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The absorption of 6-mercaptopurine from 6-mercaptopurine riboside in rat small intestine: effect of phosphate

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Abstract The intestinal absorption of 6-mercaptopurine and its nucleoside 6-mercaptopurine riboside has been studied in the rat with the in situ dual luminal and vascular perfusion. 6-Mercaptopurine is an inactive prodrug that requires intestinal absorption, cellular uptake and intracellular anabolism for cytotoxic activity. Vascular and mucosal samples were analysed by high-performance liquid chromatography (HPLC) to assess the rate of vascular appearance and amounts of thiopurine and its nucleoside in the mucosa. With 5 mmol luminal 6-mercaptopurine/l, the drug is transported across the intestine unchanged at a rate of 0.053 ± 0.006 μmol min^{-1} (g dry wt.)$^{-1}$. At concentrations below 20 mmol/l, 6-mercaptopurine riboside is not transported across the intestine intact but is split by phosphorolysis in the intestinal mucosa. The rate of vascular appearance of 6-mercaptopurine [0.043 ± 0.005 μmol min^{-1} (g dry wt.)$^{-1}$] from 5 mmol luminal 6-mercaptopurine riboside/l did not differ significantly from that seen with 5 mmol luminal 6-mercaptopurine/l. When the lumen was perfused with 6-mercaptopurine riboside the riboside appeared in the tissue together with a higher mucosal concentration of 6-mercaptopurine than in perfusions with 6-mercaptopurine. Some metabolism of 6-mercaptopurine to 6-thioguanine was also observed; however, no 6-thioguanine appeared in the vascular effluent. Increasing the luminal phosphate concentration from 2 to 10 mmol/l increased mucosal phosphorolysis of 6-mercaptopurine riboside and more than tripled the rate of vascular appearance of 6-mercaptopurine; conversion of 6-mercaptopurine to 6-thioguanine was significantly inhibited. These results suggest that with a modest increase in luminal phosphate concentration, 6-mercaptopurine riboside can be a more effective substrate than the free drug for the oral delivery of 6-mercaptopurine.

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Introduction

The purine analogue 6-mercaptopurine is a chemotherapeutic antimetabolite that is commonly given to patients by mouth [1]. Since its introduction in 1952 [2] it has been used extensively in the maintenance treatment of childhood non-B acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma. The most important mechanism of cytotoxicity of 6-mercaptopurine has been shown to be its incorporation as 6-thioguanine deoxyribonucleotides into DNA [3–5].

Pharmokinetics studies have reported that plasma levels of mercaptopurine are unexpectedly low and highly variable after oral administration of the drug [6]. The low bioavailability of oral mercaptopurine is thought to result from intestinal and 'first pass' hepatic metabolism. Both the intestinal mucosa and the liver are primary sites for the enzyme xanthine oxidase (E.C. 1232), which is known to be responsible for the catabolism of 6-mercaptopurine to the inactive metabolite 6-thiouric acid. Furthermore, thiolmethylation of 6-mercaptopurine catalysed by thiopurine methyltransferase (TPMT, E.C. 21167), is thought to be one of the key factors governing the variability in 6-mercaptopurine metabolism [7]. Although the xanthine oxidase inhibitor allopurinol has been shown to improve the bioavailability of oral 6-mercaptopurine, complications arise due to the conversion of allopurinol to oxypurinol, since the latter has been shown to be inhibitory for transmural thiopurine transport [8].

There have been a number of reports confirming that naturally occurring purines are not transported across the intestine intact but instead are extensively metabolised to uric acid by xanthine oxidase [8, 9]. 6-Mercaptopurine, however, has been reported to inhibit uric

acid production and has been used in the clinical treatment of gout [10].

More recent results also demonstrate the presence of a mucosal cytoplasmic nucleoside phosphorylase that is responsible for the post-absorptive cleavage of the purine nucleosides inosine and guanosine into their respective bases plus ribose phosphate [9]. These observations suggested the possibility that improved oral delivery of 6-mercaptopurine could be achieved by employing 6-mercaptopurine riboside, which is more soluble than the free thiopurine.

