N-Acetyl Transferase 2 Polymorphisms Associated with Isoniazid Pharmacodynamics: Molecular Features for Ligand Interaction

KAZUTO OHKURA[1], KATSUMI FUKINO[1], YASUO SHINOHARA[2] and HITOSHI HORI[3]

[1]Faculty of Pharmacy, Chiba Institute of Science, 3 Shiomi-cho, Choshi, Chiba, 288-0025, Japan;
[2]Institute for Genome Research, The University of Tokushima, 3-8 Kuramoto-cho, Tokushima 770-8503, Japan;
[3]Department of Life System, Institute of Technology and Science,
The University of Tokushima Graduate School, 2 Minamijosanjima-cho, Tokushima 770-8506, Japan

Abstract. *Background: Isoniazid is mainly metabolized by arylamine N-acetyltransferase 2 (NAT2). Rapid acetylator types have NAT2*4/*4 alleles. Intermediate acetylator types have any of the following alleles: NAT2*4/*5, *4/*6, or *4/*7. Slow acetylator types do not have the NAT2*4 allele. We examined molecular features of these NAT2 molecules. Materials and Methods: Structures of NAT2*5, *6, and *7 were constructed based on X-ray data of human NAT2*4 using a molecular modeling technique. Results: The NAT2*4 molecule mostly occupied a positive electrostatic potential field. Ile^{114} and Arg^{197}, which are mutation sites of NAT2*4 to NAT2*5 and NAT2*6, were located in the peripheral part of the positive field. Gly^{286}, the mutation site from NAT2*4 to NAT2*7, was located near coenzyme A (CoA) in the boundary of the positive and negative fields. Conclusion: Nonbinding energies between NAT2s and isoniazid were larger than those of CoA. Molecular polymorphism appears to influence the reactivity between NAT2 and the external ligand.*

Isoniazid is a key drug in antituberculosis therapy, and is mainly metabolized by the arylamine *N*-acetyltransferase 2 (NAT2) system of the liver and small intestine (Figure 1). In the *NAT2* gene, 36 haplotypes have been identified so far (1, 2). NAT2s are classified into three groups, named rapid (RA), intermediate (IA), and slow acetylators (SA). Phenotypes of NAT2s depend on the number of active alleles (wild-type *NAT2*4*); RA, IA, and SA types have two, one, and no active alleles, respectively (3-5). In SA-type patients, isoniazid metabolism is slow and the serum isoniazid level becomes high on standard administration of isoniazid. In contrast, RA-type patients might be administered an increased dosage of isoniazid to achieve a sufficient therapeutic serum level (6, 7). Half of all Japanese people are the RA type, and the optimal dosage for RA patients is an important therapeutic factor (3, 8). The dose of isoniazid recommended by the World Health Organization of 5 mg/kg, with a range of 4-6 mg/kg, represents such a compromise (9). A dose of 3 mg/kg (150 mg for a 50-kg patient) will suffice to achieve the desired therapeutic effect of antituberculosis treatment in SA patients, but RA types require a dose of 6 mg/kg (300 mg for a 50-kg patient) to ensure optimal bactericidal activity (10).

Polymorphisms of *NAT2* alleles have been reported as follows: I^{114} to S (*NAT2*5*), R^{197} to Q (*NAT2*6*), and G^{286} to E (*NAT2*7*) (11). RA types have *NAT2*4/*4* alleles. IA-type individuals have any of the following alleles: *NAT2*4/*5, *4/*6,* or **4/*7*. SA types do not have *NAT2*4* alleles, and comprise *NAT2*5, *6,* or **7* alleles.

The main metabolic pathway of isoniazid involves the metabolism of isoniazid to acetyl isoniazid by NAT2 (Figure 1). Acetyl isoniazid produced in this pathway is then hydrolyzed by amidase to acetyl hydrazine. On the other hand, there is also a pathway in which isoniazid is hydrolyzed by amidase to hydrazine. It is possible that NAT2, cytochrome P450 2E1 (CYP2E1), and glutathione-*S*-transferase (GST) affect the blood concentrations of isoniazid and hydrazine. *NAT2* gene polymorphisms are involved with metabolism of these drugs. We analyzed the correlation between the genotype-associated molecular features of NAT2 and the serum isoniazid, acetyl isoniazid, hydrazine, and acetyl hydrazine levels, and discussed the effect of site mutation within the NAT2 molecule.

