Biotransformation of moclobemide in humans

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The structure of the urinary metabolites formed after moclobemide administration in humans was elucidated, and the pattern compared with that in the plasma. The metabolic pathways of moclobemide were also compared with those of structurally related substances. After oral moclobemide administration, on average 95% of the dose was recovered in the urine within 4 days, with a mean of 92% being excreted during the first 12 h. The drug is extensively metabolized: less than 1% of the dose was excreted unchanged. A total of 19 metabolites, accounting together for about 64% of the dose, was isolated and all metabolites accounting for more than 1% of the dose were identified. Consistent with other morpholine-containing compounds, metabolic pathways of moclobemide include mainly oxidative attack on the morpholine moiety, leading to a multitude of oxidation products. Four primary metabolic reactions were identified: morpholine N-oxidation, aromatic hydroxylation, morpholine C-oxidation and deamination. The major metabolites in urine are 4 carboxylic acids (M7A and M7B, M8, M9) that account for 49% of the dose. Only 2 metabolites (M3, M10) were found to be hydroxylated on the aromatic nucleus. They were excreted completely as conjugates of glucuronic and/or sulfuric acid. Conjugation in general, however, seems to be of minor importance in the overall biotransformation of the drug. The metabolite pattern in plasma was found to be qualitatively but not quantitatively similar to that observed in urine. Almost all of the main urinary metabolites were found in plasma as well. The unchanged parent compound and 2 primary oxidation products of the morpholine ring (M1, M15), which were present in urine only in trace amounts, could easily be detected in plasma.

R. Jauch[1], E. Griesser[1], G. Oesterhelt[1], W. Arnold[1], W. Meister[1], W. H. Ziegler[2], T. W. Guentert[3]

[1]Research Department, [3]Department of Clinical Pharmacology, F. Hoffmann-La Roche, Basle, [2]Division of Clinical Pharmacology, University Hospital, Zurich, Switzerland

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R. Jauch, Research Department, F. Hoffmann-La Roche, CH-4002 Basle, Switzerland

Moclobemide belongs to a new generation of monoamine oxidase (MAO) inhibitors of the benzamide type and contains a morpholine ring as a characteristic part of its structure.

The aim of this study was to elucidate the structures of the urinary metabolites formed from moclobemide after its administration to humans and to compare the metabolite pattern in urine and plasma. In addition, a comparison of metabolic pathways between moclobemide and structurally related drugs was attempted.

Material and methods

Drug administration and sample collection

Two healthy young volunteers received 50 mg ^{14}C-labelled moclobemide (2 μCi/mg; position of radiolabel, see Fig. 1) orally in a hard gelatine capsule. Blood was taken by puncture of an arm vein at different time points up to 10 h after administration and plasma was obtained by centrifugation. Urine and faeces were collected quantitatively in fractions up to 96 h after administration.

Isolating and identifying the metabolites

Metabolites were isolated from pooled 0–12 h urine by chromatography on Amberlite XAD-2 resin and subsequent extractions with ethyl acetate at pH 9 and pH 3. These extractions were made before and after enzymatic hydrolysis with a mixture of beta-glucuronidase and arylsulfatase. Separation and purification of the metabolites were achieved by preparative thin-layer chromatography (TLC) on silica gel. Structures were identified by using radio-GC, GC/MS, MS and ^{1}H-NMR. Migration distances on TLC compared with reference compounds were an important additional tool to get a first hint for possible structures or chemical nature of a compound. Derivatization reactions on isolated metabolites and/or synthetic reference compounds facilitated structure elucidations in

several cases. All metabolites accounting for more than 1% of the dose were identified.

In plasma (0.5–4 h pool: not hydrolyzed enzymatically) the metabolite pattern was investigated by analytical TLC on silica gel.

Results and discussion

Administration of ^{14}C-labelled moclobemide allowed investigation of the mass balance of excretion, determination of major excretory routes and subsequent metabolite identification and quantification.

Mass balance of excretion

Following the oral administration of 50 mg moclobemide, a mean of 95% of the dose was excreted in urine within the 96 h collection period, 92% within the first 12 h. Combined total urinary and faecal recovery of drug-related material accounted for all of the drug dose. Before excretion the drug was almost entirely metabolized; at most 0.4% of the dose was excreted as intact moclobemide in urine.

Urinary metabolites

A multitude of different moclobemide metabolites are formed in the metabolic degradation of the drug (Fig. 1). Four distinct groups of compounds could be identified in urine by 2-dimensional TLC: acidic (49% of dose), neutral (8%), basic (2%) compounds and N-oxides (4.5%). A total of 19 metabolites were identified, accounting together for about 64% of the dose.

