Plasma concentrations of sulfadoxine in healthy and malaria infected Thai subjects

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Summary

The disposition of sulfadoxine was studied in the presence of pyrimethamine in 18 healthy Thai subjects who had been suffering from falciparum malaria in the 6 months prior to the study, and in 12 Thai patients with acute malaria. The volunteers were administered an oral dose of 500 mg sulfadoxine + 25 mg pyrimethamine (1 Fansidar tablet). They were classified retrospectively as responders (Group I, n = 8) or nonresponders (Group II, n = 10) according to previous response to treatment with Fansidar. The patients were treated with 3 Fansidar tablets corresponding to 1500 mg sulfadoxine and 75 mg pyrimethamine. Five of them were completely cured. Seven patients showed R I or R II resistance. In all cases blood samples were collected up to 288 h post dose. The resultant plasma was analyzed for active (i.e. unchanged) and total sulfadoxine using a modified Bratton-Marshall method.

In the healthy volunteers the plasma concentration time course of total sulfadoxine was similar for responding and nonresponding subjects. However, in nonresponders active sulfadoxine tended to show shorter half-lives (harmonic means were 212 h vs 267 h, respectively). Furthermore, significantly higher amounts of metabolites (mainly N_4-acetylsulfadoxine) were present in plasma of nonresponders. In contrast to these findings, in malaria patients, plasma concentrations of active and total sulfadoxine were even higher in nonresponders as compared to the subjects who could be successfully cured. Furthermore, in this case there was no increase of the amount of metabolites in plasma.

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Generally, the elimination half-lives of sulfadoxine in the patients were shorter and the plasma levels were sligthly lower than would be predicted from the results in volunteers. This difference may be due to the disease state, possibly related to a change in the protein binding. In conclusion, the present study did not reveal a direct relationship between the therapeutic response, metabolic disposition and plasma concentrations of sulfadoxine.

Key words: pyrimethamine; sulfadoxine; Fansidar; malaria; bioavailability.

Introduction

An increasing number of failures with Fansidar treatment in patients suffering from falciparum malaria have been reported from South East Asia, South America and East Africa (De Souza, 1980; Fernex, 1981; Höfler, 1980; Holzer et al., 1980; Holzer, 1985; MMWR, 1980). These failures to antiparasitaemic response may be attributed to the development of resistance in the malaria parasite or to host factors which can influence the therapeutic efficacy (Holzer, 1985).

The present study was designed in order to investigate to what extent the observed failures are caused by metabolic deviations of the host. If the observed therapeutic failures during Fansidar treatment are due to rapid acetylation of sulfadoxine, one should find in such subjects lower concentrations of active sulfadoxine, together with an increased ratio of metabolized compared to total sulfadoxine in plasma. Considering the incomplete knowledge about plasmodial resistance mechanisms and drug metabolism it is of particular interest to compare plasma concentrations and pharmacokinetic parameters of sulfadoxine in responding and non-responding volunteers and in patients suffering from symptomatic falciparum malaria.

Subjects, Materials and Methods

Subjects

Eight healthy volunteers who in the last 6 months prior to this study had been suffering from falciparum malaria and who could successfully be treated with a single dose of 3 tablets Fansidar were selected and classified as responders for this trial (Group I, Table 1).

Ten volunteers who in the last six months prior to this study had been suffering from falciparum malaria and could not be successfully cured with a single dose of 3 tablets (i.e. RII or RIII response) represented the group of "nonresponders" (Group II).

At the beginning of this study the blood films of all participants were negative for plasmodia. Physical examination and clinical data showed all subjects to be in good health.

In addition, 12 patients suffering from symptomatic falciparum malaria (proven by positive blood film) were also included into this trial. The initial parasite counts ranged from 600 to 68172/μl.

