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An In-vitro Study on the Metabolism of Rokitamycin and Possible Interactions of the Drug with Rat Liver Microsomes

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Abstract

This in-vitro study was designed to identify the enzyme(s) involved in the major metabolic pathway of rokitamycin, i.e. the formation of leucomycin A7, and to assess possible interactions of the drug with rat liver microsomes.

Formation of leucomycin A7 was NADPH-independent and was not appreciably inhibited by anti-rat NADPH cytochrome P-450 reductase serum or cimetidine, a non-specific inhibitor of cytochrome P-450 isoforms. Eadie–Hofstee plots for the formation of leucomycin A7 were indicative of apparently monophasic behaviour for six rat liver microsomes tested. The mean ($\pm$ s.d.) kinetic parameters, K_m, V_{max} and V_{max}/K_m, for the formation of leucomycin A7 from rokitamycin were $47 \pm 13\,\mu$M, 390 ± 56 nmol min^{-1} (mg protein)$^{-1}$ and $8{\cdot}6 \pm 1{\cdot}6$ mL min^{-1} (mg protein)$^{-1}$, respectively. Three esterase inhibitors (100 μM), bis-nitrophenylphosphate, physostigmine and metrifonate inhibited the formation of leucomycin A7 by more than 60%. Metabolism of rokitamycin was inhibited by terfenadine, but not by mequitazine, whereas chlorpheniramine and theophylline activated the formation of leucomycin A7. Rokitamycin, leucomycin A7, leucomycin V, erythromycin and clarithromycin were weak inhibitors of CYP3A-catalysed 3-hydroxylation of quinine with mean IC50 values ranging from 71 to $> 100\,\mu$M.

It is concluded that in rat liver microsomes the formation of leucomycin A7 from rokitamycin is catalysed mainly by an esterase (possibly cholinesterase, EC3.1.1.8), but not by cytochrome P-450 enzyme(s). Although in this in-vitro animal study CYP3A activity was barely inhibited by rokitamycin, the possibility cannot be totally discounted in man when rokitamycin is co-administered with drugs metabolized by CYP3A.

Rokitamycin (Figure 1) is a new 16-membered ring macrolide antibiotic that is not only more potent but also has a wider spectrum than other macrolides (Morohoshi et al 1984). Because rokitamycin can be absorbed even when gastric juice is hypoacidic or anacidic (Morishita et al 1984b), it is considered a suitable antibiotic for treatment of infectious respiratory diseases in the elderly, whose gastric juice is often hypoacidic. In addition, the use of macrolide antibiotics has recently been extended to the treatment of *Helicobacter pylori* infection, a common cause of gastritis and peptic ulcers (Walsh & Peterson 1995; Penston & McColl 1997). Thus, these drugs, including rokitamycin, can be administered concomitantly with other clinically relevant drugs, possibly resulting in drug–drug interactions in man.

It has been reported that rokitamycin, both in man and in other animals, is rapidly metabolized to leucomycin A7, leucomycin V (the principal metabolites), 10″-OH-rokitamycin and 14-OH-rokitamycin (Morishita et al 1984a, 1987; Suzuki et al 1987a, b; Sakai et al 1988). All the metabolites

