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Inhibition of lipopolysaccharide (LPS)-induced endothelial cytotoxicity by diosmin

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The cytotoxic effect of lipopolysaccharide (LPS) on cultivated bovine aortic endothelial cells was determined. This LPS-induced cytotoxicity was attenuated by diosmin. That means, the IC_{50}-value of LPS in the combination with 8 μmol/l diosmin was shifted from 31 to 70 ng/ml in a concentration dependent manner. As a hypothesis it was suggested, that the inhibition of LPS-induced cytotoxicity in bovine aortic endothelial cell cultures by diosmin could be probably mediated via inhibition of tyrosine kinases.

1. Introduction

Lipopolysaccharide (LPS) derived from gram-negative bacteria induces septic shock reaction leading to a damage of the vascular system. Especially the endothelium is a target for cytotoxic LPS-effects. Septic shock is characterized by severe hypotension, hyporeactivity to vasoconstrictors, profound vascular leakage, and ultimately multiple organ failure frequently culminating in death [1]. Flavonoids like quercetin and myricetin are able to attenuate the LPS-induced cytotoxicity [2]. Because of the well known antiinflammatory and blood vessel protective effects of diosmin [3], we investigated the influence of this flavonoid on the LPS-induced cytotoxicity to answer the question, if there is a beneficial effect of diosmin in LPS-damaged endothelial cells *in vitro*.

2. Investigations, results and discussion

The LPS-induced toxicity in the endothelial cell culture was attenuated by diosmin. The shift to the right of the LPS-cytotoxicity curve with diosmin is demonstrated in Fig. 1. This figure also shows that the attenuation of the LPS-induced cytotoxicity by diosmin is more or less regular over the entire concentration range of LPS, resulting in an increase of IC_{50} from 31 ng/ml for LPS alone to 70 ng/ml for the combination of LPS plus 8 μmol/l diosmin. This shift of the IC_{50}-values to higher LPS concentrations indicates that the endothelial cells were able to tolerate higher doses of LPS in the presence of diosmin. The beneficial effect of this flavonoid against the LPS-induced cytotoxicity was dose-dependent (Fig. 2). Even at low diosmin concentrations (1–10 μg/ml) the cytotoxic effect of LPS was attenuated, cytotoxic effects of diosmin itself were not observed at concentrations lower than 164 μmol/l (100 μg/ml). These results might be an explanation of the protective effect of flavonoid fractions consisting of 90% diosmin and 10% hesperidin (Daflon®) in the treatment of edema and local inflammations in the vascular system [4]. Bacterial endotoxin (LPS) has potent proinflammatory properties toward many cell types, including vascular endothelial cells resulting in gram-negative bacterial sepsis and endotoxic shock. LPS induces protein tyrosine phosphorylation and the LPS-induced toxicity could be prevented by tyrosine kinase inhibitors [5]. Recently we showed that the synthetic tyrosine kinase inhibitor Tyrphostin B46 was able to suppress the LPS induced cytotoxicity in cultivated endothelial cells [6]. The phosphorylation is an early event of the LPS-induced cytotoxicity.

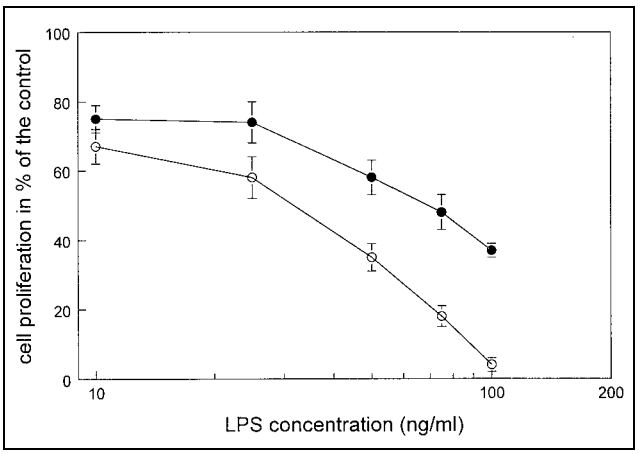

Fig. 1: Dose-effect curves of LPS alone (○) and in combination with 8 μmol/l diosmin (●) on proliferation of cultivated endothelial cells. The cells were cultivated for 4 days with the indicated concentration of LPS and the combination of LPS + 8 μmol/l diosmin, respectively

