Expression of Hsp70 in kidney cells exposed to ochratoxin A

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Abstract Ochratoxin A (OTA) is a possible etiological agent of endemic nephropathy, a chronic renal disease with high prevalence in limited geographic areas. Ochratoxicosis has many characteristics of different pathological states in which heat shock proteins (Hsps) are usually induced. The most inducible heat shock proteins belong to the Hsp70 family. We determined the level of expression of Hsp70 by the Western blot analysis in kidneys of rats treated with low doses of OTA and in LLC-PK1 and MDCK cells exposed to OTA. Estimation of cell viability and release of lactate dehydrogenase (LDH) confirmed the toxic effects of OTA on cultured cells. OTA affects the relative distribution of two Hsp70 isoforms (68-kDa and 74-kDa isoforms), but does not change total amount of Hsp70 in rat kidney. No changes in the Hsp70 level were detected in LLC-PK1 and MDCK cells treated with OTA, although the cells were seriously injured, as was seen from the reduced cell viability and increased release of LDH. Both cell lines were capable of having Hsp70 induced following a heat shock. However, exposure of the cells to OTA before the heat shock challenge prevented Hsp70 induction. Results of the study show that OTA does not induce Hsp70 in rat kidney or in cultured kidney cells. The absence of Hsp70 protective effects in the cells and tissues might be a possible explanation for the cumulative destructive effects of OTA and a silent onset of endemic nephropathy in humans and of OTA-induced experimental nephrotoxicity in animals.

Keywords Ochratoxin A · Endemic nephropathy · Nephrotoxicity · Heat shock protein 70 · Cell viability · Lactate dehydrogenase release

Introduction

Endemic nephropathy (EN) is a chronic renal disease with high prevalence in limited geographic areas (some parts of Croatia, Bosnia, Serbia, Bulgaria and Romania). The disease has a silent onset, and appears in its overt form with the symptoms of renal insufficiency, predominantly in the fourth and fifth decades of life. There are many hypotheses regarding the etiology of EN. Different etiological agents have been investigated: microorganisms, poisons of plant and animal origin, metals and non-metals originating from soil, food or water, ionizing radiation, genetic factors, etc. It is, however, not possible to explain the severity of clinical symptoms and fully understand the development of the disease with any of the agents mentioned. In numerous studies, a possible causal relationship between ochratoxin A [OTA, 5-chloro-(hydroxy-3-methyl-1-oxo-isocumarin) carbonyl-L-phenylalanine] and EN has been suggested (Plesˇtina et al. 1990; Čvorisˇćec et al. 1998; Radić et al. 1997; Pavlovic´ et al.1979). OTA is a mycotoxin, produced as a secondary metabolite by Aspergillus and Penicillium strains and it is a natural contaminant of mouldy food. Contamination of food samples with OTA and higher OTA concentrations in blood samples of people living in endemic regions have frequently been found, suggesting exposure to OTA (Radić et al. 1997; Pavlovic´ et al. 1979). OTA is also known as the causal agent of acute ochratoxicosis in animals (Pavlovic´ et al. 1979). Toxicological studies are mainly focused on the impaired renal function caused by OTA (Berndt et al. 1980; Marquardt and Frohlich 1992). After natural or experimental exposure to OTA, a number of morphological (Rutqvist et al. 1977; Elling 1983) and biochemical (Dirheimer and Creppy 1991; Gekle et al. 1993, 1998; Schwerdt et al. 1997) changes have been observed, primarily in the proximal kidney tubule and to a lesser extent in liver (Kane et al. 1986; Creppy et al. 1990; Marquardt and Frohlich 1992), brain (Zanić-Grubišić et al. 1996; Belmadani et al. 1998), and
other tissues (Creppy et al. 1985; Žanić-Grubišić et al. 1995). In addition, OTA acts as a carcinogenic (Schlatter et al. 1996; Castegnaro et al. 1998) genotoxic (Creppy et al. 1985) and mutagenic (Hennig et al. 1991) agent. At the cellular level, OTA decreases the rate of protein synthesis (Creppy et al. 1984), as well as the rate of DNA and RNA synthesis (Stetina and Votava 1986). OTA causes DNA damage (single-strand breaks) in vitro and in vivo (Creppy et al. 1985; Stetina and Votava 1986).

OTA has immunosuppressive effects (Creppy et al. 1983). A decrease in immunoglobulins IgG, IgA and IgM levels in lymphoid tissue and serum (Dwivedi and Burns 1984) and reduced natural killer cell activity have been reported (Luster et al. 1987), suggesting that natural protection of the affected organism might be compromised. However, not much data are available about the influence of OTA on the cellular self-protective and repairing mechanisms. Delayed onset of the symptoms in the EN might be connected with the accumulation of intracellular damage that has not been properly corrected during the long silent period of the disease.