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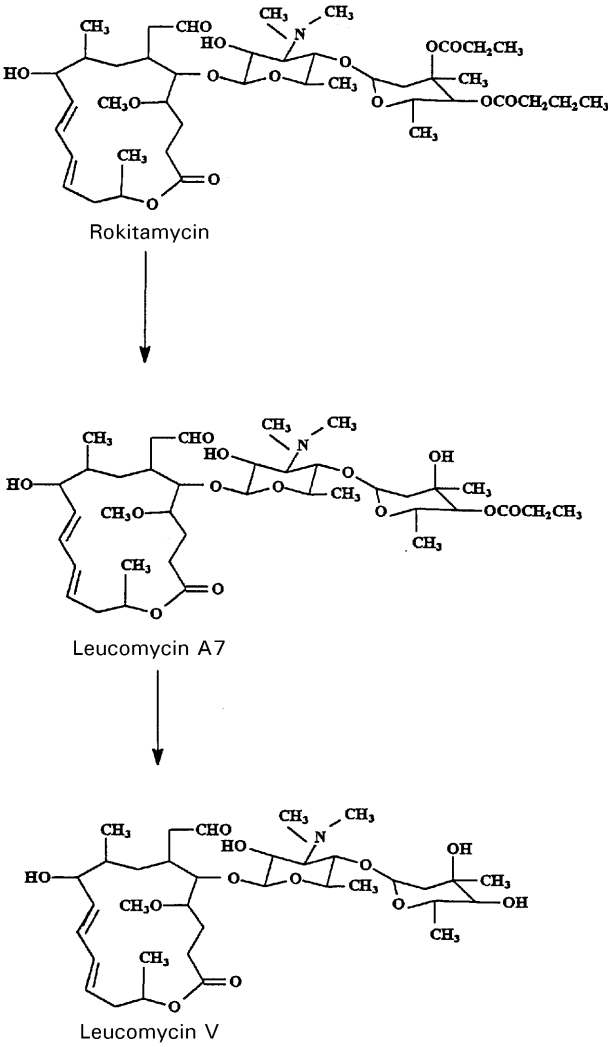

Figure 1. The chemical structure and major metabolic pathways of rokitamycin in rats.

have been reported to have some antibacterial activity (Goto et al 1984). Our recent study (Zhao et al 1999) showed that in man an esterase (possibly cholinesterase, EC3.1.1.8) is the main enzyme involved in the formation of leucomycin A7 from rokitamycin and of leucomycin V from leucomycin A7.

In this extended in-vitro study we have investigated the kinetics of formation of leucomycin A7 from rokitamycin and attempted to identify specific enzyme(s) involved in this major metabolic pathway of rokitamycin in rat liver microsomes; examined possible rokitamycin–drug interactions in-vitro with rat liver microsomes; and compared these data from the rat liver microsomal study with those derived from liver microsomes from man (Zhao et al 1999), to examine species differences or similarities with regard to the in-vitro metabolism of rokitamycin.

Materials and Methods

Chemicals and reagents

Rokitamycin, leucomycin A7, leucomycin V, mequitazine, theophylline and clarithromycin were provided by Asahi (Tokyo, Japan). bis-*p*-Nitrophenylphosphate, physostigmine, metrifonate, terfenadine, cimetidine, SKF-525A (2-diethylaminoethyl-2,2-diphenyl valerate) and quinine were purchased from Sigma (St Louis, MO). Synthetic 3-hydroxyquinine was a generous gift from Dr P. Winstanley (University of Liverpool, Liverpool, UK). Chlorpheniramine, acetonitrile, methanol and other reagents of analytical grade were purchased from Wako (Osaka, Japan). $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan). Antiserum and pre-immune serum for NADPH P-450 reductase were obtained from Daiichi (Tokyo, Japan).

Preparation of microsomal fractions

Liver samples were obtained from six male Wistar rats. Animals were fed a standard diet and had free access to drinking water. The animals were also left to acclimatize in our animal facility for one week before the liver microsomal samples were prepared.

Microsomal preparations from rat liver tissues used herein were made according to standard procedures described elsewhere (Chiba et al 1993; Echizen et al 1993). After the determination of protein concentration (Lowry et al 1951), the individual microsomal samples were frozen and stored at −80°C until used.

