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OXIDATIVE DENITROSATION AND ACTIVATION OF N-NITROSODIMETHYLAMINE[+]

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The NADPH - dependent microsomal metabolism of N-nitrosamines (N-NA) has been shown to result in the formation of nitrite in addition to the concomitant generation of aldehydes and reactive metabolites (1). The mechanisms of these reactions, however, appear to be distinct since the activation of N-NA is linked to the liberation of nitrogen in its molecular state (2). Accordingly, the denitrosation of N-NA deserves particular attention as a potential detoxifying metabolic pathway for this class of carcinogens. Reductive reactions catalyzed by cyt. P-450 either directly (3) or via superoxide anion ($O_2^{\cdot-}$) generation (4) have been proposed as mechanisms for the formation of nitrite. Hydroxyl (OH -) radicals, on the other hand, which may be formed as a consequence of $O_2^{\cdot-}$ generation, remained to be examined regarding their function as oxidants in this mode of N-NA degradation. The present results obtained with an artificial OH - radical generating system (5) provide evidence for oxidative reactions resulting in the concurrent denitrosation and activation of N-nitrosodimethylamine.

MATERIALS AND METHODS

The incubation media were composed of 0.025 units ml^{-1} of xanthine oxidase, 0.5 mM xanthine, 10 μM Fe^{3+}-EDTA and 50 mM N-nitrosodimethylamine (NDMA) in 50 mM potassium phosphate, pH 7.5, - unless stated otherwise. The reactions were followed at 37°C over 7 min by repetitive transfer of aliquots to trichloracetic acid (TCA). Nitrite and formaldehyde were determined according to the principles of published procedures (6,7). The formation of reactive metabolites was analyzed under identical conditions except that 10 mg ml^{-1} of bovine serum albumin (essentially fatty acid free) and 1.5 μCi ml^{-1} of ^{14}C-NDMA were additionally present. The TCA - precipitated protein was resuspended and reprecipitated trice in 100 mM potassium phosphate, pH 7.5, to remove the unmetabolized nitrosamine. The final pellet was subjected to Protosol digestion prior to counting. The rate of OH - radical production under the experimental conditions described was estimated to be 15 μM min^{-1} using methanol as a substrate.

RESULTS AND DISCUSSION

The supplementation with N-nitrosodimethylamine (NDMA) of a reaction system known to generate hydroxyl (OH -) radicals via an iron - catalyzed Haber - Weiss

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Table 1. Characteristics of the hydroxyl radical - mediated degradation of NDMA

a. Product formation (V_{max}):	
Nitrite (NO_2^-)	2.1 µM min^{-1}
Formaldehyde (HCHO)	4.1 µM min^{-1}
BS-Albumin - binding*	1.8 µM min^{-1}
b. Concentrations required for half - maximal velocities (NO_2^- & HCHO):	
NDMA	5.5 mM
Fe^{3+}-EDTA**	2 µM
c. Concentrations required for half - maximal inhibition (NO_2^- & HCHO):	
Superoxide dismutase	12 nM
Catalase	5 nM
Benzoate	5 mM
Ethanol	25 mM

* expressed as ^{14}C-methyl equivalents
** after prior removal of contaminating iron with Chelex 100

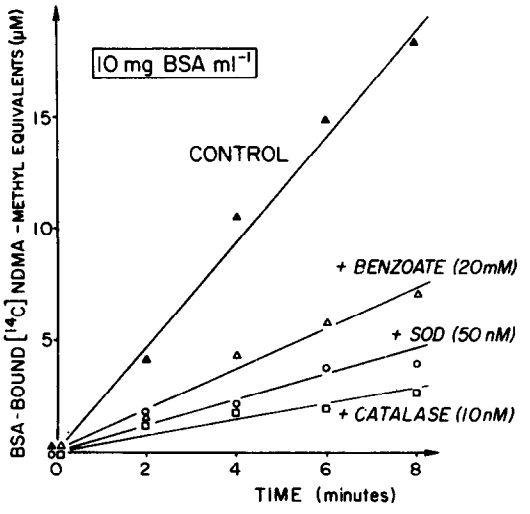

Fig. 1. Modification by inhibitors of the binding of NDMA-metabolites to BSA

reaction (5) was found to result in the formation of nitrite, formaldehyde and reactive metabolites (cf., Table 1). The dependence of nitrite and formaldehyde formation on the presence of chelated iron and the fact that only low levels of iron were required for half - maximal saturation are considered as evidence for the degradation of NDMA via OH - radical - catalyzed oxidative reactions (cf., Table 1). The indirect participation of both superoxide anions and hydrogen peroxide was verified by the demonstration of a high sensitivity of product formation to inhibition by superoxide dismutase and catalase, respectively (cf., Table 1 and Fig. 1). Consistent with these inhibitory effects of enzymes known to interfere with OH - radical formation are those observed with OH - radical scavenging agents such as benzoate and ethanol (cf., Table 1 and Fig. 1). From the results described it is concluded that oxidative as well as reductive reactions have to be considered as possible mechanisms for the NADPH - dependent microsomal denitrosation of N-nitrosamines. Although the involvement of OH - radicals in cyt. P-450 - catalyzed reactions is disputed at present (8,9,10), their participation in the metabolism of NDMA will be the cause of an additional formation of as yet unidentified reactive metabolites.

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