Comparison of metoprolol/α-hydroxy-metoprolol metabolic ratio after a single dose and in steady state

ĎURICOVÁ J., PEŘINOVÁ I., KACÍŘOVÁ I., GRUNDMANN M.

Department of Clinical Pharmacology, University Hospital Ostrava, and Faculty of Medicine, University of Ostrava, Czech Republic

SUMMARY

Comparison of metoprolol/α-hydroxymetoprolol metabolic ratio after a single dose and in steady state

Metoprolol/α-hydroxymetoprolol metabolic ratio (MR) 3 hours after a single metoprolol dose is used for cytochrome P450 2D6 phenotyping. The aim was to compare cytochrome P450 2D6 metabolic activity after the first metoprolol dose and in steady state. Thirteen adult hypertensive patients (7 females) in whom an introduction of the beta-blocker metoprolol was indicated were included. Age (mean ± SD) was 42.8 ± 12.9 years, weight (mean ± SD) was 94.0 ± 25.4 kg. Metoprolol dose was chosen based on clinical grounds on the day of metoprolol first ingestion. Blood samples were drawn after the first dose and at least 2 weeks since metoprolol introduction to ensure steady state. The patients were phenotyped as extensive metabolizers in both periods, after metoprolol first ingestion and in steady state. We observed a significant correlation ($r_s = 0.8418$, P = 0.0003) between the metoprolol/α-hydroxymetoprolol MRs. All the patients were phenotyped as extensive metabolizers in both periods, despite statistically significant differences between the median MRs (0.59 versus 0.81, P = 0.0266). The differences were not of such an extent so as to assign subjects to different phenotypic groups. Metoprolol/α-hydroxymetoprolol MR in steady state is an appropriate alternative to metoprolol/α-hydroxymetoprolol MR after a single dose.

Key words: cytochrome P450 2D6 – phenotype – probe drug – metoprolol

SÚHRN

Porovnanie metabolického pomeru metoprolol/Š-hydroxymetoprolol po jednorázovom a opakovanom užití

Metabolický pomer (MR) metoprololu/α-hydroxymetoprololu za 3 hod po užití jednorázovej dávky metoprololu sa používa na stanovenie fenotypu enzýmu cytochrómu P450 2D6. Cieľom tejto práce bolo porovnať metabolickú aktivitu cytochrómu P450 2D6 po prvom a opakovanom užití metoprololu. Do tejto práce bolo zahrnutých 13 pacientov s hypertenziou (7 žien), u ktorých bolo plánované nasadenie beta-blokátora metoprololu. Vek (priemer ± SD) bol 42,8 ± 12,9 rokov, telesná hmotnosť (priemer ± SD) bola 94,0 ± 25,4 kg. Dávka metoprololu bola u každého pacienta zvolená na základe klinického stavu pacienta v deň užitia prvej tablety. Krvné odbery boli urobené po užití prvej dávky metoprololu a najmenej 2 týždne od nasadenia metoprololu k zaručeniu ustáleného stavu (opakované užívanie). Na základe hodnôt MR boli všetci pacienti po jednorázovom i opakovanom užití zaradení do tej istej fenotypovej skupiny. Pozorovali sme tesnú koreláciu medzi MR po jednorázovom a opakovanom užití metoprololu ($r_s = 0,8418$, P = 0,0003). Všetci pacienti boli v oboch odberoch zaradení do rovnakej fenotypovej skupiny, extenzivný metabolizátor i napriek signifikantnému rozdielu medzi MR metoprololu/α-hydroxymetoprololu po jednorázovom

Address for correspondence:

PharmDr. Jana Ďuricová

Department of Clinical Pharmacology, University Hospital Ostrava, and Faculty of Medicine, University of Ostrava

17. listopadu 1790, 708 52 Ostrava

e-mail: jankaduricova@seznam.cz, jana.duricova@fno.cz

a opakovanom užití (medián 0,59 vs. 0,81, P = 0,0266), Využitie MR metopro-
lolu/α-hydroxymetoprololu u pacientov na dlhodobej terapii metoprololom je vhodné pre
stanovenie fenotypu enzýmu cytochrómu P450 2D6.

