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EFFECT OF SULFAMERAZINE ON MULTIPLE FORMS OF CYTOCHROME P-450 IN RATS

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ABSTRACT:

Sulfamerazine effect was studied on different isoforms of CYP 450 induced by phenobarbital (CYP 2B1, 2B), benzo[a]pyrene (CYP 1A1), isoniazid (CYP 2E1), clofibrate (CYP 4A) and clotrimazole (CYP 3A) pretreatment. Significant decrease in the level of cytochrome P-450 and drug metabolizing enzyme activities was observed due to sulfamerazine treatment of inducer pretreated rats, indicates destructive effect of sulfamerazine on all isoforms studied. Phenobarbital post-treatment of sulfamerazine pre-treated rats could not affect the inducing mechanism of phenobarbital. Pre-treatment of sulfamerazine affected inducing mechanism of benzo[a]pyrene, isoniazid, clofibrate and clotrimazole, however, post treatment of these inducers showed protection with respect to the levels of electron transport components and drug metabolizing enzymes activity.

KEYWORDS: Sulfamerazine, inducers, Drug metabolizing enzymes.

INTRODUCTION:

Sulfonamides are venerable group of antimicrobials, in the antimicrobial revolution in 1935. Major development is a universal use of sulfa drugs in the form of either sulfamethoxazole (Human dose form) or sulfadaizine (Veterinary dose) combined with 2, 4, diaminopyrimidine derivative, trimethoprim. Sulfonamides were amongst the first clinically useful antimicrobial agent and also a component of dye prontosil in the synthesis of clinically useful analogues, in treatment of pneumonia caused by fungus, which is common condition in AIDS patients. Their synergism has made the combinations extremely popular in both veterinary and human medicines. Sulfamerazine acting individually as competitive inhibitors of specific enzymatic reaction.

MATERIALS AND METHODS

Animals

Male Wistar rats (200-220 g, 3 months old) from Hoffkine Institute, Mumbai. The animals were housed in plastic cages and were fed on appropriate standard laboratory diet (Lipton India Ltd., Mumbai, INDIA) and tap water ad libitum. Nicotinamide adenine dinucleotide phosphate (NADPH) Nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate dehydrogenase, glucose 6-phosphate disodium salt, aminopyrine, aniline hydrochloride, N-[2-hydroxyethyl] piperazine-N-[2 ethane sulfonicacid] (HEPES), sodium salt of sulfamerazine were obtained from Sigma Chemical Company (St. Louis , MO, USA). Phenobarbital was obtained from E Merck (Mumbai, INDIA). Sucrose, phenol, trichloroaceticacid, sodium chloride, potassium chloride, calcium chloride, ethylenediaminetetra acetic acid disodium salt and other chemicals were of analytical grade obtained from Qualigens Fine Chemicals (Mumbai, INDIA).

Treatment of animals

In these experiments standard doses of the inducers viz. phenobarbital (80 mg/kg in saline, i.p., for 3 days), clotrimazole (100 mg/kg in saffola oil, i.p., for 3 days), clofibrate (100 mg/kg in saffola oil, i.p., for 3 days), benzo[a] pyrene (20 mg/kg in saffola oil, i.p., for 2 days) and isoniazid (100 mg/kg in saline,i.p., for 3 days) were used to create induced status of mixed function oxidase system in rats. The dose of 100 mg/kg in saline (i.p.) for 3 days, was used as an inhibitory dose of sulfamerazine.

Adult male rats were divided into 5 groups of 4 animals each. Group 1 injected with saline or saffola oil and served as control. Group 2 was injected with standard dose of respective inducer. Group 3 was treated with a standard dose of sulfamerazine. Group 4 received standard dose of respective inducer prior to sulfamerazine treatment and group 5 received a standard dose of respective inducer after sulfamerazine treatment.

The Male Wistar rats were injected intraperitoneally (i.p.) in the morning between 8.00 and 9.00 a.m. The volume injected into the rats of body weight of 200 g was 1 ml.