In this paper, we address the question of the appropriate functioning of cellular defensive mechanisms when experimental rats or cell lines are exposed to OTA. We assumed that OTA might activate these mechanisms and in particular the heat shock response, which is considered to be a conserved, immediate but transient cellular response to elevated temperature and different kinds of harmful environmental effects. It involves transcriptional activation and subsequent synthesis of heat shock proteins (Hsps). Hsps regulate the fundamental cellular processes such as protein folding, protein sorting, degradation, assembly of proteins into larger complexes, and resolubilization of aggregates (Benjamin and McMillan 1998; Jensen and Johnson 1999; Clark and Muchowski 2000; Feldman and Frydman 2000). These proteins are induced following exposure of the cell to hyperthermia, surgery, anesthesia, restraint, tissue damage, ischemia-reperfusion, inflammation, oxidative injury and a variety of other stimuli such as heavy metal intake, administration of amino acid analogs, different pharmacological agents, etc. (Christman et al. 1985; Welch 1992). Since ochratoxicosis in vitro and in vivo has many characteristics of the above-mentioned physiological/pathological states, we wanted to find out whether expression of Hsp70, a prominent member of the Hsp family, would be changed by OTA exposure.

Hsps comprise a family of proteins constitutively expressed in all cells under normal conditions. According to their respective molecular mass, Hsps are divided into six subfamilies: large Hsps (110 kDa), Hsp90, Hsp70, Hsp60, Hsp40 and small Hsps (30 to 18 kDa; Lindquist and Craig 1988). The most inducible Hsps belong to the Hsp70 family that includes both the constitutive proteins (Hsp73) essential for cellular function and the inducible form (Hsp72), which increases in response to environmental stress (Blake et al. 1990). The third major form of Hsp70 is referred to as glucose-regulated 78-kDa protein (GRP78), localized within the lumen of the endoplasmic reticulum. A fourth form of Hsp70 is found within mitochondria and is referred to as GRP75 (Welch 1992).

This study was designed to examine the effects of OTA on expression of members of the Hsp70 family, Hsp73 (constitutive) and Hsp72 (inducible), in two experimental models. In the in vivo model, Wistar rats were subjected to treatment with a low dose of OTA, simulating natural exposure in areas of risk, and the level of Hsp70 expression was examined in kidney tissue. In the in vitro model, two kidney cell lines LLC-PK1 (pig kidney cell line, corresponding to the proximal tubule epithelial cell) and MDCK (Madin-Darby canine kidney cell line, corresponding to the distal tubule epithelial cell) (Gstraunthaler et al. 1985) were exposed to OTA and the level of Hsp70 expression was determined either immediately after the exposure or after a specific recovery period.

**Materials and methods**

**Experimental animals**

Male rats (n = 32) of the Wistar strain, mean body weight 250 g, aged 12 weeks, were used in the experiment. The animals were kept in standard laboratory conditions, with a light/dark cycle of 12 h/12 h and at a constant temperature of 24°C. The animals had free access to food (standard laboratory pellets, Pliva d.d., Zagreb, Croatia) and water. Rats were allocated to three experimental groups and a control group. Rats from the first group (n = 8) were given a daily dose of 120 μg/kg OTA (Sigma, St. Louis, Mo., USA) in 0.5 ml olive oil, per os by gastric intubation for 10 days; rats from the second group (n = 8) were given the same OTA dosage for 30 days, and finally, rats from the third group (n = 8) were given the same OTA dosage for 60 days. The control animals (n = 8) were given 0.5 ml olive oil during the experiment and were killed at time points 0, 10, 30 or 60 days.

**Tissue preparation**

At the end of the experiment rats were killed and kidneys were immediately excised, freed from the connective and fatty tissue and washed in ice-cold saline. Kidney homogenates (10 g/l) were prepared in cold 0.14 M KCl, pH 7.4, using an Ultra-Turrax homogenizer and filtration. The samples for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were prepared according to Laemmli (1970).

**Cell cultures**

**Culture of kidney cells**

LLC-PK1 and MDCK cells were cultured at 37°C in MEM medium (Sigma) containing 10% fetal bovine serum (BSA; Sigma). Cells were exposed to OTA for different periods according to experimental protocols described below. OTA was dissolved in ethanol at a concentration of 10 mg/ml, and the final concentration was obtained by appropriate dilution in the respective culture medium. The final ethanol concentration in the medium was 0.025% in LLC-PK1 culture and 0.005% in MDCK culture. Ethanol was added to the control cells in a final concentration 0.025% for LLC-PK1 and 0.005% for MDCK.

Cells were harvested by removing the medium, washing in phosphate-buffered saline (PBS; 20 mM phosphate buffer, 150 mM