J Pharm Pharmaceut Sci (www.cspsCanada.org) 9(1):101-112, 2006

Contribution of Polymorphisms in *UDP-Glucuronosyltransferase* and *CYP2D6* to the Individual Variation in Disposition of Carvedilol

Yoh Takekuma[1], Toru Takenaka[2], Masami Kiyokawa[2], Koujiro Yamazaki[2], Hiroshi Okamoto[3], Akira Kitabatake[3], Hiroyuki Tsutsui[3], Mitsuru Sugawara[2]

[1]Department of Clinical Pharmaceutics and Therapeutics, Graduate School of Pharmaceutical Science, Hokkaido University, Sapporo, Japan
[2]Department of Pharmacy, Hokkaido University Hospital, Sapporo, Japan
[3]Department of Cardiovascular Medicine, Hokkaido University Hospital, Sapporo, Japan

Received 17 December 2005, Revised 22 February 2006, Accepted 23 February 2006, Published 1 March 2006.

ABSTRACT PURPOSE. It has been reported that carvedilol, which has beta-adrenergic blocking and vasodilating activities, is mainly metabolized by UDP-glucuronosyltransferase (UGT) 1A1, UGT2B4, UGT2B7 and *CYP2D6*. The aim of this study was to determine whether the activity of glucuronidation has an influence on the area under the curve (AUC) of carvedilol and whether polymorphisms in *UGTs* and *CYP2D6* contribute to individual variation in disposition of carvedilol in Japanese. **METHODS**. Plasma concentrations of carvedilol and its glucuronide were determined by reversed-phase high-performance liquid chromatography (HPLC). Genotyping of *UGT1A1, UGT2B4* and *UGT2B7* genes was carried out by the direct sequence method. *CYP2D6* genotyping was carried out using an amplification refractory mutation system (ARMS) assay and PCR-restriction fragment length polymorphism (RFLP). **RESULTS**. The level of carvedilol glucuronidation ability in the high-level AUC group was significantly lower than that in the low-level group. The frequencies of *UGT1A1*6*, *UGT2B7*3* and *CYP2D6*10* in the low level ability of glucuronidation group were significantly higher than those in the high level group, and the same tendency was found in the frequency of *CYP2D6*5*, though there was no significant difference. **CONCLUSION**. Polymorphisms of *UGT1A1, UGT2B7* and *CYP2D6* strongly affect the pharmacokinetics and disposition of carvedilol in Japanese.

Corresponding Author: Yoh Takekuma, Department of Clinical Pharmaceutics and Therapeutics, Graduate School of Pharmaceutical Science, Hokkaido University, Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan. y-kuma@pharm.hokudai.ac.jp

INTRODUCTION

Generally, orally administered drugs are absorbed by the small intestine and then metabolized in the liver. Metabolism includes phase I (oxidation, reduction, hydrolysis, etc.) and phase II (conjugation) reactions. The phase I reaction introduces a functional group such as a hydroxyl group onto the molecule or exposes a pre-existing functional group, and the phase II reaction connects the functional group to an endogenous species such as a glucuronic acid. Modified drug molecules are hydrophilic and are excreted into bile and urine. However, some drugs do not undergo the phase I reaction and are conjugated directly. It is possible that individual variations in enzyme activity for conjugation affect the pharmacokinetics of these drugs.

Carvedilol ((±)-1-carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]-amino]-2-propanol) has β-adrenergic blocking and vasodilating activities [1, 2]. This drug is used to treat angina pectoris and hypertension and has recently been used to treat chronic heart failure (CHF). However, for treatment of CHF, it is recommended that the dose of carvedilol should be gradually and carefully increased because of its negative inotropic activity [2-5].

It has been reported that carvedilol is metabolized by both oxidation and conjugation pathways in the liver into various metabolites and that the main pathway is direct glucuronidation of carvedilol because the main metabolite in plasma and urine was found to be the glucuronide of unchanged carvedilol (22% and 32%, respectively) [6, 7]. Three UDP-glucuronosyltransferase (UGT) isoforms have been reported to be capable of conjugating carvedilol

into two forms of its glucuronides (G1 and G2) [8]. UGT2B4 formed both glucuronides, whereas UGT1A1 (G2) and UGT2B7 (G1) formed either one. On the other hand, oxidation pathways are mainly catalyzed by CYP2D6 [9]. CYP2D6 is responsible for the formation of 4'-hydroxy carvedilol and 5'-hydroxy carvedilol, and both metabolites are excreted into urine (6.4%) [7]. Therefore, we should not disregard the influence of CYP2D6 in discussing the disposition of carvedilol, although glucuronidation is the major metabolic pathway of carvedilol in humans.

The aim of this study was to clarify whether polymorphisms in *UDP-Glucuronosyltransferase* and *CYP2D6* contribute to individual variation in disposition of carvedilol.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the Graduate School of Medicine, Hokkaido University. Written informed consent for participation in the study was obtained from all subjects.

Chemicals and regents

(±) -Carvedilol was kindly supplied by Daiichi Pharmaceutical Co. (Tokyo, Japan). Dihydroergotamine was obtained from Sigma-Aldrich (St. Louis, MO). β-glucuronidase was purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents were of the highest grade available.

Patients and blood sampling

Forty-six patients (8 females and 38 males; median age, 65.5 (26-83) years; median body weight, 61.4 (32.0-98.9) kg) with CHF or angina pectoris who were being treated with carvedilol were enrolled in this study. The patients with CHF were classified into New York Heart Association (NYHA) class II-III. The daily doses of carvedilol ranged from 1.25 to 20 mg, and the drug was taken in one or two doses daily. The median creatinine clearance (Ccr) in the patients was 63.0 (15.9-156.6) mL/min. No patients had clinically overt hepatic failure. There were no concomitantly used drugs that have been reported to strongly influence plasma concentration of carvedilol. After a fixed dose of carvedilol had been administered for 6 to 10 days, venous blood samples were collected just before drug administration and at 1, 2, 4, 6, and 10 h after administration.