RESULTS DISCUSSION

Our earlier studies on inhibition of catalytic and spectral activity of cytochrome P-450 due to sulfamerazine treatment and a little decrease in phenobarbital sleeping time due to prior administration of sulfamerazine in male rats encouraged us to study the effect of sulfamerazine on various form of cytochrome P-450 enzymes(data was not shown).

Administration of sulfamerazine caused a significant decrease in the level of cytochrome P-450 (of 62%), cytochrome b5 (of 61%), and activities of aminopyrine N-demethylase (of 71%) and aniline hydroxylase (of 80%). Phenobarbital treatment resulted increase in microsomal protein (of 27%), significant induction in cytochrome P-450 (of 90%), cytochrome b5 (of 95%), activities of aminopyrine N-demethylase (of 67%) and aniline hydroxylase (of 64%).

Sulfamerazine treatment of phenobarbital-pretreated rats caused marginal decrease in cytochrome b5 (of 10%) and significant decrease in the level of cytochrome P-450 (of 51%), activities of aminopyrine N-demethylase of 26%) and aniline hydroxylase (of 50%), when compared with phenobarbital treatment alone. Phenobarbital treatment of sulfamerazine-pretreated rats showed no significant change in microsomal protein significant increase was observed in electron transport components, cytochrome b5 (of 75%), cytochrome P-450 (of 177%) and drug metabolism enzymes aminopyrine N-demethylase (of 277%), aniline hydroxylase (of 391%), when compared to sulfamerazine treatment alone. Clotrimazole treatment resulted significant increase in microsomal protein (of 120%), cytochrome P-450 (of 224%), activities of aminopyrine N-demethylase (of 60%) and aniline hydroxylase (of 106%), while cytochrome b5 was unchanged.

Table 1: Alteration in rat liver microsomal protein and electron transport components due to SMR phenobarbital, isoniazid, benzo(a)pyrene, clotrimazole, and clofibrate treatment in male rats.

Groups	Microsomal protein	Cytochrome b5	Cytochrome P-450
Control	11.60±0.58	0.21±0.02	0.38±0.02
Phenobarbital	14.60±0.50	0.41±0.01***	0.72±0.01***
SMR	12.60±0.60	0.16±0 02*	0.27± 0.01**
Phenobarbital + SMR	14.80±1.64	0.37±0.03**	0.35±0.01$
SMR + Phenobarbital	12.70±1.30	0.28± 0.02#	0.75± 0.02***
Control	12.33±0.31	0.25±0.01	0.45±0.02
Clotrimazole	27.1±0.14***	0.25±0.01	1.46 ± 0.02***
SMR	14.62±1.20	0.13± 0.01***	0.28±0.01**
Clotrimazole + SMR	24.70 ±1.57***	0.20±0.01*#$	0.71± 0.02*** $$
SMR + Clotrimazole	29.03±1.25##	0.20 ± 0.01*#$	1.30± 0.03***$#
Control	12.53 ± 0.25	0.24 ± 0.02	0.40± 0.01
Clofibrate	12.13 ± 0.50	0.27 ± 0.01	0.62± 0.01***
SMR	9.45 ± 0.15**	0.12 ± 0.01***	0.18± 0.01***
Clofibrate +SMR	10.60 ±0.30*	0.18 ± 0.01* $	0.14± 0.01***#
SMR + Clofibrate	14.13 ±0.24*	0.24 ± 0.01#	0.24± 0.01#
Control	13.50 ± 0.35	0.20 ± 0.01	0.51 ± 0.02
Isoniazid	17.57 ±0.75**	0.32 ± 0.01***	0.75 ± 0.02***
SMR	16.03 ±0.9	0.10 ± 0.01***	0.24 ± 0.01***
Isoniazid + SMR	15.0 ± 0.8	0.14 ± 0.01* $	0.33 ± 0.01*** $
SMR + Isoniazid	14.33 ± 0.22	0.30 ±0.01***#	0.51 ± 0.02#
Control	14.56 ± 0.19	0.18 ± 0.01	0.44 ± 0.02
Benzo(a)pyrene	9.20 ± 0.18***	0.24 ± 0.01*	0.84± 0.02***
SMR	15.85 ±0.67	0.0 ± 0.01***	0.32± 0.01**
Benzo(a)pyrene+ SMR	8.52 ± 0.21***	0.14 ± 0.01$#	0.44 ± 0.01$##
SMR +Benzo(a)pyrene	18.03± 0.12***	0.10 ±0.01***$	0.32± 0.02** $

Values are mean of three experiments ±SEM, four animals in each group.

