6-Mercaptopurine: high-dose 24-h infusions in goats *


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Summary. In vitro investigations have indicated the need for both prolonged exposure to 6-mercaptopurine (6MP) and the use of high concentrations to achieve maximal cell kill. After the customary oral administration the bioavailability of 6MP appeared to be low, and i.v. bolus injections resulted in short-lived high concentrations of 6MP, so prolonged infusions seemed rational. To test the feasibility of this approach 24-h infusions were given to goats. We used our improved HPLC method to quantitate 6MP and 6MP riboside (6MPR) in plasma, CSF, and urine. The concentrations of 6MPR were in excess of those of 6MP. Since 6MPR can easily be converted to 6MP, 6MPR acts as a depot for 6MP. Penetration of both 6MP and 6MPR into CSF was excellent. Of the total dose administered, 38% to 68% could be accounted for in the urine, with about equal amounts of 6MP and 6MPR. At doses of 20 and 10 mg kg\(^{-1}\) h\(^{-1}\) total concentrations of 6MP and 6MPR in excess of 100 \(\mu\)M were reached during 24-h infusions. However, all three experimental animals died due to toxicity. A dose of 2 mg kg\(^{-1}\) h\(^{-1}\) was tolerated; the total steady state concentration of 6MP and 6MPR in two experiments was about 10 \(\mu\)M. We conclude that the prolonged infusion of 6MP is feasible, and in view of the excellent penetration of 6MP and 6MPR into CSF, studies using prolonged infusions of thiopurines are warranted in man.

Key words: 6-Mercaptopurine - 6-Mercaptopurine riboside - Thiopurines

Introduction

For three decades the thiopurines, 6MP and 6TG, have been used in the treatment of malignant diseases, in particular the leukemias. The route of administration and the dose have been based on empirical grounds and clinical experience. The recent availability of sensitive and specific analytical methods has made it possible to perform sophisticated pharmacokinetic studies on these antimetabolites (De Abreu et al. 1982; Van Baal et al. 1984). The results of these studies led many investigators to question the rationale of the customary oral administration (Brox et al. 1981; Ding and Benet 1979; Zimm et al. 1983).

Metabolic activation of 6MP and 6TG is necessary in each individual target cell. This involves the transfer of ribose phosphate to 6MP or 6TG with phosphoribosylpyrophosphate as a co-substrate and hypoxanthine guanine phosphoribosyl transferase as the enzyme. 6MP, an analog of hypoxanthine, is transformed to 6-thio-inosine monophosphate (6-thio-IMP), which can be further metabolised to 6-thio-GMP by two rate-limiting key enzymes. On the other hand 6-thio-IMP can be dephosphorylated to 6MPR by 5′-nucleotidase. 6TG, an analog of guanine, is transformed directly to 6-thio-guanine monophosphate (6-thio-GMP), which can be further metabolised to 6-thio-GMP by two rate-limiting key enzymes. On the other hand 6-thio-IMP can be dephosphorylated to 6MPR by 5′-nucleotidase. 6TG, an analog of guanine, is transformed directly to 6-thio-guanine monophosphate (6-thio-GMP). Ultimately 6-thio-GMP can be incorporated into DNA and RNA. 6-Thio-IMP and its methylated analog 6-methyl-thio-IMP have profound inhibitory effects on purine de novo synthesis. Which of these biochemical pathways, either incorporation into DNA and RNA or blocking the purine de novo synthesis, is responsible for cell kill is still a point of discussion after so many years (Le Page 1977; Paterson and Tidd 1975). It cannot be excluded that more than one mechanism is involved and that these mechanisms differ in various tumor (cell) models. Also the method by which a cytotoxic effect is measured may be of importance. For instance, growth inhibition may correlate...
with the well-known immunosuppressive effects of thiopurines. However, clonogenic assays are the methods of choice for assessing cytotoxic effects of anticancer drugs (Mackillop et al. 1983; Rupniak et al. 1983). In vitro studies, using clonogenic assays, have indicated the importance of the duration of exposure, in addition to the effect of the concentrations of 6MP and 6TG (Tidd et al. 1972; Tidd and Paterson 1974a; Wotring and Roti Roti 1980). As a rule longer exposure and higher concentrations of 6MP or 6TG lead to greater cell kill. A plateau effect in cell kill becomes apparent at concentrations above 100–200 gM for 6MP and 1–2 µM for 6TG (Tidd and Paterson 1974b). However, a study on 9L rat brain tumor cells continuously exposed to 6MP showed paradoxical behavior of 6MP as a cytotoxic agent i.e., decreasing cell kill with increasing drug dose (Matsumura et al. 1983).