Digestion of RNA in the small intestine releases 3′-ribonucleotides, which are hydrolysed in the lumen to nucleosides plus inorganic phosphate by alkaline phosphatase on the apical membrane [11]. In this paper we report that the absorption and transmural transport of 6-mercaptopurine can be greatly enhanced if it is supplied to the lumen as the nucleoside together with a low concentration of inorganic phosphate.

Materials and methods

Animals

Male Wistar rats (240–260 g) bought from Harlan Olac (Oxon, UK) were fed ad libitum on a standard laboratory chow diet (Bantin and Kingman Ltd, Hull, UK) with free access to water in controlled conditions of temperature and light.

Perfusion technique

Single-pass luminal and vascular perfusions were performed in situ at 37°C inside a thermostatically controlled cabinet using a water-jacketed apparatus in a manner similar to that described by Hanson and Parsons [12]. The rats were anaesthetised by an intraperitoneal injection of sodium pentobarbitone (80 mg/kg body weight; Rhone Merieux Ltd.). Mid-line and lateral abdominal incisions were made to expose the jejunum. The vasculature supplying the spleen, rectum, colon, stomach, ileum and duodenum was tied off. A measured segment of jejunum (25 cm) with its associated vasculature intact was then flushed from the oral end with 30 ml of warmed Krebs-Ringer buffer (120 mmol NaCl/l, 4.5 mmol KCl/l, 1 mmol $MgSO_4$/l, 1.8 mmol Na_2HPO_4/l, 0.2 mmol NaH_2PO_4/l, 1.25 mmol $CaCl_2$/l, 25 mmol $NaHCO_3$/l) gassed (19:1, O_2:CO_2) to pH 7.4 before use, followed by 20 ml air and cannulated. The vascular perfusate, containing 5 mmol glucose/l and bovine serum albumin fraction V (5% w/v) in Krebs-Ringer buffer, was perfused from the main reservoir into two channels. The first channel recirculated the perfusate (7 ml/min) through a multibulb oxygenator back into the reservoir. The second introduced oxygenated perfusate at 1.5 ml/min through the vasculature supplying the jejunum via the superior mesenteric artery. Once the vascular circulation had been established, the animal was killed by a lethal dose of anaesthetic into the heart. Collection of the vascular perfusate was performed via a cannula inserted into the portal vein.

The luminal perfusate consisting of previously gassed Krebs-Ringer buffer was segmented with gas, the flow rates of liquid and gas being constant throughout the perfusion at 2.0 and 2.0 ml/min, respectively, as determined by the peristaltic pump. Luminal perfusate containing the drugs to be studied was contained in a separate luminal pot kept at 37°C and continually gassed. Timed sampling of both the vascular (1 min in every 2 min) and the luminal effluent (1 min in every 5 min) began with the onset of the luminal flow. After a steady-state control period of 5–15 min had been established, the luminal perfusate containing the drug to be studied was allowed to flow through the intestinal lumen. For those experiments in which raised luminal phosphate concentrations were employed, previously gassed Krebs-Ringer buffer (112 mmol NaCl/l, 4.5 mmol, KCl/l, 1 mmol $MgSO_4$/l, 9.0 mmol Na_2HPO_4/l, 1.0 mmol NaH_2PO_4/l, 1.25 mmol $CaCl_2$/l, 25 mmol, $NaHCO_3$/l) at pH 7.4 was used for the luminal perfusate throughout the experiment.