Assay for metabolism of rokitamycin with rat liver microsomes

Microsomal fractions were incubated in the absence or presence of an NADPH-generating system at 37°C for 2 min in test tubes. The incubation mixture (250 μL) consisted of microsomal protein (0·025 to 0·05 mg mL^{-1}), potassium phosphate buffer (pH 7·4, 100 mM), EDTA (0·1 mM) and rokitamycin (2·5–400 μM). All reactions were performed in the linear range with regard to protein concentration and incubation time. After termination of the reaction by addition of ice-cold acetonitrile (100 μL), nitrazepam (25 μM in 25 μL methanol) was added to the samples as an internal standard for assay of leucomycin A7. The mixture was centrifuged at 10 000 *g* for 10 min, and the supernatant (50 μL) was analysed by high-performance liquid chromatography (HPLC).

HPLC conditions

The amount of leucomycin A7 formed from rokitamycin was determined in the incubation mixture by HPLC with UV-detection. HPLC was performed with a model L-7200 pump, model L-7400 UV detector, model L-7200 autosampler, model D-7500 integrator (all from Hitachi, Tokyo, Japan) and a 250 mm × 4·6 mm i.d. Capcell Pak C_{18} UG120 column (Shiseido, Tokyo, Japan). Column temperature was maintained at 30°C with a model SM-05 water circulator (Taitec, Tokyo, Japan). The mobile phase was 33 : 67 (v/v) acetonitrile–0·01 M potassium phosphate buffer, containing 1-heptanesulphonic acid (5 mM) and phosphoric acid (4 mL in 2000-mL mobile phase). The mobile phase was delivered at a flow rate of 1·0 mL min^{-1}. The eluate was monitored at 229 nm. Inter- and intra-assay coefficients of variation for each procedure (n = 6) were < 10%, and the lowest limit of detection for leucomycin A7, defined as the concentration with a signal-to-noise ratio of 10, was 0·1 μM.

Assays for quinine 3-hydroxylation, a CYP3A4-catalysed reaction in rat liver microsomes (Zhao & Ishizaki 1997), was performed according to the HPLC assay method reported elsewhere (Wanwimolruk et al 1996; Zhao & Ishizaki 1997).

Kinetics of formation of leucomycin A7 from rokitamycin

Preliminary results indicated that the rate of formation of leucomycin A7 from rokitamycin was linear at 37°C for incubation times up to 10 min and for microsomal protein concentrations up to 0·15 mg mL^{-1} at a substrate (rokitamycin) concentration of 50 μM. Accordingly, kinetic studies were performed at 37°C with an incubation time of 2 min at microsomal protein concentrations of 0·025–0·05 mg mL^{-1}.

Because the formation of leucomycin A7 by microsomes obtained from six rat livers occurred monophasically, consistent with simple Michaelis–Menten kinetic behaviour, the one-component enzyme kinetic parameters (K_m, V_{max} and V_{max}/K_m without the numerical sub-indices) for the formation of leucomycin A7 from rokitamycin (2·5–400 μM) were estimated by linear regression analysis of unweighted raw data. The kinetic parameters were estimated initially by graphic analysis of Eadie–Hofstee plots, and the values obtained were used as the first estimate for non-linear least-squares regression analysis, by MULTI (Yamaoka et al 1981), in which unweighted raw data were fitted to the model equation.

Inhibition study

The effects of co-incubation of inhibitors specific for cytochrome P450 or esterases in the microsomal metabolism of rokitamycin were studied separately. Two so-called non-specific inhibitors of cytochrome P-450 in man, cimetidine (Somogyi & Muirhead 1987) and SKF-525A (Rossi et al 1987), were used to test possible inhibition of the formation of leucomycin A7 from rokitamycin in three different rat liver microsomes. The esterase inhibitors used were bis-nitrophenylphosphate, physostigmine and metrifonate (Hallak & Giacobini 1987; Iatsimirskaia et al 1997). The concentration (50 μM) of substrate rokitamycin was chosen according to the mean apparent Km value obtained from six rat liver microsomes tested. Rokitamycin was incubated with or without one of the inhibitors for cytochrome P-450 isoforms or esterases, under the incubation condition described above. The effects of each compound on the formation of leucomycin A7 were compared with control values determined by the incubation of rokitamycin alone, and the inhibition values were expressed as a percentage of the respective control values.

