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Interaction of rocuronium with human liver cytochromes P450

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ABSTRACT

Rocuronium is a neuromuscular blocking agent acting as a competitive antagonist of acetylcholine. Results of an inhibition of eight individual liver microsomal cytochromes P450 (CYP) are presented. As the patients are routinely premedicated with diazepam, possible interaction of diazepam with rocuronium has been also studied.

Results indicated that rocuronium interacts with human liver microsomal CYPs by binding to the substrate site. Next, concentration dependent inhibition of liver microsomal CYP3A4 down to 42% (at rocuronium concentration 189 μM) was found. This effect has been confirmed with two CYP3A4 substrates, testosterone (formation of 6β-hydroxytestosterone) and diazepam (temazepam formation). CYP2C9 and CYP2C19 activities were inhibited down to 75–80% (at the same rocuronium concentration). Activities of other microsomal CYPs have not been inhibited by rocuronium.

To prove the possibility of rocuronium interaction with other drugs (diazepam), the effect of rocuronium on formation of main diazepam metabolites, temazepam (by CYP3A4) and desmethyldiazepam, (also known as nordiazepam; formed by CYP2C19) in primary culture of human hepatocytes has been examined. Rocuronium has caused inhibition of both reactions by 20 and 15%, respectively.

The results open a possibility that interactions of rocuronium with drugs metabolized by CYP3A4 (and possibly also CYP2C19) may be observed.

1. Introduction

Rocuronium is a neuromuscular blocking agent (NMBA) acting as a competitive antagonist of acetylcholine on the postsynaptic nicotinic receptor of neuromuscular junction (1). Its advantage is relatively quick onset of the neuromuscular block (the most quick among currently used non-depolarizing NMBA, 60 s after 0.6 mg/kg dose) and an intermediate duration of its action (35–50 min after the same dose) (1,2). Rocuronium is predominantly used to facilitate endotracheal intubation, to provide skeletal muscle relaxation during surgery, or to facilitate mechanical ventilation in intubated, critically ill patients (1–3).

Rocuronium is according to its structure (Fig. 1) an aminosteroid compound similar to vecuronium or pancuronium (1,2). It is in principle a basic derivative of androstane with acetylated hydroxyl group and with a substituent containing a quaternary nitrogen atom; this is why the bromide salt is used in drug formulation. Steroid nature of the molecule is an advantage as it is not a substrate of pseudocholinesterase whose genetically determined deficiency is a serious complicating factor causing prolonged muscle relaxation e.g. after suxamethonium (succinylcholine) (1–4). Primary route of rocuronium elimination is the liver reuptake and biliary excretion (5). Rocuronium is excreted from human organism in the urine (26% in 48 h), in the bile (7% in 48 h) and in faeces (up to 31% during 7 days). Two metabolites of rocuronium were found (5); 17-desacetylrocuronium is formed in relatively small amount, this metabolite was found in the plasma, urine, bile and faeces and in amount less than 10%. It was suggested that a

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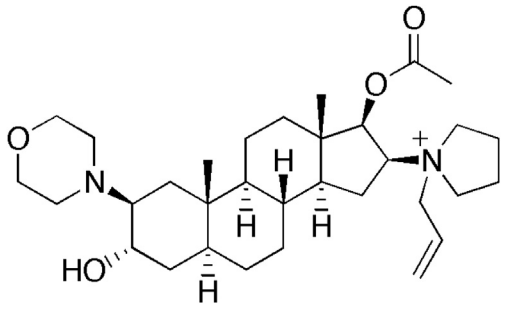

Fig. 1. Structure of rocuronium (1-allyl-1-(3α, 17β–dihydroxy-2β-morpholino-5α-androstan-16β-yl)pyrrolidinium bromide 17-acetate).

cytochrome P450 enzyme, presumably CYP3A4, takes part in this reaction (6,7). An N-desallylrocuronium metabolite was found to be even less formed than the previous compound as it was detected in the bile only, however, a study on formation of this metabolite is lacking. As the amount of totally excreted rocuronium was below 75% of the doses, a formation of other (more polar) metabolites as glucuronides cannot be ruled out (5).

Rocuronium, however, exhibits a great variability in the effect and in the course of neuromuscular block as well in the time of recovery. Variability of response to rocuronium is according to the literature primarily caused by pharmacokinetics of this drug. In the literature there are reports indicating that an interaction of rocuronium with liver monooxygenase system of cytochromes P450 (CYP) (8) may contribute to these differences (9). Also, in patients with liver dysfunction or after application of inhalational anesthetics, a decrease of the rocuronium clearance and increase of elimination half-time have been observed (2); these effects may be due to CYP-mediated formation of reactive species known to be formed from inhalation anesthetics (10).