Determination of carvedilol and its glucuronide

Plasma concentrations were determined by reversed-phase high-performance liquid chromatography (HPLC) with a fluorometric detector. The separation was performed on a GL-Pak Nucleosil 100-5C_8 (4.6 mm I.D. x 250 mm) column (GL Science Inc., Tokyo, Japan). The mobile phase was a mixture of acetonitrile and 50 mM potassium dihydrogenphosphate (28:72) containing a final concentration of 5 mM tetra-n-butylammonium chloride. The flow rate was 1.0 mL/min and column temperature was 40ºC. Excitation and emission wavelengths of 240 nm and 340 nm, respectively, were used for fluorometric detection. For the determination of unchanged carvedilol in plasma, 200 μL of a sample was mixed with 2 mL of 1 M sodium hydroxide and 200 μL of dihydroergotamine solution (20 μg/mL in methanol) as an internal standard and then vortexed for 20 sec. Five mL of diethyl ether was added to the mixture, and the mixture was shaken for 20 min. After centrifugation at 1,800 x g for 5 min, the upper organic layer (4 mL) was evaporated to dryness. The residue was reconstituted with 200 μL of the mobile phase and 40 μL of them were injected into the HPLC system. The lower limit of quantitation for carvedilol was 0.5 ng/mL. Coefficients of variation were 2.62% and 9.38% at 50 ng/mL and 0.5 ng/mL, respectively (n = 5). Determination of carvedilol glucuronide was done after converting the glucuronide to parent carvedilol by β-glucuronidase; that is, 200 μL of plasma was mixed with 20 μL of β-glucuronidase solution (98,000 units/mL) and then incubated at 46ºC for 1.5 h. After the reaction, total carvedilol was determined as described above. The concentration of the glucuronide was calculated by subtracting the concentration of the unchanged form from the total

concentration. The area under the curve (AUC_{0-10}) was calculated from the plasma concentration of carvedilol or its glucuronide using the linear trapezoidal rule.

Genotyping

We obtained written informed consent from 40 of 66 patients and genotyped their UGTs and CYP. Genomic DNA was prepared using standard methods. The exons of *UGT1A1, UGT2B4* and *UGT2B7* genes (containing the promoter region of *UGT1A1*) in 40 patients from whom written informed consents were obtained for genotyping were sequenced.

Each exon was amplified from genomic DNA (20-60 ng) using 0.5 units of Ex-Taq (Takara Bio Inc., Shiga, Japan) with 1 μM of the primers shown in Table 1.

Table 1. Primers for amplification and sequencing of *UGT1A1, UGT2B4* and *UGT2B7*.

Gene	Use	Site	Direction	Primer Name	Sequence
UGT1A1	Amplification	Exon1	Forward	TA-F	5'-AAGTGAACTCCCTGCTACCTT-3'
			Reverse	E1-R	5'-GCTTGCTCAGCATATATCTGGG-3'
		Exon2-4	Forward	E2-F1	5'-CTCTATCTCAAACACGCATGCC-3'
			Reverse	E2-R	5'-TTTTATCATGAATGCCATGACC-3'
		Exon5	Forward	E5-F	5'-GAGGATTGTTCATACCACAGG-3'
			Reverse	E5-R	5'-GCACTCTGGGGCTGATTAAT-3'
	Sequence	Exon1	Reverse	UGT1A1e1seq1R	5'-ATGTTCTTCACCCGCTGC-3'
			Forward	E1-F2	5'-GAAGACGTACCCTGTGCCATT-3'
		Exon2	Reverse	UGT1A1e2seq1	5'-AATCATAGTCTTAAGAGG-3'
		Exon3-4	Forward	E2-F2	5'-CCTTCAGAGGACCCCTGTTTT-3'
		Exon5	Reverse	UGT1A1e5seq1R	5'-GCAAAGTATTTCCTTAATAAG-3'
UGT2A4	Amplification	Exon1	Forward	UGT2B4e1-F	5'-GGCAGGTGCCTGTCTGTAGT-3'
			Reverse	UGT2B4e1-R	5'-CAAAAATACCCCACTACCCTGA-3'
		Exon2	Forward	UGT2B4e2-F	5'-TCTCTCACCACTTTGCCTTTC-3'
			Reverse	UGT2B4e2-R	5'-CCACTTCCACCTTTCTTCCA-3'
		Exon3	Forward	UGT2B4e3-F	5'-GACCCTTAACAGAGGCAACC-3'
			Reverse	UGT2B4e3-R	5'-TGGGTTCTTTACAAACTTTAACAGC-3'
		Exon4-5	Forward	UGT2B4e4-5-F	5'-TGAACTTTTCATTGATTATCTTATTTG-3'
			Reverse	UGT2B4e4-5-R	5'-TTCTTTCGAAATCAGTCGCTTA-3'
		Exon6	Forward	UGT2B4e6-F2	5'-TTTACTGGAGACGGGGTGAC-3'
			Reverse	UGT2B4e6-R2	5'-GTTGAAGCACGATGCACAAT-3'
	Sequence	Exon1	Reverse	UGT2B4e1seq1R	5'-GGAACAGAAGTCCTCCACTA-3'
			Forward	UGT2B4e1seq2	5'-TGAAGTTTATCCTGTATC-3'
		Exon2	Forward	UGT2B4e2seq	5'-ACAAACACTTTGCCTACA-3'
		Exon3	Reverse	UGT2B4e3seq	5'-CCTGATAAAACTTGATTTTCTC-3'
		Exon4	Forward	UGT2B4e4seq	5'-TATTTACATCAGTCTGAG-3'
		Exon5	Forward	UGT2B4e5seq	5'-TTAATTCAGTTCAGTGTG-3'

