diminish its ability to activate the immune cells.
- No modulation of HLA-DR or CD86 expression was induced by pCS.

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ABSTRACT

Immune system dysfunction is a common condition in chronic kidney disease (CKD). The present study investigated the effect of *p*-Cresyl sulfate (*p*CS) on human cell line U937 monocyte-derived macrophages (MDM) activity. MDM (1×10^6 cells/mL) were incubated with *p*CS (10, 25, or 50 μg/mL), with or without lipopolysaccharide (LPS; 25 ng/mL) and then evaluated NO production, phagocytosis and antigen-presenting molecules expression (HLA-ABC, HLA-DR, CD80 and CD86). All analyses were performed by flow cytometry. All *p*CS concentrations were able to increase NO production ($49 \pm 12.1\%$, $39.8 \pm 7.75\%$, $43.7 \pm 11.9\%$, respectively) compared to untreated cells ($4.35 \pm 3.34\%$) after 6 h incubation but only the lowest concentration increased this production after 12 h ($82.9 \pm 8.6\%$, $61 \pm 7.2\%$, $40.8 \pm 11.7\%$). Combined with LPS, the same results were observed. Regarding to phagocytosis, all concentrations were able to induce bead engulfment ($35.4 \pm 2.71\%$, $30 \pm 3.04\%$, $23.28 \pm 4.58\%$). In addition, *p*CS (50 μg/mL) was able to increase HLA-ABC and CD80 expression, showed a slight effect on HLA-DR expression and, no difference in basal CD86 levels. *p*CS can induce an increased oxidative burst and phagocytosis by human macrophages while no modulation of HLA-DR or CD86 expression was induced. Together, these results suggest that *p*CS induces macrophage activation but interfere in antigen processing, leading to a failure in adaptive immune response in CKD.

1. Introduction

Uremic toxicity is caused by a progressive decline in kidney function due to the accumulation in the body of potentially toxic compounds with biological and/or biochemical activity, collectively called uremic retention solutes or uremic toxins (Vanholder et al., 2003; Yavuz et al., 2005). *p*-cresyl sulfate (*p*CS) levels, an uremic toxin, increases with the worsening of renal function (Liabeuf et al., 2010), and has been associated with an imbalance of the immune system response, atherosclerosis, and cardiovascular disease (Dou et al., 2004; Vanholder et al., 2011). Studies indicate that *p*CS seems to induce leukocyte activation (Schepers et al., 2007). A growing body of evidence from *in vivo* studies indicates that *p*CS plays significant pathological roles in chronic kidney

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disease (CKD) by causing renal tubular cell damage by inducing oxidative stress (Watanabe et al., 2014), promoting endothelial dysfunction and the suppression of respiratory burst action in the blood. Furthermore, *p*CS has been found to be associated with cardiovascular disease and mortality in CKD patients (Liabeuf et al., 2010; Meijers et al., 2008).

The derangement of the immune system in CKD patients leads, for example, to an increased susceptibility to infections and unresponsiveness to vaccination. In fact, it has been reported that both adaptive and innate immune responses seem to be affected adversely by uremic toxicity (Haag-Weber and Horl, 1993; Lewis et al., 1998). The uremia-related immune deficiency involves mainly the antigen-presenting cells (APC) as well as T lymphocytes but many pieces are missing to complete the puzzle of the immune response in CKD.

*p*CS is already known for its effects in several biological conditions in CKD patients due to their high levels of this uremic toxin in blood. However, a lack of study on *p*CS effects on immunological responses led our group to evaluate this uremic toxin in the activation of human monocyte-derived macrophages, reaching a better understanding of the effects of *p*CS on immune system dysfunction. Thus, this study aimed to evaluate the monocyte-derived macrophage (MDM) response to the uremic toxin *p*CS, as well as to determine the cellular events involved in the innate immune response in CKD.