The metabolic cascade is initiated by 4 primary reactions: morpholine N-oxidation, aromatic hydroxylation, morpholine C-oxidation and deamination. Quantitatively, the products of the oxidation of the morpholine ring are particularly prominent. They can all be considered to be derived from a metabolic product with a single hydroxyl group in the morpholine moiety (M1). The position of this substituent has not been established. By further oxidation of the alcoholic group and additional oxidative attacks in the heterocyclic ring several metabolites with a lactam structure (neutral compounds, M11, M13–M16) are formed. Ring opening via hypothetical intermediate aldehydes leads to carbamides, tertiary and secondary amines. The

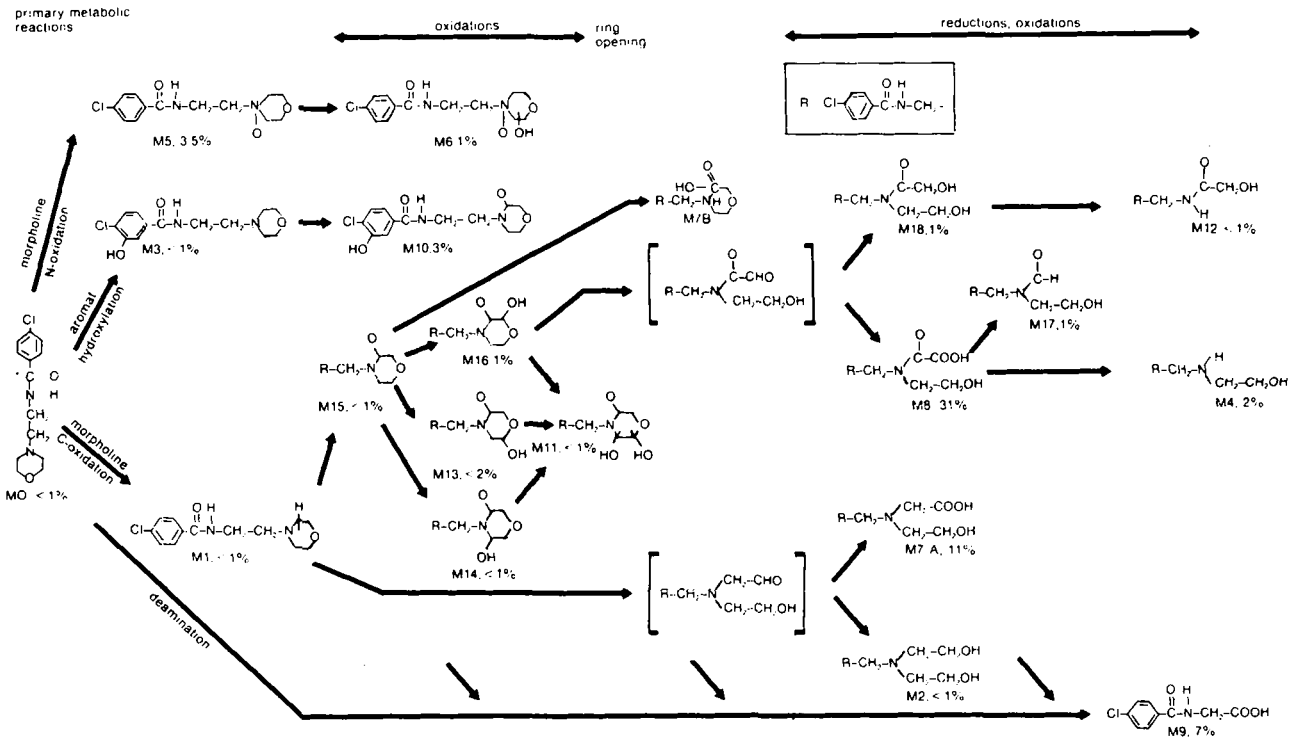

Fig. 1. Structures and possible biogenetic pathways of the metabolites of moclobemide in humans. The scheme relates to urinary metabolites. Structures with framed designation were found in plasma as well. Percentages relate urinary recovery of a metabolite to the dose administered. [ ] hypothetical intermediate; * position of the ^{14}C-label.

4 most important excretion products in the urine (M8, M7A and M7B, M9) are all carboxylic acids accounting together for 49% of the administered dose (Fig. 1). Besides oxidation of the aliphatic part of the molecule, oxidation of the aromatic ring was also observed, albeit to a lesser extent. The phenols thus formed do not appear as free compounds. They are excreted into the urine almost exclusively in the form of conjugates with glucuronic and/or sulfuric acid.

Metabolites in plasma

A comparison of the concentrations of parent drug and total radioactivity in plasma indicated that a large fraction of the radioactivity could not be accounted for by unchanged drug. The metabolic pattern of drug-related compounds in the plasma pool revealed, besides parent drug, a variety of circulating metabolites. Overall, the pattern of metabolites in plasma was qualitatively similar to that in urine. Among the metabolites identified in plasma were the main urinary metabolites, namely the acids M8, M7A, M9, and, in traces, the MAO-A-inhibiting N-oxide M5. The MAO-B-inhibiting M4 was also detected in plasma, but it is not clear at present whether the concentrations found for this product were real or artifactually enhanced because of a labile precursor. Nevertheless, as judged from autoradiograms of the TLC plates, in plasma one of the major metabolites was the lactam derivative M15, a compound accounting for less than 1% of the dose in urine. This supports the assumption that M15 is an early but still relatively lipophilic metabolite undergoing further degradation to mainly acids (Fig. 1).

