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Livestock Production Science 76 (2002) 245–250

LIVESTOCK PRODUCTION SCIENCE

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Mycotoxins in the food chain: the role of ochratoxins

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Abstract

Mycotoxins are widespread contaminants of food and feeds, of meat, cheese, spices, fruits, and grapes and also of some beverages. The toxins are mainly produced by the four genera of fungi *Fusarium*, *Claviceps*, *Aspergillus* and *Penicillium* that grow on almost every kind of nourishing medium. In the past, the main concerns about OTA addressed the carcinogenic and nephrotoxic potential of this mycotoxin; however, recent studies have documented significant alterations of immune responses. Since these effects were observed even at OTA concentrations far below the doses used in cancerogenicity studies, such additional effects by OTA have gained increased attention now. Since the immune response processes are inevitably involved in the defence against microbial invasion and tumour cell propagation, it is predictable that this research provides important new information about OTA and other mycotoxins. This report deals with OTA food contamination, consumer exposure and recent toxicological data related to effect on cytokine release in the liver by OTA.

Keywords: OTA; Exposure; Immune suppression; Tumour necrosis factor α

1. Introduction: ochratoxin A

Contamination of foodstuff with mutagenic and carcinogenic mycotoxins such as aflatoxins, sterigmatocystin, ochratoxins or fumonisins is a major matter of concern for human health. Toxins with oestrogenic effects, such as zearalenone, impede animal breeding. In central European countries probably the most ubiquitous carcinogenic mycotoxin is ochratoxin A (OTA), which can be detected at levels greater than 0.1 ppb in more than 90% of human and swine blood samples. The toxin exposure is exclusively based on the ingestion of contaminated food and feed; however, airborne invasion may also rarely occur, e.g., with people working on waste fields (Richard et al., 1999). OTA-contamination of grain is most prominent, although highly variable and directly influenced by storage conditions after harvest. The toxin is transmitted to swine by mouldy fodder from which carry-over to humans has been demonstrated. Very recently the German Federal Ministry for Health has initiated a study aimed to determine the exposure of the consumer to OTA and the level of contamination of foods. This study entitled *Ochratoxin A: Contamination of Foods and*

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0301-6226/02/$ – see front matter
PII: S0301-6226(02)00124-0

Consumer Exposure was conducted by six federal and one private institution over a period of 2.5 years and was published in the year 2000 (Wolff et al., 2000). It represents the most recent and comprehensive survey on various aspects of OTA in food.

1.1. OTA and food

OTA-contaminated foods are abundantly found in Europe. About 57% of 6476 food samples that were examined contained OTA above the detection limit of 0.01 μg/kg (Wolff et al., 2000). Most prominent examples are cereals and cereal products for which a maximal residue level of 5 μg OTA/kg has been recommended by European legislation. With the exception of one sample of breakfast cereal (muesli) with an OTA concentration of 31.8 μg/kg (Fig. 1), the 5-ppb level was exceeded in only very few samples in the study noted above.

Among 2374 samples of grain and grain products from rye, wheat, barley, oats, maize, buckwheat, and millet, levels above 3 ppm OTA were reached only in 1.4% of the samples (Wolff, 2000). Flour contained half of the level of OTA present in grain. Some infant cereal foods from soy products were atypically contaminated above the average. No evidence was obtained for a relationship between seasonal or regional characteristics during harvest and the OTA content in cereals, flour, bread, and other cereal products. Since bread and rolls contribute to 31 and 9% of OTA intake, respectively, of the total OTA dietary intake of non-vegetarian adults (Cholmakov-Bodechtel et al., 2000), a major impact from this study is that storage, transport, and processing conditions for grain and grain products have to be significantly improved in the future to assure consumer protection.