Kľúčové slová cytochróm P450 2D6 – fenotyp – substrátová látka – metoprolol

Introduction

The human cytochrome P450 2D6 (P450 2D6) is involved in the oxidative metabolism of about 25 % of all commonly prescribed drugs [1]. The enzyme is characterized by a high range of interindividual variability [2]. It is highly polymorphic with more than 100 variant alleles described so far [3]. However, beside the genetic polymorphism environmental factors may also play important role in metabolic activity [4]. Individual P450 2D6 activity can be affected by gender [5], liver diseases [6], pregnancy [7] and nutritional habits [8]. Administration or discontinuation of an enzyme inhibitor can significantly change the metabolic activity; a phenotypic shift may even occur [9–11]. Thus, the ability to measure the activity of P450 2D6 enzyme is of high significance. Genotyping alone is not sufficient to accurately predict an individual's actual P450 2D6 activity, phenotyping on the other hand can determine the exact enzymatic activity as it also reflects non-genetic factors. Phenotyping is usually based on single administration of a probe drug, a compound that is predominately or exclusively metabolized by the enzyme. The resulting metabolic activity is determined from the metabolic ratio MR (maternal compound/metabolite) in urine or in blood [12]. Bimodal or trimodal distributions of the MR are typically seen in European populations with the poor metabolizer phenotype representing a separate subgroup, which can be clearly defined by an antimode [13]. Metoprolol serves as one of the probe drugs with 70–80% of its metabolism mediated by P450 2D6, of which α-hydroxylation is exclusively mediated by P450 2D6 [14]. The metabolic ratio of metoprolol over its metabolite α-hydroxymetoprolol in plasma 3 hours after a single metoprolol administration has been validated under standard conditions for the measurement of enzyme activity of P450 2D6 *in vivo* [15]. A single dose of metoprolol is usually used for P450 2D6 phenotyping. Use of metoprolol MR in patients routinely treated with metoprolol would eliminate the need for administration of an additional probe drug. The fraction of metoprolol dose available systematically may increase during long-term treatment with metoprolol [16]. P450 2D6 has a high affinity and a low capacity and P450 2D6 dependant metabolism could saturate during chronic exposure leading to a reduction of metabolic capacity [17]. To our knowledge, no direct comparison of metopro-lol/α-hydroxymetoprolol MR after a single dose and in steady state has been performed. The aim of the present study was to compare P450 2D6 metabolic activity using metoprolol as a probe drug in patients after first metoprolol dose and in metoprolol steady state.

MATERIALS AND METHODS

Thirteen adult patients (7 females) attending outpatient clinic for hypertension treatment at the Department of Clinical Pharmacology, University Hospital Ostrava, were included. Age (mean ± SD) was 42.8 ± 12.9 years, body weight (mean ± SD) was 94.0 ± 25.4 kg. In all of these patients, an introduction of the beta-blocker metoprolol to their therapy was indicated due to non-satisfactory treated hypertension. Two of the patients had already been on the beta-blocker atenolol, however with persisting higher pulse, thus a shift to metoprolol was indicated. At the time of admission, one patient had been treated with 6 antihypertensive drugs, three of the patients had been treated with 5 anti-hypertensive agents, one patient had 3 antihypertensive drugs, three patients had been on 2 antihypertensive drugs and three patients on antihypertensive mono-therapy, two patients had been without previous antihypertensive medication. To optimize the metoprolol therapy, therapeutic drug monitoring (TDM) of metoprolol was recommended as well as P450 2D6 phenotyping.

The patients were invited to our department on the day of their first ingestion of metoprolol. They were instructed to take other "morning" medications (if they were on poly-therapy) at home on the study day. The dose of metoprolol was chosen based on clinical grounds (7 patients were prescribed 200 mg of metoprolol, 5 patients were given 100 mg of metoprolol and one patient was given 50 mg of metoprolol). Blood samples (4.5 ml each) were drawn into a neutral tube at 1, 3 and 4 hours after metoprolol intake. Baseline data including renal function test (serum creatinine, urea) and liver function test (ALT, AST, γ-GT) were obtained on the study day or were recorded if they were not older than 3 months. The patients further continued in the prescribed therapy with metoprolol. Blood samples in metoprolol steady state were drawn on the next check-up, at least 2 weeks since metoprolol introduction to ensure metoprolol steady state. The scheme of blood collections was the same as at first metoprolol ingestion including blood sample before metoprolol intake. Patients' medical history was recorded and screened for the presence of the P450 2D6 inhibitor. In 5 patients a change in medication was reported between the two periods of blood collections. In most cases a new antihypertensive agent was added (doxazosin, rilmenidine, telmisartan, trandolapril and hydrochlorothiazide), further sibutramin, allopurinol and tamsulosin were also prescribed in one case.

Assay of metoprolol and α-hydroxymetoprolol

Serum concentrations of metoprolol and α-hydroxy-metoprolol were measured by means of high-performance liquid chromatography (HPLC) with fluorescence detection at 230–300 nm, as described previously [18].