Gene	Use	Site	Direction	Primer Name	Sequence
		Exon6	Forward	UGT2B4e6seq	5'-TGATCTCCTGACCTCGTG-3'
UGT2A7	Amplification	Exon1	Forward	UGT2B7e1-F	5'-TTGTCTCTTTGCCATCCACA-3'
			Reverse	UGT2B7e1-R	5'-CAAAACCCCACTACCCTGAC-3'
		Exon2	Forward	UGT2B7e2-F2	5'-GATATTTGCCTACATTTTTGCC-3'
			Reverse	UGT2B7e2-R2	5'-CCCTTTGTAAATATTATTTGATTGG-3'
		Exon3	Forward	UGT2B7e3-F	5'-AAAAACTGAGTGATTGGGTCAG-3'
			Reverse	UGT2B7e3-R	5'-TCCACACCAGTAAGGCACTTC-3'
		Exon4	Forward	UGT2B7e4-F	5'-GTTGGCCACACGTAGGTTTT-3'
			Reverse	UGT2B7e4-R	5'-ACATTTTGTTTTTCCTTAACAAAT-3'
		Exon5	Forward	UGT2B7e5-F	5'-TCACACACACCGTATAGCCTTC-3'
			Reverse	UGT2B7e5-R	5'-AAAAAGGATGAAACTCACACTCA-3'
		Exon6	Forward	UGT2B7e6-F2	5'-AGCCCTTAAAAGGGACAGGA-3'
			Reverse	UGT2B7e6-R2	5'-CAGTGGACTTCTTAATGATCTTGTG-3'
	Sequence	Exon1	Forward	UGT2B7e1seq1	5'-CTCAGACTGTTGATTTAA-3'
			Forward	UGT2B7e1seq2	5'-TACAGGAAATCATGTCAA-3'
		Exon2	Reverse	UGT2B7e2seqR2	5'-ACTCATAAAACTCATATACGTGTGA-3'
		Exon3	Forward	UGT2B7e3seq	5'-AATTCCTCAAAATACTGG-3'
		Exon4	Forward	UGT2B7e4seq	5'-CTTATTTACTAACATCCC-3'
		Exon5	Forward	UGT2B7e5seq	5'-AAGTACGTGTTTTTTCCT-3'
		Exon6	Forward	UGT2B7e6seq	5'-TGAGAGAGGAGTCTTGCC-3'

The conditions of polymerase chain reaction (PCR) were as follows: denaturation at 94ºC for 5 min, followed by 30 cycles comprising denaturation at 94ºC for 30 sec, annealing at 54-64ºC for 10 to 30 sec, and extension at 72ºC for 30 to 80 sec, and then a final extension at 72ºC for 7 min. The PCR products were treated by ExoSap-IT (Takara Bio Inc.) at 37ºC for 15 min and at 80ºC for 15 min to degrade the excess primers and dNTP. The products were directly sequenced with the primers listed in Table 1 using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommended protocol.

For *CYP2D6* variants, the same 40 patients were genotyped. Mutant alleles that have been reported to have high frequencies in the Japanese population were selected in this study [10]. Genotyping of *CYP2D6*5* (deletion of the *CYP2D6* allele) was carried out using an amplification refractory mutation system (ARMS) assay as described by Johansson et al. [11] and Steen et al. [12] with minor modification. Genotyping of 100C>T (common SNP to *CYP2D6*4, *10, *14, *36, *37, *47* and **49*) was carried out using the ARMS assay as described by Johansson et al. [13]. Then samples that had the 100T allele were genotyped on *CYP2D6*4* and *CYP2D6*14*. Genotyping of *CYP2D6*4* was carried out using the ARMS assay as described by Heim et al. [14]. Genotyping of *CYP2D6*14* was carried out using the PCR-restriction fragment length polymorphism (RFLP) assay as described by Wang et al. [15]. In this study, subjects with 100C>T mutation were classified into *CYP2D6*10* except for *CYP2D6*4* and **14* because frequencies of *CYP2D6*18, *21, *36, *37, *47* and **49* are rare in the Japanese population [10].

Statistical analysis

Data are expressed as mean ± SD. Differences in the ability of glucuronidation between low and high level AUC groups were measured using student's t-test. Differences between allele frequencies in the two groups were measured using Fisher's exact test. Correlation between the numbers of mutant alleles and the ability of glucuronidation was analyzed using Spearman's rank correlation test. A p value below 0.05 was considered statistically significant.

RESULTS

Pharmacokinetics property of carvedilol

Plasma concentrations of carvedilol and its glucuronide in the 46 patients were determined, and 66 profiles of $AUC_{0\text{-}10}$ were obtained. Pharmacokinetic parameters are listed in Table 2.

Table 2. Pharmacokinetic parameters of carvedilol in tested patients.

Groups of usage	Normalized AUC (hr/kL)	C_{max}/dose (kL^{-1})	t_{max} (h)	$t_{1/2}$ (h)
Once a day (n = 17)	17.8±22.6	3.35±3.79	3.23±1.02	2.99±1.30
Twice a day (n = 49)	28.7±23.8	4.95±4.20	3.49±1.95	6.32±5.75

Normalized AUC: ratio of $AUC_{0\text{-}10}$ to dose of carvedilol.
Data are given as means±SD.

No difference was found between the tested subjects and Japanese healthy subjects in t_{max} and $t_{1/2}$ [16]. However, ratios of $AUC_{0\text{-}10}$ to dose of carvedilol (normalized AUC) and C_{max}/dose in the subjects were higher than those in Japanese healthy subjects [16].

Normalized AUCs of each subject are shown in Figure 1.

The range of normalized AUCs was very wide in both the once a day and twice a day group (2.69-85.1 and 2.83-108.9, respectively) and it was independent of dose. It was ascertained whether individual variation of ability to glucuronidate was responsible for this individual variation of normalized AUCs. In general, the ratio of metabolite to parent drug AUC is used as indicator of metabolization ability. However, in this study, we defined the metabolic index (MI) as follows because clearance of carvedilol glucuronide depends on renal function:

$$MI = (AUC\ glucuronized \times Ccr) / AUC\ unchanged, \quad (1)$$

where AUC glucuronized and AUC unchanged are AUC of glucuronized carvedilol and AUC of unchanged carvedilol, respectively and Ccr is creatinine clearance.