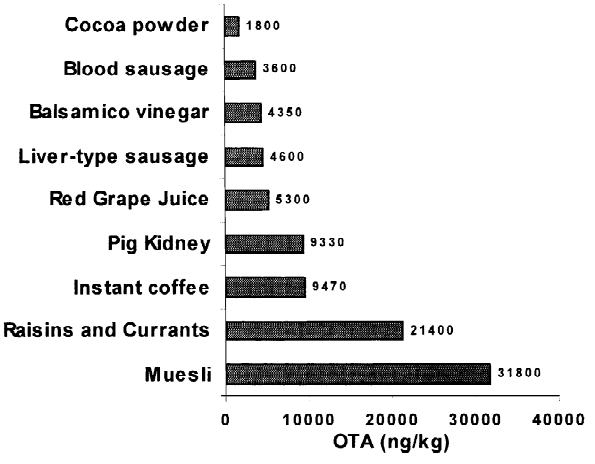

Fig. 1. Peaks of OTA contamination in foods from Germany in 1996–1999 (Arch. Lebensmittelhyg. 51 (2000) 84–117).

A significant but often unrecognised toxin burden comes from coffee (Studer-Rohr et al., 1995), beer, and juices. With regards to the mean population of adult Germans these beverages contribute to approximately 14.2–14.5, 7–9.8, and 6.6–7,5%, respectively, of the total OTA intake. According to that report 50–75% of 240 boiled samples of roasted coffee contained $\geq 0.3 \leq 0.6$ μg OTA/l coffee. Instant coffee is even more critical since it was shown to contain significantly higher levels of OTA than coffee prepared from roasted beans (Fig. 1) (Bresch et al., 2000). Black tea was found not to be contaminated by OTA, whereas 42% of children's herbal teas contained relatively high toxin concentrations of up to 10 μg/kg.

Among the German beer varieties *Pils*, *Export*, *Weizen*, and *Starkbier*, more than 70% contained the toxin at average concentrations of about 0.03 μg/l due to the use of contaminated barley. A recent study of Danish beers confirms this level of contamination for most local beer varieties. In that study a mean concentration of 0.049 μg OTA/l Danish beer were reported (Jørgensen, 1998).

OTA in meat and meat products presents a special problem (Gareis and Scheuer, 2000), as OTA-carry over from feed to meat has been shown experimentally (Madsen et al., 1982). The kidney is highly contaminated followed by the liver, muscle, and fat. Considerable amounts of OTA have also been found in blood (Mortensen et al., 1983), the incidence of OTA in blood sausages from swine being 77.2%, followed by liver-type sausage (67.9%) and raw sausages (46.7%). This corresponds to maximum OTA levels of 4.6 and 3.2 ppb in blood- and liver-type sausages, respectively. It was noted that OTA contamination of meat products, e.g., beef sausages, may also arise from spices carrying the mycotoxin. In lean pork, only small amounts of the mycotoxin, at a maximum concentration of 0.14 μg/kg, were detected, in as many as 17.2% of the samples. OTA was essentially absent in meat from poultry, whereas low-level OTA was present at levels around the

detection limit of 0.01 μg/kg in poultry sausages. OTA was incidentally detected in one out of 58 beef samples. This may be due to the well-known complete destruction of OTA by the rumen microflora.

1.2. Human exposure

Currently available exposure data and human OTA blood levels in Germany from the years 1996–1999 show that the absolute OTA intake was approximately 28.7–290.8 ng/day, corresponding to a daily intake of 0.44–3.6 ng/kg BW (Cholmakov-Bodechtel et al., 2000; Rosner et al., 2000). Only in a worst-case scenario will the higher level be reached. Young children of 4–6 years showed to be higher risk groups, their daily intake being 16 times higher than the average intake of adults. From a recent study on the OTA burden of Europeans from 13 EU member states (SCOOP, 1995), the calculated total daily intake of OTA by food varied between 0.9 ng/kg BW in Germany and 4.6 ng/kg BW in Italy. These values are below the tolerable daily intake (TDI value) of 5 ng OTA/kg BW per day suggested by WHO, indicating that, with the exception of long-term effects of OTA in kidney patients and immune suppressed patients, OTA borne risks for man may be calculable. However, it was already noted that repeated uptake of food with OTA concentrations very close to or just above the recommended limit of 5 μg OTA/kg cereal or cereal products means that the acceptable daily intake (ADI) of 5 ng OTA/kg BW per day may be rapidly achieved (Petzinger and Ziegler, 2000). Elimination of OTA in humans is extremely slow, since the toxin has the longest half-life known for living mammals (see below). Repeated, almost daily uptake of OTA, therefore, will cause low albeit toxicologically relevant toxin concentrations in blood.

