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Kinetic Profiles of Diacetoxyscirpenol and Two of Its Metabolites in Blood Serum of Pigs

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Orally administered diacetoxyscirpenol (2 mg/kg of body weight) was rapidly absorbed into the blood serum of pigs; within 1 h, the highest amounts of diacetoxyscirpenol (9.6 to 21.9 ng/ml) were detected. Two metabolites of diacetoxyscirpenol were identified by gas chromatography-mass spectroscopy as monoacetoxyscirpenol and scirpenetriol. The three trichothecenes were present in the blood serum of pigs for only 24 h, indicating a rapid metabolism of these compounds.

Diacetoxyscirpenol (3-hydroxy-4,15-diacetoxy-12,13-epoxytrichothec-9-ene) is a potent mycotoxin produced by certain *Fusarium* strains (1, 16). This compound shows a wide-ranging biological activity, including toxicity to fungi, plants, animals, and various mammalian tissue cultures (1, 2, 5, 12, 17). Diacetoxyscirpenol, T-2 toxin, deoxynivalenol, and nivalenol are some of the most important trichothecene mycotoxins in agricultural products (3, 4, 7, 9, 14). Consequently, there is a possibility of human consumption of animal products contaminated with trichothecenes or their metabolites. Studies on the metabolism of trichothecenes could provide important information for both evaluating and controlling human exposure to residual trichothecene metabolites in foods of animal origin. The metabolic fate of T-2 toxin has been studied in rats, mice, chickens, and pigs as well as in a cow (8, 13, 18, 19). However, very little is known about the fate of diacetoxyscirpenol in animals (10). This report describes the pharmacokinetic profiles of diacetoxyscirpenol and two metabolites in the blood serum of pigs after oral administration of this trichothecene.

MATERIALS AND METHODS

Mycotoxins. Diacetoxyscirpenol was purchased from Sigma Chemical Co., Taufkirchen, Federal Republic of Germany. Monoacetoxyscirpenol and scirpenetriol were prepared by hydrolysis of diacetoxyscirpenol in a methanolic ammonia solution (11). 4-Acetoxyscirpenediol was prepared by hydrolysis of diacetoxyscirpenol in sulfuric acid (6). The identities of the trichothecenes were confirmed by mass spectroscopy.

Animals. Five female pigs of 20 ± 1 kg were housed in separate metabolic cages. The animals were provided with water ad libitum and fed twice a day with a commercial pelleted pig ration (800 g/day). Five days before the administration of diacetoxyscirpenol, each pig was generally anesthetized, and a catheter (no. 2 ERU; Rüsch, Waiblingen, Federal Republic of Germany) was surgically inserted into the left vena jugularis externa. Catheters were rinsed with 20 ml of a 0.9% sodium chloride solution containing 10 IU of sodium heparinate (Hoffmann-La Roche, Grenzach/Wyhlen, Federal Republic of Germany) per ml twice a day to prevent blood coagulation.

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Administration of diacetoxyscirpenol. Crystalline diacetoxyscirpenol (2 mg/kg of body weight) was placed in stomach-soluble gelatin capsules and administered to four pigs by intubation. A capsule without diacetoxyscirpenol was given to one animal for control purposes.

Sampling. Blood samples (20 ml) were collected at 0, 0.5, 1, 2, 4, 6, 12, 24, 48, 72, and 96 h after the administration of diacetoxyscirpenol and centrifuged (1,500 × *g*, 10 min) to obtain sera. After sampling, the catheters were rinsed in the same manner as described above.

Mycotoxin analysis. (i) Extraction and purification. Serum (10 ml) was extracted with 50 ml of ethyl acetate. The further purification steps were carried out by the method of Swanson et al. (15). For that purpose, 45 ml of the ethyl acetate layer was concentrated. The residue was dissolved in 1 ml of methanol-water (1:9 [vol/vol]) with an ultrasonic apparatus (Bransonic 12; Bransonic Europa, Soest, The Netherlands) and chromatographed on a Sep-Pak C 18 cartridge (Waters Associates, Inc., Millford, Mass.). The cartridge was rinsed with 1 ml of methanol-water (1:9 [vol/vol]), and the trichothecenes were eluted with 2 ml of methanol-water (8:2 [vol/vol]). The solvent was evaporated under a gentle stream of nitrogen. The remaining residue was dissolved in 8 ml of toluene and added to a preconditioned Florisil column (10 by 30 mm, 100/200 mesh; Paesel, Frankfurt, Federal Republic of Germany) (15). The column was rinsed with 10 ml of dichloromethane, and the mycotoxins were eluted sequentially with 15 ml of chloroform-methanol (95:5 [vol/vol]) and 15 ml of chloroform-methanol (50:50 [vol/vol]). The chloroform-methanol eluates were combined and concentrated to dryness.

