Distribution of 6-Mercaptopurine Ribonucleoside and Other Purine Analogs to Brain

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SUMMARY

Experiments are reported that compare the distribution to brain of purine base and ribonucleoside pairs labeled in the purine moiety. A procedure for the facile preparation of radioactive 6-mercaptopurine ribonucleoside from 6-mercaptopurine-8-14C using purine nucleoside phosphorylase is also described. Radioactivity in plasma, brain, and muscle was determined after administration of 6-mercaptopurine, 6-mercaptopurine ribonucleoside, 6-thioguanine, or 6-thioguanine ribonucleoside in mice or rats. At the times chosen for tissue sampling, there were no major differences in brain uptake between the base and ribonucleoside pairs. Muscle radioactivity ranged from 3 to 7 times that in brain, indicating a barrier to the penetration of these agents into brain. Brain to plasma concentration ratio after 6-mercaptopurine ribonucleoside administration was independent of dose (0.02 to 50 ¡¿moles/mouse; 0.03 to 20 ¡¿moles/rat), route of administration (i.p., p.o., i.v.), and experimental duration (0.5 to 2 hr). Analyses of cerebrospinal fluid radioactivity in rats indicate that 6-mercaptopurine ribonucleoside penetrates this compartment only to a limited extent.

INTRODUCTION

Toyoshima et al. (8) have reported that 6-MPR4 may offer therapeutic advantage over the free base, 6-MP, by virtue of a high distribution to brain. This observation has recently been quoted in a major clinical text concerning cancer medicine (3). We have examined the distribution of 6-MP, 6-MPR, 6-TG, and 6-TGR using drugs labeled in the purine moiety. In contrast to Toyoshima et al. (8), we found no major differences in the distribution to brain between base and nucleoside pairs. The earlier observation (8) that 6-MPR enters brain more readily than 6-MP may relate to the use of uniformly labeled 6-MPR-1H.

MATERIALS AND METHODS

Preparation of Labeled Compounds. 6-MPR-8-14C was synthesized from 6-MP-8-14C (27 ¡¿Ci/¡¿mole; Schwarz/Mann, Orangeburg, N. Y.) in the following manner. A solution containing 1 ¡¿mole of 6-MP-8-14C and 5 ¡¿moles of ¡-D-ribose 1-phosphate in 2 ml of 10 mM Tris-HCl at pH 7.4 was treated with 0.8 ¡¿M units (1) of crystalline purine nucleoside phosphorylase (EC 2.4.2.1). The reaction was allowed to proceed at room temperature for 30 min, after which it was stopped by heating on a boiling water bath for 1 min. The entire sample was then placed on a PEI-cellulose (Bio-Rad Laboratories, Richmond, Calif.) column (25 x 1 cm) and eluted with water. Complete separation of 6-MP and 6-MPR was effected, as determined with cold material by the UV absorption at 320 nm. The 6-MPR-8-14C peak was lyophilized and stored as a frozen solution in 0.9% NaCl. Conversion of 6-MP-8-14C to 6-MPR-8-14C was virtually complete under these conditions.

6-TG-35S and 6-TGR-35S were prepared by isotopic exchange (5) using rhombic 35S obtained from New England Nuclear, Boston, Mass. The purity of the labeled compounds was determined by thin-layer chromatography using PEI cellulose eluted with water or cellulose eluted with 1-butanol:acetic acid:water (4:1:1). Strips of the thin-layer plastic sheets were cut at 1-cm increments, and the radioactivity in each strip was determined and compared to the RF of added, cold material. The purity of 6-MP-8-14C and 6-MPR-8-14C was greater than 92%, and the purity of 6-TG-35S and 6-TGR-35S was greater than 98% by these criteria.

Chemicals and Reagents. Unlabeled reagents and drugs were products of Sigma Chemical Co., St. Louis, Mo. Crystalline purine nucleoside phosphorylase was purified from human erythrocytes by Chong Kong of this laboratory using the method of Agarwal and Parks (1). PEI-cellulose and cellulose-coated plastic sheets were obtained from Brinkmann Instruments, Inc., Westbury, N. Y.

Distribution Studies. Female CD1 mice (25 to 30 g) and male rats (150 to 350 g) from Charles River Laboratories, North Wilmington, Mass., were used. Drugs were administered i.p., p.o., or i.v. in 0.9% NaCl solution. Animals were sacrificed between 0.5 and 2 hr after drug administration, and samples of plasma, whole brain, leg muscle, and CSF were assayed for radioactivity.
For i.p. administration, radiolabeled drug was diluted with cold compound to give a total dose of 1.8 μmoles/mouse (~ 0.5 μCi/mouse) or 20 μmoles/rat (~ 5 μCi/rat). The p.o. dose of 6-MPR was 50 μmoles/mouse, a dose comparable to that used by Toyoshima et al. (8). Animals were sacrificed by cervical dislocation 1 hr after i.p. or 2 hr after p.o. administration. Blood samples were then obtained rapidly by cardiac puncture using heparinized syringes.

For i.v. administration, rats were anesthetized with pentobarbital, 30 mg/kg i.v. The jugular vein and carotid artery were then exposed and cannulated with No. 50 polyethylene tubing. Isotope solution was infused into the venous cannula using a Harvard Model 940 infusion/withdrawal pump, and serial blood samples were drawn via the arterial cannula. A priming dose of 1 to 2 μCi was infused over a period of 7.5 min, followed by a slow, sustaining infusion which delivered one-half of the priming dose in 75 min, a time that corresponds to the biological half-life of 6-MPR in plasma as determined in 2 experiments. This procedure maintained relatively constant plasma isotope levels throughout the experiment. Rats were sacrificed 30 (3 animals) or 60 (2 animals) min after beginning the infusion. Immediately prior to sacrifice, 50 to 100 μl of CSF were collected stereotaxically from the cisterna magna by puncturing the exposed atlantooccipital membrane with a 27-gauge needle attached to No. 20 ethylene tubing.

