A Revision of the Metabolic Disposition of Amantadine

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Amantadine is one of the most commonly used drugs for the control of tremor in Parkinson's disease. Additionally, it has an antiviral action in the prevention of type A influenza. It has been previously reported that amantadine is nearly completely eliminated in the urine. No metabolites have been detected. Surprisingly, in a case of amantadine overdose, several metabolites could be identified by gas chromatography/mass spectrometry. This finding prompted us to re-investigate the metabolism of amantadine under a therapeutic dosing regimen. The bulk of the dose was eliminated unchanged. However, eight metabolites could be identified. Besides N-acetylation which is the major metabolic pathway, several rather unusual metabolic pathways were observed: N-methylation, formation of Schiff bases and N-formiates. No metabolites with a hydroxylated adamantane ring system could be detected.

INTRODUCTION

Amantadine (1-aminoadamantane) is one of the most commonly used drugs for the control of tremor in Parkinson's disease. Additionally, it has an antiviral action in the prevention of type A influenza. The pharmacokinetics of amantadine have been thoroughly studied by several authors.[1-8] Amantadine has a plasma half-life of 11.8 ± 2.1 h and is almost completely eliminated via the kidneys. The elimination kinetics of amantadine strongly depend on the renal function. Bleidner *et al.*[1] studied the amantadine metabolism in mice, dogs and humans. They found that the dose was almost completely eliminated unchanged with the urine in all species. After an oral dose of 2–4 mg kg^{-1} to human volunteers, 62–93% of the drug was excreted unchanged within four days. No metabolites have been detected so far in mice, monkeys and man. However, small amounts of *N*-methylamantadine were identified in the urine of dogs.

Surprisingly, in a case of suicidal poisoning with an unknown dose of amantadine and bromazepam, we were able to detect several metabolites using our standard capillary gas chromatographic/mass spectrometric screening procedure.[9] This finding prompted us to re-investigate the amantadine metabolism under a therapeutic dosing regimen.

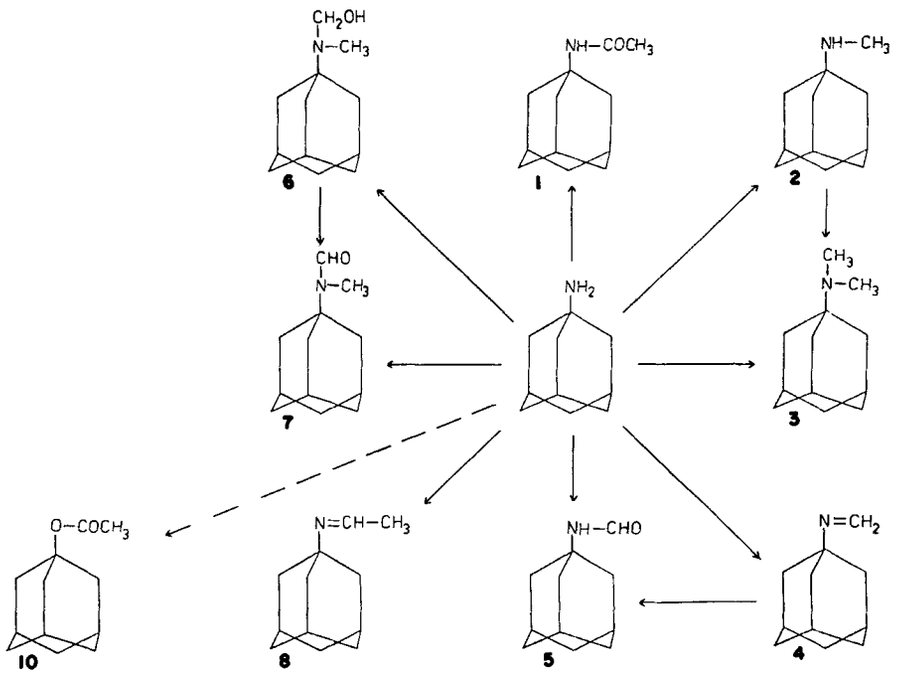

Figure 1. Metabolism of amantadine

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Table 1. Mass spectra and retention times of amantadine and its metabolites

	Retention time (s)	Mass spectra (m/z (intensity %))
Amantadine	211	$[M]^{+\cdot}$151 (51), 136 (6), 108 (19), *94* (100), 91 (11), 79 (14), 77 (20), 67 (11), 58 (11), 57 (48)
1	556	$[M]^{+\cdot}$193 (47), 150 (8), 137 (7), *136* (100), 135 (8), 108 (6), 94 (48), 79 (12), 77 (12), 67 (9), 57 (8)
2	282	$[M]^{+\cdot}$165 (18), 150 (2), 135 (27), 124 (6), 122 (7), 110 (10), 109 (18), *108* (100), 95 (11), 83 (21), 71 (15)
3	341	$[M]^{+\cdot}$179 (22), 164 (3), 123 (11), *122* (100), 107 (4), 93 (4), 91 (4), 85 (14), 79 (6), 55 (8)
4	239	$[M]^{+\cdot}$163 (8), 136 (3), *135* (100), 120 (2), 107 (13), 106 (8), 93 (28), 91 (10), 81 (12), 79 (37), 77 (12), 67 (18), 55 (12)
5	440	$[M]^{+\cdot}$179 (63), 150 (2), 136 (18), 135 (8), 134 (18), 123 (5), *122* (100), 121 (11), 110 (8), 94 (32), 92 (43), 79 (28), 77 (22), 67 (16), 55 (11)
6	351	$[M]^{+\cdot}$195 (10), 163 (2), 136 (11), *135* (100), 134 (3), 120 (2), 108 (6), 107 (10), 106 (4), 93 (22), 91 (4), 79 (23), 77 (8), 67 (13), 55 (12)
7	424	$[M]^{+\cdot}$193 (8), 177 (2), 163 (1), 150 (2), 136 (70), *135* (100), 122 (4), 120 (4), 107 (11), 94 (11), 93 (23), 79 (28), 77 (8), 67 (13), 55 (9)
8	267	$[M]^{+\cdot}$177 (12), 136 (11), *135* (100), 120 (8), 107 (10), 93 (18), 91 (6), 79 (19), 77 (6), 67 (9), 55 (5)
9	465	$[M]^{+\cdot}$201 (51), 158 (1), 144 (6), 136 (12), *135* (100), 117 (4), 107 (13), 93 (20), 91 (10), 79 (23), 77 (11), 67 (18), 55 (7)
10 (artefact)	367	$[M]^{+\cdot}$194 (22), 164 (3), 152 (6), 139 (63), *134* (100), 121 (43), 119 (68), 105 (41), 95 (68), 92 (59), 71 (58), 67 (45), 55 (38)