[a] mg protein/ g liver.

[b] nmol/mg microsomal protein.

Significantly different from control at *P< 0.05, **P< 0.01, ***P< 0.001, by One way analysis of variance and Tukey-Kramer multiple comparisons test.

Significantly different from sulfamerazine group # P< 0.001

Sulfamerazine treatment after clotrimazole-pretreatment showed a little decreases in microsomal protein (of 9%) and cytochrome b5 (of 20%), while significant decrease was observed in the level of cytochrome P-450 (of 51%), aminopyrine N-demethylase (of 25%), and aniline hydroxylase activities (of 61%). Sulfamerazine treatment prior to clotrimazole treatment resulted significant increase in cytochrome b5 (of 54%), cytochrome P-450 (of 364%) and activity of aniline hydroxylase (of 57%) while no noticeable change was observed in the level of microsomal protein and activity of aminopyrine N-demethylase Clofibrate treatment showed significant increase in the level of cytochrome P-450 (of 55%), activities of aminopyrine N-demethylase (of 252%) and the aniline hydroxylase (of 130%). While a little decrease in the microsomal protein (of 3%) and marginal increase (of 12%) in cytochrome b5 was observed. Clofibrate treatment prior to sulfamerazine treatment resulted an increase in cytochrome b5 (of 33%), cytochrome P-450 (of 77%) and activity of aminopyrine N-demethylase (of 77%), while significant decrease in activity of aniline hydroxylase (of 64%) and little decrease in microsomal protein.

Clofibrate treatment after sulfamerazine treatment also resulted in significant increase in microsomal protein (of 49%), cytochrome b5 (of 65%), cytochrome P-450 (of 100%) and activity of aniline hydroxylase (of 65%) when compared to sulfamerazine treatment alone. No appreciable change was observed in aminopyrine N-demethylase Isoniazid treatment resulted significant induction in microsomal protein (of 30%), cytochrome b5 (of 60%), cytochrome P-450 (of 47%), activities of aminopyrine N-demethylase (of 33%) and aniline hydroxylase (of 46%). Post treatment of sulfamerazine to isoniazide showed significant decrease in cytochrome b5 (of 56%), cytochrome P-450 (of 56%), aminopyrine N-demethylase (of 48%), and aniline hydroxylase (of 31%) while little change (of 15%) was observed in microsomal protein. However pretreatment of sulfamerazine to isoniazide showed significant increase in cytochrome b5 (of 200%), cytochrome P-450 (of 112%) and in aniline hydroxylase (of 114%). No noticeable change was observed in microsomal protein and aminopyrine N-demethylase activity.

Benzo[a]pyrene treatment showed significant increase microsomal protein (of 32%), cytochrome b5 (of 33%), cytochrome P-450 (of 91%), activities of aminopyrine N-demethylase (of 144%) and aniline hydroxylase of 68%), respectively. Sulfamerazine administration of benzo[a]pyrene pretreated rats showed significant decrease in cytochrome b5 (of 42%), cytochrome P-450 (of 48%), aminopyrine N-demethylase (of 52%) and aniline hydroxylase (of 25%). No appreciable changed was noticed in microsomal protein. Post administration of benzo[a]pyrene to sulfamerazine showed significant increase in cytochrome b5; aminopyrine N-demethylase (of 57%) and aniline hydroxylase (161%), microsomal protein and cytochrome P-450 were unchanged.