A satisfactory parasitaemic response occurred in five cases (Group III). In these individuals, clearance of the asexual blood forms of *P. falciparum* was obtained within one day. No recrudescence occurred in these cases. Four patients showed a clearance of asexual parasitaemia within two to six

Table 1. Subject characteristics, mean and range

| | Volunteers* | | Patients** | |
	Group I Responders (n = 8)	Group II Nonresponders (n = 10)	Group III Responders (n = 5)	Group IV Nonresponders (n = 7)
Age (years) ...	27 (19–37)	30 (19–42)	21 (16–27)	23 (19–36)
Weight (kg) ...	58 (48–75)	59 (54–70)	50 (41–53)	57 (52–62)
Height (cm) ..	164 (149–180)	167 (158–175)	160 (158–170)	168 (164–170)
Gender	♂	♂	♂	♂

* Response to Fansidar treatment as assessed on a previous infection.
** Response of parasitaemia to treatment with Fansidar as assessed in the present study.

days, followed by recrudescence on day 11 to 22 (R I type of resistance). The remaining three patients did not exhibit a satisfactory response (R II - type). These latter 7 patients were in Group IV.

The participants were male, ranging in age from 16 to 42 years and in weight from 41 to 75 kg (Table 1). This study was conducted in accordance with the guidelines of the declaration of Helsinki, concerning ethics in experimentation in humans. Informed consent was obtained prior to the study. Persons consuming more than 10 cigarettes per day and with impaired renal and hepatic function or gastrointestinal disorders were excluded. Volunteers in Groups I and II were selected from records in Rajavidhi Hospital and in malaria centres at Chiang Mai, Phrabuddhabat Hospital, Saraburi, Thailand. Volunteers as well as patients were instructed to avoid co-medication, especially sulfon-amides. Subjects vomiting within 21 h following administration of test drugs were excluded from this study. Consumption of alcoholic beverage was avoided the day before receiving the test drug and during the first two days of the study.

Experimental procedure

Fansidar tablets containing 500 mg sulfadoxine and 25 mg pyrimethamine were used as the test drug. One tablet Fansidar was administered to each volunteer following an overnight fast. (One subject was given 2 tablets corresponding to 1000 mg sulfadoxine. In this case, the resultant plasma levels were normalized to a 500 mg dose for further evaluation.) For malaria patients 3 tablets of Fansidar were given as a single dose. Blood samples (10 ml) were collected immediately before drug administration and 0.5, 1.5, 3, 6, 8, 24, 32, 48, 72, 120, 168, 216 and 288 h after the medication using EDTA as an anticoagulant. The plasma was separated by centrifugation and stored deep frozen until analyzed.

Analytical methods

Total sulfadoxine (unchanged and metabolites, mainly represented by N-acetylsulfadoxine) and active sulfadoxine were determined in plasma using a modified Bratton-Marshall method (Rieder, 1972). The lower limit of sensitivity was 1 μg/ml. The coefficient of variation was 3 to 7% in the concentration range from 10 to 100 μg/ml.

