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Interleukin 1β Decreases the GSH Content and Catalase Activity in the Human Peritoneal Mesothelial Cells *in Vitro*

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Abstract. The object of this study was to assess the effects of the inflammatory cytokine interleukin 1β (IL-1β) (0.01–1.0 ng/ml) on the activity of catalase (CAT), superoxide dismutase (SOD) and the level of glutathione (GSH), all being antioxidant mechanisms, in human peritoneal mesothelial cells (HPMC) in *in vitro* culture. HPMC were obtained from the omenta of nonuremic donors. The activity of the antioxidant mechanisms was studied on monolayers of HPMC, which were deprived of serum 48 h prior to experiment. The effect of the cytokine was tested in a medium with low serum concentration (0.1%) or in a medium with 10% fetal calf serum (FCS). Activity of the antioxidant mechanisms was determined by spectrophotometry. The GSH level was decreased in mesothelial cells (MC) after 24 h of exposition to IL-1. However, after 72 h of incubation with IL-1 the GSH level increased in MC in the presence of 10% FCS, $p<0.05$. The activity of CAT was inhibited after 72 h exposure to IL-1. Interleukin 1 did not affect SOD activity in MC. However, when supplemented with 10% FCS, IL-1 decreased the activity of SOD after 24 and 72 h of incubation. We conclude that the activity of antioxidant mechanisms in MC is decreased by IL-1β in ways that might increase their vulnerability to the cytotoxic effect of free radicals.

Key words: dialysis; inflammation; interleukin 1; antioxidants; catalase; superoxide.

Introduction

Peritoneal dialysis (PD) is a well-known alternative to hemodialysis in the treatment of patients with end-stage renal failure. A wider application of this method of treatment, however, is limited by its complications, such as acute and chronic inflammation processes, also due to the bio-incompatibility of peritoneal dialysis fluids[26, 28]. It was shown that PD might induce a chronic inflammation of the peritoneal cavity as a result of peritoneal macrophage activation[5, 16, 32] and enhanced migration of polymorphonuclear (PMN) cells to the peritoneal cavity with increased generation and release of free radicals, prostaglandins and cytokines[2, 9, 22, 31, 33]. Viability of the mesothelial cells (MC) is crucial for the efficiency of PD and during inflammation they are exposed to many possibly harmful factors, e.g. free radicals (FR). Thus, the antioxidant status of MC seems to be important for their longevity, especially when FR are overproduced during inflammation.

Abbreviations used: MC – mesothelial cells, GSH/GSSG – total intracellular glutathione, CAT – catalase, SOD – superoxide dismutase, IL-1 – interleukin 1, FCS – fetal calf serum, CAPD – continous ambulatory peritoneal dialysis, FR – free radicals, H_2O_2 – hydrogen peroxide.

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However, the antioxidant status of these cells might be influenced by many factors, such as cytokines, generated in the peritoneal cavity during its inflammation. It is well documented that cytokines influence the cellular antioxidant status in different cell types[15, 24, 34]. One of the most potent pro-inflammatory cytokines is interleukin 1β (IL-1β) [6]. Its level was shown to be increased during PD and during inflammation within the peritoneal cavity[2]. Thus, the aim of this study was to evaluate the influence of this cytokine on the activity of antioxidant processes, such as the concentration of intracellular glutathione (GSH/GSSG), the activity of catalase (CAT) and superoxide dismutase (SOD) in MC *in vitro*.

Materials and Methods

In vitro culture of mesothelial cells. A number of experiments were conducted on human peritoneal MC, which were isolated and cultured *in vitro* according to the method described by VAN BRONSWIJK et al. [28] A fragment of the human omentum obtained during an abdominal surgical procedure was washed in calcium and magnesium-free Hanks solution, and then incubated in a shaker in 0.05 g% Trypsin – 0.02 g% EDTA solution for 30 min at 37ºC. After incubation, the tissue was removed and the solution was centrifuged at 150 g for 10 min. The supernatant was discarded, the cells were washed once and suspended in medium M199 with hydrocortisone (1 μg/ml) and 10% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 μg/ml), amphoterycin (0.25 μg/ml) (all chemicals provided by Sigma) and seeded into 75 cm^2 culture flasks (Costar, USA). The cells were cultured in a humid air atmosphere with 5% CO_2 at 37ºC. The medium was changed every 3 days. In all the experiments we used cells from passages 1 to 3, and all measurements were made in duplicate.

Identification of the cells. Confluent cells were identified as mesothelial on account of their cobblestone appearance in an inverted microscope[23]. In addition, the immunohistochemical staining was performed with monoclonal antibodies to the human cytokeratin, which were made visible by horseradish-peroxidase--conjugated second antibody (Amersham, UK) [30]. All the cells were stained positively for cytokeratins.

Measured antioxidant mechanisms. Level of total intracellular glutathione. The level of total (reduced and oxidized) glutathione was measured by spectrophotometry according to the enzymatic method described by TIETZE[25] and modified by GRIFFITH[12].

Activity of catalase was determined by spectrophotometry according to the method described by AEBI[1].