2. Toxicological concerns about OTA

The toxicology of OTA has been already reviewed (Boorman, 1989; DFG, 1990; Kuiper-Goodman, 1996; Petzinger and Ziegler, 2000), but some recent developments should be considered. In man OTA exhibits unusual toxicokinetics, with a half-life in blood of 840 h (35 days) after oral ingestion (Schlatter et al., 1996). The delayed excretion of the toxin in man may be due to reabsorption during an enterohepatic circulation, due to reabsorption from the urine after tubular secretion, and due to extensive protein binding. Since the toxin is ingested with almost every meal, humans may not be free of toxin for very long periods. The toxin has been considered by the International Agency for Research on Cancer to be possibly carcinogenic (group 2B) for humans (IARC, 1993), meaning that steady toxin exposure must be considered as a cause for serious concern. With respect to chemical carcinogens, not only dosage but, more importantly, the time–dosage profile ($c \times t$ product) has to be considered as relevant for tumour development. In this respect the ubiquitous presence of OTA is a subject of a toxicological debate presently (Petzinger and Ziegler, 2000).

Liver elimination of OTA is maintained by protein carriers that shuffle the toxin from its protein-bound form in blood into the hepatocyte and subsequently secrete the toxin into bile. The uptake carrier has been identified (Kontaxi et al., 1996) but less is known about the mechanism involved in the release into bile. A carrier system is also involved in the uptake of OTA by proximal tubule cells, which secrete the toxin into urine (Tsuda et al., 1999). Such systems are biological entrance gates that determine the elimination toxicokinetics of OTA and therefore have a major impact on half-life times and selective organ exposure.

2.1. OTA and the immune system

Toxic effects resulting from very low concentrations of OTA in the ng/ml range affect the immune system. It appears that this system is by far the most sensitive among all other sensitive organs. OTA is clearly an immunosuppressive agent (Harvey et al., 1992; Størmer and Lea, 1995; Müller et al., 1999). Concentrations as low as 5 ng OTA per kg BW suppressed immune responses in mice (Haubeck et al., 1981). Concerning the cellular immunity OTA leads to inhibition of immune responses transmitted by B- and T-lymphocytes. In relation to the humoral immunity OTA induces a regression of IgG-, IgA-, and IgM-immunoglobulins (Müller et al., 1995). To date there is no information regarding an OTA-induced cytokine release.

2.2. OTA and cytokines

Since this data was not available our recent interest focussed on the release of cytokines from the liver under OTA exposure. The immediate aim was to demonstrate a modulatory effect of OTA on liver defence mechanisms, when these are required as defence against bacteria and endotoxins after their intestinal absorption. The delivery of mycotoxins from the ingesta to the liver means that this organ is also an early protection barrier against a systemic toxin distribution throughout the entire body. Therefore, two factors were considered to be of importance for the acute toxin burden in the liver: the OTA elimination capacity of the liver (reported previously by Kontaxi et al., 1996) and the acute response by cytokine effects (this report).

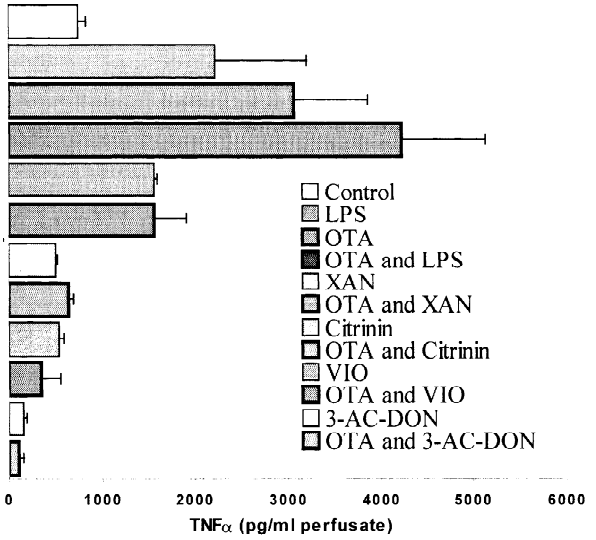

Fig. 2. TNFα release from the isolated and perfused rat liver by LPS and mycotoxins.

