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Urinary Excretion of O6-Butylguanine after the Administration of N-Nitroso-N-butylurea in Rats

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Abstract

O6-Butylguanine was detected in the urine of rats given the butylating agent N-nitroso-N-butylurea. O6-Butylguanine contents in the 24-h rat urinary samples after i.p. doses of 50, 100, and 200 mg/kg N-nitroso-N-butylurea were 1.03 ± 0.41 (SE), 8.30 ± 1.70, and 59.53 ± 6.52 pmol, respectively. This suggests that O6-butylguanine formation in nucleic acids might be repaired in vivo, possibly by base excision, besides other mechanisms. After i.v. doses of 0.1 and 1 mg/kg of O6-butylguanine to rats urinary excretion did not exceed 2% of the administered dose, suggesting that the amount of O6-butylguanine effectively released by base excision might be much larger than that detected in the urine after N-nitroso-N-butylurea. Inhibition of the enzyme O6-alkyl-DNA transferase by N-nitrosodimethylamine increased urinary O6-butylguanine resulting from exposure to N-nitroso-N-butylurea (100 mg/kg i.p.) up to four times, thus confirming an alternative DNA repair mechanism.

Introduction

Small alkylating agents such as N-nitrosamides and N-nitrosamines give rise to the formation of a series of alkylated DNA bases. O6-Alkylguanine is the primary promutagenic lesion produced and its persistence correlates with the carcinogenicity of the alkylating compound (1).

O6-Alkylguanines are readily repaired in bacteria and mammals by the activity of AT an enzyme that transfers the alkyl group from DNA to one of its own cysteine residues and is inactivated during this process (2). The protein most actively removes methyl groups, but larger alkyl groups such as ethyl, propyl, and butyl are also substrates, although the rate of removal drops as the alkyl chain becomes longer (3). AT is the O6-alkylguanine repair mechanism that has been most extensively studied, but alternative mechanisms may be involved in the removal of this lesion (4–7). We postulate that O6-alkylguanines, particularly those with alkyl chains longer than methyl, which are repaired by AT at a slower rate might also be repaired by base excision, as reported for other types of DNA lesions such as 7-methylguanine or 3-methyladenine (8). O6-Alkylguanine base excision in vivo might result in urinary excretion of the alkylated base, but the presence of this adduct in the urine of animals given alkylating agents has never been reported, although it has been considered in a few studies (9–11). This might be because of either the prompt repair by AT or the lack of highly sensitive and specific methods.

We tested this by measuring the urinary excretion of O6-Butylguanine in rats given the butylating agent NBU. O6-Butylguanine was quantitatively analyzed using a previously developed method which combines immunoaffinity extraction and high resolution gas chromatography-negative ion chemical ionization mass spectrometry with selected ion recording (12).

AT activity can apparently be modulated in vivo by various compounds including O6-methylguanine, O6-benzylguanine, and NDMA (13–15). The inhibition of AT might unmask alternative DNA repair mechanisms including base excision. This paper also presents the effect of inhibition of AT by NDMA on O6-Butylguanine urinary excretion resulting from exposure to NBU in rats.

Materials and Methods

Chemicals. O6-Butylguanine was synthesized as described previously (12); NBU was obtained from Serva Feinbiochimica GMBH and Co., Heidelberg, Germany, and NDMA was from Aldrich Milwaukee, WI. Guanine was purchased from Fluka, Buchs, Switzerland.

Animal Treatments. Male CD (SD)BR rats [body weight, 200 ± 10 (SE) g], obtained from Charles River (Calco, Italy), were housed under constant conditions with a 12/12-h light/dark cycle and free access to food and water, in metabolic cages for collection of urine samples.

The urinary excretion of O6-Butylguanine was tested in rats given the compound i.v. at doses of 0.1 and 1 mg/kg/0.5 ml DMSO. Urine was collected at 24-h intervals for 72 h after dosing. NBU was administered i.p. at doses of 50, 100, and 200 mg/kg/2 ml DMSO after an overnight fast. Urine was collected for 24 h and analyzed for O6-Butylguanine content.

To test whether circulating guanine could be alkylated by NBU, animals were given guanine 1 mg/kg p.o suspended in DMSO (2 ml/kg), followed, 1 h later, by either NBU (100 mg/kg/2 ml DMSO i.p.) or vehicle. Urine was collected over the next 24 h and analyzed for O6-Butylguanine content.

Each experiment included a group of animals treated with vehicle alone.

O6-Butylguanine Analysis. O6-Butylguanine was extracted and quantitatively analyzed as described previously, with minor modifications (12). Briefly, urine samples (5 ml) were diluted with 30 ml of 0.05 M phosphate buffer, pH 7.4, and the deuterated analogue of O6-Butylguanine was added as internal standard for O6-Butylguanine quantitation. Samples were then loaded on columns containing polyclonal antibodies raised against O6-Butylguanine coupled to Sepharose 4B and percolated at a rate of about 0.5 ml/min. Column washing, O6-Butylguanine elution, sample derivatization, and high resolution gas chromatography-negative ion chemical ionization mass spectrometry with selected ion recording were as described previously (12).