At the end of the 50-min perfusion the perfused segment of the intestine was removed, blotted, measured and cut into 4-cm segments. Weighed mucosal scrapes were taken for analysis by HPLC and determination of the mucosal wet weight/dry weight ratio; the remainder was dried to a constant weight in an oven (90°C). The viability of the preparation was assessed by the capacity of the intestine to take up vascular glucose and produce lactate and by the constancy of the vascular flow rate. Samples were analysed for glucose and lactate by established methods, modified for use with the COBAS MIRA autoanalyser (Roche, UK). Phosphate concentrations in the samples were also assessed in a number of perfusions using a standard phosphate kit (Roche, UK) on the COBAS MIRA.

Chromatography

Vascular, luminal and mucosal samples were analysed by isocratic HPLC with a Kontron automated HPLC system fitted with a Hypersil 5-μm ODS C18, 250 × 46 mm column and a 10-mm precolumn (Jones Chromatography Ltd.). The mobile phase was 25 mmol $NH_4H_2PO_4$/l (pH 4.5) in 10% (v/v) methanol and analyses were carried out at a flow rate of 0.5 ml/min. Detection was performed at both 254 and 310 nm. The quantities of metabolites in the samples were determined by comparison of peak area measurements with reference to external standards of known concentrations [13]. With the exception of 6-thiouric acid , the peaks obtained in chromatograms of the vascular, luminal and mucosal samples were identified by co-chromatography with reference compounds. Chromatography of 6-thiouric acid was determined when 6-thioguanine was treated with xanthine oxidase. The percentage of recovery of the thiopurine compounds in the vascular, luminal and mucosal samples was determined to be 98% ± 1.0%, 99% ± 0.8% and 97% ± 1.4%, respectively.

Materials

All chemicals and reagents were of the highest purity available. 6-Mercaptopurine, 6-mercaptopurine riboside, uracil, uric acid and hypoxanthine were obtained from Sigma Chemical Company Ltd (Poole, UK).

Expression of results

The appearance of 6-mercaptopurine in the vascular effluent is expressed as a rate [μmol min^{-1} (g dry wt.)$^{-1}$] calculated by regression-line analysis from 25 to 45 min of the perfusion using an Excel spreadsheet written for the Apple Macintosh PC and that in the mucosa is expressed in terms of concentration in micromoles per gram or millimoles per liter. All values are reported as means (± SEM) of the results of three perfusions unless otherwise indicated. Statistical comparisons of the cumulative plots were made using covariance analysis. Regression lines and the associated standard errors were calculated from the individual values in the cumulative plots.

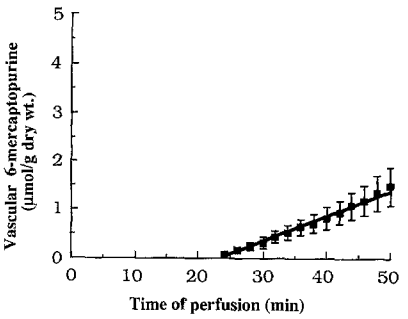

Fig 1 Cumulative vascular appearance of 6-mercaptopurine. The values are the cumulative mean (± SEM) vascular appearance for three perfusions at a luminal concentration of 5 mmol 6-mercaptopurine/l. The regression line calculated over the indicated interval gave the rate of 0.053 ± 0.006 μmol min^{-1} (g dry wt.)$^{-1}$ of vascular 6-mercaptopurine appearance

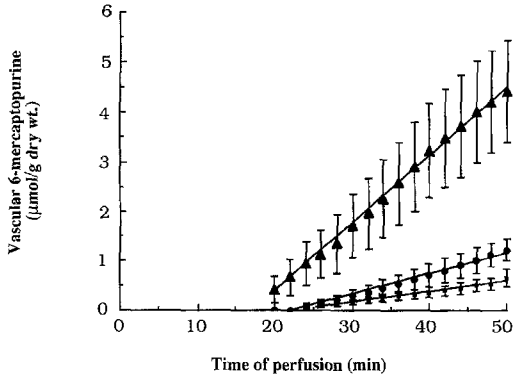

Fig. 2. Cumulative vascular appearance of 6-mercaptopurine. The values are the cumulative mean (± SEM) vascular appearance for three perfusions at each of the following luminal concentrations of 6-mercaptopurine riboside: (■) 1 mmol/l, (●) 5 mmol/l, and (▲) 10 mmol/l. Regression lines calculated over the indicated intervals gave the following rates [in μmol min^{-1} (g dry wt.)$^{-1}$] of vascular appearance of 6-mercaptopurine: (■) 0.021 ± 0.002, (●) 0.043 ± 0.005, (▲) 0.139 ± 0.018

