

Spontaneous Occurrence of Ochratoxin A Residues in Porcine Kidneys in Belgium

Dominique M. Rousseau, and Carlos H. Van Peteghem

Laboratory of Food Analysis, Department of Pharmaceutical Sciences, State University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium

Ochratoxin A is a nephrotoxic fungal secondary metabolite of several fungal species included in the genera Aspergillus and Penicillium. This mycotoxin has been found as a natural contaminant of plant products, especially cereals, in several countries (Harwig 1974) and has been observed as a causal determinant of mycotoxic porcine nephropathy, a naturally occurring disease in pigs (Krogh 1977, Rutqvist et al. 1977).

It has been experimentally observed that slaughter animals (pigs, poultry) exposed to ochratoxin A contaminated feed contain residues of ochratoxin A in tissues at slaughter (Krogh et al. 1976). The highest concentration of ochratoxin A was found in the kidneys, but the toxin was also present in adipose, liver and muscular tissues. Clinical symptoms in pigs caused by experimentally induced ochratoxicosis have been described in detail (Krogh et al. 1974).

Data concerning spontaneous occurrence of ochratoxin in Belgium were not available. Consequently a broad survey macroscopically suspected porcine kidneys was performed in our laboratory. In a study in collaboration with the Institute for Animal Pathology of the State University of Ghent and the Institute of Animal Disease Prevention of the Province of West-Flanders at Torhout, kidneys were analyzed which were suspected of ochratoxicosis at autopsy, by the veterinarians of the institutes. During these investigations two herds were discovered in which several kidneys of piglets, suffering from nephropathy, contained ochratoxin A. Consequently ochratoxin A analysis was also performed in the serum of sows and in the herd's feed. As ochratoxin A residues were found in a large number of suspected kidneys, analyzed in this study, the investigations were extended to a broader survey of suspected kidneys of slaughtered pigs.

Send reprint requests to Dominique M. Rousseau at the above address.

MATERIALS AND METHODS

Kidneys from 95 pigs were collected at autopsy in the institutes mentioned above in the period of February 1986 to February 1987.

Kidneys from 385 slaughtered pigs were collected in 6 slaughter-houses in various districts of northern Belgium in the period of February to June 1987. The choice of this sampling period was based on the observation of Szebiotko et al. (1981) that the occurrence and concentration of ochratoxin A in grain were higher in the spring than directly after harvest.

All kidneys were selected on the basis of the following macroscopical characteristics: colour change ("pale kidneys"), enlargement, mottled renal surface, cysts and cortical fibrosis (Krogh et al. 1974). The kidneys were transferred from the pathology institutes or the slaughterhouses to our laboratory within 24-48 hours and stored at -20° C until analysis.

Analysis of ochratoxin A residues in kidneys, serum and feed samples was carried out by previously described radioimmunological methods (Rousseau et al. 1985, Rousseau et al. 1986, Rousseau et al. 1987).

An analytical standard of ochratoxin A was obtained from Janssen Chimica (Belgium). All solvents and chemicals were of analytical grade.

RESULTS AND DISCUSSION

Results from ochratoxin A analysis of porcine kidneys suspected of ochratoxicosis and collected at autopsy in the mentioned institutes are shown in Table 1.

Table 1. Occurrence of ochratoxin A residues in macroscopically suspected porcine kidneys, selected at autopsy in the mentioned institutes.

ochratoxin A ng/g	A No.of kidneys	%	B No. of kidneys	%
< 0.20 ^a	54	73.0	13	62.0
0.20 - 0.99	6	8.1		_
1.00 - 4.99	12	16.2	7	33.3
5.00 - 9.99	2	2.7	1	4.7
> 10.00	-		-	_
T otal	74	100.0	21	100.0

detection limit

A = Institute for Animal Pathology of the State University of Ghent.

B = Institute of Animal Disease Prevention at Torhout.

A large number of suspected kidneys which contained ochratoxin A residues were isolated cases. In two cases, however, ochratoxin A was found in kidneys of several pigs from the same herd. Consequently an extended investigation of both herds was undertaken.

At the beginning of 1986 herd 1 had to contend with an increased neonatal mortality. A great number of piglets died ± 5 days after birth. Autopsy revealed pale, swollen and degenerated kidneys. Ochratoxin A residues were found in 6 out of 13 analyzed kidneys (concentrations: 1.3, 1.1, 1.6, 0.8, 1.0 and 1.5 ng/g). Subsequently serum samples of some sows were analyzed. Two out of 4 analyzed serum samples contained ochratoxin A residues (concentrations: 3.7 and 3.1 ng/ml).