Materials and Methods

Molecular modeling and analysis. Molecular model of NAT2*5, *6, and *7 were constructed based on X-ray data of human wild-type

Correspondence to: Dr. Kazuto Ohkura, Faculty of Pharmacy, Chiba Institute of Science, 3 Shiomi-cho, Choshi, Chiba 288-0025, Japan. Tel: +81 479304785, Fax: +81 479304785, e-mail: kohkura@sag.bekkoame.ne.jp

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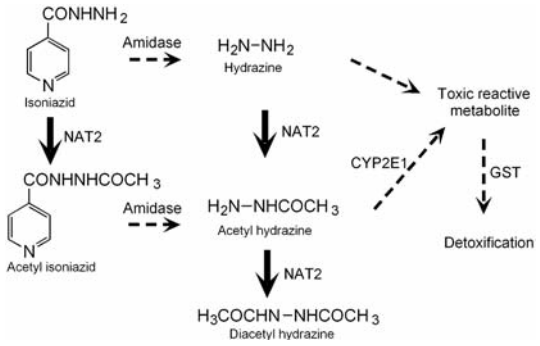

Figure 1. *Metabolic pathways of isoniazid in humans.*

Table I. *Genotype distribution of NAT2 in tuberculosis patients.*

	Genotype	n	%	
RA	*NAT2*4/*4*	60		46.5
IA	*NAT2*4/*5B*	2	1.6	
	*NAT2*4/*6A*	31	24.0	44.2
	*NAT2*4/*7B*	24	18.6	
SA	*NAT2*6A/*6A*	5	3.9	
	*NAT2*6A/*7B*	6	4.6	9.3
	*NAT2*7B/*7B*	1	0.8	
Total		129	100	

Table II. *Serum concentration of isoniazid (INH), hydrazine (Hz), acetyl isoniazid (AcINH), and acetyl hydrazine (AcHz). Samples were collected from the patients 2 hours after administration of INH (5 mg/kg), and the metabolites were detected (16). Results are the mean (±SE) of 129 patients.*

	RA	IA	SA
INH (μg/ml)	2.07±1.12	3.22±1.73	4.70±1.13
Hz (ng/ml)	22.15±9.23	27.02±12.46	35.23±14.44
AcINH (μg/ml)	3.68±1.59	2.55±1.30	1.00±0.65
AcHz (μg/ml)	2.46±1.98	2.15±1.30	1.03±0.62

Acetylator group: RA, rapid; IA, intermediate; SA, slow.

NAT2*4 (2PFR) using insightII-discover with homology module (Accelrys Inc., USA) (12). The energy minimization of models was performed using a consistence valence forcefield. Electrostatic potential fields of NAT2s were calculated, and the +1.0 kT/e (gray) and −1.0 kT/e (dark gray) contour was displayed (13). The z-matrix data for single nucleotide polymorphism (SNP)-involved regions of NAT2*4, *5, *6, and *7 (site 1: L^{110} - N^{118}, site 2: T^{193} - D^{201}, and site 3: K^{282} - I^{290} in Figure 2) were extracted from each NAT2, and molecular orbital analysis was performed with PM3 Hamiltonian using MOPAC (Fujitsu Ltd., Japan) (14). Solvation free energies (dGW) were determined from molecular orbital parameters (15). Nonbinding energies between ligands (acetyl CoA (2PFR), isoniazid (1W6F)) and NAT2s were determined using the discover module.

Results

Genotype distribution of NAT2s. The *NAT2* gene distribution of Japanese tuberculosis patients is summarized from reference 16 (Table I). The percentages of RA, IA, and SA were 46.5, 44.2, and 9.3%, respectively. For each acetylator type, the serum level of isoniazid and metabolites is summarized in Table II (16). In SA-type patients, hydrazine accumulation was observed, with a mean of 35.23±14.44 ng/ml being detected.

Molecular features of NAT2 models. The amino acid sequence of NAT2*4 and three mutation sites (bold letters) are shown in Figure 2. Molecular models of NAT2*5, *6, and *7 were constructed based on X-ray data of human NAT2*4. The wild-type NAT2*4 mostly occupied the positive electrostatic potential field, and the negative field was covered by the positive field (Figure 3). The CoA molecule was located in the boundary between the positive and negative electrostatic potential fields. Ile^{114} and Arg^{197}, which are mutation sites of NAT2*4 to *5 and *6, were located in the peripheral part of the positive field. Gly^{286}, the mutation site from NAT2*4 to *7, was located near CoA. NAT2*5, *6, and *7 molecules had the same electrostatic potential fields in each model (data not shown). Solvation free energy, which is an index of stereo-hydrophobicity (15), of the three mutation sites of NAT2*4 (underlined in Figure 2) was −1132.1 (site 1), −1900.5 (site 2), and −1498.7 (site 3) kJ/mol. At NAT2*5, *6, and *7 mutation sites, dGW values decreased to −1155.4, −2370.6, and −1843.2 kJ/mol, respectively, and the hydrophobicity of these sites significantly increased compared to NAT2*4. The differences in dGWs between wild-type NAT2*4 and mutated NAT2*5, *6, and *7 were 23.3, 470.1, and 344.5 kJ/mol, respectively. The absolute differential dGW value between CH_4 and $C_{120}H_{242}$ (model compounds) was 6.616 kJ/mol; thus, the dGW difference between wild-type and mutant NAT2s was very large.

