DRUG METABOLISM AND DISPOSITION

Abstract

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Drug Metabolism and Disposition May 1988, 16 (3) 512-514;

0090-9556/88/1603-0512$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 16, No. 3
Printed in U.S.A.

Short Communication

Intervention of Nitrite in the Metabolism of Quaternary Ammonium Compounds

QACs[1] are xenobiotics by virtue of their wide environmental utilization (1–4) and their toxicological and pharmacological effects (5–8). They are nitrosatable *in vivo* (9) and *in vitro* (10), and their metabolites are primarily excreted in rat bile after ip administration (9). Rat liver microsomal preparations demethylate QACs (9), and concurrent administration of $NaNO_2$ and ADB-9 damaged rat liver, presumably from formed nitrosamine (9). This paper follows up these findings with an examination, in Wistar rats and *Buffo vulgaris* toads, of the implication of the presence of nitrite ion on ADB-9 metabolism *in vivo*. Unpublished data from this author show that toad liver also demethylates QACs.

Healthy male animals (150–200 g) were given 0.5 ml of aqueous solutions of pure ADB-9 by gastric intubation at 0.145, 0.29, 0.87, and 1.45×10^{-4} mol/kg, and 24-hr urine samples were obtained. Toad urine was collected using a special technique (11). A second group was pretreated with 1.5×10^{-3} mol of $NaNO_2$/kg and 5 min later was again intubated with 1.45×10^{-4} mol of ADB-9/kg, and urine was collected. Blood plasma was drawn from all animals after 4 hr using heparinized pipettes and assayed for volatile N-nitrosamines. All urine samples were investigated for amines and nitrosamines. Samples for nitrosamine analysis were collected in glass tubes containing 0.2–1 ml of 0.5 NaOH to prevent *in situ* nitrosation. A modified Sloneker method (12) was adopted for examining the ADB-9 content of urine. An aliquot of 0.5–5 ml of urine was vigorously shaken for 1 min in a clean and dry 25-ml separating funnel containing 1 ml of 1% aqueous picric acid solution. The ADB-9-picric acid complex was extracted into 5 ml of $CHCl_3$. The extract was dried over anhydrous Na_2SO_4, and its UV spectrum and absorbance at 360 nm were determined in a Unicam SP 800 UV spectrometer. Standard ADB-9 solutions in distilled water and urine samples spiked with ADB-9 (0.1–5 μg/ml) were similarly assayed. (These showed a characteristic absorption peak at 360 nm due to ADB-9-picric acid complex.) The limit of detection was 0.3 μg of ADB-9. Analyzing pooled and concentrated $CHCl_3$ extracts increased the sensitivity of the method. Urine samples were extracted on an Extrelut column (Merck, Darmstadt, FRG) with CH_2Cl_2. This was concentrated to 0.5 ml and examined by both unidirectional and two-dimensional TLC or by paper chromatography for ADB-9 and lower amine species. The chromatograms were developed in one or more of the solvent mixtures described in table 1 and scrutinized for UV fluorescent metabolites. Secondary amine content was quantified in rat urine as dimethylamine (13). Blood plasma and urine samples were diluted to 20 ml each with 0.9% saline and extracted directly on Extrelut columns with CH_2Cl_2 (14). Quantification of 1-ml CH_2Cl_2 concentrates for volatile nitrosamines was by GLC, after clean-up, (15) using pure reference compounds.

Received April 28, 1986; accepted December 3, 1987.

[1] Abbreviations used are: QAC, quaternary ammonium compound; ADB-9, nonyl dimethylbenzylammonium bromide; NDMA, nitrosodimethylamine.

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Recovery of the internal standard (100 ng of nitrosodiisopropylamine) was >90%. Nitrosamine presence was counterchecked by denitrosation of the CH_2Cl_2 extracts (16) after double dilution with ethyl acetate, evaporation and concentration to 0.5 ml, and mixing with an equal amount of glacial acetic acid. The denitrosating agent was 0.2 ml of 3% (v/v) HBr in glacial acetic acid. Nitrite released was determined spectrophotometrically (17).

No urine sample exhibited the characteristic absorption peak of ADB-9-picric acid complex thereby indicating that, within the detection limit, ADB-9 was not excreted into rat or toad urine. However, only the urine of ADB-9-dosed animals contained primary, secondary, and tertiary amine derivatives, whereas urine of animals given $NaNO_2$ plus ADB-9 was lacking in these entities (table 1). A dose-dependent excretion rate of secondary amines in rat urine, after ADB-9 administration, was emergent (table 2). Only blood collected from $NaNO_2$-pretreated animals produced any GLC peak (fig. 1) of retention time identical with NDMA or any reference nitrosamine. No urine sample exhibited any like peak. The mean NDMA concentration in 10 pooled samples was 0.08 μg/100 ml in rats and 0.05 μg/100 ml in toads. The detection limit was 2 ng of nitrosamine, and the very low levels of NDMA in blood precluded its mass spectrometric confirmation. Nevertheless, nitrite ion was detectable in blood extracts after treatment with HBr/glacial acetic acid. The production of this nitrosating species by the reaction is convincing evidence of the splitting of a nitrosamine and, therefore, of its presence *in situ* (18).

The results support an earlier finding (9) that quaternary ammonium surfactants are degraded *in vivo* and their metabolites excreted in bile. However, the significance of the present work resides in the indicated interaction *in vivo* between nitrite (or its derivative) and ADB-9 amine metabolites, resulting in the nonappearance of the latter in urine and presumably in nitrosamine formation. This dual and complementary role of nitrite ion, as an amine scavenger and a nitrosating agent *in vivo*, could be of pharmacological and toxicological significance in the metabolism of amines. The occurrence of amine entities in urine of animals given ADB-9 is in line with the proposed predealkylation mechanism in the nitrosative cleavage of QACs (10) and tertiary amines (19, 20). The proposed effect of nitrite on ADB-9 metabolism and subsequent reactions of the metabolite overlap. The detection of a nitrosamine in the urine of animals given ADB-9 plus $NaNO_2$ distinguishes the latter whereas the absence of the amine metabolites in the fluid differentiates the former. Although spontaneous nitrosation *in vivo* occurs preferentially in the stomach, as a result of gastric acidity, the phenomenon would be uncommon in a situation in which nitrite disappears from the stomach fairly rapidly (21–23) and intubated ADB-9 is not equally degraded.

The whole study underlines the possible contribution of quaternary ammonium compounds in nitrosamine carcinogenesis.

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PreviousNext
Back to top

In this issue

Drug Metabolism and Disposition
Vol. 16, Issue 3
1 May 1988

ISSN 1521-009X (Online)