EXPERIMENTAL

Three healthy, male volunteers received an oral dose of 200 mg of amantadine. Urine was collected for 72 h and kept at −20 °C before analysis. Additionally, morning urine was collected from hospitalized patients with a permanent medication of 2 × 100 mg amantadine per day for Parkinson's disease. 20 ml aliquots of the urine samples were extracted twice with 20 ml diethyl ether (nanograde, Mallinckrodt) at pH 2 and pH 9. For the cleavage of glucuronides, 20 ml of urine was incubated with 0.5 ml of glucuronidase/sulphatase (Merck, Darmstadt) at 37 °C for 12 h. The urine samples were then extracted as described above. The organic solvent was removed with a dry stream of nitrogen. The dried extract was then dissolved in 500 μl methanol and a 1 μl aliquot was used for gas chromatographic/mass spectrometric analysis.

Analysis was performed on a Finnigan 4021 gas chromatograph/mass spectrometer. The gas chromatographic conditions were: injection port 285 °C; split 1:100; 30 m DB-5 capillary column (J & W Scientific, Rancho Cordova), temperature programme 75–300 °C with 15 °C min^{-1}; direct coupling to the mass spectrometer. The mass spectra were run in the electron impact and chemical ionization mode: ion source temperature 250 °C, ion source pressure $\sim 2 \times 10^{-7}$ Torr (electron impact) and $\sim 4 \times 10^{-5}$ Torr (chemical ionization with methane), multiplier voltage 2000 V.

Additionally, the extract was subjected to methylation, acetylation and silylation using standard derivatization techniques. Besides the corresponding derivatives of **1–8**, 1-adamantol acetate (**10**) could be identified after acetylation (cf. Fig. 1). The identification of amantadine metabolites was based on comparison with reference compounds which were synthesized from amantadine using standard procedures.

Quantification of unchanged amantadine in urine was performed using a Perkin-Elmer F22 gas chromatograph equipped with a nitrogen detector (2 m OV17 column; column temperature 230 °C) according to Biandrate *et al.*[10]

RESULTS AND DISCUSSION

The fragmentation of amantadine and its derivatives (Table 1) mainly follows two pathways: (a) The molecular ion loses C_4H_7. This is the main fragmentation pathway of amantadine[10,11] and its methylated and acylated analogues **1**, **2**, **3** and **5**, (b) The Schiff bases **4** and **8** as well as **7** and **9** mainly eliminate the substituted amino function forming a prominent adamantyl ion (m/z 135). Under chemical ionization conditions the corresponding $[M+1]^+$ ions and a more or less abundant adamantyl ion at m/z 135 were observed.

In agreement with previous results,[1] we found that 65–85% of the dose of amantadine was excreted unchanged with the urine. Additionally, eight metabolites and an artefact could be identified (Fig. 1). The main metabolite is *N*-acetylamantadine (**1**) (5–15%). N-Methylation leads to *N*-methylamantadine (**2**) and *N,N'*-dimethylamantadine (**3**). *N*-Methyleneamantadine (**4**) is most probably formed by hydroxylation of the methyl group of **2** and subsequent loss of water. Further oxidation probably leads to *N*-formylamantadine (**5**). In analogy, **6** and **7** are most probably generated by stepwise oxidation of a methyl group of **3**. Additionally, small amounts of *N*-ethylideneamantadine (**8**) could be identified. This rather unusual metabolite is probably formed by condensation of endogenous acetaldehyde with amantadine or by reduction of *N*-acetylamantadine and subsequent loss of water. A further metabolite (**9**) which was present in all urine samples could not be identified unequivocally. Possibly, this metabolite with a molecular ion at m/z 201 and a base peak at m/z 135 is *N*-adamantylpyrrole.

Surprisingly, 1-adamantol acetate (**10**) could be detected after acetylation of the urine extracts with acetic anhydride. By reaction of the reference compounds with acetic anhydride the possibility that **10** is generated from **1–8** could be excluded. No 1-adamantol could be detected prior to acetylation. Therefore, 1-adamantol acetate

is most probably an artefact generated from an amantadine metabolite which could not be identified under the experimental conditions applied here.

No glucuronidation was observed except for **6**. The main metabolite **1** as well as **3**, **4** and **9** were present in all urine extracts. The other metabolites could only be detected in patients on permanent amantadine medication.

Except for the acetylation, the metabolic disposition of amantadine follows rather unusual pathways such as N-methylation and formation of Schiff bases and *N*-formiates. There was no evidence for formation of more 'usual' metabolites by oxidation of the adamantane ring system to hydroxyl or keto derivatives.

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