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N-Acetyltransferase 2 Genotype-Related Efficacy of Sulfasalazine in Patients with Rheumatoid Arthritis

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Purpose. For the individual optimization of drug therapy with sulfasalazine (SASP), we studied the influence of the *N-acetyltransferase 2* (*NAT2*) genotype on the pharmacokinetics, efficacy, and incidence of adverse reactions of SASP in patients.

Methods. Ninety-six rheumatoid arthritis (RA) patients were treated or had been treated with 0.5 and/or 1.0 g/day of SASP. The wild-type allele (*NAT2*4*) and three variant alleles (*NAT2*5B*, **6A,* and **7B*) of *NAT2* were determined by the polymerase chain reaction-restriction fragment length polymorphism method. Plasma concentrations of SASP and its two metabolites, sulfapyridine (SP) and *N*-acetylsulfapyridine (AcSP), were estimated by HPLC. Therapeutic efficacy and incidence of adverse reactions were also monitored as recommended by the American College of Rheumatology.

Results. Patients were classified into three groups by *NAT2* genotyping: Rapid Type (homozygote for *NAT2*4*), Intermediate Type (heterozygote for *NAT2*4* and variant alleles), and Slow Type (homozygote for variant alleles). There was no clear difference in the genotype frequencies between RA patients and healthy subjects. *NAT2* genotypes significantly affected both the plasma concentration ratios of SP to AcSP (SP/AcSP) and the efficacy of SASP ($p < 0.05$). Adverse reactions to SASP were found in 26 (27.1%) out of 96 patients, and there was no difference among the three genotype groups.

Conclusions. *NAT2* gene polymorphism is related to the plasma SP/AcSP ratio and the efficacy of SASP.

KEY WORDS: *N*-acetyltransferase 2; genotype; sulfasalazine; rheumatoid arthritis.

INTRODUCTION

Sulfasalazine (SASP), one of the disease-modifying antirheumatic drugs (DMARDs), has long been used in the treatment of rheumatoid arthritis (RA) (1). Many placebo-controlled studies have shown that SASP improves the erythrocyte sedimentation rate, duration of morning stiffness, pain (as assessed by visual analogue scale), articular index, number of swollen joints, number of painful joints, and patient's global assessment.

SASP is 5-aminosalicylic acid (5-ASA) linked by an azo bond to sulfapyridine (SP) (2). When orally administered, 30% of SASP is absorbed in the upper gastrointestinal tract, and the remainder is split in the colon by bacterial action into 5-ASA and SP (2,3). The 5-ASA remains largely within the large bowel, but the SP is totally absorbed and metabolized to N-acetylsulfapyridine (AcSP) predominantly by hepatic arylamine N-acetyltransferase 2 (NAT2). Both SASP and SP are considered to have a variety of actions, including immunomodulatory effects, antibacterial activity, and inhibition of folate-dependent enzymes (2–5). Adverse reactions such as nausea, vomiting, headache, malaise, hemolytic anemia and reticulocytosis appear to be dependent on the serum SP concentration (3,6–9). The *NAT2* gene exhibits a hereditarily determined polymorphism, and the individual phenotypes can be classified as rapid, intermediate, and slow acetylators by using isoniazid and sulfametazine as probe drugs (10). Several reports have described the relationship between the acetylator phenotypes and the pharmacokinetics, efficacy, and toxicity of SASP in the treatment of RA and inflammatory bowel diseases (IBD) (6–9,11–16).

In 1990, Deguchi *et al*. suggested that four *NAT2* alleles, including a wild-type allele (*allele1*) and three variant alleles (*alleles 2*, *3,* and *4*) with a single nucleotide polymorphism (G857A, G590A, and C481T, respectively), could predict the acetylator phenotypes of isoniazid in healthy Japanese subjects (17,18). Then, we reported that individual acetylator phenotypes for procainamide and SASP in healthy subjects, and also for isoniazid in tuberculous patients, correlated with the combination of these four alleles (19–22). In addition, *NAT2* genotypes were shown to be associated with the capacity for SP acetylation and the incidence of adverse reactions in the SASP treatment of 13 patients with IBD (21).