Activity of superoxide dismutase was determined by spectrophotometry according to the method described by MCCORD and FRIDOVICH[18].

The concentration of GSH/GSSG in the sample (and the activity of CAT and SOD, respectively) was derived from the standard curve for glutathione (CAT and SOD, respectively), and presented in nmol (international units of activity for CAT and SOD) per 1 mg of intracellular protein. The concentration of protein was determined according to the method described by LOWRY et al.[17].

Effect of IL-1β on antioxidant activity of MC. To deprive cells of the serum effect, cell monolayers in 24-well plates were exposed for 48 h to M199 medium supplemented with a reduced concentration of FCS (0.1%). Thereafter, the cells were exposed for 6, 24 and 72 h to a medium with 0.1% FCS or a medium with 10% FCS, supplemented in the individual groups with IL-1β (Sigma, USA) at concentrations of 0.01, 0.1 and 1.0 ng/ml.

After the above described time intervals, the medium was discarded, the cells washed with Hank's solution and lysed with destilled water. Thereafter, the level of GSH/GSSG and the activity of CAT and SOD in MC were measured. In the control groups, cells were exposed to medium + 0.1 FCS and to medium + 10% FCS that did not contain tested cytokines.

Statistical analysis. The results were expressed as the mean ± standard deviation (SD); $n = 6$ in each group. The data were submitted to statistical analysis using the non-parametric Wilcoxon test for paired data. A p value less than 0.05 was considered significant.

Results

IL-1β in a concentration of 1.0 ng/ml in the medium with 0.1% FCS decreased the level of GSH/GSSG after 24 h exposure (–10.6% vs. control, $p<0.05$; Fig. 1). However, in the presence of 10% FCS, IL-1β showed a quite different effect. After 24 h of incubation, IL-1β in the highest concentration decreased the level of GSH/GSSG in MC (–33.3% vs. control, $p<0.05$), but after 72 h the level of GSH/GSSG increased in the concentration of 0.1 ng/ml (+47% vs. control, $p<0.05$) and in the concentration of 1.0 ng/ml (+141.3% vs. control, $p<0.05$; Fig. 1).

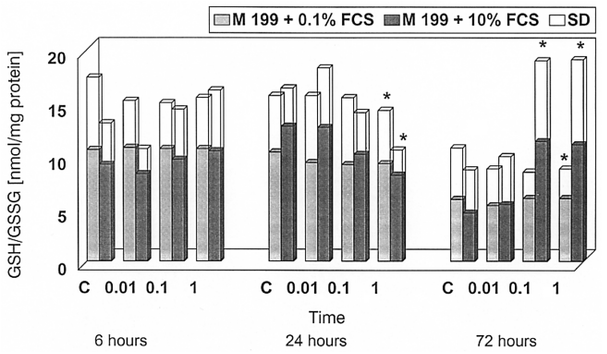

Fig. 1. The effect of IL-1β [0.01, 0.1 and 1.0 ng/ml] with 0.1% FCS or 10% FCS on the GSH/GSSG level in MC after 6, 24 and 72 h of exposure. Values are the mean ± SD; presented results are mean from 6 experiments performed on mesothelial cells from nonuremic donors. * – indicates that a change of studied group versus the control [(C): M199 + 0.1% FCS or M199 + 10% FCS respectively] at that time point is significant ($p < 0.05$)

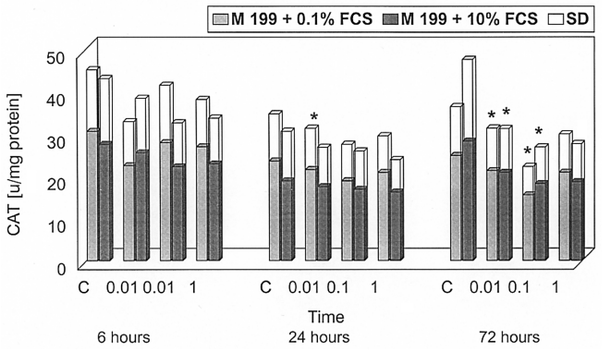

Fig. 2. The effect of IL-1β [0.01, 0.1 and 1.0 ng/ml] with 0.1% FCS or 10% FCS on the CAT activity in MC after 6, 24 and 72 h of exposure. Explanations see under Fig. 1

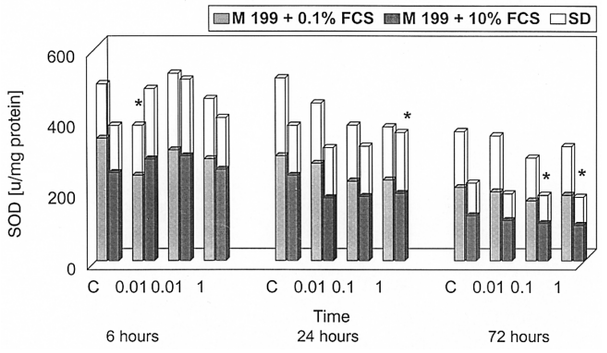

Fig. 3. The effect of IL-1β [0.01, 0.1 and 1.0 ng/ml] with 0.1% FCS or 10% FCS on the SOD activity in MC after 6, 24 and 72 h of exposure. Explanations see under Fig. 1