To determine the origin of this ochratoxicosis the feed of the pigs was also analyzed. Feeding was carried out with corn grown on the home farm and mixed with a commercial pig feed concentrate. The 3 analyzed corn samples contained ochratoxin A residues (concentrations: 3.6, 6.5 and 7.2 ng/g).

It is unlikely that the ochratoxin A contamination of the feed was the only cause of the increased mortality. It is more likely that the immunosuppressive effect of ochratoxin A (Dwivedi and Burns 1985, Haubeck et al. 1981) reduces the natural resistence of the pigs and makes them more sensitive to all kinds of diseases.

In May 1986 analogous problems occurred in herd 2. Ochratoxin A residues were found in 3 out of 4 analyzed piglet kidneys (concentrations: 0.5, 1.7 and 1.8 ng/g). Again serum samples of sows and feed samples were taken. The 4 analyzed serum samples contained ochratoxin A (concentrations: 2.3, 3.7, 2.8 and 3.0 ng/ml). Feeding was carried out with compounded feed purchased in France, containing barley as the main constituent. Ochratoxin A residues were found in the 3 analyzed compounded feed samples (concentrations: 4.6, 5.6 and 5.8 ng/g).

The first survey evidenced that problems concerning ochratoxicosis were likely to occur in Belgium. Consequently a broad survey of macroscopically suspected kidneys of slaughtered pigs was performed. Results of ochratoxin A analysis of 385 suspected kidneys, collected in 6 slaughterhouses of several districts of northern Belgium are shown in Table 2.

Table 2. Ochratoxin A residues in macroscopically suspected porcine kidneys, collected at slaughter.

Ochratoxin A ng/g	N° of kidneys	%
< 0.20 ^a	317	82.3
0.20 - 0.99	24	6.3
1.00 - 4.99	35	9.1
5.00 - 9.99	4	1.0
> 10.00	5	1.3
Total	385	100.0

detection limit

Table 3. Occurrence of ochratoxin A in macroscopically suspected porcine kidneys in various European countries.

Country	N° of analyzed kidneys	kidneys containing ochratoxin A %	concentration of ochratoxin A ng/g
Denmark	60	35	2 - 68 (a)
Sweden	129	25	2 - 10 (b)
WGermany	104	21	0.1 - 1.8 (c)
Poland	73	45	2 - 23 (d)
Hungary	197	39	5 - 100 (e)
Belgium	385	17.7	0.2 - 12

⁽a) (Krogh 1977)

In Table 3 the results of the broad survey in Belgium were compared with results of surveys of suspected kidneys in other European countries.

This comparative study pointed out that the percentage of suspected porcine kidneys containing ochratoxin A was lower in Belgium than in other countries but still was comparable with the percentages in West-Germany and in Sweden. The concentrations of the toxin were similar to the concentrations found in Sweden, West-Germany and Poland but were significantly lower than the concentrations found in Denmark and Hungary. The percentage of pigs with macroscopical changes characteristic of mycotoxic porcine nephropathy was about 0.09 %. This percentage was similar to that reported in other countries: 0.07 % in Denmark (Krogh 1977), 0.05 % in Poland (Golinski et al. 1984) and 0.02 % in Hungary (Sandor et al. 1982).

No significant correlation was observed between the apparent

⁽b) (Rutqvist et al. 1977)

⁽c) (Bauer et al. 1984)

⁽d) (Golinski et al. 1984)

⁽e) (Sandor et al. 1982)

severity of the macroscopical lesions and the ochratoxin A concentrations in the kidneys. This observation corresponds with investigations in other countries (Golinski et al. 1984).

In 82.3 % of the macroscopically suspected porcine kidneys no ochratoxin A was found. This phenomenon can be explained by assuming that ochratoxin A present in tissue is metabolized down to nondetectable levels when the pigs are kept on a toxin-free diet for several weeks before slaughter (Krogh et al. 1976), or on the basis that other components in feed, such as nephrotoxic quinones of fungal origin (Hald & Krogh 1982), can induce the observed macroscopic changes.

From the 385 kidneys examined 5 contained more than 10 ng ochratoxin A/g kidney (concentrations: 10.1, 10.6, 10.8, 11.3 and 12.1 ng/g). These 5 kidneys were collected at the same time in the same slaughterhouse. Consequently the surmise was raised that these kidneys belonged to pigs of a same herd. Unfortunately the identity of the herd's owner could not be traced.