Although several reports of the importance of acetylator phenotype or *NAT2* genotypes in SASP therapy have involved RA and IBD patients, there was no obvious conclusion concentrating the classification of subjects based on their *NAT2* genotype (11–14,21). To achieve this, further investigations of the relationships between the *NAT2* genotypes and

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ABBREVIATIONS: SASP, sulfasalazine; RA, rheumatoid arthritis; PCR-RFLP, polymerase chain reaction- restriction fragment length polymorphism; SP, sulfapyridine; AcSP, *N*-acetylsulfapyridine; DMARDs, disease-modifying antirheumatic drugs.

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efficacy or incidence of adverse reactions during SASP treatment were required in a large numbers of patients. In the present study, we studied the influence of the *NAT2* genotype on the pharmacokinetics, efficacy, and/or rate of adverse reactions of SASP in 96 patients with RA.

METHODS

Patients and Treatment

Ninety-six patients were randomly selected from medical records at five hospitals. All patients fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 criteria for RA and were being treated or had been treated with 0.5 g/day and/or 1.0 g/day of SASP in the form of enteric-coated Azulfizine® EN tablets (Pharmacia K. K., Tokyo, Japan). Nonsteroidal antiinflammatory drugs, prednisolone less than 7.5 mg/day, and methotrexate were coadministrated with SASP in the case of 69, 58, and 20 patients. Patients taking other DMARDs or immunosuppressive drugs were excluded from the study. Significant liver damage was not observed before treatment in all patients.

For 89 patients, the efficacy of SASP was determined by the physician's global assessment using a 6-scale grade according to the ACR criteria, and both grades 1 and 2 were considered to be effective (23). Adverse reactions were obtained from clinical symptoms and laboratory test data in the medical records. The aims of this study were fully explained to each patient who gave written informed consent. About 2 ml blood and plasma samples were stored below −20°C and sent to Kobe University Hospital. The following study protocol was approved by the Ethics Committee of each University.

NAT2 Genotyping

The most common mutations in the Japanese population at positions C481T, G590A, and G857A of the *NAT2* gene were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described previously (19,21,22). According to the nomenclature of the *NAT2* gene, wild-type and three variant alleles were defined as *NAT2*4* and **5B*, **6A*, **7B*.

Briefly, each genomic DNA was extracted from 0.5 ml whole blood using a DNA Extractor WB Kit (Wako Pure Chemical Industries Ltd., Osaka, Japan). The three parts of the *NAT2* gene were amplified with a pair of primers for each, using a programmable heat block (Program Temp Control System PC-700, Astec Co., Fukuoka, Japan). To establish the presence or absence of each single nucleotide polymorphism, PCR products were digested with *Kpn* I, *Taq* I or *BamH* I, and the fragments were separated on 1.5%, 2%, or 2% agarose gels, respectively. Patients can be stratified by this genotyping into three groups: the homozygote for the wild-type allele *NAT2*4/*4* (named Rapid Type), the compound heterozygote for the wild-type and variant alleles *NAT2*4/*5B*, *NAT2*4/*6A,* and *NAT2*4/*7B* (named Intermediate Type), and the homozygotes for the variant alleles *NAT2*5B/*5B*, *NAT2*5B/*6A*, *NAT2*5B/*7B*, *NAT2*6A/*6A*, *NAT2*6A/*7B,* and *NAT2*7B/*7B* (named Slow Type). One hundred eighty healthy subjects also acted as controls for *NAT2* genotyping.

Determination of SASP, SP, and AcSP Concentrations

Twenty-seven blood samples (n = 17, 7, and 3 for Rapid, Intermediate, and Slow Types) were collected once 1–5 h after SASP administration and were immediately centrifuged at 3000 rpm (950 × *g*) for 5 min to separate the plasma, which was stored at −20°C until assay of the SASP, SP, and AcSP concentrations. It was confirmed that there was no alteration in the concentrations during freezing, and SP and AcSP concentrations in plasma were measured by HPLC (LC-10A series, Shimadzu Co., Kyoto, Japan) (21,22). The separation was carried out using a reversed-phase column (Nucleosil 10 C_{18}, 250 mm × 4.0 mm i.d., Chemco Chemical Co. Ltd., Osaka, Japan). The mobile phase was methanol/20 mM NaH_2PO_4 containing 20 mM tetra-*n*-butylammonium (30/70, vol/vol). The flow rate was 1.5 ml/min, and the column temperature was maintained at 40°C in a column oven (CT10A, Shimadzu). The calibration curves were linear over a concentration range of 0.5 to 100.0 μg/ml ($r^2 > 0.997$) for SASP, SP, and AcSP.