Immunoinhibition study

Anti-rat NADPH cytochrome P-450 reductase serum was used to investigate possible cytochrome P-450-mediated inhibition of rokitamycin metabolism. This antiserum (50 μL) substantially (by more than 70%) inhibited cytochrome P-450 reductase, and potentially (according to the Daiichi, Tokyo, Japan, product instructions) inhibits the metabolism of several different substrates of the respective cytochrome P-450 isoforms from man, including CYP3A4 and 2D6 in liver microsomes.

Rat liver microsomes (0·1 mg protein mL^{-1}) were first incubated for 30 min at room temperature in the absence or presence of anti-NADPH cytochrome P450 reductase antisera (25 and 50 μL) to enable antigen–antibody complex formation. The substrate (rokitamycin, 10 μM) was then added; the assay conditions were the same as those described above.

Effects of drugs on rokitamycin metabolism

Several drugs, which have been used for the treatment of chronic obstructive pulmonary diseases (e.g. chronic bronchial asthma) and are co-administered with rokitamycin in other clinical settings, were assessed, by use of rat liver microsomes, for possible inhibition of the formation of leucomycin A7 from rokitamycin. The drugs tested included

theophylline and three H_1-receptor antagonists, mequitazine, terfenadine and chlorpheniramine.

All the drugs tested were dissolved in methanol. The inhibitory potency of the respective substrates was, where appropriate, defined by IC50 values (the concentrations resulting in 50% inhibition). IC50 values were identified by use of drug concentrations ranging from 0 to 200 μM and two different concentrations (5·0 and 50 μM) of rokitamycin. A solution of each drug in methanol (50 μL) was evaporated to dryness before addition of the other reactants. Pooled microsomes obtained from six different rat livers were used to determine IC50 values, and inhibition activity was compared with that from the respective control incubation.

Effects of macrolide drugs on CYP3A activity

Macrolide drugs, including rokitamycin and its two active metabolites (leucomycin A7 and leucomycin V), erythromycin and clarithromycin, were used to test the possible inhibition of the metabolism of the specific substrate mediated by CYP3A (i.e. quinine 3-hydroxylation) in rats (Zhao & Ishizaki 1997). IC50 values were determined by use of macrolide drug concentrations from 0 to 100 μM and a quinine concentration of 20 μM, which is (approx.) the mean apparent Km value of quinine 3-hydroxylation obtained from six different rat liver microsomes (Zhao & Ishizaki 1997).

All numerical values are expressed as means ± s.d.

Results

Rokitamycin metabolism

The major pathway of rokitamycin metabolism (i.e. formation of leucomycin A7) was examined by use of rat liver microsomes. Preliminary studies revealed that the formation of leucomycin A7 was NADPH-independent; moreover, the addition of anti-rat NADPH cytochrome P-450 reductase serum (up to 50 μL) and cimetidine, a non-specific inhibitor of cytochrome P-450 isoforms, (Somogyi & Muirhead 1987), to the incubation mixture in the presence of an NADPH-generating system, did not result in inhibition of the formation of leucomycin A7 (results not shown). These results strongly suggest that the formation of leucomycin A7 from rokitamycin is not catalysed by rat cytochrome P-450 isoform(s) or other NADPH-dependent enzyme(s), e.g. flavin-containing monooxygenase (FMO).

Kinetic study

Because metabolism of rokitamycin was NADPH-independent, we performed a kinetic study in the absence of the NADPH-generating system. Typical Eadie–Hofstee and Michaelis–Menten plots for the formation of leucomycin A7 from rokitamycin are shown in Figure 2. For all the rat liver microsomes used, the Eadie–Hofstee plots for the formation of leucomycin A7 were indicative of apparently monophasic behaviour, suggesting the involvement of a single enzyme in the metabolism of rokitamycin in rat liver microsomes. Accordingly, a simple Michaelis–Menten kinetic analysis (i.e. a one-enzyme kinetic approach) was used to estimate the kinetic parameters (i.e. K_m, V_{max} and V_{max}/K_m). The individual and mean kinetic parameters for this metabolic pathway of rokitamycin obtained from six different rat liver microsomes are listed in Table 1.