Although all analyzed kidneys were declared unfit for consumption by the meat inspection services in the slaughterhouses because of the macroscopical lesions, the remaining organs and the carcass of the slaughter animals entered commercial channels. Based on toxin distribution constants, experimentally obtained by Krogh et al. (1976), these organs and tissues of the carcasses contained detectable levels of ochratoxin A, e.g. a concentration of 12.1 ng ochratoxin A/g kidney corresponds with a concentration of 7.8 ng ochratoxin A/g liver, 4.2 ng ochratoxin A/g muscular tissue and 2.8 ng ochratoxin A/g adipose tissue. Ordinary cooking does not eliminate the toxin since ochratoxin A is relatively heat stable (Trenk et al. 1971). This means that the toxin also can occur in prepared food.

A transfer of ochratoxin A from contaminated feed to food of animal origin is thus possible. This involves an important risk for the public health. A regular survey of suspected porcine kidneys in slaugtherhouses is therefore useful.

Acknowledgments. The authors are indebted to Drs. F. Castryck, J. Hoorens and P. Vandekerckhove for collecting kidneys at autopsy and to the veterinary meat inspectors at the slaughterhouses in Torhout, Wijnendale, Zedelgem, Velzeke, Herzele and Achterbroek for submitting kidney materials. This work was supported by grant 3.0033.83 from the National Fund for Scientific Research, Belgium. D.M.R. is a research assistant of the National Fund for Scientific Research.

References

Bauer J, Gareis M, Gedek B (1984) Zum Nachweis und Vorkommen von Ochratoxin A bei Schlachtschweinen. Berl Münch Tierärztl Wschr 97: 279-283

- Dwivedi P, Burns R B (1985) Immunosuppressive effects of ochratoxin A in young turkeys. Avian Pathol 14: 213-225
- Golinski P, Hult K, Grabarkiewicz-Szczesna J, Chelkowski J, Kneblewski P, Szebiotko K (1984) Mycotoxic porcine nephropathy and spontaneous occurrence of ochratoxin A residues in kidneys and blood of Polish swine. Appl Environ Microbiol 47: 1210-1212
- Hald B, Krogh P (1982) Natural occurrence of nephrotoxic quinones and quinone-producing fungi in foodstuffs. Proceedings of the fifth IUPAC symposium on mycotoxins and phycotoxins,

 Vienna: 84-87
- Harwig J (1974) Mycotoxins. Elsevier, Amsterdam
- Haubeck H D, Larkowski G, Kölsch E, Röschenthaler R (1981)
 Immunosuppression by ochratoxin A and its prevention by
 phenylalanine. Appl Environ Microbiol 41: 1040-1042
- Krogh P, Axelsen N H, Elling F, Gyrd-Hansen N, Hald B, Hyldehaard-Jensen J, Larsen A E, Madsen A, Mortensen H P, Möller T, Petersen P K, Ravnskov U, Rostgaard M, Aalund O (1974) Experimental porcine nephropathy. Changes of renal function and structure induced by ochratoxin A contaminated feed. Acta Path Microbiol Scand Sect A 246: 1-21
- Krogh P, Elling F, Hald B, Larsen A E, Lillehoj E B, Madsen A, Mortensen H P (1976) Time-dependent disappearance of ochratoxin A residues in tissues of bacon pigs. Toxicology 6: 235-242
- Rousseau D M, Slegers G A, Van Peteghem C H (1985) Radioim-munoassay of ochratoxin A in barley. Appl Environ Microbiol 50: 529-531
- Rousseau D M, Slegers G A, Van Peteghem C H (1986) Solidphase radioimmunoassay of ochratoxin A in serum. J Agric Food Chem 34: 862-865
- Rousseau D M, Candlish A A G, Slegers G A, Van Peteghem C H, Stimson W H, Smith J E (1987) Detection of ochratoxin A in porcine kidneys by a monoclonal antibody based radio-immunoassay. Appl Environ Microbiol 53: 514-518
- Rutqvist L, Björklund N E, Hult K, Gatenbeck S (1977) Spontaneous occurrence of ochratoxin residues in kidneys of fattening pigs. Zentralbl Veterinaermed Reihe A 24: 402-408
- Sandor G, Glavits R, Vajda L, Vanyi A, Krogh P (1982) Epidemiologic study of ochratoxin A - associated porcine nephropathy in Hungary. Proceedings of the fifth IUPAC Symposium on Mycotoxins and Phycotoxins, Vienna: 349-352
- Szebiotko K, Chelkowski J, Dopierala B, Godlewska B, Radomyska W (1981) Mycotoxins in cereal grain. Part I. Ochratoxin, citrinin, sterigmatocystin, penicillic acid and toxigenic fungi in cereal grain. Die Nahrung 25: 415-421
- Trenk H L, Butz M E, Chu F S (1971) Production of ochratoxins in different cereal products by <u>Aspergillus ochraceus</u> Appl Microbiol 21: 1032-1035
- Received April 16, 1988; accepted August 15, 1988.