Tumour necrosis factor α (TNFα) is among the early response cytokines, released following exposure of the liver to bacterial endotoxins, e.g., *E. coli*-lipopolysaccharides. We have recently demonstrated that this cytokine is also released in significant amounts from a blood-free perfused rat liver following OTA exposure (Weidenbach et al., 2000). In addition, another pro-inflammatory cytokine, IL-6, was present in the perfusate. This cytokine release was additive to a prevailing TNFα release induced by *E. coli*-LPS (Fig. 2). Therefore, OTA-related TNFα release in blood may provide an additional risk for sepsis patients (Fig. 3).

In contrast, other mycotoxins such as 3-acetoxy-deoxynivalenol (3-Ac-DON), xanthomegnin (XAN), citrinin (CIT), and viomellein (VIO) even sup-

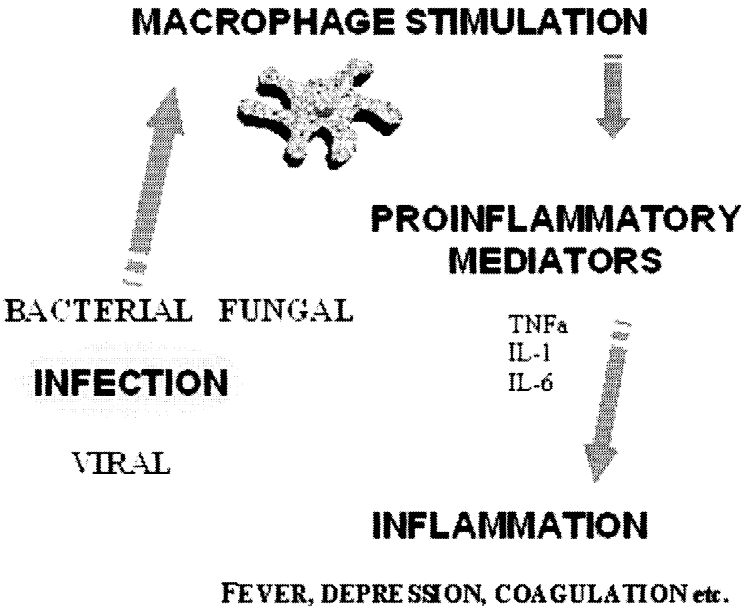

Fig. 3. Cytokine release during the sepsis cascade caused by bacterial LPS and mycotoxins.

pressed OTA-induced TNFα release when present simultaneously in the perfusate (Fig. 2).

Since the cytokine release was exclusively dependent upon extracellular calcium and was inhibited by the phosphodiesterase IV-inhibitor rolipram (Weidenbach et al., 2000), we conclude that OTA represents a receptor-dependent trigger of a signal cascade leading eventually to TNFα secretion by liver cells (Kupffer cells and parenchymal cells). This is the first report of a significant immunostimulatory and modulating effect of this mycotoxin in the liver. With respect to TNFα it is tempting to speculate that this cytokine may induce apoptosis in the liver, as has previously been reported in a kidney cell line (Schwerdt et al., 2000) and in various other cells (Seegers et al., 1994a,b).

Acknowledgements

We wish to express our gratitude to Dr. Uwe Lauber, Department of Animal Nutrition, Hohenheim University, Stuttgart, Germany and Professor Dr. Rudolf Krska, IFA, Tulln, Austria for their kind supply of OTA and several other mycotoxins.

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