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Original Contribution

Biomarkers of oxidative stress study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl_4 poisoning

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Abstract

Plasma and urinary levels of malondialdehyde-like products (MDA) and isoprostanes were identified as markers of in vivo lipid peroxidation in an animal model of CCl_4 poisoning. We sought to determine the extent to which the formation of these oxidation products is influenced by inhibition of the cyclooxygenase enzymes which catalytically generate proinflammatory lipid peroxidation products known as prostaglandins and thromboxane. In the present studies, after induction of oxidant stress in rats with CCl_4, lipid peroxidation products measured in plasma and urine demonstrate that isoprostanes and MDA can be partially inhibited by cyclooxygenase inhibitors, albeit to different extents. The lowering of isoprostane and MDA formation, however, may not to due primarily to the diminution of catalytic generation of isoprostanes or MDA by the cyclooxygenases but, rather, may be the result of the suppression of nonenzymatic lipid peroxidation. This is suggested since 8,12-iso-$iPF_{2\alpha}$−VI is also reduced by indomethacin, yet, unlike other isoprostanes and MDA, it is not generated catalytically by the cyclooxygenase. Thus, although the two cyclooxygenase inhibitors we tested have statistically significant effects on the measurements of both isoprostanes and MDA in this study, the results provide evidence that these lipid-degradation products primarily constitute markers of oxidative stress.
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Keywords: CCl_4; Indomethacin; Meclofenamic Acid; Rat; Plasma; Urine; MDA; Isoprostanes; Free radicals

Introduction

This article is the third report of the Biomarkers of Oxidative Stress Study (BOSS) using acute CCl_4 poisoning as a rodent model for oxidative stress. Oxidation products of

Abbreviations: BOSS, Biomarkers of Oxidative Stress Study; MDA, malondialdehyde-like products; COX, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs; PG, prostaglandin; GC/NICI-MS, gas chromatography/negative ion chemical ionization mass spectrometry.

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lipids in plasma and urine of rats treated with CCl_4 were measured as a part of the validation study, searching for noninvasive biomarkers to assess oxidative stress accurately. In our previous report, we concluded that measurements of two lipid peroxidation products, malondialdehyde-like products (MDA) and isoprostanes, are potential candidates for general biomarkers of oxidative stress in plasma and urine.

MDA is a product of both free radical-initiated lipid peroxidation and the enzymatic activity of thromboxane synthase, the latter of which converts the cyclooxygenase (COX)-generated, unstable endoperoxide intermediate prostaglandin (PG)H_2 in equimolar quantities to thromboxane A_2 and to 15-hydroxyheptadecatrienoate and MDA [1]. Isoprostanes belong to a family of PG-like compounds generated primarily from arachidonic acid in vivo through nonenzymatic, free radical-catalyzed oxidation [2–6], although there is evidence for COX participation in the production of one of these compounds, 8-iso-$PGF_{2\alpha}$ [6–9]. Further, COX-dependent prostaglandin $PGF_{2\alpha}$ as a secondary product following F_2-isoprostane generation in CCl_4-induced oxidative damage was also described in vivo [10]. In this regard, it has been previously reported that the COX enzyme can catalytically generate small amounts of the isoprostane 8-iso-$PGF_{2\alpha}$, although the majority of studies suggest that COX does not significantly contribute to the formation of this compound in urine in normal humans or animals [11–15]. Experiments were conducted with rats administered CCl_4 to elucidate the mechanism of the in vivo formation of isoprostanes and MDA and to explore the use of measuring these compounds as an index of endogenous lipid peroxidation. Rats were pretreated with one of two nonsteroidal anti-inflammatory drugs (NSAIDs), indomethacin or meclofenamic acid, to determine the extent to which NSAIDs might affect the generation of these compounds under settings of oxidant stress. Indomethacin and meclofenamic acid are used primarily for the treatment of pain and inflammation [16–18]. They are competitive inhibitors of COX, which, as noted, is the enzyme that mediates biosynthesis of PGs and thromboxanes from arachidonic acid [19–24]. COX activity originates from two distinct and independently regulated isozymes, COX-1 and COX-2 [17,25–27], both of which are inhibited in vivo by indomethacin and meclofenamic acid [16,25,26]. In addition to the ability of these agents to inhibit the catalytic activity of COX, it has also been proposed that some NSAIDs exert antioxidant properties independent of COX inhibition [17,28]. In the present studies, we report that after CCl_4-induction of oxidant stress in rats the lipid peroxidation products, plasma MDA and F_2-isoprostanes can be significantly decreased by pretreatment with cyclooxygenase inhibitors. However, even after the pretreatment, these markers still show significant responses related to both the dose and timing of CCl_4. The results for the markers measured in urine were more variable.

Materials and methods

Materials, experimental treatment of the animals, specimen collection, and methods are described in detail previously [29]. In this publication, only a brief description is given.

Chemicals and reagents

Carbon tetrachloride, indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid), meclofenamic acid (2-[2,6-dichloro-3-methyl-phenyl)amino] benzoic acid), and all other chemicals and reagents used in the study were obtained from Sigma-Aldrich Corporation (St. Louis, MO).

Animals and treatment protocol

Male Fisher 344 rats (260–280 g) obtained from Charles River Laboratories (Raleigh, NC) were used in all experiments.

Indomethacin pretreatment

The indomethacin regimen used was previously shown to inhibit COX activity in rats by 90% (dose of 5 mg/kg sc 24, 12, and 2 h prior to the injection of CCl_4) [30]. An additional booster dose was injected 8 h after the last injection of CCl_4 for the measurements performed 16 h after treatment with CCl_4.

Meclofenamic acid pretreatment

Meclofenamic acid was injected at a dose of 5 mg/kg ip 12 and 2 h prior to the injection of CCl_4.

CCl_4 treatment

Animals from each of the 3 dose groups (Control—canola oil, 120 mg/kg CCl_4 ip and 1200 mg/kg CCl_4 ip) were investigated at 3 time points (2, 7, and 16 h after CCl_4 injection) for indomethacin-pretreated rats and at 1 time point (7 h) for meclofenamic acid-pretreated groups. Each group consisted of 5 rats for indomethacin pretreatment and 6 rats for meclofenamic acid pretreatment. Animal treatment, sample preparation, liver histology, and serum enzyme activities were done at NIEHS, Research Triangle Park (RTP), NC, USA [29,31].

Each 1 ml sample of plasma or urine was marked with a code number so that those conducting the assays were not aware of the treatment status of the animals.

Specimen collection

Plasma sample preparation

Blood (5 ml) was drawn from the dorsal aorta through single draw vacutainer needles (21 gauge) into open vacutainer blood collection tubes containing heparin. Blood was centrifuged (2000 rpm for 10 min at 4°C) no more than