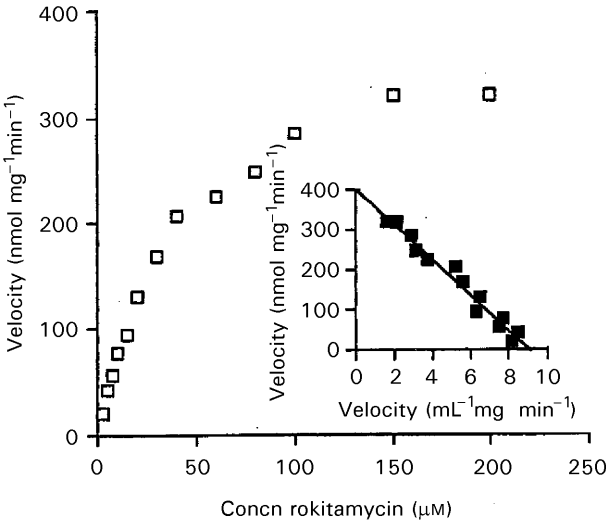

Figure 2. Representative Michaelis–Menten and Eadie–Hofstee (inset) plots for the formation of leucomycin A7 from rokitamycin in rat liver microsomes.

Table 1. Kinetic parameters for the formation of leucomycin A7 from rokitamycin in rat liver microsomes.

Rat liver microsome sample	K_m (μM)	V_{max} (nmol min^{-1} (mg protein)$^{-1}$)	V_{max}/K_m (mL min^{-1} (mg protein)$^{-1}$)
1	44	400	9·1
2	53	393	7·4
3	42	405	9·6
4	58	456	7·9
5	59	400	6·8
6	26	285	11
Mean ± s.d.	47 ± 13	390 ± 56	8·6 ± 1·6

Inhibition study

Our preliminary studies have shown that the formation of leucomycin A7 was not catalysed by rat cytochrome P-450 isoform(s) or by other NADPH-dependent enzyme(s). In addition, a recent study (Zhao et al 1999) demonstrated that an esterase might be the main enzyme involved in the formation of leucomycin A7 from rokitamycin in liver microsomes from man. Thus, several inhibitors of esterases were used in an inhibition study with rat liver microsomes. Three esterase inhibitors, bis-nitrophenylphosphate, physostigmine and metrifonate (100 μM) (Hallak & Giacobini 1987; Iatsimirskaia et al 1997), markedly (by more than 60%) inhibited the formation of leucomycin A7 with metrifonate resulting in the greatest (approx. 97%) inhibition. However, a so-called non-specific inhibitor of cytochrome P-450 isoforms in man, SKF-525A (Rossi et al 1987), also (by approx. 70%) inhibited the formation of leucomycin A7 (results not shown).

Effects of drugs on rokitamycin metabolism

Possible metabolic interactions between rokitamycin and four other drugs (theophylline, chlorpheniramine, mequitazine and terfenadine) that are co-administered with rokitamycin in some clinical settings were assessed by using a pooled microsomal sample obtained from six different rat livers. The results, shown in Figure 3, indicate that terfenadine inhibited the formation of leucomycin A7 with mean IC50 ($\pm$ s.d.) values of 45 ($\pm$5·0) and 18 ($\pm$10) μM, respectively, when rokitamycin at concentrations of 50 μM (Figure 3A) and 5 μM (Figure 3B) was used as the substrate. However, inhibition of the formation of leucomycin A7 by mequitazine was weaker (by up to 40%). In contrast, chlorpheniramine and theophylline activated the metabolism of rokitamycin (50 and 5 μM) in a concentration-dependent manner.

