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Short communication

Effect of lipopolysaccharide (LPS)-evoked host defense activation on hepatic microsomal formation and reduction of sulfamethoxazole hydroxylamine in the rat

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Abstract

The incidence of adverse reactions to sulfamethoxazole-trimethoprim (SMX-TMP) combination products is higher in patients with AIDS than in the general population. Idiosyncratic adverse reactions to SMX are believed to be dependent upon the formation of the reactive intermediate, sulfamethoxazole hydroxylamine (SMX-HA), and its further oxidation product, nitroso-SMX. Changes in the disposition of SMX have been proposed to contribute to the increased risk of SMX adverse reactions in patients with AIDS. Activation of host defense mechanisms is known to alter drug metabolism and could decrease the enzymatic reduction of SMX-HA to the parent SMX, causing an imbalance in bioactivation and detoxification. We tested this hypothesis in a rat model of lipopolysaccharide (LPS)-evoked host defense activation. Rats were treated i.p. with 1 mg/kg of LPS, and hepatic microsomes were isolated 24 hr after treatment. The bioactivation of SMX to SMX-HA was reduced 50% by pretreatment with LPS (113 ± 10 vs 65 ± 4 pmol/min/mg; $P < 0.05$). However, the NADH-dependent reduction of SMX-HA to SMX was reduced by over 80% (454 ± 90 vs 81 ± 48 pmol/min/mg; $P < 0.05$). A decreased ability to reduce SMX-HA to SMX could predispose patients with systemic activation of host defense mechanisms, such as those with AIDS, to the occurrence of SMX-associated adverse reactions.

Keywords: Sulfamethoxazole; Sulfamethoxazole hydroxylamine; Idiosyncratic reactions; Hydroxylamine reductase; Inflammation; Cytochrome P450

1. Introduction

SMX-TMP combination products are associated with a low incidence in the general population of idiosyncratic hypersensitivity syndrome reactions characterized by fever, skin rash, and multi-organ toxicity occurring 7–14 days after the start of therapy [1]. In HIV-infected patients with AIDS, the incidence of clinically similar ADR is much higher, approaching up to 50% [1]. While TMP may be implicated in some cases, it is believed that SMX is responsible for the majority of adverse reactions.

Oxidation of SMX at the N_4-position produces hydroxylamine (SMX-HA) and nitroso (SMX-NO) metabolites, believed to be responsible for initiating idiosyncratic hypersensitivity syndrome reactions [2,3]. Although these metabolites will react with glutathione [2], the net result is predominantly the reduction of SMX-NO to SMX-HA rather than the formation of stable glutathione conjugates that are excreted. While it has been hypothesized that low levels of plasma and cellular glutathione [4,5] may be responsible for the increased susceptibility to SMX ADR in patients with AIDS, the depletion of glutathione is not required for the cellular toxicity associated with SMX-HA [2], and the treatment of HIV-infected individuals with *N*-acetylcysteine does not reduce the incidence of ADR [6]. Recently, we and others [3,7] have hypothesized that the reduction of SMX-HA back to the parent SMX may be a critical protective pathway. We have demonstrated that CYP and

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Abbreviations: ADR, adverse drug reaction; CYP, cytochrome P450; LPS, lipopolysaccharide; NMHR, NADH-dependent microsomal hydroxylamine reductase; SMX, sulfamethoxazole; SMX-HA, sulfamethoxazole hydroxylamine; and SMX-TMP, sulfamethoxazole-trimethoprim.

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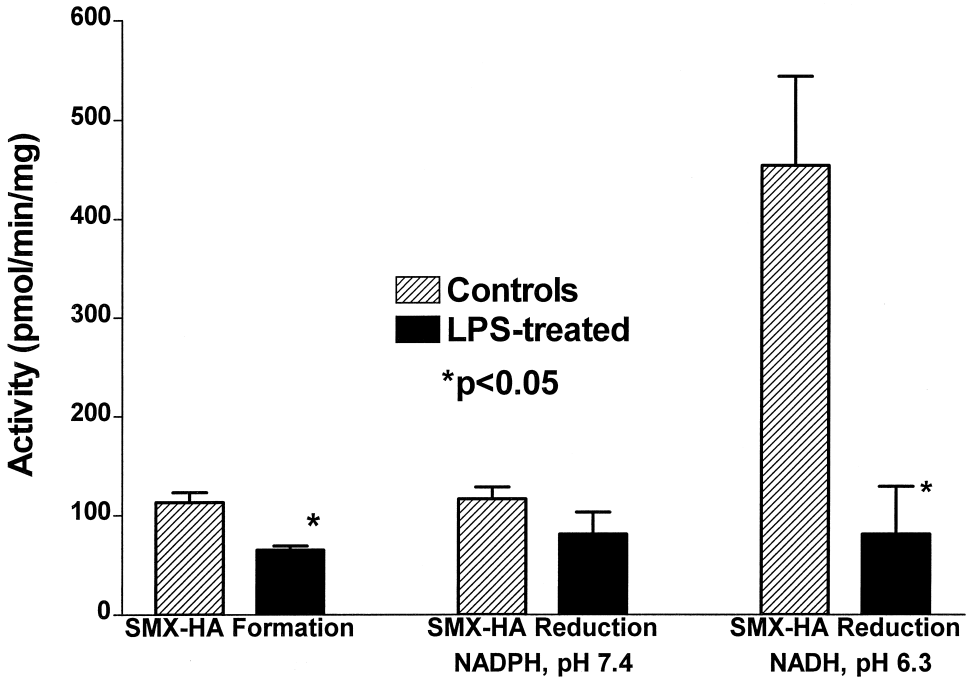