Data evaluation

Drug disposition was characterized by model independent parameters. The maximum plasma concentrations of total and active sulfadoxine ($C_{m(T)}$ and $C_{m(A)}$, respectively) and the time of their occurrence (t_m) were taken directly from the observed plasma concentrations. The rate constant of the terminal elimination phase (λ_z) was determined by linear regression from the log-linear slopes of the plasma concentration curves of the active compound. The corresponding apparent half-life was calculated according to $t_{1/2 (A)} = \ln 2 \lambda_z$.
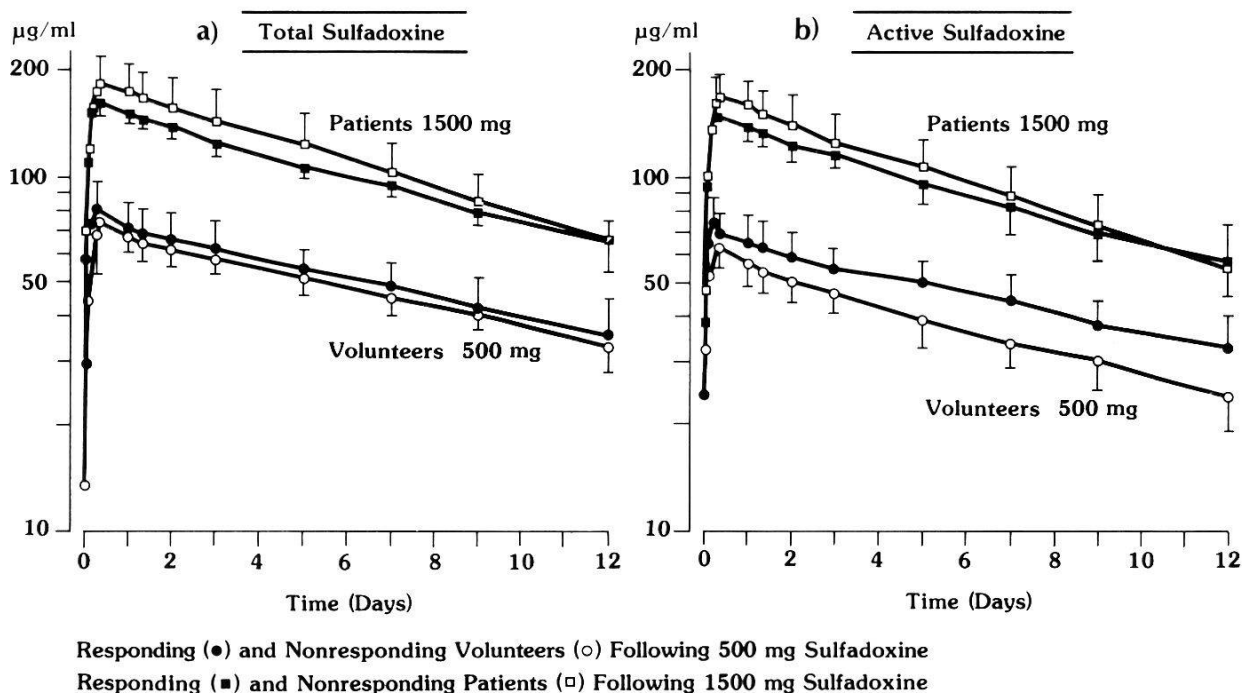
Responding (●) and Nonresponding Volunteers (○) Following 500 mg Sulfadoxine
Responding (■) and Nonresponding Patients (□) Following 1500 mg Sulfadoxine

Fig. 1. Plasma concentrations of total and active sulfadoxine (mean values ± S. D.) in responding and nonresponding volunteers and patients following an oral dose of 500 mg and 1500 mg, respectively.

The areas under the plasma concentrations of total and active drug (AUC_T and AUC_A) were obtained using the trapezoidal rule up to the last measured time. The percentage of sulfadoxine metabolized was defined as the ratio of the average concentrations of metabolized and total drug within the period up to the last measuring time: Perc. met. = $[(\overline{C}_{tot} - \overline{C}_{active})/\overline{C}_{tot}) * 100$. This relationship was obtained from the corresponding AUC values according to the equation $\overline{C}_{met}/\overline{C}_{tot} = (AUC_T - AUC_A)/AUC_T$.

For the calculation of the disposition parameters of active sulfadoxine, the AUC was extrapolated to infinity using the parameters of the regression line of the terminal plasma level time course. The oral plasma clearance was derived from the equation $Cl/F = Dose/AUC_0^\infty$, where F represents the fraction of the oral dose absorbed. Accordingly, the apparent volume of distribution was obtained using the relationship: $V_d/F = Dose/AUC_0^\infty * \lambda_z$).

Cl/F and V_d/F were normalized per kg bodyweight. The results were presented both as mean ± S. D. and as median and range. The average elimination half-life was described using the harmonic mean. The pharmacokinetic parameters determined in the groups of responding and nonresponding volunteers and patients were compared using nonparametric methods (Kruskal-Wallis analysis of variance). Subsequently, a multiple range test was performed according to Nemenyi to show which groups were different from the others (Sachs, 1984).