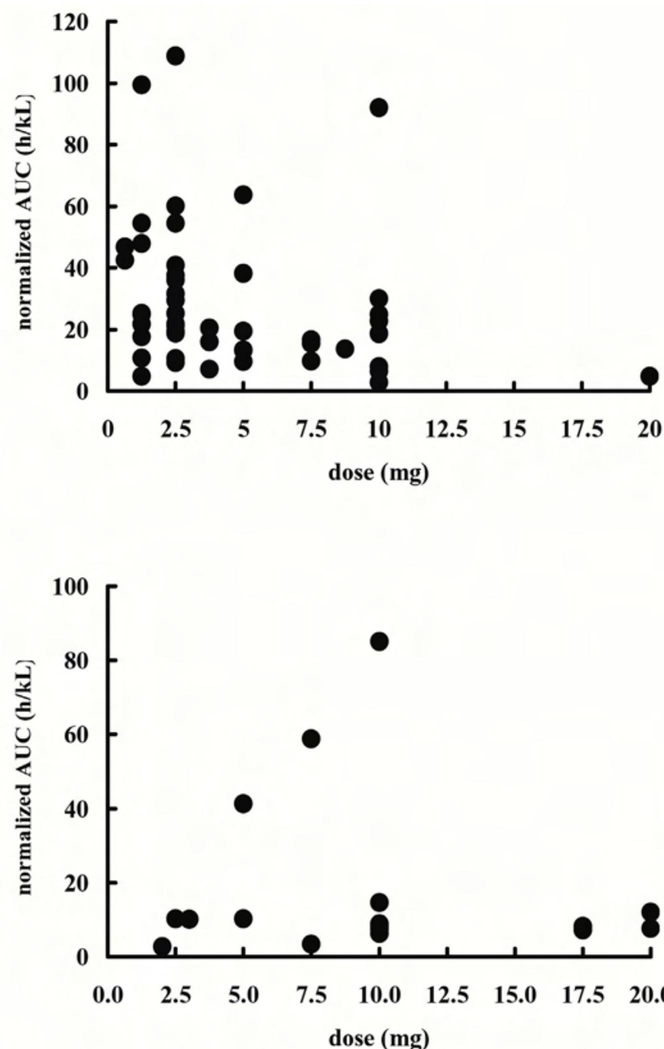

Figure 1: Distribution of ratios of $AUC_{0\text{-}10}$ to dose of carvedilol (normalized AUC) at each dose in the patients taking carvedilol. *The top graph is the group of twice a day (n=49) and bottom graph is the group of once a day (n=17).*

The subjects were classified into low and high level normalized AUC groups, and the metabolic indexes in these two groups were compared (Figure 2).

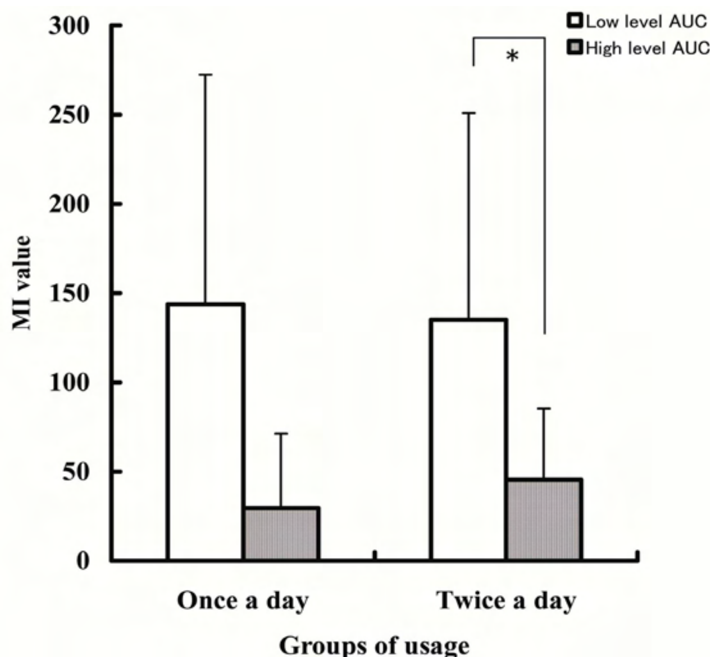

Figure 2: Comparison of the ability of carvedilol glucoronidation in the low and high level AUC groups. *MI value: ratio of carvedilol glucuronide to unchanged carvedilol AUCs which was multiplied by creatinine clearance. Each column represents the mean with SD. *; p<0.001.*

In the twice a day group, MI value was significantly lower in the high level AUC group than in the low level AUC group (p<0.01). In the once a day group, the same tendency was found, but though there was no significant difference.

Genotyping

Genotyping of *UGT1A1, UGT2B4*, and *UGT2B7* in the 40 patients that gave written informed consent for genotyping was carried out. For reference sequences, AF297093 was used for *UGT1A1*, and NT_077444.2 (GenBank accession numbers) was used for *UGT2B4* and *UGT2B7*.

Table 3 shows the results of genotyping. The subjects were classified into low and high level MI groups. DNA sequence analysis confirmed the presence of variants of *UGT1A1*6* (211G>T, G71R), *UGT1A1*28* (A (TA) 6TAA to A (TA) 7TAA), *UGT2B7*2* (802C>T, H268Y) and *UGT2B7*3* (211G>T, A71S) in *UGT2B7*. In *UGT2B4*, no SNP was found except for a silent mutation (1212A>T).

Frequencies of *UGT1A1* and *UGT2B7* alleles in the 40 subjects are shown in Table 4. No significant differences were found between low and high level MI groups with *UGT2B7*2*. However, the frequencies of *UGT1A1*6*, *UGT1A1*28* and *UGT2B7*3* were significantly different between the two groups. The frequencies of *UGT1A1*6* and *UGT2B7*3* in the low level MI group were higher than those in the high level MI group, but the frequency of *UGT1A1*28* in the low level MI group was lower than that in the high level MI group.

As shown in Tables 3 and 4, genotyping for four *CYP2D6* alleles was carried out in the same 40 patients genotyped for *UGT*. Variants of *CYP2D6*4*, **5* and **10* were detected. Only one subject had the *CYP2D6*4* allele. The frequency of *CYP2D6*10* in the low level MI group was two-times higher than that in the high level group, and the same tendency was found in the frequency of *CYP2D6*5*, though there was no significant difference.