(ii) GC-MS. Extracts (equivalent to 9 ml of serum) and standard trichothecenes (5 μg) were derivatized to trifluoroacetic acid esters (TFA) by reaction with 20 μl of *n*-methyl-bis(trifluoroacetamide) (Merck E. AG, Darmstadt, Federal Republic of Germany). The analysis was carried out in a Finnigan MAT 1020 gas chromatography (GC)-mass spectroscopy (MS) system (Finnigan MAT, Bremen, Federal Republic of Germany) equipped with a computer-based data system software. Two-microliter splitless injections were made into a fused silica capillary column (DB 5, 15 m by 0.322 mm, 0.25-μm film thickness; ICT, Frankfurt, Federal Republic of Germany) with helium (15 lb/in^2) as the carrier gas. The injection temperature was set at 205°C and the oven

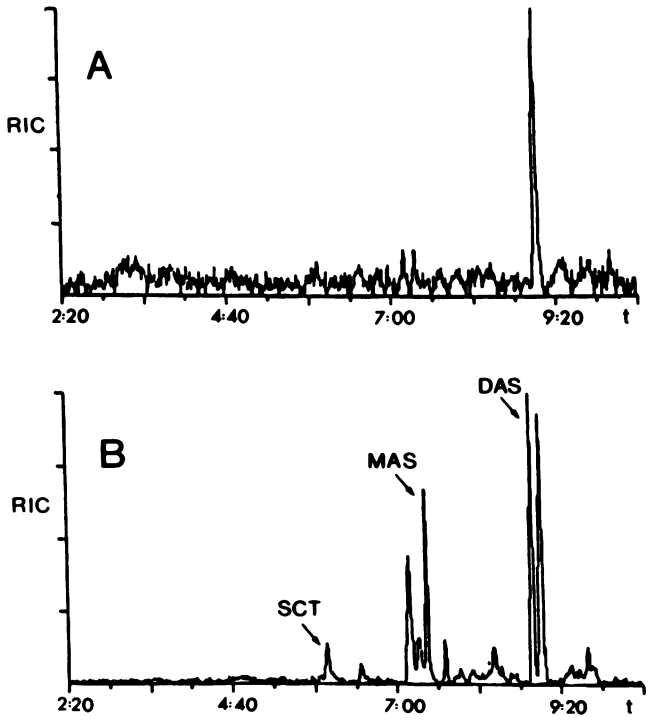

FIG. 1. Total ion current profile of derivatized extracts of the blood serum collected from one pig immediately before (A) and 0.5 h after (B) oral administration of diacetoxyscirpenol (DAS). MAS, Monoacetoxyscirpenol; SCT, scirpenetriol. t, Time, in minutes. RIC, Reconstructed ion chromatogram.

programmed from 140°C (3 min) to 260°C at 15°C/min. Analyses were carried out in the positive chemical ionization mode in methane. The mass spectrometer was operated in the selected-ion-monitoring mode and was switched on 90 s after sample injection. Two ions were monitored for each mycotoxin: scirpenetriol TFA; m/e^+ 457 (base peak), m/e^+ 571 (M + H); monoacetoxyscirpenol TFA, m/e^+ 457 (base peak), m/e^+ 517 (M + H); 4-acetoxyscirpenediol TFA; m/e^+ 403 (base peak), m/e^+ 517 (M + H); and diacetoxyscirpenol TFA; m/e^+ 403 (base peak), m/e^+ 463 (M + H). Criteria for identification were the detection of the two key ions at the corresponding retention times of the standard toxins and the relative intensities of these ions. Quantification of the trichothecenes was made by comparing the computer-integrated peak areas of the total ion current to those of the corresponding external standards.