Results

Plasma concentration vs time-profiles of total and active drug in responding and nonresponding volunteers (Groups I and II) and patients (Groups III and IV) after an oral dose of 500 mg and 1500 mg, respectively, are depicted in Figs. 1a and 1b. The pharmacokinetic parameters are presented in Table 2. Furthermore, the individual values of the elimination half-life of sulfadoxine and the percentage of metabolites present in plasma are illustrated in Figs. 2 and 3.

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Table 2. Pharmacokinetic parameters (mean ± S.D., median and range) of sulfadoxine in responding and nonresponding volunteers and patients following an oral dose of 500 mg and 1500 mg, respectively (for definition of parameters see Data evaluation)

		Healthy subjects		Patients	
		Group I Responders (n = 8)	Group II Nonresponders (n = 10)*	Group III Responders (n = 5)	Group IV Nonresponders (n = 7)
Dose (mg)	$\bar{x}$	500	500	1500	1500
t_m (h)	$\tilde{x}$ (range)	6 (6–6)	6 (3–8)	6 (6–8)	8 (6–8)
C_m (A) (μg/ml)	$\bar{x} \pm$ S.D.	70±11	65±10	148±10	169±34
	$\tilde{x}$ (range)	71 (53–87)	64 (55–88)	148 (138–163)[b]	175 (132–222)
C_m (T) μg/ml	$\bar{x} \pm$ S.D.	77±15	77±10	162±9	184±38
	$\tilde{x}$ (range)	73 (52–102)	72 (68–94)	160 (151–176)[b, e]	192 (143–243)
AUC_0^∞ (A) (μg h/ml)	$\bar{x} \pm$ S.D.	26689±6271	18893±3870	44844±10838	44095±7343
	$\tilde{x}$ (range)	26637 (18245–40144)	18438 (12864–25909)	45783 (34654–61559)	43383 (32258–55341)
Perc. metab. (%)	$\bar{x} \pm$ S.D.	8.6±3.3	20.6±10.8	10,4±5.9	12.4±2.6
	$\tilde{x}$ (range)	9.7 (4.1–14.9)	18.4 (7.4–34.8)[a]	11.4 (0.8–16.2)	13.0 (8.9–16.6)
$t_{1/2}$(A) (h)	harm. mean	267	212	210	185
	$\tilde{x}$ (range)	256 (215–375)	217 (135–330)	223 (166–274)	172 (167–253)[d]
(Cl/F)/BW (ml h^{-1} kg^{-1})	$\bar{x} \pm$ S.D.	0.333±0.067	0.463±0.109	0.714±0.149	0.610±0.095
	$\tilde{x}$ (range)	0.350 (0.235–0.435)	0.451 (0.278–0.648)	0.792 (0.487–0.832)[c]	0.622 (0.475–0.756)[d]
(V$_d$/F)/BW (l/kg)	$\bar{x} \pm$ S.D.	0.129±0.020	0.153±0.038	0.218±0.028	0.166±0.036
	$\tilde{x}$ (range)	0.139 (0.112–0.170)	0.137 (0.120–0.240)	0.202 (0.193–0.256)[c]	0.717 (0.114–0.214)

significant differences between groups:

a) I vs II; $p < 0.05$
b) I vs III; $p < 0.05$
c) I vs III; $p < 0.01$
d) I vs IV; $p < 0.05$
e) II vs III; $p < 0.05$

(results of Kruskal-Wallis-test and subsequent multiple comparison).