Effects of macrolide drugs on CYP3A activity

The potential inhibitory effects of macrolide derivatives (rokitamycin, leucomycin A7, leucomycin V, erythromycin and clarithromycin) on quinine 3-hydroxylation (CYP3A) are shown in Table 2. All five derivatives tested slightly inhibited CYP3A-catalysed 3-hydroxylation of quinine by rat liver microsomes with mean IC50 values ranging from

Table 2. Inhibition by five macrolide drugs of 3-hydroxylation of quinine (20 μM) by CYP3A in rat liver microsomes.

Drug	IC50 (μM)[a]
Rokitamycin	> 100
Leucomycin A7	97 $\pm$ 3
Leucomycin V	> 100
Erythromycin	73 $\pm$ 18
Clarithromycin	71 $\pm$ 21

[a]Concentration resulting in 50% inhibition; mean $\pm$ s.d. obtained from microsomes from three different rat livers.

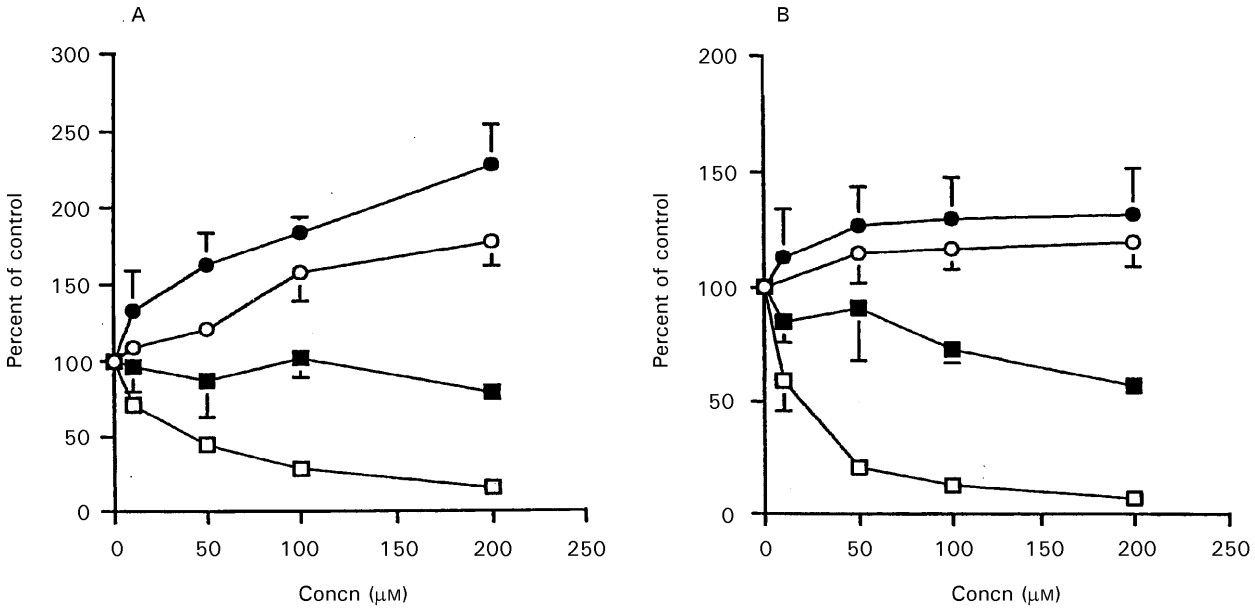

Figure 3. Effects of theophylline (○) and three histamine H_1-receptor antagonists (●, chlorpheniramine; ■, mequitazine; □, terfenadine) on the formation of leucomycin A7 from rokitamycin (A. 50 μM, B. 5 μM). Data are means $\pm$ s.d. from three different determinations with a pooled microsomal sample obtained from six different rat livers.

71 to $>100\,\mu$M, suggesting that these drugs are weak in-vitro inhibitors of CYP3A activity in rat liver microsomes.