TABLE 1. Diacetoxyscirpenol (DAS) concentrations in the blood serum of pigs given DAS (2 mg/kg of body weight) orally

Time (h) after DAS administration	DAS concn (ng/ml) in pig no.:			
	1	2	3	4
0	ND[a]	ND	ND	ND
0.5	21.9	ND	ND	14.9
1	7.9	12.8	9.6	1.1
2	1.3	6.0	1.6	1.3
4	ND	NA[b]	3.2	1.3
6	ND	1.2	1.2	ND
12	ND	ND	ND	0.7
24	ND	ND	ND	1.4
48 to 96	ND	ND	ND	ND

[a] ND, None detected.
[b] NA, Not analyzed.

In spiked blood sera, the recovery rates and detection limits, respectively, of three trichothecenes were as follows: diacetoxyscirpenol, 61%, 10 ng/ml; monoacetoxyscirpenol, 45%, 10 ng/ml; and scirpenetriol, 40%, 25 ng/ml.

RESULTS AND DISCUSSION

Three of four pigs showed strong salivation ca. 10 min after diacetoxyscirpenol was administered, and all animals vomited within the first 63 min. The period of emesis lasted for 30 to 60 min. Afterwards, apathy, anorexia, and posterior paresis were observed for ca. 12 h. None of the pigs showed any clinical sign of intoxication 24 h after diacetoxyscirpenol was administered.

The analysis of blood serum extracts by GC-MS revealed the presence of diacetoxyscirpenol and two metabolites (Fig. 1). The suspected toxin peaks showed retention times identical to those of the corresponding standards of diacetoxyscirpenol TFA (8.9 min), monoacetoxyscirpenol TFA (7.4 min), and scirpenetriol TFA (6.0 min). Figure 2, representing a serum sample collected 0.5 h after toxin administration, shows the confirmation of the two ions of scirpenetriol TFA (m/e^+ 457, m/e^+ 571), monoacetoxyscirpenol TFA (m/e^+ 457, m/e^+ 517), and diacetoxyscirpenol TFA (m/e^+ 403, m/e^+ 463). The characteristic ions of these trichothecenes were not detected at the corresponding retention times in extracts either of blood serum collected

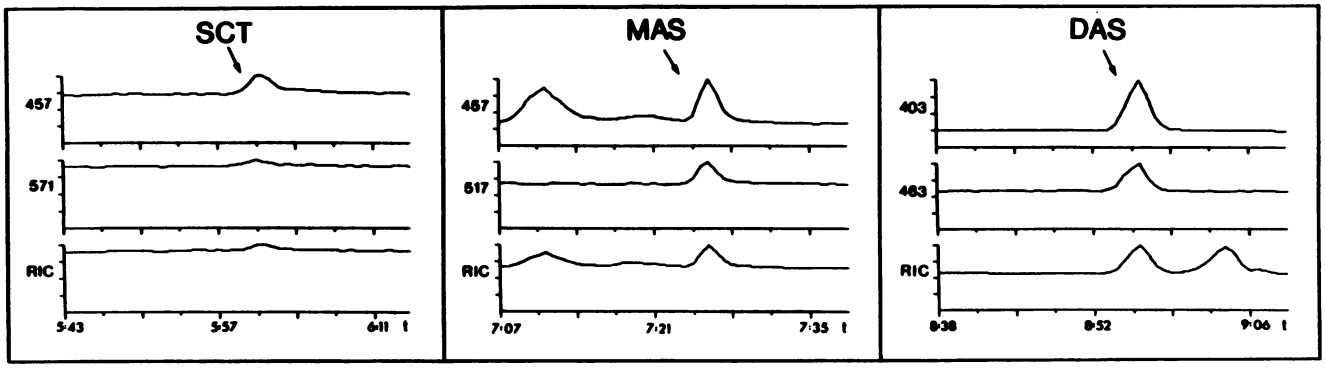

FIG. 2. Selected-ion-monitoring plots for confirmation of scirpenetriol (SCT), monoacetoxyscirpenol (MAS), and diacetoxyscirpenol (DAS) in a blood serum sample collected 0.5 h after diacetoxyscirpenol administration. t, Time, in minutes. RIC, Reconstructed ion chromatogram.

TABLE 2. Monoacetoxyscirpenol (MAS) concentrations in the blood serum of pigs given diacetoxyscirpenol (DAS) (2 mg/kg of body weight) orally

Time (h) after DAS administration	MAS concn (ng/ml) in pig no.:			
	1	2	3	4
0	ND[a]	ND	ND	ND
0.5	5.9	ND	ND	8.7
1	0.7	13.2	1.9	0.5
2	1.1	7.3	1.4	0.8
4	ND	NA[b]	3.1	0.1
6	ND	1.2	2.0	ND
12	ND	6.2	ND	1.1
24	ND	3.4	ND	0.7
48 to 96	ND	ND	ND	ND

[a] ND, None detected.
[b] NA, Not analyzed.

immediately before toxin administration or of blood serum from the control animal.