* Mean values were calculated after normalization of C_{max} and AUC_{values} of one volunteer, who was given 1000 mg to a 500 mg dose.
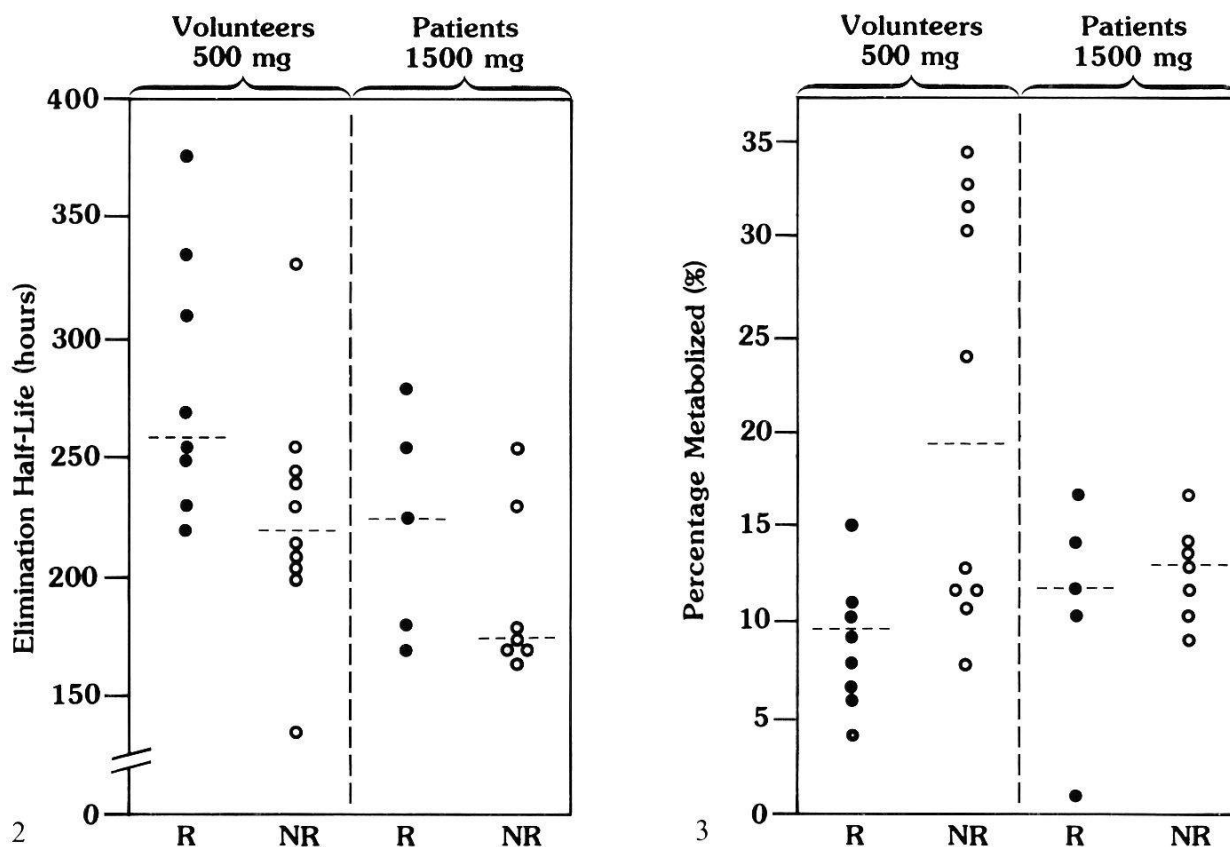
Fig. 2. Elimination half-life of active sulfadoxine in responding (R) and nonresponding (NR) volunteers and patients. (The median value of each group is indicated by a horizontal dotted line.)

Fig. 3. Percentage of sulfadoxine metabolized in plasma in responding (R) and nonresponding (NR) volunteers and patients. (The median value of each group is indicated by a horizontal dotted line.)