Radioactivity was determined by liquid scintillation counting. Duplicate aliquots (10 to 50 μl) of plasma and CSF were transferred to scintillation vials. Minced samples of whole brain and of muscle were transferred to tared scintillation vials and weighed. All samples were solubilized by incubation in 0.5 ml of Protosol (New England Nuclear) overnight at 40°. After cooling, 15 ml of Liquiflor (New England Nuclear) were added to each vial for subsequent radio assay. Quench corrections were made with an automatic external standard.

Calculation of Results. Plasma and CSF concentrations are expressed in dpm/ml; brain and muscle concentrations are recorded in dpm/g. Total drug concentration of plasma (μmoles/ml) was calculated, assuming no in vivo drug metabolism, according to the relationship: μmole/ml plasma = dpm/ml plasma ÷ dpm/μmole injected drug. All values are mean ± S.E.

RESULTS AND DISCUSSION

Table 1 summarizes results of distribution studies in mice following i.p. administration of the 2 base nucleoside pairs, i.e., 6-MP, 6-MPR and 6-TG, 6-TGR. Results are expressed as the ratio of isotope concentrations, brain:plasma and muscle:plasma. Distribution ratios for muscle are significantly greater than those for brain for all 4 compounds, indicating a barrier to drug penetration into the central nervous system; however, other factors, e.g., cellular uptake, could be involved in the difference between brain and muscle levels. Comparison of brain distribution ratios for the 2 base nucleoside pairs indicates that neither nucleoside penetrates into brain to a greater extent than its corresponding base. In fact, the distribution of 6-MPR to brain appears to be less than that of 6-MP. The nucleoside analogs shown are cleaved to the corresponding bases by purine nucleoside phosphorylase (6), and the similar tissue handling may be due to this cleavage. The distribution of 6-MP is similar to that reported by Elion et al. (2). A single dose of 6-MPR is less toxic than an equimolar, single dose of 6-MP (7, 8). The present observation that lower levels of the purine moiety persist 1 hr after administration of 6-MPR than after an equimolar dose of 6-MP may indicate that certain tissues, i.e., bone marrow, are exposed to less drug when the nucleoside rather than the base is used. 6-MP and 6-MPR also penetrate brain to a similar extent in rats after i.p. administration. The ratio of isotope concentrations, brain:plasma, for 6-MP and 6-MPR in rats was 0.126 ± 0.014 (N = 3) and 0.121 (0.102, 0.140) (N = 2), respectively.

In their study, Toyoshima et al. (8) gave a large p.o. dose of 6-MPR. Table 2 summarizes results of studies examining the effects of drug dose, route of administration, and experimental duration on 6-MPR penetration into the central nervous system. In addition to i.p. administration, the drug was given as a single p.o. dose, following the protocol of Toyoshima et al., and by continuous i.v. infusion, to maintain a constant plasma concentration. Rats were used for the latter experiments, since their larger size facilitated the processes of CSF collection and i.v. infusion. The ratio of isotope concentrations between CSF and plasma agrees with the value given by Loo et al. (4) for 6-MPR-35S in dogs and indicates limited penetration of 6-MPR into this compartment. Brain distribution ratios for 6-MPR are similar for all experimental conditions, suggesting that the discrepancy between ours and previous experiments cannot be explained by differences in the method of drug administration.

In summary, distribution studies of the 2 base nucleoside pairs, 6-MP, 6-MPR and 6-TG, 6-TGR, indicate that neither nucleoside penetrates from plasma into the central nervous system to a greater extent than its corresponding base. Test compounds used in the present study were labeled in the purine moiety using 14C (6-MP, 6-MPR) or 35S (6-TG, 6-TGR). The previous observation (8) that uniformly labeled 6-MPR-3H penetrates readily into brain may have resulted from uptake by brain of a tritiated compound other than the analog ribonucleoside, e.g., H2O-3H or ribose-3H.
Distribution of 6-MPR as a function of dose, route of administration, and time

6-MPR was administered i.p., p.o., or i.v. to groups of 4 to 5 animals as shown. Total drug dose and experimental duration are indicated. 6-MPR was given p.o. according to the protocol of Toyoshima et al. (8); the rate of i.v. infusion was adjusted to maintain a relatively constant plasma isotope concentration.

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Dose (mmoles/animal)</th>
<th>Time (hr)</th>
<th>Species</th>
<th>Distribution ratios (dpm/g tissue or ml CSF:dpm/ml plasma)</th>
<th>Plasma drug concentration (nmoles/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>1.8</td>
<td>1</td>
<td>Mouse</td>
<td>Brain:plasma 0.089 ± 0.007, Muscle:plasma 0.092 ± 0.010</td>
<td>5.5 ± 2.1</td>
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<tr>
<td>i.p.</td>
<td>0.02</td>
<td>1</td>
<td>Mouse</td>
<td>Brain:plasma 0.103 ± 0.015, Muscle:plasma 0.138 ± 0.008</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>i.v. infusion</td>
<td>0.03-0.1</td>
<td>1/2-1</td>
<td>Rat</td>
<td>Brain:plasma 0.53 ± 0.04, Muscle:plasma 0.69 ± 0.16</td>
<td>30 ± 20</td>
</tr>
</tbody>
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REFERENCES

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