Results and Discussion

Table 1 reports the urinary excretion of O6-Butylguanine in rats given the compound i.v. at doses of 0.1 and 1 mg/kg. No background levels of O6-Butylguanine were detected in the urine of animals given the vehicle alone, the limit of sensitivity of the

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: AT, O6-alkylguanine-DNA alkyltransferase; O6-Butylguanine, O6-butylguanine; NBU, N-nitroso-N-butylurea; NDMA, N-nitrosodimethylamine; DMSO, dimethyl sulfoxide.

Table 1 reports the urinary excretion of O6-Butylguanine in rats given the compound i.v. at doses of 0.1 and 1 mg/kg. No background levels of O6-Butylguanine were detected in the urine of animals given the vehicle alone, the limit of sensitivity of the
method being 0.35 pmol O6-BuGua/24-h urine sample. O6-BuGua administered i.v. at 0.1 and 1 mg/kg was excreted unchanged in the urine, 0–72-h overall excretion being about 2% of the administered dose. In the urine of animals given 1 mg/kg O6-BuGua, excretion was highest 24 h after treatment (1.82 ± 0.28% of the dose) and declined steeply to 0.006 ± 0.001% of the dose at 72 h. The percentage of excretion was similar after the 10 times lower dose; in this group of animals the compound was detectable in urine only after the first 24 h (data not shown). These findings are at variance with those reported by Mandel et al. (9) who found a high percentage of O6-methylguanine in rat urine. Because of the presence of a larger alkyl group, O6-BuGua might possibly undergo faster degradation, but this was not verified.

The low urinary recovery of administered O6-BuGua was not due to poor stability of the compound. We checked this by adding known amounts of O6-BuGua to blank urine samples. The samples were kept at room temperature for 0, 4, and 24 h before O6-BuGua extraction and analysis. The results indicated that the compound was stable over the intervals considered (77 ± 2% recovered).

Urine O6-BuGua in rats 24 h after different doses of NBU is shown in Table 2. Excretion did not increase linearly with the dose but showed a 60-fold increment as the dose quadrupled. This has already been observed with other N-nitroso compounds (16, 17) and leads us to assume that at the lowest NBU dose, the O6-BuGua formed in DNA is mainly repaired by AT, but with higher doses of NBU the amount of O6-BuGua in DNA requiring to be repaired exhausts the suicide AT protein and saturates this process, so that more O6-BuGua can be repaired by other mechanisms. Although by no means proved in this study, the results suggest that O6-BuGua in urine is possibly due to a base excision repair process. Since only 2% of the administered O6-BuGua was excrated unchanged in the urine, we assume that the amount of urinary O6-BuGua detected after NBU was only a fraction of the alkylated base effectively released by base excision.

A dose of 20 mg/kg NDMA p.o. reportedly inhibits AT in the rat liver for about 24 h (15). We used this schedule of NDMA treatment to study the effect of inhibition of AT on the urinary excretion of O6-BuGua after NBU. As shown in Fig. 1, NDMA pretreatment modulated the urinary excretion of O6-BuGua during the 24 h after the NBU dose. The effect was greatest when NBU was administered 1 h after NDMA. At this time urinary O6-BuGua was 4 times higher than after NBU alone, shown as control in Fig. 1. Then the effect of NDMA declined and urinary O6-BuGua returned to control levels when the compound was administered 6 or 24 h before NBU.

The inhibition of AT, which is assumed to be the major repair mechanism for O6-alkylguanines, enhances the amount of urinary O6-BuGua that follows treatment with NBU, thus confirming the presence of repair mechanisms other than AT.
1 h and then processed as described in “Materials and Methods.” No significant differences were observed in O6-BuGua content before and after mild acid hydrolysis (data not shown), again supporting the suggestion of a base excision process.

To our knowledge, this is the first report showing that an O6-alkylguanine is excreted in the urine of animals exposed to small alkylating agents. Our study focused on O6-BuGua, but this allowed us to suppose that other O6-alkylguanines might be excreted in urine. This could be of particular interest if we take into account that human cancer can be induced by chemicals and that the evaluation of the exposure to chemical carcinogens becomes an important goal in cancer risk assessment. The presence of O6-alkylguanines in urine indicates not only that a repair process has occurred but also that a certain carcinogen that entered the body has been metabolized to alkylating agents able to react with nucleic acids. As previous studies have suggested, urinary excretion of these DNA adducts might be used as a biological marker of the exposure to alkylating agents in animal models or in humans (20).

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References

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