Figure 3 shows the relation between numbers of mutant alleles except for *UGT2B7*2* and MI values. One *CYP2D6*5* or **4* allele is counted as two alleles because both alleles lack catalytic activity and it is thought that influence of those alleles on metabolic activity is greater than that of the other alleles. MI values showed a tendency to decrease with increase in the number of mutant alleles. (p<0.001).

DISCUSSION

This is the first study to demonstrate the relationship between polymorphisms of *UGTs* and *CYP2D6* and disposition of carvedilol at the same time. Our results indicated large variations in the normalized AUC of carvedilol. These variations were found in all groups of patients receiving carvedilol of doses of 1.25 mg to 10 mg (Figure 1).

Table 3 Results of genotyping in the 40 patients.

Low level of glucuronidation ability						High level of glucuronidation ability					
Subjects	**Ability of glucuronidation**	***UGT1A1***	***UGT2B4***	***UGT2B7***	***CYP2D6***	**Subjects**	**Ability of glucuronidation**	***UGT1A1***	***UGT2B4***	***UGT2B7***	***CYP2D6***
A	3.5	*1/*28	*1/*1	*2/*3	*1/*10	a	86.5	*1/*28	*1/*1	*1/*2	*1/*1
B	5.1	*1/*1	*1/*1	*1/*2	*10/*10	b	90.1	*1/*28	*1/*1	*1/*1	*1/*1
C	10.5	*1/*6	*1/*1	*1/*1	*5/*10	c	92.2	*1/*1	*1/*1	*1/*2	*1/*10
D	10.6	*1/*6	*1/*1	*1/*1	*10/*10	d	100.5	*1/*28	*1/*1	*1/*2	*1/*1
E	17.0	*1/*1	*1/*1	*1/*3	*1/*5	e	107.9	*1/*28	*1/*1	*1/*1	*1/*5
F	18.7	*1/*6	*1/*1	*1/*3	*1/*1	f	117.5	*1/*1	*1/*1	*2/*3	*1/*10
G	24.7	*1/*1	*1/*1	*1/*2	*10/*10	g	119.5	*6/*28	*1/*1	*1/*1	*1/*1
H	25.6	*1/*6	*1/*1	*1/*2	*10/*10	h	126.6	*1/*28	*1/*1	*1/*1	*1/*1
I	26.1	*1/*6	*1/*1	*3/*3	*1/*10	I	139.8	*1/*1	*1/*1	*1/*1	*4/*10
J	28.2	*6/*6	*1/*1	*1/*1	*1/*1	j	140.8	*1/*1	*1/*1	*2/*3	*1/*1
K	34.8	*1/*6	*1/*1	*2/*3	*1/*10	k	143.2	*1/*28	*1/*1	*2/*2	*1/*10
L	38.3	*1/*6	*1/*1	*1/*1	*1/*10	l	157.9	*1/*1	*1/*1	*1/*2	*1/*1
M	40.7	*1/*1	*1/*1	*1/*2	*1/*10	m	223.1	*1/*28	*1/*1	*1/*2	*1/*10
N	41.0	*1/*28	*1/*1	*1/*2	*1/*1	n	240.9	*1/*1	*1/*1	*2/*2	*1/*1
O	46.8	*1/*1	*1/*1	*2/*2	*1/*5	o	269.3	*1/*1	*1/*1	*2/*3	*1/*1
P	49.1	*1/*6	*1/*1	*1/*2	*1/*10	p	387.9	*1/*1	*1/*1	*1/*2	*1/*10
Q	56.0	*1/*1	*1/*1	*3/*3	*1/*1	q	504.4	*1/*1	*1/*1	*1/*2	*1/*10
R	65.1	*1/*6	*1/*1	*2/*3	*1/*10						
S	73.6	*1/*6	*1/*1	*1/*1	*1/*10						
T	77.5	*1/*28	*1/*1	*2/*3	*1/*10						
U	82.9	*1/*6	*1/*1	*1/*2	*10/*10						
V	84.0	*1/*6	*1/*1	*1/*1	*1/*10						
W	86.1	*1/*1	*1/*1	*3/*3	*1/*10						

Table 4. Comparison of allele frequencies in subjects with low and high levels of glucuronidation ability.

	Low level of glucuronidation ability		High level of glucuronidation ability	
Allele	**No. of alleles**	**% of allele**	**No. of alleles**	**% of allele**
*UGT1A1*1*	29	63.0	25	73.6
*UGT1A1*6#*	14	30.5	1	2.9
*UGT1A1*28#*	3	6.5	8	23.5
*UGT2B7*1*	21	45.6	17	50.0
*UGT2B7*2*	13	28.3	14	41.2
*UGT2B7*3#*	12	26.1	3	8.8
*CYP2D6*1#*	15	45.7	25	73.6
*CYP2D6*4*	0	0	1	2.9
*CYP2D6*5*	3	6.5	1	2.9
*CYP2D6*10#*	22	47.8	7	20.6
#; p<0.05				

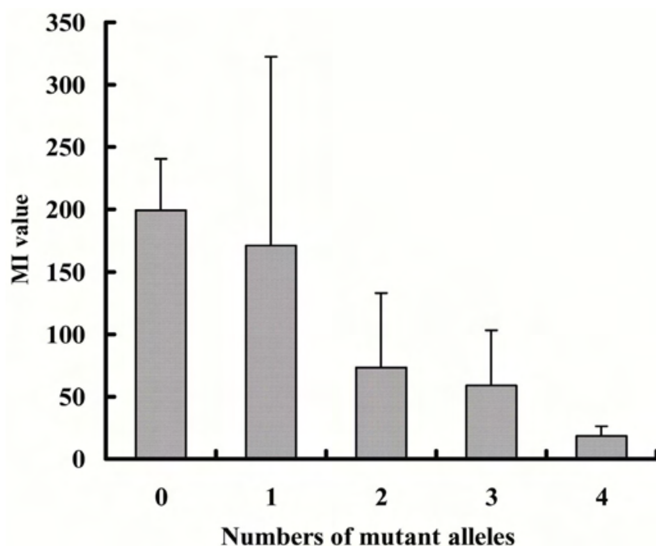

Figure 3: Relationship between numbers of mutant alleles except for *UGT2B7*2* and ability of carvedilol glucoronidation. *One CYP2D6*5 or *4 allele is counted as two alleles because both alleles lack catalytic activity. M1 values: ratio of carvedilol glucoronide to unchanged carvedilol AUCs which was multiplied by creatinine clearance. Each column represents the mean with SD. Significant correlation by Spearman's rank correlation (p<0.001).*