Fig. 1. Effect of treatment of rats with LPS (1 mg/kg, i.p.) on the formation of SMX-HA and on the reduction of SMX-HA to SMX. SMX-HA reduction was assessed at pH 7.4 with NADPH as a co-factor and at pH 6.3 with NADH as a co-factor to differentiate between CYP-mediated and NMHR-mediated reactions, respectively. Results are shown as means ± SEM; N = four animals per group. The unpaired Student's *t*-test was used to compare groups, with significance set at $P < 0.05$.

an NMHR can mediate the enzymatic reduction of SMX-HA by hepatic microsomes [3].

It is well known that activation of host defense mechanisms leads to the down-regulation of the major hepatic CYP [8]. Down-regulation occurs in response to a variety of cytokines and is known to involve regulation at the level of both transcription and mRNA stability. It also has been shown that cytokines are elevated in patients with the HIV/AIDS complex and that the drug-metabolizing capacity is altered [9]. Thus, changes in the bioactivation and reduction of SMX-HA could occur in response to host defense activation and influence the risk of adverse reactions in HIV/AIDS patients. We therefore hypothesized that the reduction of SMX-HA back to SMX may be decreased during host defense activation, leading to an imbalance in the formation and detoxification of SMX-HA. We elected to test this hypothesis in the rat, using LPS-evoked host defense activation as a model.

2. Materials and methods

Sprague–Dawley rats were treated i.p. with bacterial LPS (serotype 0127:B8) at 1 mg/kg to activate systemic host defense mechanisms [8]. Twenty-fours later, the rats were killed, and hepatic microsomes were prepared as previously described [10]. Total protein, CYP, SMX oxidation to SMX-HA, and reduction of SMX-HA were determined as previously described [3,10]. All materials were obtained from standard sources as noted in previous publications [3,8,10].

3. Results and discussion

Total CYP was reduced following LPS treatment, as previously reported (1.01 ± 0.14 vs 0.86 ± 0.10 nmol P450/mg protein) [8]. Oxidation of SMX to SMX-HA was reduced significantly by approximately 50% (113 ± 10 vs 65 ± 4 pmol/min/mg; $P < 0.05$; Fig. 1). At pH 7.4, NADPH-dependent reduction of SMX-HA appears to be mediated primarily by CYP enzymes [3], while when NADH is a co-factor, NMHR is the primary enzyme involved. This latter enzyme is most active *in vitro* at a pH of 6.3, and this pH is therefore commonly used to assay its activity [3]. NADPH-dependent reduction at pH 7.4 (predominantly CYP-mediated) was not reduced significantly (although this may be attributable, in part, to a low turn-over via this pathway and a small sample size). However, NADH-dependent reduction of SMX-HA at pH 6.3 (predominantly NMHR-mediated) was reduced significantly by over 80% (454 ± 90 vs 81 ± 48 pmol/min/mg; $P < 0.05$; Fig. 1). Under the aerobic conditions encountered *in vivo*, the NADH-dependent microsomal hydroxylamine reductase would be expected to be predominantly responsible for the reduction of SMX [3].

The majority of CYPs are expressed predominantly in the liver, but the systemic distribution of NMHR is not

known. The identity of this enzyme system also is not clear, and currently there are no available probes to measure protein or mRNA levels. While its properties would suggest that it is not a CYP, it has been reported recently that the pig form of the enzyme is, in fact, a member of the CYP2D subfamily [11]. Its identity in human liver has not been determined. Thus, mechanistic investigations of its down-regulation are not possible currently. Nevertheless, our data indicate that NMHR activity can be decreased significantly during systemic inflammation.

The greater decrease in the reductive pathway compared with the oxidative pathway could result in a relative imbalance in the formation and detoxification of SMX-HA. As the reductive pathway, at least *in vitro*, shows a greater activity and has a lower k_m [3] than the bioactivation pathway, it is expected to play a significant role in determining the concentration of SMX-HA achieved in the body or in the cell. A greater relative decrease in the activity of NMHR, therefore, could result in an increased exposure to SMX-HA *in vivo*. We have only looked at one time point, but at least part of the time an imbalance may exist. If a similar down-regulation of NMHR occurs in patients with AIDS, it could be a partial explanation of the increased risk of SMX ADR and warrants further investigation. As the rat does not experience SMX hypersensitivity syndrome reactions, we cannot explore this possibility in this model. Further studies of the importance of changes in this pathway in AIDS must be carried out in patients.

Acknowledgments

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