Statistical Analysis

Fisher's exact test and χ^2 test were used to compare the frequencies of efficacy and adverse reactions, and the Mann-Whitney *U* test was used to compare the other parameters among patient subgroups.

RESULTS

NAT2 Genotype

The frequencies of the *NAT2* genotypes in 96 Japanese RA patients and 180 healthy Japanese subjects are shown in Table I. The allele frequencies of *NAT2*4*, *NAT2*5B*, *NAT2*6A*, and *NAT2*7B* were 73.5, 0.0, 20.8, and 5.7% in RA patients and 71.1, 1.9, 18.1, and 8.9% in healthy subjects, respectively. The genotype and allele frequencies of the *NAT2* gene were not significantly different between the groups. The frequencies of the three *NAT2* genotype groups, Rapid Type, Intermediate Type, and Slow Type, in RA patients (55.2, 36.4, and 8.3%) were also similar to those in healthy subjects (48.3, 45.5, and 6.2%, respectively).

Table I. Frequencies of *NAT2* Genotypes in 96 Patients with RA and 180 Healthy Subjects

NAT2 genotype		Number (%)	
Group	Genotype	RA patients	Healthy subjects
Rapid	*NAT2*4/*4*	53 (55.2)	87 (48.3)
Intermediate	*NAT2*4/*5B*	0 (0.0)	6 (3.3)
Intermediate	*NAT2*4/*6A*	27 (28.1)	54 (30.0)
Intermediate	*NAT2*4/*7B*	8 (8.3)	22 (12.2)
Slow	*NAT2*5B/*7B*	0 (0.0)	1 (0.6)
Slow	*NAT2*6A/*6A*	5 (5.2)	3 (1.7)
Slow	*NAT2*6A/*7B*	3 (3.1)	5 (2.8)
Slow	*NAT2*7B/*7B*	0 (0.0)	2 (1.1)
	Total	96 (100)	180 (100)

Plasma Concentrations of SASP, SP, and AcSP

Figure 1 shows the plasma concentrations of SASP, SP, and AcSP obtained 1–5 h after SASP administration in 27 RA patients. In each *NAT2* genotype group, these plasma concentrations exhibited substantial interindividual variation. Plasma concentration ratios of SP/AcSP less than 0.2 were seen in 82.4% of the Rapid Types and in 42.9% of the Intermediate Types; however, the figure for the Slow Types was 0.0% (Fig. 2). In contrast, plasma SP/AcSP ratios over 0.4 were seen in 0.0% of the Rapid Types, 28.6% of the Intermediate Types, and 33.3% of the Slow Types. Factorial analysis by the variance method showed that the *NAT2* genotype affected the plasma SP/AcSP ratios significantly (p = 0.049); however, the dose of SASP had no effect (p = 0.6847).

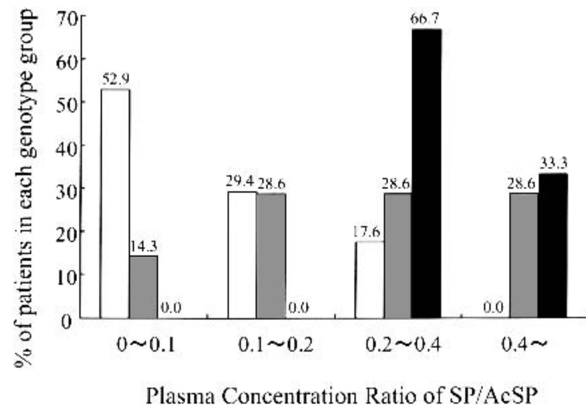

Fig. 2. Plasma concentration ratio of SP/AcSP from 1 to 5 h after SASP administration in three groups classified according to the *NAT2* genotypes: 17 Rapid Types (open bars), 7 Intermediate Types (gray bars), and 3 Slow Types (solid bars). Each column shows the percentage of patients in each genotype group.