Volunteers

As shown by Fig. 1a (lower part), the disposition of total sulfadoxine was similar for responding and nonresponding volunteers. Furthermore, the maximum plasma levels of active sulfadoxine were not significantly different between both groups. In the responding subjects the proportion of metabolites (mainly N_4-acetylsulfadoxine) in plasma ranged between 4 and 15%. However, the nonresponders showed a tendency to shorter elimination half-lives compared to the responders (212 vs 267 h, harm. means). Due to a considerable overlap of values, this difference was not statistically significant. However, the amount of metabolites present in plasma was significantly higher in nonresponders (7–35%).

Patients

In patients, the elimination half-live was slightly reduced in the group of nonresponders compared to the responders (185 vs 210 h, respectively, harm. means). However, as in the case of responding and nonresponding volunteers, this was not statistically significant due to a considerable overlap in the values of both groups (Fig. 2). The degree of metabolization was comparable for the

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responding and nonresponding patients and ranged in the same order as the corresponding value for the responding volunteers (Fig. 3). A remarkable finding in the patients was that, in contrast to the volunteers, the plasma concentrations of total and active sulfadoxine were even higher in the nonresponding patients throughout a period of 216 h (Figs. 1a and 1b, upper part). This difference may be due to an increased volume of distribution or a slightly reduced extent of drug absorption in the responding patients.

Discussion

The wide range of values and the indication of a bimodal distribution of the metabolic ratio in nonresponding volunteers (Fig.2) could be very tentatively associated with the occurrence of slow and rapid acetylators. Due to the very limited number of cases this assumption cannot be confirmed on the basis of the present study. However, it would be of great interest to correlate these findings with the respective acetylator status of the same individuals by using a test drug such as sulfamethazine (Vree et al., 1980). In patients, the elimination half-lives were shorter and the maximum plasma levels and AUC values were lower than predicted from the results in volunteers. One of the reasons for these changes in drug disposition may be the disease state, resulting in an altered protein binding and tissue distribution. Accordingly, the increase in the volume of distribution and the oral plasma clearance as well as the slight reduction of the elimination half-life can be readily associated with an increase in the fraction of unbound drug in plasma (Svensson et al., 1986).

Initially, it might be compelling to associate the higher metabolic inactivation of sulfadoxine observed in those volunteers who were retrospectively classified as nonresponders, with therapeutic failures which occurred on a previous occasion. According to this assumption, a successful therapeutic response to the combination should be closely related to the plasma levels of active sulfadoxine. However, this hypothesis is contradicted by the findings in patients under therapeutic conditions. In this case, the plasma concentrations of the active compound were even higher in those patients who could not be successfully cured with a single dose of 1500 mg sulfadoxine +75mg pyrimethamine.

Furthermore, in nonresponding patients there was no increase in the metabolites present in plasma as was expected from the results in nonresponding volunteers. These results demonstrate that the failure to the treatment with the combination of pyrimethamine and sulfadoxine can not be attributed to metabolic deviations of the host resulting in decreased plasma levels of active sulfadoxine.

In a previous study with the combination of pyrimethamine and sulfalene the pyrimethamine plasma levels were comparable in responders and nonresponders (Trenholme et al., 1975). The plasma levels of pyrimethamine, obtained in the present study, confirm these results. The concentration was in

the predicted range for both responding and nonresponding subjects (Sari-kabhuti, to be published). Therefore, a direct correlation between therapeutic failure and insufficient pyrimethamine levels could be excluded.

As previously discussed (Trenholme et al., 1975; Williams et al., 1975, 1978) and on the basis of the present study other host factors or the development of parasite resistance could give rise to the lack of therapeutic efficacy of combinations of pyrimethamine and long acting sulfonamides. Qualitative and quantitative modifications in the enzyme dihydrofolate reductase and/or an increased synthesis of folinic acid within the hosts erythrocytes, enabling the malaria parasites to bypass the sequential blockade of the folate biosynthesis may be an explanation for the impaired antimalarial activity (Trenholme et al., 1975).

In conclusion, the present study did not reveal an unequivocal relationship between the therapeutic response, acetylator phenotype and plasma concentrations of sulfadoxine.

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