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Original Contribution

Biomarkers of Oxidative Stress Study II. Are oxidation products of lipids, proteins, and DNA markers of CCl_4 poisoning?

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Abstract

Oxidation products of lipids, proteins, and DNA in the blood, plasma, and urine of rats were measured as part of a comprehensive, multilaboratory validation study searching for noninvasive biomarkers of oxidative stress. This article is the second report of the nationwide Biomarkers of Oxidative Stress Study using acute CCl_4 poisoning as a rodent model for oxidative stress. The time-dependent (2, 7, and 16 h) and dose-dependent (120 and 1200 mg/kg ip) effects of CCl_4 on concentrations of lipid hydroperoxides, TBARS, malondialdehyde (MDA), isoprostanes, protein carbonyls, methionine sulfoxidation, tyrosine products, 8-hydroxy-2′ -deoxyguanosine (8-OHdG), leukocyte DNA–MDA adducts, and DNA-strand breaks were investigated to determine whether the oxidative effects of CCl_4 would result in increased generation of these oxidation products. Plasma concentrations of MDA and isoprostanes (both measured by GC-MS) and urinary concentrations of isoprostanes (measured with an immunoassay or LC/MS/MS) were increased in both low-dose and high-dose CCl_4-treated rats at more than one time point. The other urinary markers (MDA and 8-OHdG) showed significant elevations

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with treatment under three of the four conditions tested. It is concluded that measurements of MDA and isoprostanes in plasma and urine as well as 8-OHdG in urine are potential candidates for general biomarkers of oxidative stress. All other products were not changed by CCl_4 or showed fewer significant effects.
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Keywords: CCl_4; Rat; Plasma; Urine; Lipid hydroperoxides; TBARS; MDA; Isoprostanes; Protein carbonyls; Methionine sulfoxidation; Tyrosine products; 8-OHdG; M_1G; DNA strand breaks; Free radicals

Introduction

Radical-mediated oxidation products of lipids, proteins, and DNA have been studied for many years [1–3] though the use of the products of these reactions as specific markers of oxidative damage in vivo has been developed only in the past decade. Because one of the greatest needs in this field of research has been to assess reliably oxidative stress status in animal models and in humans [4], a variety of methods for the measurement of oxidative stress have been proposed and a number of review articles addressing this issue have been written [5–10]. Currently, however, there is no consensus on which methods are the most useful, reliable, accurate, or specific for different types of oxidative insults [11]. There has been little direct comparison between the different methods using identical samples [12]. Therefore, it is extremely difficult to provide absolute reference values for the specific markers in different living systems [4,11].

The National Institute of Environmental Health Sciences (NIEHS) has organized and sponsored a multilaboratory validation study for determining which of the biomarkers used for noninvasive measurement of oxidative stress are most specific, sensitive, and selective for different types of oxidative insults [11,13]. This article is the second report of the nationwide Biomarkers of Oxidative Stress Study using acute CCl_4 poisoning as a rodent model for oxidative stress. During the past decades, application of this compound has been shown to be an excellent tool for the study of experimental oxidative injury due to its rapid metabolism in the liver to a trichloromethyl radical metabolite ($^{\bullet}CCl_3$) and subsequent initiation of lipid peroxidation. The first report with the results for several antioxidant biomarkers has already been published [14].

In this comprehensive study, the time- and dose-dependent effects of CCl_4 on the oxidation products of lipids, proteins, and DNA were measured in blood, plasma, and urine collected from rats treated under the same protocol as the previous study [14]. The criterion used to identify a good marker for the measurement of oxidative stress was a significant effect seen at both doses at more than one time point. It was hypothesized that plasma and urine levels of different oxidation products would increase due to the oxidative stress induced by CCl_4 in a time- and dose-dependent pattern.

Materials and methods

Chemicals and reagents

Carbon tetrachloride and all other chemicals and reagents used in the study were obtained from Sigma–Aldrich Corp. (St. Louis, MO, USA).

Animals and treatment protocol

Male Fisher 344 rats (260–280 g) obtained from Charles River Laboratories (Raleigh, NC, USA) were used in all experiments. The animals were housed three to a cage. Autoclaved hardwood bedding was used in solid-bottom polycarbonate cages with filter tops. Animal rooms were maintained at 20–25°C with 35–70% relative humidity with alternating 12-h light and dark cycles. The rats had free access to deionized, reverse-osmosis-treated water and received autoclaved NIH 31 rodent chow (Zeigler Bros., Gardners, PA, USA) ad libitum. For all experiments, rats were fasted overnight and then administered intraperitoneal (ip) injections of carbon tetrachloride in canola oil. Control rats received an equal volume of canola oil. Fasting continued through the experiment. Rats were anesthetized with Nembutal (0.1 ml/100 g body wt) and 5 ml blood was removed from the dorsal aorta proximal to its bifurcation into the common iliac arteries.

CCl_4 treatment

Animals from each of the three dose groups (control–canola oil, 120 mg/kg CCl_4 ip, and 1200 mg/kg CCl_4 ip) were investigated at three time points (2, 7, and 16 h after CCl_4 injection). Each group consisted of five rats for each analysis. Animal treatment, sample preparation, liver histology, and serum enzyme activities were described elsewhere [14,15] and carried out at NIEHS (Research Triangle Park (RTP), NC, USA).

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

Specimen collection

Whole blood sample preparation

Blood (5 ml) was drawn through single-draw 21-gauge vacutainer needles into open vacutainer blood collection