Results

Transport of 6-mercaptopurine and 6-mercaptopurine riboside

Perfusions were performed at luminal 6-mercaptopurine and 6-mercaptopurine riboside concentrations of 5 and 1, 5, 10 and 20 mmol/l, respectively. The drug-containing perfusate began to flow through the lumen of the intestine at 15 min after the start of the perfusion. Figures 1 and 2 show the mean cumulative appearance of 6-mercaptopurine at concentrations of 5 mmol luminal 6-mercaptopurine/l and 1, 5, and 10 mmol luminal 6-mercaptopurine riboside/l, respectively. At the lower concentrations of 6-mercaptopurine (< 5 mmol/l) no vascular appearance of the drug was detected and at higher concentrations (> 5 mmol/l), 6-mercaptopurine was not soluble in the luminal perfusate. The transepithelial transport of 6-mercaptopurine riboside was detected only when 20 mmol 6-mercaptopurine riboside/l was luminally perfused, (see Fig. 4B).

Mucosal thiopurine concentrations at the end of the perfusions

Table 1 shows the tissue thiopurine content (in micromoles per gram) measured at the end of the perfusions. The mucosal concentration of 6-mercaptopurine with 5 mmol luminal 6-mercaptopurine/l was determined to be 0.38 ± 0.02 mmol/l. However, this was significantly increased to 0.81 ± 0.04 mmol/l ($P < 0.001$) when 5 mmol 6-mercaptopurine riboside/l was luminally perfused. The mucosal concentration of 6-mercaptopurine riboside under these conditions was 1.99 ± 0.09 mmol/l. In addition, mucosal scrape analysis carried out post-perfusion indicated the presence of the thiopurine metabolite 6-thioguanine at a concentration of 0.46 ± 0.02 and 0.41 ± 0.03 mmol/l, respectively, when 5-mmol/l concentrations of 6-mercaptopurine and 6-mercaptopurine riboside were luminally perfused. The 6-thioguanine formed from 6-mercaptopurine did not appear in the vascular effluent.

Effect of increased luminal phosphate concentration on the transport of 6-mercaptopurine and its riboside

When the phosphate concentration of the luminal perfusate was increased from the control value (2 mmol/l) to 10 mmol/l, no additional phosphate appeared in the vascular perfusate. In the presence of 10 mmol luminal phosphate/l the rate of vascular 6-mercaptopurine appearance observed when using 5 mmol luminal 6-mercaptopurine riboside/l was significantly increased ($P < 0.001$) from 0.0435 ± 0.005 to 0.162 ± 0.013 μmol min^{-1} (g dry wt.)$^{-1}$ (Fig. 3), whereas the rate of intestinal transport of 6-mercaptopurine with 5 mmol 6-mercaptopurine/l in the lumen was reduced ($P < 0.05$) to 0.020 ± 0.001 from 0.0534 ± 0.006 μmol min^{-1}(g dry wt.)$^{-1}$ (data not shown). At 20 mmol 6-mercaptopurine riboside/l, the higher level of luminal phosphate caused a significant increase in the vascular appearance of 6-mercaptopurine from 0.045 ± 0.006 to 0.104 ± 0.01 μmol min^{-1} (g dry wt.)$^{-1}$, whereas the appearance of 6-mercaptopurine riboside was reduced (Fig. 4). The initial rates of appearance of vascular 6-mercaptopurine riboside are shown in Fig. 4. From the graph it is evident that two possible rates could have been calculated. The discrepancy in linearity of this cumulative plot may be representative of an intestinal leak or increased paracellular movement occurring in the system. However, the latter proposals were tested by repeating the perfusions with the non-transported purine 6-thioguanine in the lumen, and the absence of 6-thioguanine in the vascular effluent indicated that

