Oncogenicity of Purine 3-Oxide and Unsubstituted Purine in Rats

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ABSTRACT

Injection s.c. of purine 3-oxide into Wistar rats resulted in the appearance of sarcomas and fibromas at the interscapular site of administration, carcinomas in the liver, and a high incidence of s.c. fibromas in the hip at a distance from the site of injection. A small number of liver tumors but no tumors at the injection site appeared in rats to which the parent compound, purine, was administered. Oxidation of purine 3-oxide by xanthine oxidase was found to occur in two steps to yield the potent oncogen 3-hydroxyxanthine. A similar process may occur in vivo since a protein preparation from rat s.c. tissue has similar oxidizing activity.

INTRODUCTION

The potent oncogenicity of the N-oxides of such important substituted purines as guanine, xanthine, and adenine (1, 15-18) stimulated our interest in the study of purine 3-oxide as well as of the parent unsubstituted purine. Both the position of the N-oxide in the purine ring and various substituents influence the oncogenicity or potency of the particular purine (1, 17). Thus, while 3- and 7-hydroxyxanthines are highly oncogenic, 1-hydroxyxanthine and adenine 1-oxide are much less potent (16, 17). This difference can be attributed to enzymatic transformation of some purine N-oxides to chemically reactive sulfate esters (9) (guanine 3-oxide, 3-hydroxyxanthine, 7-hydroxyxanthine) or to a nonreactive ester [9-hydroxyxanthine (17)]. With respect to the parent compounds, Sugiuara et al. (16) reported xanthine, guanine, and adenine as nononcogenic. However, Haddow and Hornung (6) did report 1 sarcoma in 12 rats treated with xanthine.

Purine itself is not a component of nucleic acid; it is found in nature only as the nucleoside antibiotic nebularine (2), first isolated from certain mushrooms. Of the possible N-oxides of unsubstituted purine, only purine 3-oxide has been isolated, fully characterized, and prepared in amounts sufficient for assay.

This report details assays for oncogenicity of purine and purine 3-oxide in rats.

MATERIALS AND METHODS

Bioassays for Oncogenicity. Assays for oncogenicity and maintenance of animals were carried out as described (1, 18). Weanling male Wistar rats (CFN; Carworth Farms, New City, N. Y.) were given injections s.c. in the interscapular area of 0.5 ml of a homogenate of test compound in CMC on alternate days 3 times weekly for 8 weeks. Groups of 20 or 21 rats were given 7.5 or 0.75 mg of purine 3-oxide or, at a molar equivalent of base, 6.5 or 0.65 mg of purine. Controls were given CMC alone.

Animals were palpated for tumor masses weekly beginning the third month of the experiment and were killed generally within 4 to 8 weeks of palpation of a nodule at the s.c. site of injection because of enormous or ulcerating tumors. Surviving rats were killed at the end of the 18th month of the experiment. Autopsies were performed on all animals. Samples of tumors, various abnormalities, and liver were fixed in 10% formalin, prepared histologically, stained with hematoxylin and eosin, and examined microscopically. Tumor latency is the time from the first injection of test compound to palpation of a mass locally. Latencies for liver tumors could not be determined because they were not palpable early.

Chemical Synthesis. The synthesis of purine 3-oxide has been described (4).

Oxidation of Purine Oxides by Xanthine Oxidase. Purine 3-oxide (50 mg) was purified on a 10-ml column of BioRad AG-50 equilibrated with ammonium acetate, pH 4.95 (0.2 M NH₄⁺), and then washed with H₂O. Pure purine 3-oxide eluted between 10 and 16 ml H₂O. Oxidation was carried out in UV cuvets containing the substrate, 0.1 μmol/ml, in 0.05 M potassium phosphate, pH 7.5, containing 10⁻⁶ M EDTA. Xanthine oxidase, 0.05 ml, was added as the commercial preparation, and reaction rates were measured at the wavelengths indicated in Table 2. Aliquots of the reaction mixture were analyzed on a 5-ml column of BioRad AG-50 (H⁺), equilibrated with H₂O and eluted with 5 ml of H₂O followed by 0.1 N HCl. A micropreparative run was analyzed on the same column.

Reaction of Hypoxanthine 3-Oxide with Acetic Anhydride. A solution of 15 μmol of hypoxanthine 3-oxide (2.57 mg), 10 mg of sodium bicarbonate, and 3 x 10⁻⁶ cpm of [methyl-¹⁴C]methionine, 50 mCi/mmol, was heated to 90° with stirring. To the clear solution was then added 0.01 ml acetic anhydride, and the reaction was allowed to proceed for 5 min. The mixture was then separated on a 10-ml column (BioRad AG-X8, minus 400 mesh) after markers of 2-methylmercaptohypoxanthine (3) and 8-methylmercaptop-hypoxanthine (12) had been admixed. Elution volumes in 1.0 N HCl were 6,8-dihydroxypurine, 17.6 ml; xanthine, 45.5 ml; hypoxanthine 3-oxide, 63 ml; hypoxanthine, 76 ml; 8-methylmercaptopxanthine, 110 ml; and 2-methylmercaptop-hypoxanthine, 180 ml.