Reactivity between NAT2s and ligands. Nonbinding energy between wild-type NAT2*4 and CoA, which is the native cofactor for NAT2, was −41.7 kcal/mol. The nonbinding energies between mutated NAT2*5, *6, and *7 and CoA was the same as that of NAT2*4. The mutation of NAT2 sites 1-3 did not affect reactivity of NAT2 with the CoA molecule. Nonbinding energies between NAT2s and isoniazid were larger than those of CoA, and the order of energy was NAT2*4 (1.91×10^3)<*5, *6 (8.83×10^5)<*7 (1.34×10^6 kcal/mol). Thus, the molecular polymorphism influenced the reactivity between NAT2 and the external ligand.

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  1 SDIEAYFERI GYKNSRNKLD LETLTDILEH QIRAVPFENL NMHCGQAMEL GLEAIFDHIV 60
 61 RRNRGGWCLQ VNQLLYWALT TIGFQTTMLG GYFYIPPVNK YSTGMVHLLL QVTIDGRNYI 120
121 VDAGSGSSSQ MWQPLELISG KDQPQVPCIF CLTEERGIWY LDQIRREQYI TNKEFLNSHL 180
181 LPKKKHQKIY LFTLEPRTIE DFESMNTYLQ TSPTSSFITT SFCSLQTPEG VYCLVGFILT 240
241 YRKFNYKDNT DLVEFKTLTE EEVEEVLKNI FKISLGRNLV PKPGDGSLTI 290

NAT2*5(site 1): I114→S, NAT2*6(site 2): R197→Q, NAT2*7(site 3): G286→E
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Figure 2. *Amino acid sequence of NAT2. Mutation sites of NAT2*4 are shown bold letters. The regions for which solvation free energies were determined are shown underlined.*

Discussion

The titer is a very important factor in drug therapy (*e.g.* tuberculosis treatment). Some types of SNP exist in NAT2 due to gene mutation (1, 2). Subtypes of NAT2s have a low isoniazid-metabolizing ability, and difficulty arises from serum hydrazine accumulation (6, 7). *NAT2* gene polymorphism analysis revealed that 46.5% of tuberculosis patients were RA, 44.2% were IA, and 9.3% were SA. NAT2*5, *6, and *7 are typical in that mutations of *NAT2* genes are reflected in the protein level. These mutant NAT2 molecules were modeled based on the wild-type NAT2*4 structure, and their molecular features were analyzed. As a result, the electrostatic potential field distribution was almost unchanged on site mutation, but the stereo-hydrophobicity of mutated regions (sites 1-3) significantly increased. This result indicates that the reactivity between NAT2s and ligands should also be changed. Indeed, the reaction (nonbinding energy) toward isoniazid of wild-type NAT2*4 (1.91×10^3 kcal/mol) decreased by an order of 2 to 3 (8.8×10^5 - 1.34×10^6 kcal/mol) in mutated NAT2*5, *6, and *7. Polymorphism of *NAT2* is a prominent example of the fact that drug-metabolizing ability is markedly altered by mutation of only one amino acid. The difference in the dGW value between CH_4 and $C_{120}H_{242}$ is 6.616 kJ/mol. In *NAT2* mutants, the stereo-hydrophobic parameter dGW significantly changed, and the difference in dGW values between wild-type and mutated NAT2 was 23.3-470.1 kJ/mol. The effect on stereo-hydrophobicity due to the NAT2 mutation site is great regarding reactivity to the ligand. Mutation of site 3, located at the boundary region between positive and negative electrostatic potential fields, mainly influenced the NAT2 activity, and the nonbinding energy significantly changed (NAT2*7: 1.34×10^6 kcal/mol). From these findings, the boundary region of the electrostatic field in the NAT2 molecule seems to affect drug metabolism. At present, this is being investigated.

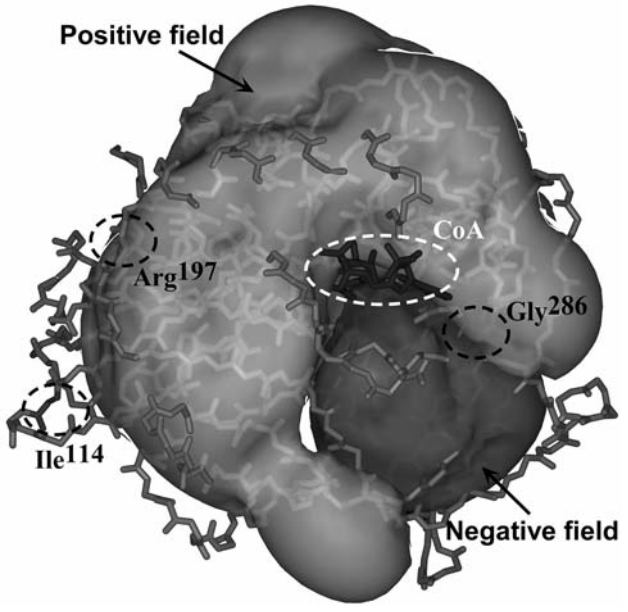

Figure 3. *Electrostatic potential field of NAT2*4. Positive and negative fields are shown gray and dark gray clouds, respectively. Ligand CoA molecule and three mutation sites (Ile^{114}, Arg^{197}, Gly^{286}) are shown.*

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