Efficacy

Patient profiles of each *NAT2* genotype group are shown in Table II. Sex, disease duration, and SASP dose were not significantly different in the three groups. The duration of SASP treatment in the Slow Types was much shorter compared with the other two types ($p < 0.05$ by Mann-Whitney *U* test). In Table III, the physicians' global assessment showed that SASP was effective in 48 of 89 patients (53.9%). In each genotype group, all patients who were Slow Types benefited, and this was significantly higher than in the case of the Rapid Types ($p < 0.01$) or Rapid Types plus Intermediate Types ($p < 0.05$).

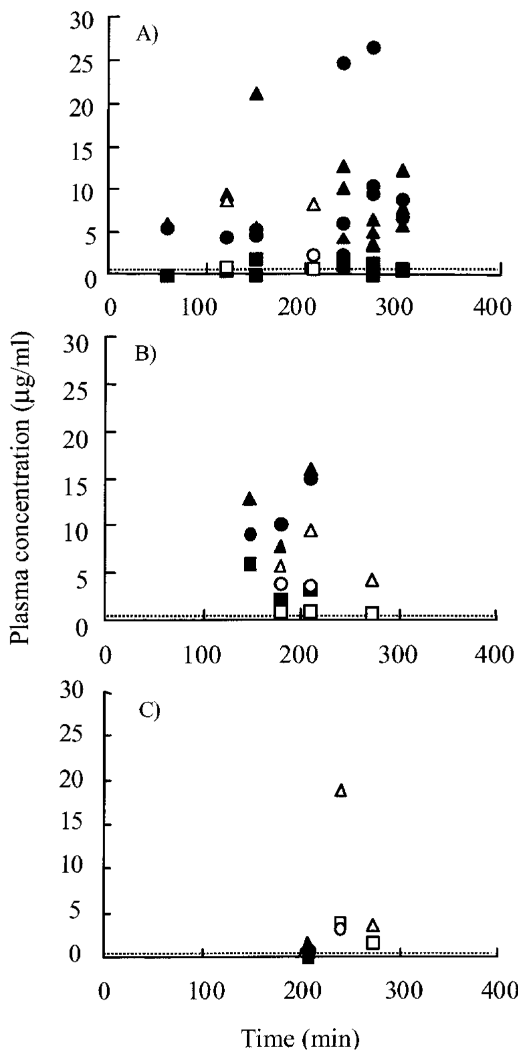

Fig. 1. Plasma concentrations of SASP (circles), SP (squares), and AcSP (triangles) after SASP administration in three groups classified according to *NAT2* genotypes: 17 Rapid Types (A), 7 Intermediate Types (B), and 3 Slow Types (C). Doses of SASP, 0.5 g/day and 1.0 g/day, are shown by open and closed symbols, respectively. Dotted lines (0.5 μg/ml) show the lower limits of SASP, SP, and AcSP.

Adverse Reactions

The total number of adverse reactions found in 96 patients was 34 (Table IV). Adverse reactions associated with SASP were found in 26 patients (27.1%), and the patients withdrawing from treatment because of adverse reactions were as follows: seven Rapid Types (13.2%), two Intermediate Types (5.7%), and two Slow Types (25.0%). Cutaneous symptoms (skin rash and/or itching) occurred in seven Rapid Types (13.2%), eight Intermediate Types (22.8%), and one Slow Type (12.5%). In contrast, gastrointestinal symptoms (nausea and/or vomiting) occurred in three Rapid Types (5.7%), three Intermediate Types (8.6%), and one Slow Type (12.5%). Systemic hypersensitivity with high fever, dyspnea, and decreased consciousness level was seen in one Rapid Type patient. The ratio of each adverse reaction was not significantly different among the three groups. Combination with methotrexate and/or prednisolone did not affect the occurrence of adverse reactions.