Oxidation of Hypoxanthine 3-Oxide by s.c. Tissue. Scrapings of s.c. tissue from a male Wistar rat were imm}

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the rats, a phenomenon not previously observed with any
dose. Tumors also appeared in the hip area of 35% of
other oncogenic purine derivative. At the 0.75-mg dose 1
dose induced tumors in 60% of the rats (Table 1). One
was immediately passed over an 80-ml column of
volume was then used to oxidize hypoxanthine 3-oxide in a
buffer as described above for xanthine oxidase.

RESULTS

Bioassays for Oncogenicity. Purine 3-oxide at 7.5 mg/
dose induced tumors in 60% of the rats (Table 1). One
sarcoma (fibrosarcoma) and 4 fibromas were present locally
in 5 rats at the interscapular s.c. site of injection, with
latencies of 9 to 18 months. Hepatocellular carcinomas
were found in livers of 9 rats, 7 of which had no local
tumors. Fibromas also appeared in the hip area of 35% of
the rats, a phenomenon not previously observed with any
other oncogenic purine derivative. At the 0.75-mg dose 1
sarcoma and 1 fibroma (7- and 16-month latencies, respec-
tively) were induced locally; no liver tumors were seen at
this lower dose.

For confirmation of the unusual appearance of fibromas
in the hip area of many of the rats at a distance from the
injection site, a replicate assay was carried out at the 7.5-
mg dose schedule. Of 15 rats 5 (33%) had tumors at the site
of injection (Table 1), with latencies of 10 to 18 months. In
to addition to the local tumors, hepatocellular carcinomas
were found in 3 of the 5 rats, and 6 hepatocellular carcinomas
and 1 cholangiocarcinoma were present in livers of 6
rats that had no local "site" tumors. Again, fibromas ap-
ting in the hip area of 7 rats (47%), 1 of which had no
tumor at the site of injection.

Reactivity of Esters of Purine 3-N-Oxide and Hypoxan-
thine 3-N-Oxide. Neither the ester formed from purine 3-N-
oxide* nor the 1 from hypoxanthine 3-N-oxide showed any
of the electrophilic and oxidizing activity (14) associated
with the esters of the oncogenic purines such as 3-hy-
droxynanthine. However, the ester from hypoxanthine 3-N-
oxide was found to have some reactivity at elevated temper-
atures. This was investigated further since it is conceivable
that this reactivity also occurs at 37° but at a rate escaping
detection. When 15 µmol of hypoxanthine 3-N-oxide were
treated with acetic anhydride at 90° (for details see "Mate-
rials and Methods"), the reaction products included 1.18
µmol of 6,8-dihydroxypurine, 2.07 µmol of xanthine, 1.08
µmol of hypoxanthine 3-N-oxide, and 2.82 µmol of hypo-
oxanthine. Methionine, 14C-labeled in the methyl group, was
also included in this experiment, but less than 1% of the
total radioactivity was found associated with 2-methylmer-
capto- or 8-methylmercaptoxanthine, both of which had
been added as markers after the reaction was completed.
Under the conditions of this experiment, 3-hydroxyxanthine
yields over 90% of radioactive 8-methylmercaptoxanthine.

Oxidation of Purine Oxides by Xanthine Oxidase. Purine
3-N-oxide is oxidized by milk oxidase first to hypoxanthine
3-oxide and then to 3-hydroxyxanthine and 3-hydroxyuric
acid, as shown in Chart 1. [The last step in this reaction has
been studied by Myles and Brown (11.) There is no chro-
matographic evidence for the formation of the conceivable
oxidation intermediates, the known 3-hydroxy-2,3-dihydro-
2-oxopurine (7) or the unknown 3-hydroxy-7,8-dihydro-8-
oxopurine. Initial rates of the oxidation have been deter-
mined for all steps and are given in Table 2. Among this
group of purine derivatives, purine 3-oxide is the poorest
substrate, comparatively, while 3-hydroxyxanthine is the
best. The pathway shown in Chart 1 was verified by ion-
exchange chromatography of an enzymatic reaction mix-
ture where the products hypoxanthine 3-N-oxide and 3-
hydroxyxanthine (coinciding with 3-hydroxyuric acid) were
well resolved.

Oxidation of Hypoxanthine 3-Oxide by Tissue Homoge-
Nate. A protein extract of s.c. tissue (see "Materials and
Methods") can also catalyze oxidation of hypoxanthine 3-
oxide. The rate of oxidation with this preparation at 25° was
about 0.5 nmol of hypoxanthine 3-oxide oxidized per min
per 0.4 mg of protein. This is roughly 10% of the activity per
unit of protein of purified milk xanthine oxidase. The

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* Purine 3-oxide was not mentioned in Table 2 of Ref. 14 since all
measurements were 0.
Oxide.

tissues can oxidize purine 3-oxide, as does xanthine oxidase, which produces 3-hydroxyxanthine, is transported from the liver.

The appearance of large numbers of s.c. fibromas in the hip area of rats treated with purine 3-oxide is a phenomenon that might be expected to appear (17).

The lack of reactivity of esters of purine 3-oxide and hypoxanthine 3-oxide was further demonstrated in a Bacillus subtilis transforming mutagenesis assay (8). In that assay 3-acetoxyxanthine was a strong mutagen, but the esters of purine 3-oxide and hypoxanthine 3-oxide were not mutagenic, nor did they decrease the transforming activity of the DNA. Thus, purine 3-oxide must be presumed to be oxidized in 2 steps to yield the known highly oncogenic 3-hydroxyxanthine.

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**REFERENCES**


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