None of the patients receiving doses of more than 15 mg had particularly high AUC. Since the dose of carvedilol in patients who showed reductions in blood pressure and heart rate was not increased, patients administered such a high dose of carvedilol may not a show high plasma concentration. Normalized AUC in the present study (2.69-85.1 hr/kL in the once a day group) was much higher than that in healthy adults (2.56-15.0 hr/kL) reported by Neugebauer et al. [6]. It has been reported that plasma concentrations of carvedilol were increased in patients with CHF compared with concentrations in healthy volunteers (50-100% higher values in patients with NYHA class Ⅳ CHF) [17]. The increase in plasma concentration is thought to be caused by a reduction in uptake of carvedilol to the liver accompanied by a decrease in the bloodstream.

However, this cannot account for our results because some of the AUC values in the present study were six-times higher than those in healthy adults, whereas patients with CHF show values only 3-4 times higher than those in healthy adults.

Figure 2 shows that MI, an indicator of the ability of glucuronidation of carvedilol, was associated with normalized AUC. In twice a day group, the MI value was significantly lower in the high level AUC group than in the low level AUC group (p<0.01). In once a day group, the same tendency was found, though there was no significant difference because the number of subjects was too small. These results suggest that the ability of glucuronidation affects the AUC of carvedilol, in accordance with results of previous studies showing that the main metabolic pathway is glucuronidation of carvedilol [6, 7].

Ohno et al. [8] showed by using a recombinant UGT assay that UGT1A1, UGT2B4 and UGT2B7 are responsible for glucuronidation of carvedilol. Therefore, these three UGT isoforms were sequenced in the 40 patients to determine whether polymorphisms of these genes are responsible for the variation in the ability of glucuronidation of carvedilol. No missense mutation in *UGT2B4* was found in the 40 patients (Table 3).

Only a few mutations in *UGT2B4*, namely, *UGT2B4*2* (1374T>A, D458E), *3 (325T>T and 1186T>C, F109L and F369L), *4 (1364A>G, K455R) and *5 (1531T>C, C511R), have been reported [18-20]. The frequencies of the *UGT2B4*2* allele in Caucasian and African populations have been reported to be 20.0% and 15.0%, respectively, by Lampe et al. [21] and Riedy et al. [22]. However, this mutation in Japanese is rare [20]. Our results are in agreement with those reports. In *UGT1A1*, many variant alleles have been reported (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/). In this study, *UGT1A1*6* (211G>A, G71R) and *28 (A (TA) 6TAA to A (TA) 7TAA) were detected. Of the 40 patients in this study, the genotypes of UGT1A1*6* were homozygous in 1 patient and heterozygous in 13 patients. All of them except for one patient were in the low level MI group (Table 3).

The frequency of this mutation in Japanese has been reported to be 13-16% [23, 24]. Yamamoto et al. [25] reported that the catalytic activity level of the *UGT1A1* enzyme was reduced to 30% in subjects with a homozygote for the *UGT1A1*6* allele.

Therefore, *UGT1A1*6* was thought to reduce the activity of carvedilol glucuronidation. The allele frequency of *UGT1A1*28* in the 40 patients was 13.7%. This finding is in agreement with results of previous studies [24, 26, 27]. However, the allele frequency of *UGT1A1*28* in the high level MI group was higher than that in the low level MI group (Table 4).

*UGT1A1*28* has been reported to be associated with a 20-80% reduction in gene expression [28, 29]. Our results do not reflect these reports. On the other hand, it has reported that a homozygote for *UGT1A1*28* reduced the activity of estradiol glucuronidation in microsomes from the human liver to 23%, whereas a heterozygote reduced the ability to 82% [30]. All of the subjects with *UGT1A1*28* in this study were heterozygous. Therefore, it is thought that a heterozygote for *UGT1A1*28* had little effect of carvedilol glucuronidation. As for *UGT2B7, UGT2B7*2* (802C>T, H268Y) and **3* (211G>T, A71S) were found in this study (Tables 3 and 4). *UGT2B7*2* was the most frequently found variant allele in Japanese in previous studies [20, 31]. Although no remarkable functional difference between *UGT2B7*1* and *UGT2B7*2* alleles was found in several studies [32-35], one study has shown that subjects who had a *UGT2B7*2* allele showed a significantly higher morphine-6-O-glucuronide / morphine ratio than did subjects with *UGT2B7*1* [36]. Our results showed that there was no significant difference between the low and high level MI groups in the allele frequency of *UGT2B7*2* (Table 4). On the other hand, the frequency of *UGT2B7*3* in the low level MI group was significantly higher than that in the high level group. *UGT2B7*3* has been reported by Hirota et al. [31] and Saeki et al. [20]. The effect of this allele on catalytic activity is still unknown. Our results suggest that *UGT2B7*3* allele reduces the activity of carvedilol glucuronidation. Therefore, it is possible that *UGT1A1*6* and *UGT2B7*3* are responsible for the low level of glucuronidation activity of carvedilol.

With regard to CYP2D6 as an oxidative enzyme, the frequencies of *CYP2D6*4, *5* and **10* in the 40 patients were 1.25%, 5.0% and 36.3%, respectively. These results are in agreement with results of previous study [10]. The frequency of *CYP2D6*10* in the low level MI group was two-times higher than that in the high level group, and the same tendency was found in the frequency of *CYP2D6*5*, though there was no significant difference.