Table 1 Tissue content (μmol g^{-1}) of 6-mercaptopurine, 6-mercaptopurine riboside and 6-thioguanine when the lumen was perfused with 6-mercaptopurine or 6-mercaptopurine riboside with either 2 or 10 mmol phosphate/l. Eavh value is the mean ± SEM from 3 perfusions

	5 mmol 6-mercaptopurine/l		5 mmol 6-mercaptopurine riboside/l		20 mmol 6-mercaptopurine riboside/l	
	2 mmol phosphate/l	10 mmol phosphate/l	2 mmol phosphate/l	10 mmol phosphate/l	2 mmol phosphate/l	10 mmol phosphate/l
6-Mercaptopurine	2.87 ± 0.19	8.7 ± 0.45	6.27 ± 0.31	25.5 ± 0.44	4.21 ± 0.22	16.4 ± 1.07
6-Mercaptopurine riboside	–	–	15.30 ± 0.69	18.8 ± 0.29	52.10 ± 2.0	35.6 ± 3.6
6-Thioguanine	3.39 ± 0.43	–	3.05 ± 0.25	–	4.81 ± 0.51	0.77 ± 0.08

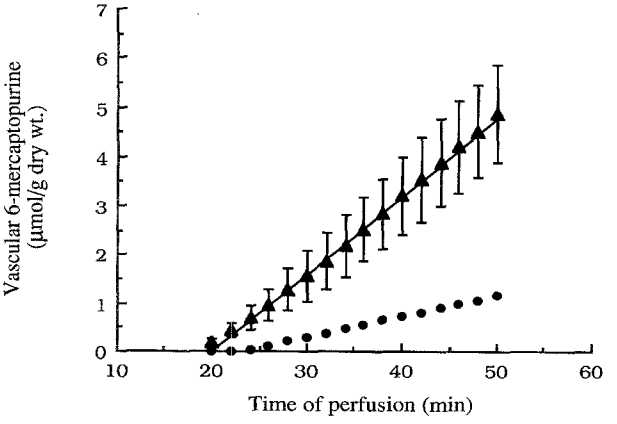

Fig 3. Cumulative vascular appearance of 6-mercaptopurine (▲). The values are the cumulative mean (± SEM) vascular appearance for three perfusions at luminal concentrations of 5 mmol 6-mercaptopurine riboside/l and 10 mmol phosphate/l; for comparison the cumulative vascular appearance of 6-mercaptopurine at luminal concentrations of 5 mmol 6-mercaptopurine riboside/l, and 2 mmol phosphate/l are shown from Fig. 2 (●). The regression line calculated over the indicated time interval gave the rate of 0.162 ± 0.013 μmol min^{-1} (g dry wt.)$^{-1}$ of vascular 6-mercaptopurine appearance