An increase in effect of rocuronium described after the use of anesthetics (11) is not only the single example of interaction of other drugs with rocuronium. Also, antibiotics as aminoglycosides (e.g. gentamicin) and lincosamides (as clindamycin) poteniate neuromuscular blockade and should be used with caution (12,13). Interestingly, clindamycin, metabolized by cytochrome P450 form 3A4 (CYP3A4), was found also to be an inhibitor of this form (14). On the contrary, drugs known as inducers of CYP enzymes, e.g. carbamazepine or phenytoin, appear to cause a decrease of the effect of rocuronium (15). In general, the key for understanding drug interactions may often be a dependence of drug (e.g. rocuronium) levels on the activity of liver drug metabolizing enzymes (mostly CYPs). In vitro studies on drug interactions are commonly used to get an insight into the nature of these unwanted effects (16).

The present work is aimed to investigate the influence of rocuronium on the activities of the eight principal drug-metabolizing enzymes, CYPs, of human liver microsomes (CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4). As diazepam (substrate of CYP3A4 and CYP2C19 (17)) is often used in premedication of the patients, in addition, microsomes and human hepatocytes were also used for studying of possible drug interaction of rocuronium and diazepam.

2. Material and methods

2.1. Materials

Rocuronium was obtained from N.V. Organon, Oss, Netherlands (10 mg/ml). The structure of tested compound is shown in Fig. 1. 7-Ethoxy-4-(trifluoromethyl)coumarin was supplied by Fluka (Buchs, Switzerland). Acetonitril, methanol and HPLC column were from Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma Aldrich CZ (Prague, Czech Republic). Pooled human liver microsomes were obtained from Biopredic International (Rennes, France). Microsomes were from 36 donors (25 males and 11 females) with a total cytochrome P450 content 582 pmol/mg of protein.

2.2. Spectral study of interaction of rocuronium with hepatic microsomal CYP

Binding difference spectra of interactions of rocuronium with microsomal CYP enzymes were followed according to established procedure (18). The cuvette contained microsomes diluted to final concentration of CYP 1 μM in 50 mM potassium phosphate buffer (pH 7.4). The tested compound was dissolved in 50 mM phosphate buffer (pH 7.4) and the concentration range in experiments were 0–210 μM. Taking into account that rocuronium level in patients reach routinely from 33 μM in plasma to 160 μM in the bile (after an i.v. application of 0.3–0.9 mg kg^{-1}) (5), the concentration range used here can be considered as adequate.

Spectra were recorded at room temperature by repetitive scanning between 300 and 700 nm, at a scan speed of 150 nm/s. Baseline of reference cuvette contained microsomes diluted to final concentration of CYP 1 μM in 50 mM K/PO_4 buffer (pH 7.4). Spectra were recorded on a Varian 4000 UV VIS spectrophotometer (Varian, Palo Alto, USA). Absorbance change at about 418 nm was plotted against concentration of compound tested. Data were analyzed using a Sigma Plot v. 8.0 graphing software (Jandel Scientific, Chicago, IL, USA).

2.3. Enzyme assays

The microsomal CYP activities were measured according to established protocols. The following enzyme assays were performed to determine activities of specific CYP enzymes: CYP1A2, 7-ethoxyresorufin *O*-deethylation (19); CYP2B6, 7-ethoxy-4-(trifluoromethyl)coumarin *O*-deethylation (20); CYP2C9, diclofenac 4′-hydroxylation (21), CYP2D6, bufuralol 1′-hydroxylation (22); CYP2E1, chlorzoxazone 6-hydroxylation (23); CYP3A4, testosterone 6β-hydroxylation (24) and diazepam 3-hydroxylation (see Method for CYP2C19); CYP2A6, coumarin 7-hydroxylation (25). CYP2C19, diazepam N-demethylation (http://www.cypex.co.uk/2c19info.htm). Incubation mixtures contained 100 mM potassium phosphate buffer (pH 7.4) except for determination of CYP3A4 activity (with substrate testosterone), where 50 mM Tris/KCl buffer, pH 7.4 was used, NADPH-generating system (0.8 mM $NADP^+$, 5.8 mM isocitrate, 0.3 unit/mL of isocitrate dehydrogenase and 8 mM $MgCl_2$), human liver microsomes and individual probe substrate. Time of incubation was 20 min.

For determination of metabolites formed from specific substrates, HPLC system Shimadzu Class VP (Kyoto, Japan) with UV detection or fluorescence detection was used. As a rule, a reversed phase chromatography was applied with Merck (Darmstadt, Germany) LiChroCART 250 × 4 mm (i.d.) cartridges packed with a LiChrospher RP-18 silica gel (5 μm particle size) with C-18 reversed phase properties; a 4 × 4 mm (i.d.) guard column was also used. For composition of mobile phase in the respective analyses, see the original literature, further details of the analyses incl. The concentrations of the respective substrates and experimental conditions are presented in Table 1.

2.4. Enzyme inhibition studies

Initially, for each enzyme assay, a preliminary experiment was done to determine K_m and V_{max} to obtain the appropriate concentration of the specific substrates for the inhibition experiments (substrate concentration was chosen in the range corresponding to the value of the K_m for each particular reaction). Data were analyzed