In vitro metabolism of Monensin with liver microsomes: identification of metabolites by Liquid Chromatography-Mass Spectrometry

B21

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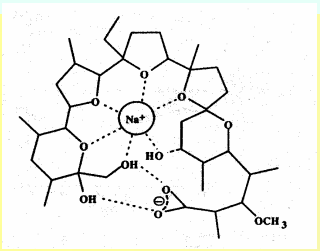
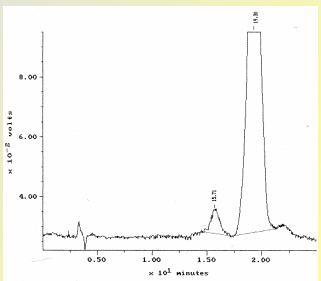
Monensin (MON, fig 1) is a ionophore antibiotic used to prevent coccidiosis in poultry. Recently, interspecies differences of MON toxicity, had been related to species variation in liver content of P4503A and in demethylation rate of MON (1, 2). Aim of the study was the identification using liquid chromatography-mass spectrometry (LC-MS) of MON metabolites, produced *in vitro* with liver microsomes from pigs, the species who mostly metabolises MON (2) and from P4503A induced rats and rabbits.



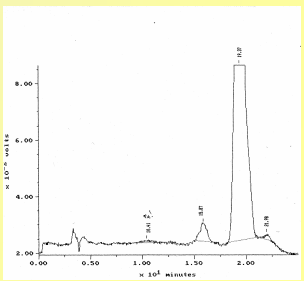
Material and methods

Microsomes, were obtained from livers of pigs (160 ± 2 kg bw), of rats (130 ± 20 g bw) induced with DEX (50 mg/kg, ip for 3 days) and of rabbits (2.0 ± 0.2 kg bw) induced with Rifampicin (RIF) (50 mg/kg ip for 4 days), and incubated with MON, as previously reported (2). HPLC elution, using post-column derivatization with vanillin was adopted to identify metabolite and assess their elution times. Then, corresponding fractions were collected without derivatization and analysed, in flow injection mode, using an ion trap/MS (LCQ-DUO Thermoquest) with positive-ion APCI. Spectra were acquired over the mass range m/z 450-750.

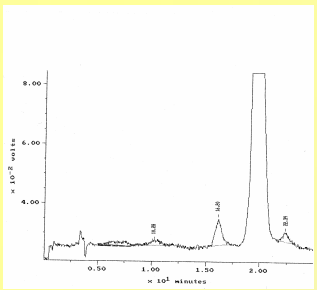
Figure 2 : HPLC elution of standard MON (2A) and of its main metabolites produced in vitro using liver microsomes from rats (2B) rabbits (2C) and pigs(2D)



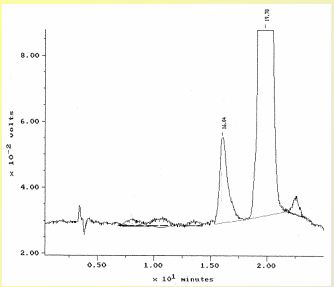
2 A



2 B



2 C



2 D

Results

Very Low amount of MON metabolites were detected by HPLC in all microsome fractions; their presence was confirmed and the structure identified by analysing with LCMS the fractions collected. All molecular ions were acquired as sodium adducts. Further to parent compound MON A (m/z 693) and MON B (m/z 679, an impuity of MON A), (see fig 2 A) three more molecular ions were detected in rat fractions (fig 2 B) at m/z 695, 649 and 665. Three also were found in rabbit fractions (fig 2 C) at m/z 695, 649 and 633 and four in pigs (fig 2 D) at m/z 709, 695, 649; the greatest one, that eluted earlier but very close to MONB, was detected at m/z 679 like MON B.

Table 1: Main metabolites produced *in vitro* from MON A

	MON A	MON 1	MON 2	MON 3	MON 4	MON 5	MON 6
m/z	693	709	695	679	665	649	633
± mol wt AMU	-	+16	-14 + 16	-14	-14, - 44, +32	-14, - 44, +16	-14, -44, -2
MON_A modifications	none	Hydroxyl-	Hydroxyl- O-demethyl-	O-demethyl-	Di-hydroxyl - O-demethyl- de-carboxyl-	Hydroxyl- O-demethyl- de-carboxyl-	O-demethyl- de-carboxyl- oxidised -

m/z = mass/charge ratio ± mol wt = mass differences from MON A AMU= atomic mass units

Discussion and Conclusions

The overall amount of MON biotransformation was the greatest in pig microsomes (2) and **MON 3** a demethylated metabolite different from MON B was the major one. The putative structures of metabolites, reported in table 1, were obtained by matching molecular ions of MS/spectra and data from literature (3). Modifications such as O-demethylation and hydroxylation supported the involvement of P4503A as reported (2, 3) while the loss of the carboxyl group could be a seen as a consequence of the hydrolysis of the ether group and should be considered the real detoxication process of MON biotransformation.

References 1. Nebbia C, Ceppa L, Dacasto M, Carletti M, Nachtmann C. Oxidative metabolism of monensin in rat liver microsomes and interactions with tiamulin and other chemoterapeutic agents: evidence for the involvement of cytochrome P-450 3A subfamily. Drug Metab Dispos 1999; 27:1039-1044. 2. Dacasto M, Eeckhoutte C, Sutra JF, Dupuy J, Nebbia C, Montesissa C, Alvinerie M, & Galtier P. CYP3A-dependent metabolism of [C]14–monensin in hepatic microsomes prepared from rat and food-producing species liver. Proceedings 12th International Symposium on Microsomal and Drug Oxidations, Montpellier July 20-24, 1998. 3. Kihel DE, Julian RK, Kennington AS. Electrospray Ionization Mass Spectrometry with In-source Collision-induced Dissociation of Monensin Factors and Related Metabolites. Rapid Commun Mass Spectrom 1998; 12: 903-910.