氧化的影响. 方法：采用离体大鼠肝线粒体膜的脂质过氧化模型和培养心肌细胞钙反常模型 结果：戈米辛 J 能抑制 Fe^{2+}-VitC 和 ADP/NADPH 所致的脂质过氧化，IC_{50} 分别为 5 5 和 4 7 μmol·L^{-1} 戈米辛 J 10 μmol·L^{-1} 对培养心肌细胞钙反常产生的丙二醛含量的增加也有抑制作用 结论：戈米辛 J 不仅能抑制大鼠肝线粒体膜的脂质过氧化而且能保护培养心肌细胞免受钙反常损伤

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Inhibitory effects of quinidine and quinine on liver microsome oxidation enzymes in man and rat[1]

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KEY WORDS quinidine; quinine; liver microsomes; pharmacogenetics; bufuralol; 7-alkoxycoumarin *O*-dealkylase; aryl hydrocarbon hydroxylases

AIM: To compare the inhibitory effects of quinidine and quinine on liver microsome bufuralol 1′-hydroxylase (BH), aryl hydrocarbon hydroxylase (AHH), and 7-ethoxycoumarin *O*-deethylase (ED) activities in man and rat. **METHODS:** A normal phase HPLC and the fluorescence spectrometry were used to assay the enzyme activities. ***RESULTS:*** *Both quinidine and quinine* produced a concentration-dependent inhibition to liver microsome BH, AHH, and ED in man and rat. Their median inhibitory concentrations (IC_{50}) on liver microsome BH, AHH, and ED low and high affinity phases were 0.2, 378, 2952, and >5000 μmol·L^{-1} for quinine in man, 290, 613, 1465, 1595, μmol·L^{-1} for quinidine in rat; 29, 207, 808, and >5000 μmol·L^{-1} for quinine in man, and 31, 54, 597, and 2508 μmol·L^{-1} for quinine in rat, respectively. **CONCLUSION:** Quinidine is a species- and stereo-selective potent inhibitor to human liver microsome BH.

The most extensively studied human genetic polymorphism of drug biotransformation is debrisoquine 4-hydroxylation, and 2 phenotypes of extensive metabolizer (EM) and poor metabolizer (PM) were identified[1]. This polymorphism is controlled by the autosomal mendelian recessive[2]. The oxidations of a number of other drugs including bufuralol 1′-hydroxylation are controlled by debrisoquine 4-hydroxylase[1,2]. On the other hand, aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase (ED), two of the other liver microsome enzymes, are not involved by the debrisoquine genetic polymorphism[3,4]. Because the frequency of PM phenotype of *debrisoquine 4-hydroxylation is low and variable in* different races[1,5], it is difficult to obtain enough PM subjects to testify whether a drug oxidation is involved by this genetic polymorphism. One reasonable approach is to look for some specific and potent inhibitor of this enzyme so that this polymorphism can be studied in some EM subjects. Quinidine was a competitive inhibitor of debrisoquine 4-hydroxylase *in vivo* and *in vitro*[6,7]. The purpose of this paper was to study the effects of quinidine and its diastereoisomer quinine on bufuralol 1′-hydroxylase (BH), AHH, and ED activities in both human and rat liver microsomes, and to determine if quinidine is a species and stereo selective inhibitor of man BH.

MATERIALS AND METHODS

Chemicals (±)-Bufuralol hydrochloride and 1′-hydroxybufuralol were generous gifts of Roche Products Plc.

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7-Ethoxycoumarin was purchased from Aldrich. Quinidine hydrochloride monohydrate, quinine hydrochloride, benzo(a) pyrene, 7-hydroxycoumarin, NADPH, NADH, and bovine serum albumin (fraction V) were purchased from Sigma. All solvent and other reagents were of AR grade.

Liver microsomes After fasting for 18 h, 6 ♂ Wistar rats weighing 204 ± 6 g were killed by stunning and exsanguination. The livers were rinsed with ice-cold normal saline twice. Six EM human livers were obtained from the victims who died of traffic accident with the permission of Research Ethical Committee. The isolation of human and rat liver microsome, and the assay of microsome protein concentration were operated exactly as previously reported[5].

Enzyme activity assays Microsome AHH and ED activities were determined by fluorescence spectrometry[8,9]. Microsome BH activity was tested by a normal phase HPLC[10]. The aqueous solutions of quinidine and quinine were freshly prepared on the day of use, and were added into the complete mixture before the addition of substrate. The samples were incubated at 37 ℃ for 2 min, and the reactions were started by adding the substrates. Neither quinidine nor quinine interfered any of the assays. All determinations were in duplicate.

Data analysis Statistical analyses were performed using the paired t test. The median inhibitory concentration (IC_{50}) and its 95 % confidence limits were calculated by weighted probit analysis method.

