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CRYPTO - OH· RADICAL PRODUCTION BY NITROFURANTOIN

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The well-known bactericidal agent, nitrofurantoin (NF), has been in widespread use for more than a quarter of a century, mainly for the treatment of acute infections of the urinary tract (1 - 3). However, pulmonary fibrosis frequently occurs on prolonged treatment with the drug, which bears a resemblance to paraquat (MV) induced damage. Previous studies have indicated that NF might be able to reductively activate oxygen (4,5) in a similar manner to MV (6). Recently, we have provided evidence for the generation of a strong oxidising species, the crypto-OH· radical, from an electron donor and H_2O_2 (7,8). In the present communication, we describe the results of an investigation to discover whether NF was able to generate this potentially damaging species. Earlier work on the reduction of NF was performed using microsomal fractions or partially purified enzyme. The studies reported here were conducted with pure preparations of NADPH - cytochrome c reductase and xanthine oxidase.

METHODS

The formation of ethylene from methionine in the presence of pyridoxal phosphate is a simple method for the determination of the crypto-OH· radical (7,8). Reactions were conducted in Fernbach flasks sealed with serum rubber stoppers as described previously and ethylene was determined by gas chromatography (9). Anaerobic conditons were achieved and maintained as described elsewhere (8). The reaction mixture for the NADPH - cytochrome c reductase experiments was composed as follows; 10 µmol glucose - 6 - phosphate; 50 µg glucose - 6 - phosphate dehydrogenase; 1 µmol NADP; NADPH - cytochrome c reductase containing 0.1 mg protein; 20 µmol methionine; 0.2 µmol pyridoxal phosphate; 200 µmol phosphate buffer pH 7.8. Other additions are as indicated in the tables. The reactionswere conducted at 22°C for the times indicated. Experiments with the xanthine oxidase system contained 1µmol xanthine; 40 µg xanthine oxidase; the same concentrations of methionine and pyridoxal

phosphate as above and 200 µmol phosphate buffer pH 6.0. The total volume of the reaction mixture was 2ml in both enzyme systems.

RESULTS AND COMMENTS

We have previously shown that paraquat was able to mediate the production of crypto-$OH^{\cdot}$ radicals in a NADPH - cytochrome c reductase dependent reaction, but only under oxygen - limiting conditions (8). Since there are close parallels between MV and NF in the damage that they cause, it was of interest to investigate whether NF also produced this reactive oxygen species.

In both NADPH - cytochrome c reductase and xanthine oxidase dependent reactions, NF was found to mediate the production of ethylene from methionine (Table I).

Table I

Ethylene Formation Mediated by Nitrofurantoin

Additions	C_2H_2 Formation NADPH-cyt c reductase pmol/45 min	Xanthine oxidase pmol/90 min
None	5	52
1µmol NF	203	250
NF + 100 catalase	19	35
NF + SOD	206 (200 U)	12 (60 U)

Reaction conditions as described in Methods.

However, although both reactions were strongly inhibited by catalase, superoxide dismutase (SOD) only inhibited in the xanthine oxidase system. This result infers that the species responsible for the oxidation of methionine was not directly derived from superoxide.

A series of experiments was undertaken to determine the roles of oxygen and H_2O_2 in the generation of the active species.

Table II

Nitrofurantoin - Dependent Ethylene Formation under Anaerobiosis

Additions	C_2H_4 Formation NADPH-cyt c reductase pmol/45 min	Xanthine oxidase pmol/6o min
1 µmol NF	2	10
10 µmol H_2O_2	168	30
NF + H_2O_2	23190	15100
NF + H_2O_2 - enzyme	155	22

Reaction conditions as described in Methods.

The results presented in Table II indicate that ethylene formation was dependent on the presence of both NF$^{\cdot}$ radical and H_2O_2. The fact that superoxide was not a prerequisite in the anaerobic formation of ethylene via xanthine oxidase is taken as further evidence that this oxygen radical did not play a direct role in the generation of a strong oxidant. A plausible explanation for the differential effect of SOD in the two enzyme systems under aerobic conditions might be that whereas NADPH - cytochrome c reductase was not autoxidisable and therefore must have reduced NF directly, xanthine oxidase - dependent reduction of NF was mediated by superoxide, which was produced by direct reduction of dioxygen. Thus, only the initial steps of generating the NF$^{\cdot}$ radical differed in each system and subsequent reactions leading to the formation of a species capable oxidising methionine appear to be similar. This species is considered to be identical with the crypto-OH$^{\cdot}$ radical formed by MV under similar circumstances. However, the NF$^{\cdot}$ radical would appear to be more stable than that of MV under aerobic conditions (8).

The results presented here are in agreement with earlier reports on the activation of NF (4,5). The crypto-OH$^{\cdot}$ radical generated via NF - dependent reactions is a highly oxidising species and should be able to cause the damage associated with NF toxicity. Reduction of NF could be readily accomplished in lung tissue via NADPH - cytochrome c reductase.

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