Ostreolysin affects rat aorta ring tension and endothelial cell viability in vitro

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Abstract

The effects of ostreolysin, a cardiotoxic cytolysin produced by the edible oyster mushroom (Pleurotus ostreatus), were studied on tension development in isolated rat aortic ring. Its cytotoxic effects on human umbilical vein endothelial cells and Chinese hamster lung fibroblasts were also studied. Ostreolysin induced a concentration-dependent increase in aortic ring tension in the range from 5 to 30 μg/ml, and was cytotoxic to both cell lines. These effects could contribute significantly to its previously observed cardiotoxicity.

Keywords: Ostreolysin; Rat; Endothelial cell; Aorta ring tension; Cardiotoxicity

Ostreolysin (Oly) is a 15-kDa cytolysin isolated from fruiting bodies of the oyster mushroom (Pleurotus ostreatus), strain Plo5 (Berne et al., 2002). It belongs to the aegerolysin protein family (Berne et al., 2005), and binds specifically to raft-like cholesterol-rich membrane domains (Sepčić et al., 2004; Rebolj et al., 2006). The binding leads to the formation of 4 nm transmembrane pores, and lysis of erythrocytes from different animal species and cell lines (Sepčić et al., 2003, 2004). Edible oyster mushroom has been recorded to occasionally induce adverse effects in animals and humans, and its aqueous extract administered intravenously (i.v.) was toxic to mice (Al-Deen et al., 1987). In the previous study, it was found that Oly induces cardiorespiratory and lethal effects in rats (Žužek et al., 2006). Its lethal dose after i.v. application to mice is 1170 μg/kg. Although a clear hyperkalemia was observed, suggesting the injury of erythrocytes and other cells, the exact mechanism of its lethal action remains unclear. In order to shed some light on this mechanism, the effects of Oly on vessel ring tension and endothelial cells viability have been evaluated.

V-79-379A (lung fibroblasts of Chinese hamster) and human umbilical vein endothelial cells (HU-VEC) were grown in Eagle MEM (Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum (FCS, Gibco), L-glutamine (2 mmol/l, Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml), in a 5% CO2 incubator at 37 °C. They were plated in

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96-well microtiter plates (Costar, USA) at a concentration of 5000 cells/well. After 3 h incubation, Oly in different final concentrations (1, 1.5, 2, 3, 5, 10, 25 and 50 μg/ml), prepared in medium without serum, was added and kept for 1 h. The cells were then washed with medium and fresh medium with 10% FCS was added for 48 h. Cytotoxicity was determined using the MTT test in which mitochondrial dehydrogenase enzymes in living cells convert water-soluble diphenyl tetrazolium bromide (MTT) to insoluble formazan (Freshney, 2000). After the addition of 20 μl of MTT (5 mg/ml) (Sigma, Germany) to each well (100 μl) for 3 h, the MTT-containing medium was carefully removed. The formazan crystals were dissolved in 100 μl DMSO (Sigma, Germany) and the absorbance was measured at 570 nm, using an Anthos microplate reader (Anthos, Australia). Viability (%) was expressed as the ratio of optical density of treated to control cells.

Rat aortas were prepared from male Wistar rats weighting 300–380 g that were anaesthetized as described previously (Zuzek et al., 2006) and sacrificed by ex-sanguination. Experiments were performed according to the previously described protocol (Kuzner et al., 2004; Boban et al., 2006). Thoracic aorta was isolated and rings of 4 mm in length (all with preserved endothelium) mounted between two stainless steel hooks in an organ bath with Krebs–Hensenleit solution of the following composition (mM): NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.6; KH2PO4, 1.2; NaHCO3, 24; d-glucose, 10, aerated with 95% O2 and 5% CO2 and maintained at 38.0 ± 0.1 °C. The aortic ring was allowed to equilibrate for at least 60 min under a basal tension of 2 g. After the equilibration period, the dose–response contractility curve of isolated rat aortic rings was determined (not shown). The maximal increase in contractile response (4.06 ± 0.13 g) was measured at 60 mM potassium, Oly induced a response of 7.6 ± 0.9% at 3.75 μg/ml, and reached a maximal contractile response of 39.4 ± 1.8% at 30 μg/ml. Furthermore, Oly diminished the endothelium-mediated relaxation of aortic rings pre-contracted with NE. This effect may derive from the injury of endothelial cells, or from the dysfunction of mechanism responsible for the ACh endothelium-mediated relaxation response. The final concentra-

The difference in sensitivity between the two cell lines was observed only at very low concentrations (1.5–5 μg/ml), where V-79 cells were slightly more affected than HUVEC. The effective concentration (ED50) producing 50% cytotoxicity was 1.3 μg/ml for V-79, and 2.2 μg/ml for HUVEC. These values are approximately 5–8-fold lower than those obtained in the previous study on human tumor cell lines (Sepčič et al., 2003). Moreover, using the same protocol, Oly cytotoxicity on HUVEC was 45 fold greater than that of a 43% identical Asp-hemolysin, an aegerolysin-like protein from the pathogenic mould Aspergillus fumigatus showing an ED50 of 100 μg/ml (Kumagai et al., 2001).

The lytic effects of Oly on erythrocytes (Sepčič et al., 2003) and endothelial cells (this work) may result in hyperkalaemia, which was proposed to play an important role in the cardiotoxicity of Oly (Zuzek et al., 2006). As shown in Fig. 2, Oly induces a dose-dependent increase in rat aorta ring tension. Expressed as percentage of the maximal rat aorta tension following the depolarization evoked by 60 mM potassium, Oly induced a response of 7.6 ± 0.9% at 3.75 μg/ml, and reached a maximal contractile response of 39.4 ± 1.8% at 30 μg/ml. Furthermore, Oly diminished the endothelium-mediated relaxation of aortic rings pre-contracted with NE. This effect may derive from the injury of endothelial cells, or from the dysfunction of mechanism responsible for the ACh endothelium-mediated relaxation response. The final concentra-

Fig. 1. Effects of different concentrations of Oly (1–50 μg/ml) on viability of V-79 (○) and HUVEC (●) cell lines expressed as % of the control (optical density of treated cells/optical density of control cells × 100%) after 48 h of growth. Results represent mean ± SD from three different experiments.
tions of Oly (15–30 μg/ml) inducing significant increase in aorta ring tension in vitro were comparable to the maximal calculated concentrations of Oly in the blood in vivo after injection of one LD50, corroborating the later mechanism as a possible cause of death in rodents. It must be noted, however, that Oly could similarly affect other vessels, including coronary arteries, causing coronary vasospasm followed by acute myocardial ischemia and injury to cardiomyocytes.

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