RESULTS

AHH activity Both quinidine and quinine at the concentrations of 0.01 – 5 mmol·L^{-1} inhibited the man and rat microsome AHH activities in a concentration-dependent manner. The IC_{50} values of quinidine for man and rat AHH were 378 and 613 μmol·L^{-1}, and the values of IC_{50} of quinine for human and rat AHH were 207 and 54 μmol·L^{-1}, respectively. Quinine was a more potent inhibitor than quinidine to both human and rat liver microsome AHH (Fig 1, Tab 1).

ED activity Quinidine and quinine produced concentration-dependent inhibitions to both high and low affinity phases of ED. There was an obvious segregation on the inhibition curves of quinidine and quinine between the high affinity and low affinity components of ED in man and rat. Nevertheless, both quinidine and quinine were weak inhibitors, especially to the human high affinity phase. Even at the concentration of 5 mmol·L^{-1}, neither quinidine nor quinine caused a half reduction of human high affinity phase of ED activity. However, quinine was a more potent inhibitor to the low affinity phase of ED in both species, and to the high affinity phase of rat ED, quinidine was a little more potent than quinine (Fig 2,3, Tab 1).

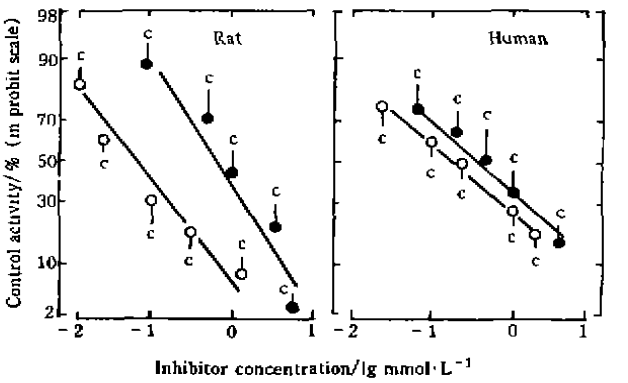

Fig 1. Inhibition of aryl hydrocarbon hydroxylase of liver microsomes by quinine (○) and quinidine (●). n = 6 rat or human livers. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Tab 1. Median inhibitory concentrations (IC_{50}) and their 95 % confidence limits of quinine and quinidine *in vitro* to aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin *O*-deethylase high (ED-H) and low (ED-L) affinity phases, and bufuralol 1′-hydroxylase (BH) in rat and human liver microsomes. n = 6 rat or human livers.

Enzyme	IC_{50} (95 % confidence limit)/ μmol·L^{-1}		$\frac{IC_{50}\text{ quinine}}{IC_{50}\text{ quinidine}}$
	Quinine	Quinidine	
Rat liver microsomes			
AHH	54 (91 – 32)	613 (940 – 399)	0.1
ED-H	2 508 (8 990 – 700)	1 595 (5 399 – 471)	1.6
ED-L	597 (1 276 – 280)	1 465 (3 822 – 561)	0.4
BH	31 (63 – 15)	290 (632 – 133)	0.1
Human liver microsomes			
AHH	207 (370 – 115)	378 (705 – 202)	0.6
ED-H	>5 000	>5 000	
ED-L	808 (2 087 – 302)	2 952 (5 632 – 1 547)	0.3
BH	29 (67 – 12)	0.20 (0.86 – 0.04)	145

BH activity Both quinidine and quinine inhibited human and rat liver microsome BH activity in a concentration-dependent manner. Quinidine was a very potent and specific inhibitor to human BH. As low as 0.1 μmol·L^{-1}, quinidine caused a 38 %-reduction of BH activity, and the IC_{50} was 0.2 μmol·L^{-1}, about 1450-fold more potent than

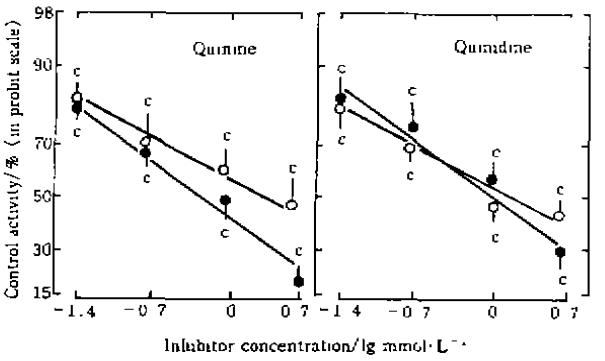

Fig 2. Inhibition of 7-ethoxycoumarin *O*-deethylase of rat liver microsomes by quinine and quinidine on the high (○) and low (●) affinity phases. $n = 6$ rat livers. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