2. Material and methods

2.1. U937 cell culture, differentiation, and stimulation

U937 cells were treated, as following describes, prior all assays performed in this study. The human monocytic cell line U937 was maintained in RPMI 1640 medium (Gibco BRL) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco BRL), 100 IU/mL penicillin, and 0.1 μg/mL streptomycin in a humidified incubator with 5% CO_2 at 37 °C. For U937 cell differentiation into adherent macrophages (MDM), cells (1×10^6 cells/mL) were cultured in a 6-well flat-bottom plate in the presence of 150 nM phorbol myristate acetate (PMA; Sigma) at 37 °C for 48 h (Picone et al., 1989) and then for further 48 h in RPMI with 2% FBS (resting conditions). For stimulation MDM were incubated for 6 h, 12 h, or 24 h with 10, 25 μg/mL (mean uremic concentration) or 50 μg/mL (maximum uremic concentration) of *p*CS (Duranton et al., 2012), which was synthesized as a potassium salt as described previously (Feigenbaum and Neuberg, 1941). Each concentration of uremic toxin was analyzed in the presence or absence of lipopolysaccharide (LPS) (25 ng/mL). Cells incubated with LPS or 2% RPMI alone were used as control.

2.2. Cytotoxicity assay (MTT assay)

MDM viability in response to differentiation and stimulation described above was estimated by the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) test. After incubation, MTT was added for 4 h incubation (37 °C at 5% CO_2) and then cells were lysed using DMSO. The supernatant was transfer to 96-well plate and analyzed at microplate reader. Viability was calculated as follows: (OD Control − OD Treated/OD Control) × 100.

2.3. Intracellular nitric oxide (NO) detection

After differentiation and stimulation for 6 or 12 h, described above, cells were stained with DAF-FM diacetate (5 μM; Molecular Probes) for 40 min at 37 °C in the dark, according to the manufacture's instructions. Then cells were washed in PBS, fixed (PBS 4% paraformaldehyde) and analyzed by flow cytometry (FACScalibur).

2.4. Phagocytosis assay

Stimulated MDM, *p*CS in the presence or absence of LPS for 12 h as described before, were incubated with fluorescent beads (20 beads:1 MDM) (FluoSpheres® polystyrene beads; Invitrogen-Molecular Probes), for 4 h at 37 °C. After incubation, cells were washed twice in cold PBS to avoid attached beads on cell surface, resuspended in PBS 4% paraformaldehyde and analyzed by flow cytometry (FACSCalibur), following instructions of the manufacturer. The phagocytosis efficiency of cells was defined as the percentage of cells that internalized beads with reference to the total cells analyzed.

2.5. Determination of the expression of MDM markers of antigen presentation

MDM were stimulated for 24 h in the presence of *p*CS with or without LPS, as described before. After stimulation, cells were stained with HLA-DR–PE, HLA-ABC–FITC, CD80–FITC, and CD86–PE (BD Bioscience) for 45 min at room temperature in the dark, washed in PBS and resuspended in PBS 4% paraformaldehyde. Cells were analyzed by flow cytometry (FACSCalibur).

2.6. Statistical analysis

All results are expressed as percentages or means ± standard deviations. The differences between groups were analyzed by parametric (analysis of variance and *t*-test) or nonparametric (Mann-Whitney and Kruskal-Wallis tests) methods, as appropriate. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences), version 13.0, for Windows. Results with P values of <0.05 were considered significant.

3. Results

3.1. Effect of pCS on MDM viability

The cytotoxicity was tested via MTT assay. No cytotoxic effect was observed after 24 h of incubation with *p*CS, at all concentrations tested, compared to that observed in untreated cells (Fig. 1).

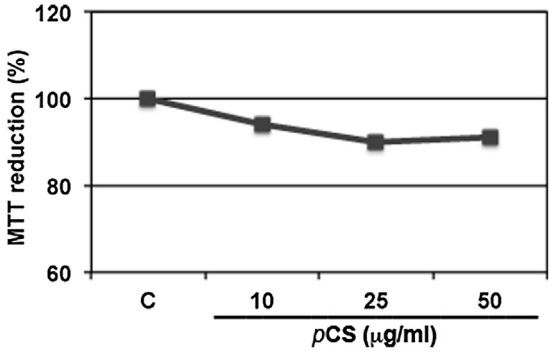

Fig. 1. Cytotoxicity assessment. MDM (1×10^4/well) were incubated for 24 h at 37 °C in a 96-well plate in the presence of *p*CS at 10, 25, and 50 μg/mL. MTT was added and incubated for an additional 3 h. After cell lysis, plates were read on a microplate reader (550 nm). Data are presented as a percentage of viable cells in comparison with untreated cells (Un) for five independent experiments performed in triplicate.