DISCUSSION

More than 50 years ago, NAT2 was first found to exhibit polymorphisms in several hepatic drug-metabolizing enzymes (24,25). Then, the phenotyping method using probe drugs was developed, and this led to studies on the relationships between the acetylator phenotypes and pharmacokinetics, efficacy, and/or toxicity. To date, at least 17 mutant alleles have been found in the human *NAT2* gene; however, genotyping of three *NAT2* point mutations was sufficient to predict the metabolism of INH in Japanese tuberculous patients as well as healthy subjects (20). Compared with phenotyping, genotyp-

Table II. Patient Profiles of Three Groups Classified According to the *NAT2* Genotypes

	Total	*NAT2* genotype: Rapid *NAT2*4/*4*	Intermediate *NAT2*4/*6A* *NAT2*4/*7B*	Slow *NAT2*6A/*6A* *NAT2*6A/*7B*
Number of patients	96	53	35	8
Male/female	20/76	13/40	5/30	2/6
Age (year)	58.0 ± 12.7	57.6 ± 13.2	58.6 ± 12.0	58.3 ± 13.7
Disease duration (year)	7.7 ± 6.4	6.3 ± 4.7	9.8 ± 8.3	8.4 ± 4.7
SASP 0.5 (g/day)/1.0 (g/day)	26/70	14/39	9/26	4/4
Duration of treatment (month)	13.1 ± 8.4	12.4 ± 8.0	15.8 ± 8.4	5.8 ± 4.6#
PSL treatment	58	31	22	6

# $p < 0.05$ compared with the Rapid Type and Intermediate Type by the Mann-Whitney *U* test.

ing is a simple and rapid technique and more reliable for typing patients with renal, hepatic, and gastrointestinal disorders. However, the relationships between the *NAT2* genotypes and pharmacokinetics, efficacy, or toxicity have not been studied in detail in the case of SASP therapy.

Both genotype and allele frequencies of the *NAT2* gene were not significantly different between RA patients and healthy subjects (Table I). Some data have indicated that acetylation influences the process of inactivation of excessive biogenic amines, including histamine, which is responsible for allergic reaction symptoms (26–28). Moreover, some research has suggested that the Slow Type is an important factor in the individual susceptibility to rheumatoid arthritis (29). However, our results show that acetylator status is not involved in the onset of RA.

In spite of the classification of *NAT2* genotypes, the plasma concentrations of SASP, SP, and AcSP (1–5 h) exhibited substantial interindividual variations (Fig. 1), which might be caused by poor (10–30%) and slow absorption of SASP (15). In contrast, the acetylation index (10), the plasma SP/AcSP ratio, correlated with the *NAT2* genotypes (Fig. 2). The period of 1–5 h represents the elimination phase of both SP and AcSP because the mean absorption lag time of SP is 6 h, and the mean time to reach the maximum plasma concentrations of SASP and SP are 6 and 12–14 h, after the multiple administration of 1.0 g SASP in healthy Japanese subjects (30). The pharmacokinetics of SASP is dose linear in the dose range of 0.5 to 2.0 g following a single administration in a healthy subject. Also, in our previous study with healthy subjects, the plasma SP/AcSP ratios of the Rapid Types [0.24 (mean value; $n = 4$)] were significantly lower than the Intermediate Types [0.53 ($n = 3$)] at 24 h after multiple administration of 1.0 g SASP (22). Therefore, the plasma SP/AcSP ratio was affected by the *NAT2* genotype and not by the dose of SASP, coadministered drugs, coexisting diseases, age, etc.

Although several reports of the dependence of acetylator phenotype or *NAT2* genotypes in the SASP therapy have involved RA and IBD patients, no conclusion was reached concentrating the classification of the subjects based on their *NAT2* genotype (11–14,21). In the present study, 0.5 and/or 1.0 g/day of SASP was administered to RA patients, which was lower than other previous reports (1.0–3.0 g/day) (12–14). As a result, the physicians' global assessment showed that Slow Types (100.0%) benefited more than Intermediate Types (63.6%) and Rapid Types (40.8%) (Table III). It should be noted that the efficacy of SASP treatment was more effective in Slow Types, although the number of subjects was small. This seems to be consistent with the shorter duration of SASP treatment in Slow Types compared with the other two Types. These findings suggest that the *NAT2* genotype is an important factor in determining the efficacy of SASP in RA patients.