The AUC of R (+)-carvedilol in patients who were poor metabolizers of debrisoquin (an indicator of low level of CYP2D6 activity) was 2.56-times higher than that of R (+)-carvedilol in patients who were extensive metabolizers of debrisoquin. In contrast, the AUC of S (-)-carvedilol in poor metabolizers of debrisoquin and that in extensive metabolizers of debrisoquin were similar [37]. Honda et al. reported of effect of *CYP2D6*10* on the pharmacokinetics of R- and S-carvedilol in healthy Japanese [38]. Accordingly, it is necessary to take polymorphisms of *CYP2D6* into consideration when investigating the pharmacokinetics and disposition of carvedilol.

Although MI is an indicator of carvedilol glucuronidation activity, the frequencies of variant alleles that affect the oxidative catalytic activity of CYP2D6 were different in the low and high level MI groups. One possible reason for this is that reduction of catalytic activity of CYP2D6 leads to an increase in the unchanged carvedilol plasma concentration and AUC as the denominator of MI. On the other hand, the absolute quantity of carvedilol glucuronides does not greatly change. As a result, the MI value as an indicator of glucuronidation ability of carvedilol decreases. It is possible that MI is a good indicator of total metabolic activity of carvedilol including UGT and CYP2D6.

Figure 3 shows the relation between numbers of mutant alleles except for *UGT2B7*2* and MI values. One *CYP2D6*5* allele is counted as two alleles because the *CYP2D6*5* allele is a whole deletion of the *CYP2D6*5* gene and it is thought that the influence of this allele on metabolic activity is greater than that of the other alleles. MI values showed a tendency to decrease with increase in the number of mutant alleles ($p<0.001$), indicating that polymorphisms of *UGT1A1, UGT2B7* and *CYP2D6* affect carvedilol disposition in cooperation.

Giessmann reported that *CYP2D6* genotype and intestinal expression of P-glycoprotein (P-gp) and multidrug-resistant protein 2 (MRP2) are the major variables in carvedilol disposition [39]. Our result is agreement with effect of *CYP2D6* genotype. However, polymorphism for UGT should be taken into consideration because carvedilol glucuronides are major metabolites. In one mutant allele group, the range of MI values was very wide compared with that in the other groups. The contribution of other metabolic pathways and intestinal expression of P-gp or MRP2 in carvedilol absorption are possible to be the reason for this. Our results demonstrated that individual variations in the disposition of carvedilol, which is metabolized by multiple pathways, are caused not only by the polymorphism for the main enzyme, UGT, but also by another enzymes such as CYP2D6.

CONCLUSIONS

Forty patients who were being treated with carvedilol were phenotyped and genotyped for *UGT1A1*, *UGT2B4, UGT2B7* and *CYP2D6*. The allele frequencies of *UGT1A1*6, UGT2B7*3* and *CYP2D6*10* in the low level MI group were significantly higher than those in the low level MI group. MI values showed a tendency to decrease with increase in the number of mutant alleles. These results suggest that polymorphisms of *UGT1A1, UGT2B7* and *CYP2D6* strongly affect the pharmacokinetics and disposition of carvedilol.

REFERENCES

[1] von Mollendorff E, Abshagen U, Akpan W, Neugebauer G, Schroter E. Clinical pharmacologic investigations with carvedilol, a new beta-blocker with direct vasodilator activity. Clin Pharmacol Ther 1986, 39: 677-82

[2] Frishman WH. Carvedilol. N Engl J Med 1998, 339: 1759-65

[3] Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. N Engl J Med 1996, 334: 1349-55

[4] Cice G, Ferrara L, D'Andrea A, D'Isa S, Di Benedetto A, Cittadini A, Russo PE, Golino P,Calabro R. Carvedilol increases two-year survival in dialysis patients with dilated cardiomyopathy: a prospective, placebo-controlled trial. J Am Coll Cardiol 2003, 41: 1438-44

[5] Keating GM, Jarvis B. Carvedilol: a review of its use in chronic heart failure. Drugs 2003, 63: 1697-741

[6] Neugebauer G, Akpan W, von Mollendorff E, Neubert P, Reiff K. Pharmacokinetics and disposition of carvedilol in humans. J Cardiovasc Pharmacol 1987, 10 Suppl 11: S85-8

[7] Neugebauer G, Neubert P. Metabolism of carvedilol in man. Eur J Drug Metab Pharmacokinet 1991, 16: 257-60

[8] Ohno A, Saito Y, Hanioka N, Jinno H, Saeki M, Ando M, Ozawa S, Sawada J. Involvement of human hepatic UGT1A1, UGT2B4, and UGT2B7 in the glucuronidation of carvedilol. Drug Metab Dispos 2004, 32: 235-9

[9] Oldham HG, Clarke SE. In vitro identification of the human cytochrome P450 enzymes involved in the metabolism of R(+)- and S(-)-carvedilol. Drug Metab Dispos 1997, 25: 970-7

[10] Soyama A, Kubo T, Miyajima A, Saito Y, Shiseki K, Komamura K, Ueno K, Kamakura S, Kitakaze M, Tomoike H, Ozawa S, Sawada J. Novel nonsynonymous single nucleotide polymorphisms in the CYP2D6 gene. Drug Metab Pharmacokinet 2004, 19: 313-9

[11] Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjoqvist F, Ingelman-Sundberg M. Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. Mol Pharmacol 1994, 46: 452-9

[12] Steen VM, Andreassen OA, Daly AK, Tefre T, Borresen AL, Idle JR,Gulbrandsen AK. Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. Pharmacogenetics 1995, 5: 215-23

[13] Johansson I, Lundqvist E, Dahl ML, Ingelman-Sundberg M. PCR-based genotyping for duplicated and deleted CYP2D6 genes.