In conclusion the effect of sulfamerazine on different isoforms of cytochrome P-450 induced by phenobarbital (CYP 2B1, 2B), benzo[a]pyrene (CYP 1A1), isoniazid (CYP 2E1), clofibrate (CYP 4A) and clotrimazole (CYP 3A) pretreatment. Significant decrease in the level of cytochrome P-450 and drug metabolizing enzyme activities was observed due to sulfamerazine treatment of inducer pretreated rats, indicates destructive effect of sulfamerazine on all isoforms studied. Phenobarbital post-treatment of sulfamerazine pre-treated rats could not affect the inducing mechanism of phenobarbital. Pre-treatment of sulfamerazine affected inducing mechanism of benzo[a]pyrene, isoniazid, clofibrate and clotrimazole, however, post treatment of these inducers showed protection with respect to the levels of electron transport components and drug metabolizing enzymes activity.The effect of sulfamerazine on different isoforms of cytochrome P-450 induced by phenobarbital (CYP 2B1, 2B), benzo[a]pyrene (CYP 1A1), isoniazid (CYP 2E1), clofibrate (CYP 4A) and clotrimazole (CYP 3A) pretreatment. Significant decrease in the level of cytochrome P-450 and drug metabolizing enzyme activities was observed due to sulfamerazine treatment of inducer pretreated rats, indicates destructive effect of sulfamerazine on all isoforms studied. Phenobarbital post-treatment of sulfamerazine pre-treated rats could not affect the inducing mechanism of phenobarbital. Pre-treatment of sulfamerazine affected inducing mechanism of benzo[a]pyrene, isoniazid, clofibrate and clotrimazole, however, post treatment of these inducers showed protection with respect to the levels of electron transport components and drug metabolizing enzymes activity (Adav and Govindwar 2003).

Phenobarbital treated rats showed elevated levels of microsomal cytochrome P-450, while synthesized hemeprotein had the same absorption maximum in its reduced carbon monoxide difference spectrum at 450 nm as microsomal cytochrome P-450 from untreated animals (Sheweita, 2000). Phenobarbital strongly induces the liver expression of many genes encoding detoxification enzyme such as aldehyde dehydrogenase, epoxide hydrolase, NADPH cytochrome P-450 reductase along with enzymes involved in phase II metabolism (Dunn *et al.*1989; Pickett and Lu 1989). Sulfamethazine treatment of phenobarbital-pretreated rats caused a significant decrease in the level of microsomal protein, cytochrome P-450 and in the activities of aminopyrine N-demethylase, aniline hydroxylase and cytochrome c-reductase which was

Table 2: Alteration in rat liver microsomal drug-metabolizing enzymes due to SMR phenobarbital, isoniazid, benzo(a)pyrene , clotrimazole , and clofibrate treatment in male rats

Groups	Aminopyrine N-demethylase [a]	Aniline hydroxylase [b]
Control	4.41 ±0.32	0.99 ± 0.09
Phenobarbital	7.37 ±1.31**	1.62 ± 0.18*
SMR	2.10 ±0.29**	0.34 ± 0.08*
Phenobarbital + SMR	5.46 ±0.97$^{\$}$	0.80 ± 0.06$^{\$}$
SMR +Phenobarbital	7.30 ±0.90**	1.67 ± 0.13*$^{\#}$
Control	4.45 ± 0.39	1.20 ± 0.12
Clotrimazole	7.14 ± 0.92*	2.47 ±0.18***
SMR	1.94 ± 0.26*	0.40 ± 0.10*
Clotrimazole +SMR	5.38 ± 0.48	0.97 ± 0.13$^{\$}$
SMR + Clotrimazole	2.17 ± 0.26	0.63 ± 0.15$^{\$}$
Control	5.29 ± 0.63	0.84 ± 0.10
Clofibrate	18.64 ± 0.15***	1.93 ± 0.15***
SMR	2.53 ± 0.10***	0.32 ± 0.08*
Clofibrate + SMR	4.18± 0.22$^{\$}$	0.69 ± 0.12$^{\$}$
SMR +Clofibrate	2.75 ± 0.10**	0.53± 0.07
Control	4.10 ± 0.10	0.97 ± 0.10**
Isoniazid	5.45 ± 0.25**	1.42 ± 0.16**
Isoniazid +SMR	2.24 ± 0.20***	0.51± 0.15**
SMR + Isoniazid	2.83 ± 0.18**	0.97 ± 0.15$^{\$}$
Control Benzo[a]pyrene	2.23 ± 0.12***	1.09 ± 0.15$^{\#}$
SMR	6.28± 0.34	1.14 ± 0.21
Benzo[a]pyrene+SMR	15.35± 0.16***	1.92 ± 0.6**
SMR + Benzo[a]pyrene	4.25± 0.16***	0.55 ± 0.08*
	7.42 ± 0.19 *$^{\$}$	1.43± 0.15
	6.67 ± 0.19 $^{\#\#}$	1.44 ± 0.05$^{\#}$