Discussion

This in-vitro study is, as far as we are aware, the first to investigate the kinetics of rokitamycin metabolism, to identify the enzyme involved in the major metabolic pathway of rokitamycin (i.e. formation of leucomycin A7), and to examine possible rokitamycin–drug interactions in rat liver microsomes. Although the metabolism of several macrolide antibiotics, including erythromycin, troleandomycin and clarithromycin, is catalysed by cytochrome P-450 enzyme(s), particularly CYP3A (Guengerich 1994; Rodrigues et al 1997), the major metabolism of rokitamycin, a new 16-membered ring macrolide antibiotic (Figure 1) (i.e. formation of leucomycin A7), is not mediated by cytochrome P-450(s) either in man (Zhao et al 1999) or in rat liver microsomes (this study). This is clear because the formation of leucomycin A7 from rokitamycin was NADPH-independent and because anti-rat NADPH cytochrome P-450 reductase serum did not have any inhibitory effect on the formation of leucomycin A7. In addition, a non-specific cytochrome P-450 inhibitor, cimetidine ($100\,\mu$M), did not inhibit rokitamycin metabolism to any appreciable extent.

Bis-nitrophenylphosphate, an inhibitor of carboxylesterases (EC 3.1.1) and cholinesterases (EC 3.1.1.8, closely related to carboxylesterases), and physostigmine and metrifonate, two specific inhibitors of cholinesterases (Hallak & Giacobini 1987; Iatsimirskaia et al 1997), strongly suppressed the formation of leucomycin A7 from rokitamycin in microsomal incubations. These observations suggest that one family of esterases (possibly cholinesterases) are the main enzymes involved in the formation of leucomycin A7 from rokitamycin in rat liver microsomes. However, whether other (i.e. extrahepatic) rat tissues other than liver also have the capacity to catalyse rokitamycin metabolism remains to be clarified, because esterases (e.g. cholinesterases) occur in several tissues (nervous system, liver, and intestinal mucosa) and in the systemic circulation (Williams 1985). In addition, another study has shown that physostigmine is a non-selective systemic inhibitor of cholinesterases and inhibits both butyrylcholinesterase and acetylcholinesterase (Tunek & Svensson 1988). Because of the difficulty in obtaining the recombinant butyrylcholinesterase and acetylcholinesterase, we could not conduct the study with these specific cholinesterases. Thus, this study gives no information about the cholinesterase(s) (butyrylcholinesterase or acetylcholinesterase, or both) involved in the metabolism of rokitamycin. In this respect, further study is definitely required for clarification.

With rat liver microsomes, several drugs that can be co-administered with rokitamycin in certain clinical settings were screened for possible interactions with rokitamycin. Although terfenadine moderately inhibited the formation of leucomycin A7 (mean IC50 ($\pm$ s.d.) values 42 ($\pm 5{\cdot}0$) and 18 (± 10) μM for rokitamycin concentrations of 50 and 5 μM, respectively) and inhibition by mequitazine was weaker (Figure 3), whether these in-vitro findings obtained with rat microsomes would be applicable to the clinical situation remains totally obscure. Nevertheless, the inhibition potencies estimated from these rat in-vitro IC50 values should not occur in man in-vivo because the peak plasma concentrations of terfen-adine and mequitazine attained in man after the usual oral therapeutic doses (Paton & Webster 1985; Sorkin & Heel 1985) are much lower than the mean IC50 values observed in this in-vitro animal study. Interestingly, this trend is consistent with our recent findings using liver microsomes from man (Zhao et al 1999).

Although study of rokitamycin metabolism with liver microsomes from man revealed no activation by chlorpheniramine and theophylline (Zhao et al 1999), in the current study we did observe relatively strong activation when chlorpheniramine and theophylline were co-incubated with rokitamycin and rat liver microsomes (especially when 50 μM rokitamycin was used as substrate) (Figure 3). These discrepant observations might be explained in part by interspecies differences in esterases, an interspecies difference in substrate affinity, and different protein binding sites in man and rats. In this respect, further studies are required.