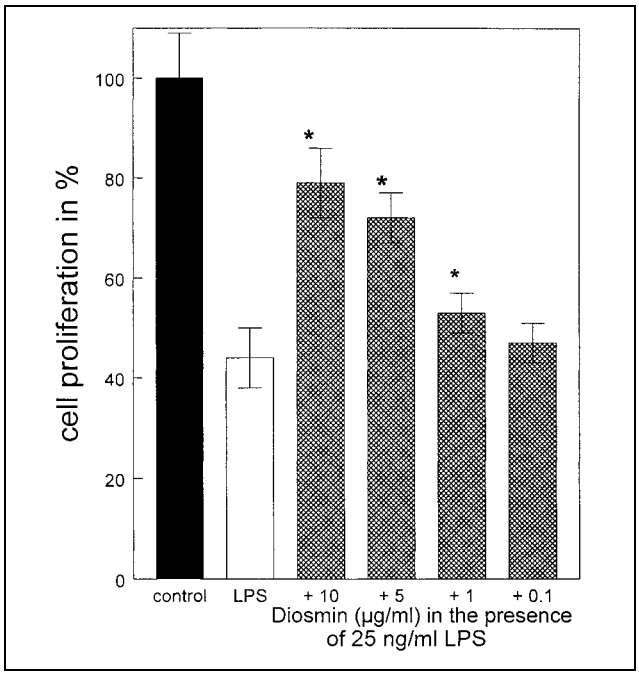

Fig. 2: Influence of different concentrations of diosmin on LPS-induced inhibition of cell proliferation in cultivated endothelial cells
* significant difference between LPS alone (25 ng/ml) and the combination between LPS and diosmin (U-test, $P < 0.05$)

Therefore the inhibition of tyrosine kinases in the vascular endothelial cells is an important element in the prevention of these damages. A tyrosine kinase inhibitory activity was reported for quercetin [7] and related flavonoids [8]. Because of the structural similarities to diosmin a similar point of attack is assumed. That means, the inhibition of protein tyrosine kinases by diosmin might be an important part of its biological activity with respect to the modulation of inflammatory cell functions in blood vessels.

3. Experimental

3.1. Chemicals

Diosmin and other reagents (including LPS: *E. coli* serotype 026:B6) were obtained from Sigma (Germany). The cell culture media and serum were purchased from Biochrom (Germany), the plastic ware from Costar (USA). A stock solution of diosmin was prepared in DMSO. The final concentration of DMSO in the cell culture medium was less than 0.5%. At this concentration DMSO did not influence the proliferation of the control cells.

3.2 Determination of cytotoxicity

Bovine aortic endothelial cells (line BKEz-7) were cultivated in Minimal Essential Medium (MEM) supplemented with 10% bovine serum [9] in 70 cm^2 culture flasks until confluence and seeded for cytotoxicity experiments in 24-well plates (inoculum 50,000 cells/well). For determination of the cytotoxicity the endothelial cells were plated without and with different concentrations of LPS (5 to 100 ng/ml) and cultivated for 4 days. In the experiments with a combination of diosmin with LPS, the cells were preincubated 2 h with the diosmin before the addition of LPS. After 4 days of cultivation the cells in each well were detached enzymatically with trypsin (0.25%)/EDTA (0.2%), and counted using the cell analyzer CASY I (Schärfe System, FRG). In comparison to the LPS-control cells (cultivated in the presence of only different concentrations of LPS) the proliferation rate in percent of the cells treated with the combination of diosmin and LPS was calculated, and IC_{50}-values were determined according to the LOGIT procedure [10].

3.3. Statistics

All the values presented are expressed as mean $\pm$ standard error of the mean of at least 3 independent experiments with 4 parallel samples. Wilcoxon's U-test was used to test significance ($P < 0.05$).

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