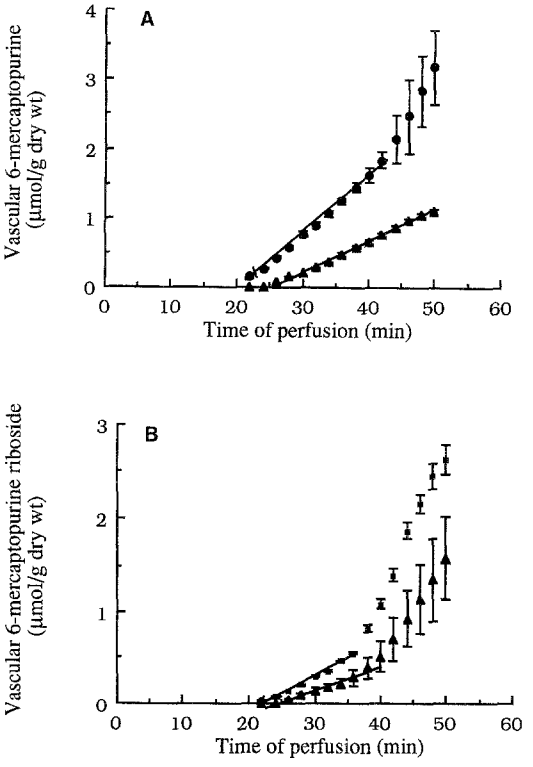

Fig. 4A Cumulative vascular appearance of 6-mercaptopurine. The values are the cumulative mean (± SEM) vascular appearance for three perfusions at a luminal concentration of 20 mmol 6-mercaptopurine riboside/l at luminal phosphate concentrations of (●) 10 mmol/l and (▲) 2 mmol/l. Regression lines calculated over the indicated intervals gave the following rates [in μmol min^{-1} (g dry wt.)$^{-1}$] of vascular appearance of 6-mercaptopurine: (●) 0.104 ± 0.01; (▲) 0.045 ± 0.006. **B.** Cumulative vascular appearance of 6-mercaptopurine riboside. The values are the cumulative mean (± SEM) vascular appearance for three perfusions at a luminal concentration of 20 mmol 6-mercaptopurine riboside/l at luminal phosphate concentrationsof (▲) 10 mol/l and (■) 2 mmol/l. Regression lines calculated over the indicated intervals gave the following rates [in μmol min^{-1} (g dry wt.)$^{-1}$] of vascular appearance of 6-mercaptopurine riboside: (▲) 0.020 ± 0.001, (■) 0.053 ± 006

there was no leak or increased paracellular movement of drugs in the system.

Effect of phosphate on the thiopurine content of the mucosa

Mucosal concentrations of 6-mercaptopurine, but not 6-mercaptopurine riboside, were increased under conditions of raised luminal phosphate concentrations when a 5-mmol/l concentration of either 6-mercaptopurine riboside or 6-mercaptopurine was perfused through the lumen (Table 1). When 5 mmol 6-mercaptopurine/l was luminally perfused the mucosal concentration was increased significantly from 0.38 ± 0.03 to 1.16 ± 0.06 mmol/l ($P < 0.001$). When 5 mmol 6-mercaptopurine riboside/l was luminally perfused at the higher luminal phosphate level the mucosal concentration of 6-mercaptopurine was increased from 0.81 ± 0.04 to 3.49 ± 0.06 mmol/l and that of 6-mercaptopurine riboside, from 1.99 ± 0.09 to 2.57 ± 0.04 mmol/l, respectively ($P < 0.01$). At 20 mmol 6-mercaptopurine riboside/l the mucosal concentrations of 6-mercaptopurine and 6-mercaptopurine riboside were 0.74 ± 0.04 and 9.14 ± 0.35 mmol/l, respectively. In the presence of 10 mmol luminal phosphate/l the mucosal concentration of 6-mercaptopurine was

significantly ($P < 0.001$) increased to 3.13 ± 0.03 mmol/l but that of 6-mercaptopurine riboside was significantly ($P < 0.05$) reduced to 6.99 ± 0.69 mmol/l. At no time did the mucosal concentration of 6-mercaptopurine or 6-mercaptopurine riboside exceed that of the luminal concentration. Table 1 also shows that with raised luminal phosphate 6-thioguanine was not detected in the mucosa when a 5-mmol/l concentration of 6-mercaptopurine or 6-mercaptopurine riboside were perfused. When 20 mmol 6-mercaptopurine riboside/l was perfused through the lumen at 10 mmol phosphate/l, 6-thioguanine was detected in the mucosa at a concentration of 0.134 ± 0.001 mmol/l, which is significantly ($P < 0.01$) lower than that observed (0.687 ± 0.074 mmol/l) at 2 m*M* luminal phosphate.