It is now evident that macrolide antibiotics can be classified into three different groups in respect of drug interactions. Rokitamycin can be assigned to the third group of macrolide antibiotics which do not inactivate cytochrome P-450 enzymes and are, therefore, unable to modify the pharmacokinetics of the relevant drugs co-administered (Periti et al 1992; Amsden 1995; von Rosensteil & Adam 1995). Indeed, a study conducted by Cazzola et al (1991) has shown that rokitamycin did not significantly alter the in-vivo pharmacokinetics of theophylline in man. The current in-vitro study also showed that rokitamycin is a poor inhibitor of 3-hydroxylation of quinine, a substrate of CYP3A (Zhao & Ishizaki 1997), by rat liver microsomes,

which is consistent with a recent study by Tsuruta et al (1997) who reported that rokitamycin, both in-vitro and in-vivo, had little inhibitory effect on the metabolism of nifedipine, another substrate for CYP3A (Guengerich et al 1986) in rats. However, two in-vitro studies with liver microsomes from man revealed that rokitamycin inhibited the CYP3A-mediated metabolism of cyclosporin (Marre et al 1993) and triazolam (Zhao et al 1999) with mean Ki (IC50) values of 30 and 2·0 ($\pm$ 5·8) μM, respectively. These data are not in accord with our current finding that rokitamycin inhibited CYP3A-mediated quinine 3-hydroxylation only weakly—mean IC50 $> 100\,\mu$M in microsomal samples obtained from three different rat livers (Table 2). Although the reason for these discrepant findings remains obscure, it might be explained in part by the interspecies differences between the structures, functions and properties of CYP3A isoform(s) that have been observed and characterized in man and rats (Nelson et al 1996). Further interspecies comparison of the mechanism(s) of the inhibition, or otherwise, of CYP3A activity against rokitamycin in microsomes from the livers of rat and man are clearly required.

For correct prediction of the in-vivo consequences of inhibition of CYP3A by rokitamycin, it is necessary to know the plasma or, more importantly, hepatic concentration of rokitamycin. In this respect, the concentration of rokitamycin found in the hepatic tissue of rats was ten times greater than that in plasma after an oral dose ($3{\cdot}3\,\mathrm{mg\,kg^{-1}}$) of rokitamycin (Morishita et al 1984a). Taking this observation into account we assume that rokitamycin will cause little inhibition of the CYP3A activity in-vivo. This is because the mean IC50 value ($> 100\,\mu$M) for inhibition by rokitamycin of CYP3A activity (i.e. quinine 3-hydroxylation) obtained from this in-vitro study is much higher than the concentration of rokitamycin in the liver in rats ($3{\cdot}5\,\mu$M (approx.) when $3{\cdot}3\,\mathrm{mg\,kg^{-1}}$ rokitamycin was given; Tsuruta et al (1997); Sakai et al (1988)). Thus, we assume that a rokitamycin–drug interaction cannot occur in-vivo when rokitamycin is co-administered with substrate drugs for CYP3A in rats. However, such a prediction based on an in-vitro animal (rat) study might not be relevant to the in-vivo situation in man. This is because we observed a marked species difference (rat compared with man) in respect of the inhibition of CYP3A activity by rokitamycin in this rat study and our previous study of microsomes from man (Zhao et al 1999), as discussed above.

In conclusion, this in-vitro study has shown that the formation of leucomycin A7 from rokitamycin is catalysed mainly by rat liver esterase(s) (possibly cholinesterase), but not by cytochrome P-450 enzyme(s) or other NADPH-dependent enzyme(s) (e.g. FMO). This study also suggests that extrapolation to man of data relating to rokitamycin drug interactions in rats might be inappropriate. Thus, whether rokitamycin inhibits CYP3A-mediated drug metabolism in man requires further investigation in-vivo.

Acknowledgements

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