Metabolite activity

Several of the key products in the moclobemide metabolism are acids with no activity. Only moclobemide-N-oxide M5 retains some MAO-A-inhibitory activity; the ring-opened metabolites M2 and M4 were found to inhibit MAO-B in rat liver. It is important to notice that M15, one of the main moclobemide metabolites in plasma, is practically inactive.

Comparison of the metabolism of moclobemide with other compounds containing the morpholine moiety

The metabolism of moclobemide was compared with that of compounds with a similar structure. From studies with a variety of compounds carrying a morpholine moiety, a general pattern of metabolic degradation reactions emerges. Carbon atoms with one or more H-atoms and alpha-positioned to heteroatoms are susceptible, in general, to metabolic oxidation. In the morpholine ring such oxidative reactions lead to lactams and lactones (1). Multiple oxidative attack leads to formation of highly oxidized morpholine ring systems and ring opening. Tatsumi et al. (2) were the first to identify dioxygenated derivatives of the morpholine ring; opening of the ring system had already been established earlier (3). The oxidative reactions at the morpholine ring result in formation of substantial amounts of acids (4). Most metabolites formed by oxidative reactions are excreted in unconjugated form.

Moclobemide metabolism in humans follows this general pattern of morpholine oxidation. Both oxidation reactions seem to occur at the morpholine substituent as intermediate steps in the metabolic cascade (see Fig. 1: formation of M15 and M7A) followed by further oxidation and ring opening. Although a 2,3-dioxo-morpholine derivative analogous to Tatsumi's structure (2) was not found with moclobemide in the ring-closed form, several closely related polyoxygenated derivatives were identified (M11, M13, M14 and M16). As expected, acids account for a large fraction of excreted oxidation products; in fact, the 2 major metabolites in urine M8 and M7A are acids.

Comparing the metabolism of moclobemide with that of other morpholine derivatives reveals a high degree of similarity. In the case of timolol, a beta-blocking agent, and molsidomine, a potent antianginal drug, the major metabolite corresponds to a major product seen with moclobemide (M7A) (5, 6). Many similarities can be found in the metabolism of moclobemide and that of trithiozine (7). Extensive oxidation of the morpholine moiety, ring-opening and stepwise breakdown was also found with this thiobenzamide derivative that reduces gastric acid secretion. Although this compound has several metabolically labile methoxy groups, at least 23% of the dose undergoes oxidative changes in the morpholine moiety (moclobemide > 55%); a considerable portion of the dose was excreted in urine as acids. The compound analogous in structure to a major moclobemide metabolite in humans (ring-opened acid M7A; 11% of the dose) was also found to be a major metabolite of trithiozine (6%). Again, as found for moclobemide, most metabolites of trithiozine with altered morpholine ring were excreted in unconjugated form. The metabolic degradation of the morpholine ring of emorfazone (8) is another example of close similarities with moclobemide. Several of the metabolites of moclobemide in humans (M1, M2, M4, M7A) were found in analogous structures of this anti-inflammatory. For both compounds the

main metabolite in humans resulted from identical metabolic degradation of the morpholine ring.

Conclusion

After oral moclobemide administration, on average 95% of the dose was recovered in the urine within 4 days, with a mean of 92% being excreted during the first 12 h. The drug is extensively metabolized: less than 1% of the dose was excreted unchanged. Nineteen metabolites accounting together for about 64% of the dose were isolated and all metabolites accounting for more than 1% of the dose were identified. Consistent with other morpholine-containing compounds, metabolic pathways of moclobemide include mainly oxidative attack on the morpholine moiety, leading to a multitude of oxidation products. Four primary metabolic reactions were identified: morpholine N-oxidation, aromatic hydroxylation, morpholine C-oxidation, deamination. The major metabolites in urine are 4 carboxylic acids (M7A and M7B, M8, M9) that account for 49% of the dose. Only 2 metabolites (M3, M10) were found to be hydroxylated on the aromatic nucleus. They were excreted completely as conjugates of glucuronic and/or sulfuric acid. Conjugation in general, however, seems to be of minor importance in the overall biotransformation of the drug. The metabolite pattern in plasma was found to be qualitatively but not quantitatively similar to that observed in urine. Almost all of the main urinary metabolites were found in plasma as well. The unchanged parent compound and 2 primary oxidation products of the morpholine ring (M1, M15), which were present in urine only in trace amounts, could easily be detected in plasma.

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