Data and statistical analysis

Data are expressed as median and range. The P450 2D6 phenotype was determined using serum metoprolol/α-hydroxymetoprolol MR 3 hours post-dose. The antimode value distinguishing between extensive metabolizers and poor metabolizers was set at MR = 10.5, in agreement with the literature [19]. Values are given as median and range. Spearman's rank correlation test was used for evaluating the relationship between MR after the first metoprolol dose and in steady state. To assess the differences between MR after the first metoprolol dose and in steady state, the Wilcoxon matched-pairs test was applied. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad

Prism for Windows version 5.0 (GraphPad Prism Software, Inc).

RESULTS

Results are summarized in Table 1. All the patients continued with the same metoprolol dose after introduction of metoprolol to their therapy. When we examined changes in patients' medication between the two sampling periods, we did not find any drug interfering with P450 2D6 enzyme. Therefore no effect of these drugs on P450 2D6 metabolic activity between the two periods is likely to occur. We observed a significant correlation ($r_s = 0.8418$, $P = 0.0003$) between the MR 3 hours after a metoprolol single dose and during repeated treatment (Fig. 1). The patients were phenotyped as extensive metabolizers in both periods, after metoprolol first ingestion and in steady state, despite statistically significant differences between the median metoprolol/α-hydroxymetoprolol MR 3 hours after the first metoprolol dose and in steady state (0.59

Table 1. Serum concentrations of metoprolol and α-hydroxymetoprolol at 3 hours post-dose, metoprolol/α-hydroxymetoprolol MR at 3 hours post-dose after the first dose and in steady state

Patient	Daily dose (mg)	Metoprolol C_F (ng/ml)	α-OH-Met C_F (ng/ml)	MR_F	Metoprolol C_{SS} (ng/ml)	α-OH-Met C_{SS} (ng/ml)	MR_{SS}	$MR_{SS} - MR_F$
1	200	43.7	210.6	0.21	131.5	323.0	0.41	0.20
2	200	47.7	93.1	0.51	95.2	52.8	1.80	1.29
3	100	60.2	80.6	0.75	78.3	117.5	0.67	-0.08
4	200	185.7	40.1	4.63	272.1	65.2	4.17	-0.46
5	100	53.2	31.6	1.68	95.7	46.2	2.07	0.39
6	100	32.1	42.2	0.76	46.6	48.1	0.97	0.21
7	200	62.2	91.8	0.68	103.2	102.2	1.01	0.33
8	100	24.2	81.9	0.30	30.4	94.7	0.32	0.02
9	200	53.9	91.0	0.59	101.5	150.0	0.68	0.09
10	50	3.2	11.4	0.28	10.1	27.9	0.36	0.08
11	200	33.6	78.1	0.43	100.4	124.1	0.81	0.38
12	100	30.3	10.1	3.00	64.0	19.6	3.27	0.27
13	200	30.0	106.9	0.28	58.5	135.1	0.43	0.15
median	**200**	**43.7**	**80.6**	**0.59**	**95.2**	**94.7**	**0.81**	**0.20**
range	**(50–200)**	**(3.2–185.7)**	**(10.1–210.6)**	**(0.21–4.63)**	**(10.1–272.1)**	**(19.6–323.0)**	**(0.32–4.17)**	**(-0.46–1.29)**

MR_F – MR after first dose, α-OH-Met – -hydroxymetoprolol, MR_{SS} – MR in steady state, C_F – concentration at 3 hours after first metoprolol dose, C_{SS} – concentration at 3 hours after metoprolol dose in steady state
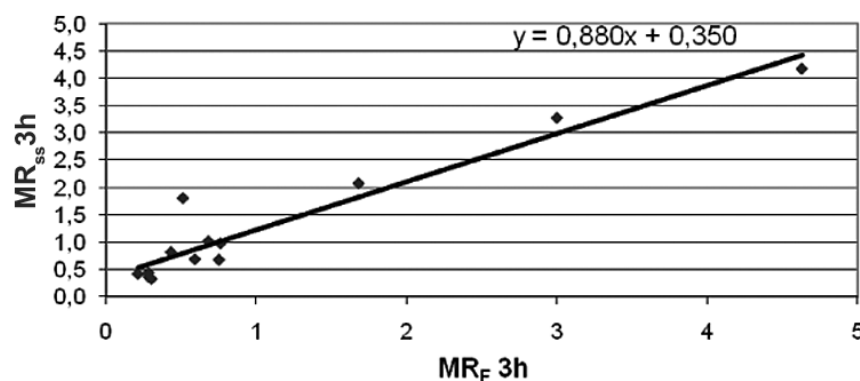
Fig. 1. Correlation between metoprolol/α-hydroxymetoprolol MR at 3 hours post-dose after the first dose and in steady state, (r_s = 0.8418, P = 0.0003)*
MR_F – MR after first dose, MR_{SS} – MR in steady state, * Spearman's rank correlation test

versus 0.81, P = 0.0266). A great variability in MR values was observed, ranging from 0.21 to 4.63 after ingestion of the first metoprolol dose and from 0.32 to 4.17 in steady state.