The in vitro data on thiopurines and the results of our recent (Schouten et al. 1985) and other pharmacokinetic studies (Brox et al. 1981; Ding and Benet 1979; Schouten et al. 1984; Zimm et al. 1983) led us to suggest that prolonged infusions of thiopurines should be studied. By prolonged infusion both the problem of the erratic absorption after oral administration (Brox et al. 1981; Ding and Benet 1979; Zimm et al. 1983) and the limited period of measurable plasma concentrations after i.v. push injections (Ding and Benet 1979; Schouten et al. 1985; Zimm et al. 1983) can be circumvented.

Our data on children (Schouten et al. 1984) and other data on monkeys (Narang et al. 1983) showed good penetration of 6MP into CSF, although an earlier study on this aspect was negative (Nelson et al. 1974). On the other hand on physicochemical grounds, like molecular weight and lipid solubility, good penetration of thiopurines into the CSF was suggested by Mellet in 1977.

Therefore it was also of interest to investigate to what extent thiopurines reach the CNS during prolonged infusions. In the present paper we report on the feasibility of prolonged infusions of 6MP in goats at different dose levels.

Materials and methods

Chemicals

6MP was obtained from Burroughs Wellcome (London, England) and from Fluka (Hioo B.V., Rotterdam, The Netherlands). The 6MP solutions for i.v. infusions were prepared on the day of administration. Helium was flushed through all solutions in which 6MP was dissolved and diluted, in order to prevent oxidation of 6MP. The desired amount of 6MP was dissolved in sodium bicarbonate 1.5% (w/v), to which sodium hydroxide was added until 6MP was completely dissolved (final pH 9.6–10.2) (Van Baal et al. 1984). The ultimate concentration of the solution was dependent on the limited solubility of 6MP, the dose to be administered, and the characteristics of the dual syringe infusion pump.

Experimental animals

Goats were chosen as experimental animals, because CSF sampling from these animals had been performed previously in our Central Animal Laboratory (Lippens 1981). Moreover, goats are rather docile, a prerequisite condition to maintain i.v. infusions for prolonged periods. During and after the experiments the goats were housed in the laboratory in separate stables. Initially catheters for i.v. infusion and for obtaining blood samples were placed percutaneously in both jugular veins on the day of the study. Later catheters were inserted by operation 4 to 5 days before the date of the infusion. To prevent catheter damage and to minimize the risk of systemic infection the catheters were tunneled subcutaneously to the posterior neck. Heparin locks were used to preserve catheter patency. Care was taken to insert the catheter for infusion some 5 to 10 cm deeper than the catheter for sampling purposes.

On the day of the experiments attempts were made to insert a thin catheter by percutaneous puncture into the lumbar spinal canal. An indwelling balloon catheter was used for urine collection. The insertion of the various catheters was accomplished during a short thiopenthal and ketamine narcosis.

Dose and administration of 6MP

In an initial experiment an i.v. push injection was given to obtain some basic pharmacokinetic parameters. In all other cases infusions were given which were planned to last 24 h. The starting dose of 6MP was 20 mg kg⁻¹ h⁻¹. The toxicity of the drug forced us to reduce the dose to 10 and 2 mg kg⁻¹ h⁻¹, respectively. We started with the higher dose, since our aim was to attain a plasma concentration of 6MP of the order of 100–200 µM. The drug was infused by a dual syringe infusion pump, loaded with two 60-ml syringes. For practical reasons the volume/h was chosen in such a way that syringes had to be changed either every 4 or every 8 h. The total amount of drug was prepared on the day of the experiment and stored in capped syringes at room temperature. The drug was infused through Millipore membrane filters, type Millex GS (pore size 0.22 µm).

Preparation of samples and 6MP determination

Blood samples of 5–6 ml were collected in tubes containing heparin and 300 µg of DTT to prevent oxidation of 6MP (De Abreu et al. 1982; Van Baal et al. 1984). After thorough mixing, the blood was chilled and centrifuged (5 min, 2000 × g).

Using a calibrated syringe 1 ml of plasma was transferred into micro test tubes (type 3810, Eppendorf, Hamburg, FRG) and kept on ice. Protein was precipitated by adding 0.1 ml recently prepared, ice-cold, trichloroacetic acid (50% w/v) (Van Baal et al. 1984). After vigorous shaking, the tubes were kept on ice and after 10 min the precipitate was sedimented by centrifugation (10 min, 2000 × g).

Samples of ±0.5 ml CSF were taken and placed in micro test tubes containing DTT.

Urine samples were taken from measured aliquots collected during specified time periods. Before samples were removed care was taken to dissolve deposits in the collecting reservoirs. Urine samples were placed in tubes containing DTT. Before analysis NaOH was added to dissolve any deposit.

6MP and 6MPR concentrations were determined on a Spectra Physics SP 8000 HPLC as previously described (Van Baal et al. 1984). The concentrations of 6MP and 6MPR are expressed in µM.