Using Nuclear Magnetic Resonance and MS/MS Spectroscopy for the Identification of Brodimoprim Metabolites in Rat Urine

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Abstract: Eight metabolites of brodimoprim (BDP) in rat urine were detected by NMR and ESI-MS/MS. They were demethyl-BDP glucuronide, demethyl-BDP sulfurate, demethyl-BDP glucuronide sulfurate, α-hydroxyl-BDP, α-hydroxyl-BDP glucuronide, BDP sulfurate, N-oxide-BDP sulfurate, and α-hydroxyl-N-oxide-BDP sulfurate. All the sulfurates are reported for the first time.

Keywords: Nuclear magnetic resonance spectroscopy, MS/MS spectroscopy, metabolites, brodimoprim.

Brodimoprim, a trimethoprim analogue diaminopyrimidine, 2,4-diamino-5-(4'-bromo-3',5'-dimethoxybenzyl) pyrimidine, is a new inhibitor of bacterial dihydro folate reductases (OHFRs), it shows activity against a broad spectrum of Gram-positive and negative-bacteria. Recently we reported the results of the study on the metabolites of brodimoprim *in vivo* with SPE-NMR method (solid phase extraction coupled with nuclear magnetic resonance), five metabolites were detected in rat urine[1]. They were demethyl BDP and its glucuronide, N-oxides BDP, α-hydroxyl-BDP and didemethyl-BDP. Though the prominent doublet signal of β-anomeric proton at δ 5.0-5.5 could indicate the presence of glucuronides, NMR has no definitive signal to prove the structure of sulfurates. So it is difficult to find sulfurates of any metabolite with the method of SPE-NMR.

In order to make up the deficiency of SPE-NMR, MS/MS method was used in this study and the rat urine was also treated as before. The molecular weights and the fragments of main components in the samples could be tested, though all the samples got from the column of SPE were mixture of metabolites. Because MS method is more sensitive than with NMR method, five new metabolites could be detected.

Experimental

Two wistar rats of approximately 250 g weight were used in the study and they were administrated orally with BDP in the dosage of 250 mg • kg^{-1}. The urine in 24 h was collected and filtered before it was loaded onto the 3 mL ENVI-18 SPE column (Supelco,

Inc., purchased from Supelco park, Bellefonte, USA, containing 300 mg of sorbent), which was activated by washing with 5 mL of methanol and then 5 mL of water. The sample was eluated with 5 mL water at first, then followed by gradient solvent of 90:10 (v/v), 80:20 (v/v), and 40:60 (v/v) of water and methanol in the same volume. Each fraction was collected and the solvent was removed in vacuum. The residues were redissolved in 0.5 mL deuterated dimethylsulfoxide, and detected by the Bruker AM-500 NMR spectrometer and AutoSpec Ultima-Tof MS/MS spectrometer.

All the sulfurates, such as: demethyl-BDP sulfurate, demethyl-BDP glucuronide sulfurate, BDP sulfurate, N-oxide-BDP sulfurate, α-hydroxyl-N-oxide-BDP sulfurate, were proved for the first time by the data of MS/MS spectroscopy, and the demethyl-BDP glucuronide sulfurate was also reported firstly based on the data of NMR and ESI-MS/MS. The MS and NMR data of eight BDP metabolites were shown in **Table 1** and **Table 2**, and the metabolic route was proposed in **Figure 1**. The signals of the metabolites were distinct in the MS spectra based on the feature of bromide isotone. The exchange between proton and deuterium which came from the solvent of deuterated dimethylsulfoxide (DMSO) also existed in MS spectra. The lowest molecular weight for every metabolite was reported in **Table 1**. All the structures of glucurinides were shown in the **Figure 1**, whether the sulfurates connected to the hydroxyl group or amino group in metabolites **4, 7, 8, 9**, could not be determined yet, except in metabolite **3**. The detail results of the study will be published later.

Table 1 Mass spectra data* of BDP and its metabolites with ESI-MS/MS

Symbol	BDP and its metabolites	*m/z*
1	BDP	339 $[M+H]^+$, 324 $[339\text{-}CH_3]^+$, 281 $[339\text{-}2\times CH_3\text{-}CO]^+$
2	Demethyl-brodimoprim-glucuronide	501 $[M+H]^+$, 325 $[501\text{-glc}]^+$
3	Demethyl-brodimoprim-sulfurate	405 $[M+H]^+$, 323 $[405\text{-}H_2SO_3]^+$
4	Demethyl-brodimoprim-glucuronide-sulfurate	581 $[M+H]^+$, 499 $[581\text{-}H_2SO_3]^+$, 323 $[499\text{-glc}]^+$
5	α-hydroxyl-brodimoprim	355 $[M+H]^+$, 338 $[355\text{-}NH_3]^+$, 243 $[338\text{-}CH_3\text{-HBr}]^+$
6	α-hydroxyl-brodimoprim-glucuronide	531 $[M+H]^+$, 355 $[531\text{-glc}]^+$, 338 $[355\text{-}NH_3]^+$
7	Brodimoprim-sulfurate	419 $[M+H]^+$, 337 $[419\text{-}H_2SO_3]^+$
8	N-oxide-brodimoprim-sulfurate	435 $[M+H]^+$, 353 $[435\text{-}H_2SO_3]^+$, 336 $[353\text{-}NH_3]^+$
9	α-hydroxyl-N-oxide-brodimoprim-sulfurate	453 $[M+H]^+$, 371 $[453\text{-}H_2SO_3]^+$

*The molecular weight, especial for sulfurates was reported except for the effect of deuterium which came from the solvent of DMSO.