Values are mean of three experiments ±SEM, four animals in each group.
[a] nmol of formaldehyde liberated/min/mg microsomal protein.
[b]nmol of p-aminophenol formed/min/mg microsomal protein.
Significantly different from control at *P< 0.05, **P< 0.01, ***P< 0.001,
by One way analysis of variance and Tukey-Kramer multiple comparisons test.
Significantly different from Sulfamerazine group $^{\#}$P< 0.01,$^{\#\#}$P< 0.001

indicated that CYP 2B and 3A are susceptible to sulfamethazine and its metabolites. Post treatment of sulfamethazine of benzo[a]pyrene treated rats showed a slight decrease in cytochrome P-450 and in the activity of aminopyrine N-demethylase compare with benzo[a]pyrene treatment alone (Kodam *et al.* 1996).

The level of protein and other parameters of mixed function oxidase system were increased by the phenobarbital treatment. SMR treatment caused significant decrease in the level of cytochrome P-450 and drug metabolizing enzymes. While SMR treatment of pretreated rats caused significant decrease in the level of cytochrome P-450 and marginal changes in the drug metabolizing enzymes. Phenobarbital treatment of SMR pretreated rats showed no significant change in microsomal protein when compared with phenobarbital alone indicates any effect of SMR on inducing mechanism of phenobarbital.

Clotrimazole, an azole antifungal drug, and 1-diphenylmethyl-imidazole preferentially induce cytochrome P-450 2B1/2 in dose dependent manner and slight induction of cytochrome P-450 1A1/2 (Kobayashi *et al.* 1993). Clotrimazole pretreatment resulted in significant increase in microsomal protein, electron transport components and drug metabolizing enzymes. But cytochrome b5 was unchanged. Treatment of SMR

after clotrimazole pretreatment showed little decrease in microsomal protein, cytochrome b5, while significant decrease in the cytochrome P-450 and drug metabolizing enzymes. This indicates inhibitory effect of SMR on induced cytochrome P-450. SMR treatment prior to clotrimazole treatment resulted in significant increase in cytochrome P-450 but significant decrease in aminopyrine N-demethylase and aniline hydroxylase activity (Adav *et al.* 2005). This result indicates inhibition of specific isoforms responsible for this activity inductive mechanism. Clofibrate treatment showed significant increase in the level of cytochrome P-450, aminopyrine N-demethylase and aniline hydroxylase activity while little effect on microsomal protein and cytochrome b5. Clofibrate treatment after SMR treatment resulted in significant decrease in the level of cytochrome P-450 and activity of induction pattern has been observed from clofibrate treatment previously (Gibson *et al.* 1982) Clofibrate treatment to male chicken showed significant increase in the electron transport components and drug metabolizing enzymes. Similar results were observed when SMR treatment of clofibrate pretreated rats. These results indicate the inhibitory effect of SMR on clofibrate-

STATISTICAL ANALYSIS

One way Analysis Variance of variance (ANOVA) with the Turkey-kramer Multiple Comparison Test.

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