DISCUSSION

Beta-blocker metoprolol is widely used in patients for the treatment of different cardiovascular diseases. Metoprolol also serves as one of the probe drugs for P450 2D6 phenotyping. However, most of the studies performed so far have used a single dose of metoprolol to determine the P450 2D6 phenotype. We compared metoprolol/α-hydroxymetoprolol MR after a single dose of metoprolol and in steady state.

First studies on P450 2D6 phenotyping were conducted by assessing the urinary MR of the probe drug derived from urine samples collections during the post-dose of 6- to 12-h period. Thus, a more practical alternative has been sought based on only one-point sample collected at a shorter time. Sohn et al. examined the utility of the single post-dose 3-plasma metabolic ratio after an oral single dose of 100 mg metoprolol in two Asian populations. He found statistically significant (r_s = 0.688 and 0,810, p < 0.0001) correlations between the post-dose urinary and plasma MR, confirming the utility of this one-point plasma MR [15]. This finding was further confirmed by Tamminga et al. who observed good correlations (r_p = 0.997, P = 0.049) between the metoprolol/α-hydroxymetoprolol MR of the AUC_{0-12} and the MR values taken from the 3-hour plasma sample after a single metoprolol dose [20]. The metoprolol/α-hydroxymetoprolol MR 3 hours after a single metoprolol dose was further used in the study of Jonkers et al. to phenotype the subjects [19]. Metoprolol MR in patients receiving the drug for several days was used in two studies. However, the MR was calculated from the area under the curve (AUC) of metoprolol to its metabolite α-hydroxymetoprolol [21, 22]. Ismail et al. studied the relevance of P450 2D6 genetic polymorphism on chronic metoprolol therapy in cardiovascular patients. P450 2D6 phenotypes were determined using metoprolol as a probe drug. The distribution of the phenotypes was calculated according to the plasma ratio of metoprolol to its metabolite α-hydroxymetoprolol at 4 hours after drug intake [23]. Rau et al. studied the effect of the CYP2D6 genotype on metoprolol metabolism and the metabolic ratio during long-term therapy. The metoprolol to α-hydroxy-metoprolol ratio was determined from two blood samples drawn 1 hour apart, the first sample was drawn at a median of 3 hours after administration of the last metoprolol dose [24]. Pronounced effects of multiple dosing on the bioavailability of metoprolol have been reported [16]. P450 2D6 is an enzyme with a high affinity and a low capacity for its substrates and it becomes saturated at relatively low concentrations [17]. Higher metoprolol systemic availability upon repeated administration of metoprolol may be the consequence of the saturation of the enzyme. Such an effect has been observed e.g. for haloperidol, where higher haloperidol doses decreased pharmacokinetic differences between P450 2D6 genotype groups [25]. The clearance of metoprolol may be also decreased during chronic treatment as a result of a potential reduction in hepatic blood flow. Hence in our study we tried to compare the metoprolol/α-hydroxymetoprolol MR after a single dose and during repeated metoprolol treatment and to assess whether the two MRs may be used interchangeably. We observed a significant correlation between the MR after a metoprolol single dose and during repeated treatment. Despite **s**tatistically significant differences between the median metoprolol/α-hydroxymetoprolol MR 3 hours after first metoprolol dose and in steady state, the patients were phenotyped as extensive metabolizers in both periods, after metoprolol first ingestion and in steady state. The differences were not of such an extent so as to assign subjects to different phenotypic groups between the two sampling periods. The differences

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between the MRs after a single dose and in steady state might by of relevance in the case of MR values close to the antimode separating the phenotypes.

The metoprolol/α-hydroxymetoprolol MR in steady state is an appropriate alternative to metoprolol/α-hydroxymetoprolol MR after a single metoprolol dose. The use of metoprolol MR in patients routinely treated with metoprolol would eliminate a need for administration of an additional probe drug for P450 2D6 phenotyping.

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