Table III. Efficacy of SASP in Three Groups Classified According to the *NAT2* Genotypes

	Total n = 89	*NAT2* genotype: Rapid *NAT2*4/*4* n = 49	Intermediate *NAT2*4/*6A* *NAT2*4/*7B* n = 33	Slow *NAT2*6A/*6A* *NAT2*6A/*7B* n = 7
0.5 g/day				
Grade 1	1/24	0/13	1/8	0/3
Grade 2	13/24	5/13	5/8	3/3
Subtotal	14/24 (58.3%)	5/13 (38.5%)	6/8 (75.0%)	3/3 (100.0%)
1.0 g/day				
Grade 1	8/65	4/36	2/25	2/4
Grade 2	26/65	11/36	13/25	2/4
Subtotal	34/65 (52.3%)	15/36 (41.7%)	15/25 (60.0%)	4/4# (100.0%)
Total	48/89 (53.9%)	20/49 (40.8%)	21/33 (63.6%)	7/7##,* (100.0%)

# $p < 0.05$ and ##$p < 0.01$ compared with the Rapid Type by Fischer's exact test.
* $p < 0.05$ compared with the Rapid Type + Intermediate Type by Fischer's exact test.

Table IV. Adverse Reactions of SASP in Three Groups Classified According to the *NAT2* Genotypes

	NAT2 genotype			
	Total (n = 96)	Rapid (n = 53)	Intermediate (n = 35)	Slow (n = 8)
Patient number of adverse reactions				
0.5 g/day SASP	11/26	4/14	6/9	1/4
1.0 g/day SASP	15/70	9/39	5/26	1/4
Total	26 (27.1%)	13 (24.5%)	11 (31.4%)	2 (25.0%)
Withdrawal because of toxicity	11 (11.5%)	7 (13.2%)	2 (5.7%)	2 (25.0%)
Adverse reactions				
Cutaneous#	16 [6]	7 [4]	8 [1]	1 [1]
Gastrointestinal##	7 [3]	3 [1]	3 [1]	1 [1]
Elevated liver enzymes	3 [1]	3 [1]	0	0
Hematological###	1	1	0	0
Cardiovascular####	2	0	1	1
Systemic hypersensitivity	1 [1]	1 [1]	0	0
Others	4	1*	3**	0

Rash and/or itching; ##nausea and/or vomiting; ###leucopenia; ####palpitation.
[ ] = numbers of patients withdrawn.
* Anemia; **dry mouth, ischemic heart disease, or diabetes mellitus.

In general, the adverse reactions of SASP were divided into two groups. One involves hypersensitive reactions, such as skin rash, aplastic anemia, and hepatic and pulmonary dysfunctions, which are independent of the dose of SASP or blood concentration (31). The other adverse reactions are dependent on the dose of SASP or blood concentration of SP (> 50 μg/ml), such as nausea, vomiting, headache, malaise, hemolytic anemia, and reticulocytosis (6). In the phenotyping study, a higher incidence of nausea/vomiting and raised hepatic enzymes was seen in slow acetylators (14). As far as our results are concerned, no correlation between the *NAT2* genotype and the overall toxic reactions of SASP was seen, although the rate exhibited a trend: Rapid Type < Intermediate Type < Slow Type (Table IV). This discrepancy may be explained by the low incidence of adverse reactions caused by the low dose (0.5 and/or 1.0 g/day) of SASP given in the form of enteric-coated tablets. Small numbers of the Slow Type, 8 out of 96 patients, make it difficult to evaluate the influence of the *NAT2* genotype on toxicity. As in previous reports, we observed hypersensitive reactions in all genotype groups with the same incidence.

In summary, we conducted the *NAT2* genotyping of wild-type and three variant alleles in Japanese RA patients. The *NAT2* gene polymorphism has no influence on the onset of RA or on the incidence of adverse reactions to SASP, although there was an effect on the plasma SP/AcSP ratio and the efficacy of SASP. These data suggest that *NAT2* genotyping could become a useful alternative to therapeutic drug monitoring for SASP. A further prospective study will be required to determine the effective and safe dosage regimen of SASP for RA, which can be decided based on the *NAT2* genotype of individual patients.

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