The quantitative results are listed in detail in Tables 1, 2, and 3. The highest amounts of diacetoxyscirpenol were measured 30 to 60 min after toxin administration, indicating a rapid absorption of this mycotoxin. At the same time, three of four pigs showed the highest amounts of monoacetoxyscirpenol, whereas the highest amounts of scirpenetriol were detected after 1 to 2 h. Only pig number 3 showed the highest levels of monoacetoxyscirpenol and scirpenetriol after 4 and 6 h, respectively. The mean values of the data for the four pigs demonstrate increasing amounts of the two metabolites and decreasing levels of diacetoxyscirpenol during the first hour; after that time, concentrations of the three trichothecenes decreased rapidly to nondetectable amounts within 48 h (Fig. 3). Because the detection limits were quite high and the recovery rates were low for monoacetoxyscirpenol and scirpenetriol, the actual levels in the blood serum were probably higher than measured.

The high metabolizing activity of animal livers in vitro was previously reported by Ohta et al. (10), but they detected monoacetoxyscirpenol as the sole metabolite of diacetoxyscirpenol. The experiments reported here show that diacetoxyscirpenol was also metabolized to the more polar compound scirpenetriol in pigs. It is supposed that diacetoxyscirpenol is deacetylated in a stepwise manner, first at C-4 and than at C-15. GC-MS showed no evidence that 4-acetoxyscirpenediol, an isomer of monoacetoxyscirpenol,

TABLE 3. Scirpenetriol (SCT) concentration in the blood serum of pigs given diacetoxyscirpenol (DAS) (2 mg/kg of body weight) orally

Time (h) after DAS administration	SCT concn (ng/ml) in pig no.:			
	1	2	3	4
0	ND[a]	ND	ND	ND
0.5	1.6	ND	ND	3.0
1	ND	14.8	1.8	ND
2	0.5	6.5	1.4	1.3
4	0.4	NA[b]	2.7	1.3
6	ND	1.6	3.1	ND
12	ND	ND	ND	0.7
24	ND	ND	ND	0.6
48 to 96	ND	ND	ND	ND

[a] ND, None detected.
[b] NA, Not analyzed.

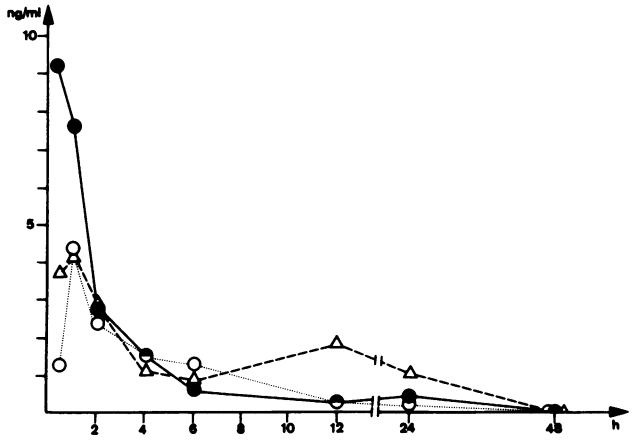

FIG. 3. Plot of concentrations of diacetoxyscirpenol (●), monoacetoxyscirpenol (△), and scirpenetriol (○) in the blood serum of pigs versus time after oral administration of diacetoxyscirpenol (2 mg/kg of body weight). The data are the mean values for four animals.

was present in the serum extracts. Although the TFA derivatives of monoacetoxyscirpenol and 4-acetoxyscirpenediol had nearly the same retention times in the GC analysis, the mass spectra of both trichothecenes obtained by chemical ionization in methane clearly differed: the base peak of monoacetoxyscirpenol TFA was m/e^+ 457 under the MS conditions described above, whereas the base peak of 4-acetoxyscirpenediol TFA was m/e^+ 403. The mass chromatograms of the serum extracts at m/e^+ 403 showed no significant increase in intensity at the corresponding retention time.

The data reported here demonstrate that measurable amounts of diacetoxyscirpenol, monoacetoxyscirpenol, and scirpenetriol were present in the blood serum of pigs for only 24 h after oral administration of diacetoxyscirpenol.

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