Luminal disappearance

As the experiments performed were of a single-pass nature, it was not possible to make an accurate estimate of the luminal disappearance of the drugs. Drug concentrations in the luminal effluent were not significantly below those in the initial perfusate.

Uric acid production

In all the experiments performed the concentration of uric acid in the vascular effluent was measured. For control perfusions during which no drug was perfused through the lumen the rate of vascular uric acid appearance was 0.072 ± 0.01 μmol min^{-1} (g dry wt.)$^{-1}$. When either 6-mercaptopurine or 6-mercaptopurine riboside was perfused through the lumen it was found that uric acid production was inhibited to such an extent that an accurate rate of vascular uric acid appearance could not be calculated.

Results obtained using this perfusion system confirmed that when either inosine or hypoxanthine were luminally perfused, neither was transported across the intestine intact but they were extensively metabolised to uric acid. The rates of vascular uric acid appearance for each of these purines at a luminal concentration of 1 m*M* were 0.358 ± 0.003 and 0.176 ± 0.06 μmol min^{-1} (g dry wt.)$^{-1}$, respectively (Fig. 5). Both results are significantly above ($P < 0.001$) the control rate of uric acid appearance. Finally, experiments were performed to investigate the vascular appearance of uric acid when both 6-mercaptopurine riboside (5 mmol/l) and hypoxanthine (1 mmol/l) were luminally perfused under conditions of raised luminal phosphate (10 mmol/l). The rate of uric acid appearance under the aforementioned conditions was 0.001 μmol min^{-1} (g dry wt.)$^{-1}$ with a rate of vascular 6-mercaptopurine appearance of 0.110 ± 0.003 μ mol min^{-1} (g dry wt.)$^{-1}$, which was significantly lower ($P < 0.01$) than the rate at which the drug appeared in the absence of hypoxanthine.

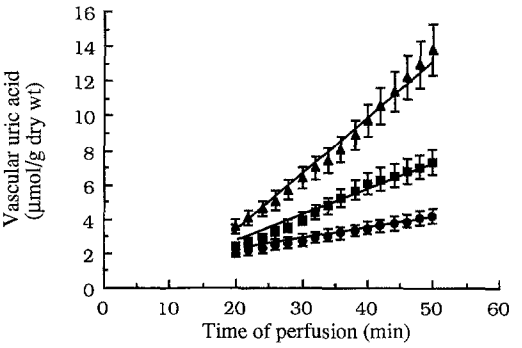

Fig. 5 Cumulative vascular appearance of uric acid. The values are the cumulative mean (± SEM) vascular appearance for three perfusions at luminal concentrations of (▲) 1.0 mmol inosine/l, (■) 1 mmol hypoxanthine/l, (●) control (no substrate in the lumen). Regression lines calculated over the indicated intervals gave the following rates [in μmol min^{-1} (g dry wt.)$^{-1}$] of vascular appearance of uric acid: (▲) 0.358 ± 0.03, (■) 0.176 ± 0.06, (●) 0.072 ± 0.01

Discussion

The results of this study provide important new evidence concerning the intestinal transport and metabolism of 6-mercaptopurine and 6-mercaptopurine riboside by rat small intestine. In addition our data show that a relatively small increase in luminal phosphate from 2 to 10 mmol/l enhances the vascular delivery of 6-mercaptopurine from luminally perfused 6-mercaptopurine riboside without increasing the transmural transport of phosphate. This observation may provide a mechanism to overcome the low bioavailability of orally delivered 6-mercaptopurine [6].