IL-1β decreased the activity of CAT in MC exposed to the medium with 0.1% FCS: after 24 h incubation in the concentration 0.01 ng/ml (–12.2% vs. control, $p<0.05$), after 72 h in 0.01 and 0.1 ng/ml (–11.8% vs. control, $p<0.05$ and –41.2% vs. control, $p<0.05$ respectively; Fig. 2). Similarly, the activity of CAT was decreased in MC exposed to IL-1β in the medium with 10% FCS after 72 h incubation: –45.6% vs. control, $p<0.05$ in 0.01 ng/ml and –54.4% vs. control, $p<0.05$ in 0.1 ng/ml (Fig. 2).

The SOD activity was decreased in MC exposed for 6 h to IL-1β in 0.01 ng/ml in the medium with 0.1% FCS (–30.5% vs. control, $p<0.05$; Fig. 3) and in MC exposed to IL-1β in the medium with 10% FCS: after 24 h in 1.0 ng/ml (–21.1% vs. control, $p<0.05$), after 72 h in 0.1 ng/ml (–16.7% vs. control, $p<0.05$) and in 1.0 ng/ml (–20% vs. control, $p<0.05$; Fig. 3).

Discussion

Free radicals and several other mediators, including cytokines, are generated during chronic inflammation in the peritoneal cavity induced by the process of peritoneal dialysis[2, 9, 22, 31, 32, 33]. One of the main components of the peritoneal membrane are mesothelial cells, which constitute the layer of cells covering the peritoneum and are preferentially exposed to inflammatory mediators present in the peritoneum. These very potent cells were shown to be a source of different mediators of inflammation, such as IL-1α and β[7], IL-6[27], TGF-α[11], prostaglandins PGI_2 and PGE_2[3] and nitric oxide[20]. It is very probable that the longevity of the peritoneum depends mainly on the preservation of the MC function.

In our study we observed that, after short-term exposition (6 h) IL-1 did not influence the antioxidant mechanisms of MC. This lack of effect was not changed in the presence of 10% FCS. After longer incubation with IL-1, however, we noted a tendency to decrease GSH levels and CAT activity in the studied MC, especially at the higher concentrations of IL-1. This might indicate that IL-1 produced in large quantities during inflammation might, besides its many other roles, decrease the antioxidant mechanisms of MC. The "prooxidative" effect of the IL-1β is well-documented in phagocytic[4] as well as in non-phagocytic cells, i.e., human mesangial[21] and murine tumor cells[34]. It was also demonstrated that erythrocytes of uremic patients have reduced CAT[8], SOD activity and GSH content[29]. Moreover, the process of PD by itself promotes the generation of FR within the peritoneal cavity[19]. Thus,

the addition of any prooxidative factors, such as IL-1, or any group of hazardous factors realised during inflammation, might become deleterious to the survival of MC. However, since the inflammation process is composed of many different factors playing multiple roles, their influence on the antioxidant mechanisms of MC should be studied more extensively in future.

An interesting finding of uncertain relevance is that exposure of MC to IL-1 for 72 h at concentrations of 0.1 and 1.0 ng/ml significantly increased the GSH level, but only when the medium was supplemented with 10% FCS. This might indicate a form of co-action between IL-1 and a factor or factors present in the serum, possibly the role of IL-1 receptor antagonist. This co-action seems to be very favorable for MC, because it has been shown that the GSH/GSSG system is of major importance in their defense against FR[13]. On the other hand, we observed that such co-action might also lead to negative results: IL-1 with 10% FCS inhibited the activity of SOD after 24 and 72 h. However, SOD is of only minor importance in MC defense against FR injury[13].

Regarding IL-1 action on the antioxidant mechanisms of MC, we consider two possible mechanisms. The first could be a direct influence on the GSH level and CAT activity. The second, preferred by us, is based on the assumption that IL-1 acts as a stimulator of free radical production, which in turn leads to a depletion of two major MC defense mechanisms: GSH level and CAT activity. It might be worth pointing out that both of these have similar scopes of action and a common substrate: hydrogen peroxide (H_2O_2). H_2O_2 can deplete cellular GSH, and reduced nicotinamides during glutathione peroxidase-catalysed H_2O_2 reduction serve as precursors of the hydroxyl radical OH* and may also inhibit cytoplasmic CuZnSOD[10]. Since H_2O_2 is generally not reactive enough to oxidize many organic molecules in an aqueous environment, but easily diffuses through hydrophobic membranes, its role has been ascribed to the initiation of FR cytotoxicity rather than to chemical reactivity. Moreover, it was shown that exogenously generated H_2O_2 or a H_2O_2 – derived product mediates release of PGI_2 from cultured endothelial cells[14]. Thus, in conclusion, we believe that the observed effect of IL-1 action was an indirect one and was due to the stimulation of FR production, possibly through generation of H_2O_2.

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