Figure 1 Structure of brodimoprim (BDP) and its metabolic route

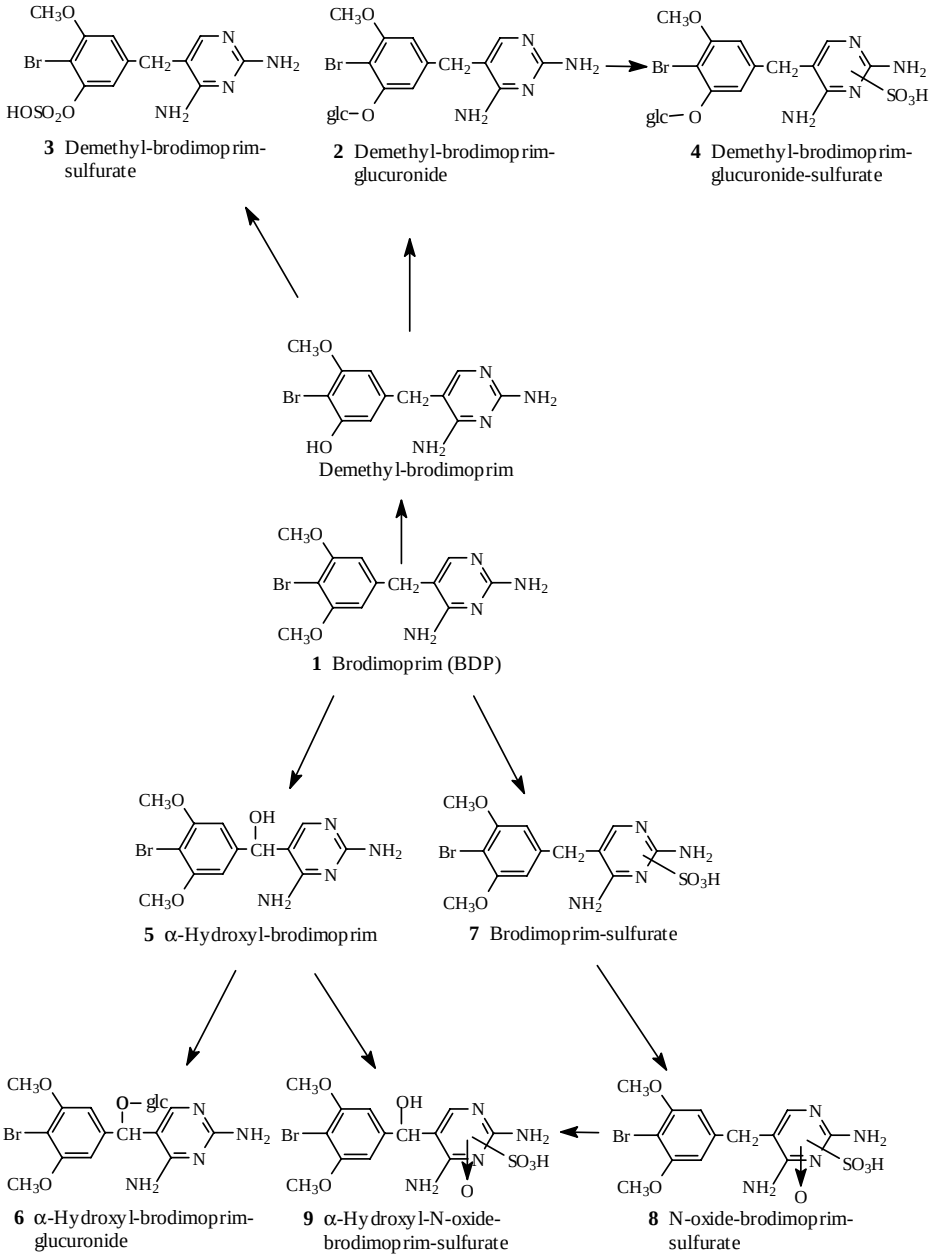

Table 2 Partial data of NMR spectra of BDP and its metabolites (δppm)

Symbol	BDP and its metabolites	Proton of benzyl	Proton of pyrimidine	Methylene	β-anomeric proton of glucuronide
1	BDP	6.67	7.51	-	-
2	Demethyl-brodimoprim-glucuronide	6.72 6.66	7.40	-	5.00
3	Demethyl-brodimoprim-sulfurate	7.03 6.72	7.36	-	-
4*	Demethyl-brodimoprim-glucuronide-sulfurate	-	-	-	-
5	α-hydroxyl-brodimoprim	6.70	7.38	5.53	-
6*	α-hydroxyl-brodimoprim-glucuronide*	-	-	-	-
7	Brodimoprim-sulfurate	6.62	7.48	-	-
8	N-oxide-brodimoprim-sulfurate	6.64	7.67	-	-
9	α-hydroxyl-N-oxide-brodimoprim-sulfurate	6.60	7.65	5.51	-

*α-hydroxyl-brodimoprim-glucuronide and demethyl-brodimoprim-glucuronide-sulfurate were detected only by MS/MS spectroscopy, no data available by NMR, as the sensitivity is different with the two methods.

Reference

1. Y. K. Si, R. M. Xu, M. Kong, W. Y. He, S. R. Zhang, *Acta Pharmaceutica Sinica*, **1998**, *33*, 697.

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