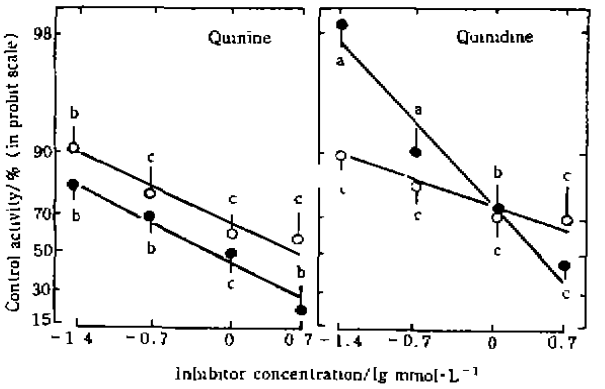

Fig 3. Inhibition of 7-ethoxycoumarin *O*-deethylase of human liver microsomes by quinine and quinidine on the high (○) and low (●) affinity phases. $n = 6$ human livers. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

to rat BH activity, and 155 and 145 times more potent than quinine to rat and human BH, respectively. In contrast, quinine was about 9-fold more potent to inhibit rat BH activity than quinidine (Fig 4, Tab 1).

DISCUSSION

The profound variance of the inhibitory potencies of quinidine or quinine in the present study further confirms that AHH, ED, and BH are different enzyme forms of liver microsome cytochrome P-450[3,4]. Comparing the IC_{50} values to these enzymes in man, it has been shown that quinidine is a specific and powerful inhibitor to

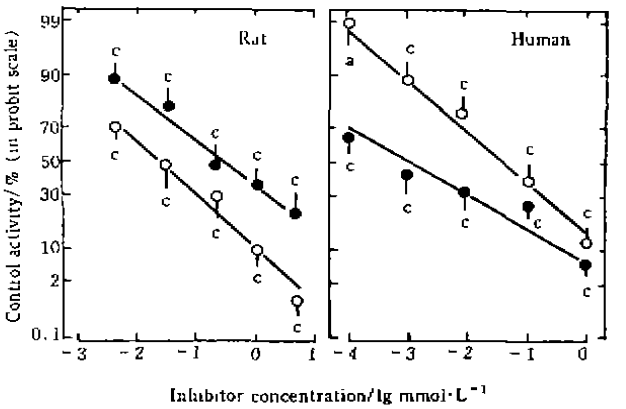

Fig 4. Inhibition of bufuralol 1′-hydroxylase of liver microsomes by quinine (○) and quinidine (●). $n = 6$ human livers. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

human BH by some 1 890 − 25 000 folds more potent than to AHH, ED high and low affinity phases, respectively. In contrast with above findings, quinidine is a weaker inhibitor to these enzymes in rat liver microsomes. While comparing the IC_{50} values of both isomers to these enzymes, quinidine has also manifested a highly stero-selective inhibition to human BH, whereas to BH in the rat, to AHH and ED in both species, such selectivity and potency of inhibition were lost or in the reverse, except the ED high affinity phase to which both isomers are poor inhibitors.

Although quinidine is a potent competitive inhibitor of human BH ie debrisoquine 4-hydroxylase[6,7,11], it is not metabolized by this enzyme[7]. On the other hand, there is a competitive inhibition interaction among the substrates of BH, but no such species and stereo differences of inhibition potency seen in this study have been observed[10]. An acceptable explanation of these phenomena is that there is some structure or conformation variance between the active sites of human and rat BH, so the stereo configuration difference of the secondary alcohol group between quinidine and quinine makes former much fitter to combine with human BH. Occupying the active sites of human BH and forming quinidine-BH complex, quinidine interferes with the binding of the substrate to the enzyme or the catalyzing process. However, the species difference in present

study further warns that it should be careful to extent directly the results observed in animal experiment to man.

In conclusion, our present study has suggested that quinidine is a highly stereo and species selective potent inhibitor to human BH, and so a small dose of quinidine can be used as a probe to screen whether a drug biotransformation pathway is involved by the polymorphism of human liver microsome debrisoquine 4-hydroxylase type in extensive phenotype subjects.

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奎尼丁和奎宁对人和大鼠肝微粒体氧化酶的抑制作用[1]

R963 R968

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关键词 奎尼丁；奎宁；肝微粒体；遗传药理学；丁呋洛尔；羟基香豆素脱羟基酶；芳基烃羟化酶类

目的：比较奎尼丁和奎宁对人和大鼠肝微粒体丁呋洛尔1'-羟化酶(BH)，7-乙氧基香豆素脱乙基酶(ED)和芳基烃羟化酶(AHH)的抑制作用 **方法**：正相HPLC或荧光分光法测定上述酶促反应. **结果**：奎尼丁和奎宁对上述酶均呈浓度依赖性抑制对人BH，AHH，ED低及高亲和力成分奎尼丁的IC_{50}分别为0.2，378，2952和>5000 μmol·L^{-1}；奎宁则为29，207，808和>5000 μmol·L^{-1}. 奎尼丁对大鼠上述酶的IC_{50}为290，613，1465和1595 μmol·L^{-1}；奎宁则为31，54，597和2508 μmol·L^{-1}. **结论**：奎尼丁是有种属和立体特异性的人肝微粒体BH强效抑制剂.