Pharmacogenetics 1996, 6: 351-5

[14] Heim M,Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. Lancet 1990, 336: 529-32

[15] Wang SL, Lai MD, Huang JD. G169R mutation diminishes the metabolic activity of CYP2D6 in Chinese. Drug Metab Dispos 1999, 27: 385-8

[16] Fujimaki M, Hakusui H, Hasegawa Y, Ajima H, Ota H, Igafashi S,Yamamura H. Pharmacokinetics of Carvedilol (DQ-2466) in Healthy Subjects. Jpn J Clin Pharmacol Ther 1990, 21: 415-424

[17] Carlson W, Oberg K. Clinical Pharmacology of Carvedilol. J Cardiovasc Pharmacol Ther 1999, 4: 205-218

[18] Jin C, Miners JO, Lillywhite KJ, Mackenzie PI. Complementary deoxyribonucleic acid cloning and expression of a human liver uridine diphosphate-glucuronosyltransferase glucuronidating carboxylic acid-containing drugs. J Pharmacol Exp Ther 1993, 264: 475-9

[19] Levesque E, Beaulieu M, Hum DW, Belanger A. Characterization and substrate specificity of UGT2B4 (E458): a UDP-glucuronosyltransferase encoded by a polymorphic gene. Pharmacogenetics 1999, 9: 207-16

[20] Saeki M, Saito Y, Jinno H, Tanaka-Kagawa T, Ohno A, Ozawa S, Ueno K, Kamakura S, Kamatani N, Komamura K, Kitakaze M, Sawada J. Single nucleotide polymorphisms and haplotype frequencies of UGT2B4 and UGT2B7 in a Japanese population. Drug Metab Dispos 2004, 32: 1048-54

[21] Lampe JW, Bigler J, Bush AC, Potter JD. Prevalence of polymorphisms in the human UDP-glucuronosyltransferase 2B family: UGT2B4(D458E), UGT2B7(H268Y), and UGT2B15(D85Y). Cancer Epidemiol Biomarkers Prev 2000, 9: 329-33

[22] Riedy M, Wang JY, Miller AP, Buckler A, Hall J, Guida M. Genomic organization of the UGT2b gene cluster on human chromosome 4q13. Pharmacogenetics 2000, 10: 251-60

[23] Akaba K, Kimura T, Sasaki A, Tanabe S, Ikegami T, Hashimoto M, Umeda H, Yoshida H, Umetsu K, Chiba H, Yuasa I, Hayasaka K. Neonatal hyperbilirubinemia and mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene: a common missense mutation among Japanese, Koreans and Chinese. Biochem Mol Biol Int 1998, 46: 21-6

[24] Maruo Y, Nishizawa K, Sato H, Doida Y, Shimada M. Association of neonatal hyperbilirubinemia with bilirubin UDP-glucuronosyltransferase polymorphism. Pediatrics 1999, 103: 1224-7

[25] Yamamoto K, Sato H, Fujiyama Y, Doida Y, Bamba T. Contribution of two missense mutations (G71R and Y486D) of the bilirubin UDP glycosyltransferase (UGT1A1) gene to phenotypes of Gilbert's syndrome and Crigler-Najjar syndrome type II. Biochim Biophys Acta 1998, 1406: 267-73

[26] Ando Y, Chida M, Nakayama K, Saka H, Kamataki T. The UGT1A1*28 allele is relatively rare in a Japanese population. Pharmacogenetics 1998, 8: 357-60

[27] Hall D, Ybazeta G, Destro-Bisol G, Petzl-Erler ML, Di Rienzo A. Variability at the uridine diphosphate glucuronosyltransferase 1A1 promoter in human populations and primates. Pharmacogenetics 1999, 9: 591-9

[28] Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci U S A 1998, 95: 8170-4

[29] Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 1995, 333: 1171-5

[30] Fisher MB, Vandenbranden M, Findlay K, Burchell B, Thummel KE, Hall SD, Wrighton SA. Tissue distribution and interindividual variation in human UDP-glucuronosyltransferase activity: relationship between UGT1A1 promoter genotype and variability in a liver bank. Pharmacogenetics 2000, 10: 727-39

[31] Hirota T, Ieiri I, Takane H, Sano H, Kawamoto K, Aono H, Yamasaki A, Takeuchi H, Masada M, Shimizu E, Higuchi S, Otsubo K. Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. Drug Metab Dispos 2003, 31: 677-80

[32] Bhasker CR, McKinnon W, Stone A, Lo AC,

Kubota T, Ishizaki T, Miners JO. Genetic polymorphism of UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significance. Pharmacogenetics 2000, 10: 679-85

[33] Holthe M, Klepstad P, Zahlsen K, Borchgrevink PC, Hagen L, Dale O, Kaasa S, Krokan HE, Skorpen F. Morphine glucuronide-to-morphine plasma ratios are unaffected by the UGT2B7 H268Y and UGT1A1*28 polymorphisms in cancer patients on chronic morphine therapy. Eur J Clin Pharmacol 2002, 58: 353-6

[34] Holthe M, Rakvag TN, Klepstad P, Idle JR, Kaasa S, Krokan HE, Skorpen F. Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. Pharmacogenomics J 2003, 3: 17-26

[35] Court MH, Krishnaswamy S, Hao Q, Duan SX, Patten CJ, Von Moltke LL, Greenblatt DJ. Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in human liver microsomes: specificity and influence of the UGT2B7*2 polymorphism. Drug Metab Dispos 2003, 31: 1125-33

[36] Sawyer MB, Innocenti F, Das S, Cheng C, Ramirez J, Pantle-Fisher FH, Wright C, Badner J, Pei D, Boyett JM, Cook E, Jr., Ratain MJ. A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. Clin Pharmacol Ther 2003, 73: 566-74

[37] Zhou HH,Wood AJ. Stereoselective disposition of carvedilol is determined by CYP2D6. Clin Pharmacol Ther 1995, 57: 518-24

[38] Honda M, Nozawa T, Igarashi N, Inoue H, Arakawa R, Ogura Y, Okabe H, Taguchi M, Hashimoto Y. Effect of CYP2D6*10 on the pharmacokinetics of R- and S-carvedilol in healthy Japanese volunteers. Biol Pharm Bull 2005, 28: 1476-9

[39] Giessmann T, Modess C, Hecker U, Zschiesche M, Dazert P, Kunert-Keil C, Warzok R, Engel G, Weitschies W, Cascorbi I, Kroemer HK, Siegmund W. CYP2D6 genotype and induction of intestinal drug transporters by rifampin predict presystemic clearance of carvedilol in healthy subjects. Clin Pharmacol Ther 2004, 75: 213-222