Previous studies have shown that the naturally occurring purines guanine, hypoxanthine and xanthine are not transported intact across isolated loops of intestine but instead are largely degraded as shown by the increase in the serosal appearance of uric acid [9]. However, it was found that when luminally perfused, adenine, unlike its nucleoside adenosine, did give rise to adenine in the serosal secretions of rat intestine as well as causing an increase in the serosal appearance of uric acid [14]. These results, together with those reported by Bronk et al. [8], who demonstrated that the intestinal transport of 6-mercaptopurine and 6-thioguanine in mouse small intestine appears to resemble that of adenine, support the findings reported in this paper. The results shown in Fig. 1 confirm that 6-mercaptopurine is transported across the intestine intact, albeit at a comparatively low rate, although in the rat no 6-thiouric acid was found. However, the thiopurines cause a significant diminution in the rate of uric acid production as compared with control levels. This inhibition of uric acid production was confirmed in perfusions with both luminal hypoxanthine and 6-mercaptopurine riboside in the lumen. When luminal

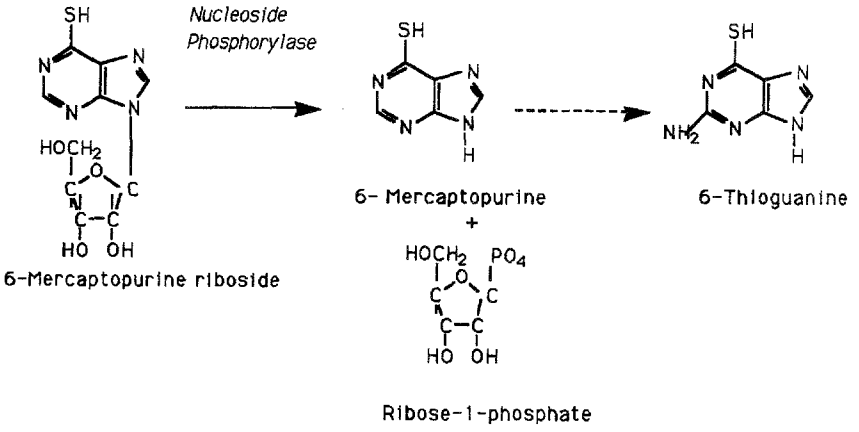

Fig. 6 Metabolic route of 6-mercaptopurine riboside in rat small intestine

6-mercaptopurine riboside was present the vascular uric acid appearance from luminal hypoxanthine was again significantly reduced from control levels, as was the rate of appearance of the drug.

Analysis of mucosal extracts at the end of perfusions using luminal 6-mercaptopurine revealed the presence of 6-mercaptopurine and the thiopurine metabolite 6-thioguanine in the tissue. The conversion of 6-mercaptopurine to 6-thioguanine has been reported and is thought to proceed via a nucleotide interconversion pathway [15]. The absence of vascular or mucosal 6-thiouric acid, which was shown to be the main metabolite of 6-mercaptopurine resulting from xanthine oxidase activity in mouse small intestine [8] as well as the reduction in uric acid production and presence of 6-thioguanine in the mucosal extract all indicate that 6-mercaptopurine is metabolised by a route that is dissimilar from that of the naturally occurring purines in the rat [9] (see Fig. 6).

The results we obtained (Fig. 2) showing that 6-mercaptopurine riboside is not transported across the intestine intact at concentrations below 20 mmol/l agree with those reported for natural purine nucleosides by Stow and Bronk [9], who demonstrated that adenosine, guanosine and inosine did not cross the epithelial layer of the rat intestine intact. The nucleosides were shown to be cleaved to their respective bases plus ribose phosphate by the action of a cytoplasmic nucleoside phosphorylase that is thought to reside in the intestinal villi [16].

The rate of vascular appearance of 6-mercaptopurine from luminally perfused 6-mercaptopurine riboside (5 mmol/l) was not significantly different from the vascular appearance of 6-mercaptopurine with 5 mmol 6-mercaptopurine/l in the lumen. Although it was apparent that 6-mercaptopurine riboside was not a better substrate for 6-mercaptopurine transport than the drug itself, the solubility of the riboside in an aqueous medium permitted higher concentrations of the riboside to be used and, hence, greater rates of 6-mercaptopurine transport to be achieved (Fig. 2).

Post-perfusion mucosal analysis revealed that both 6-mercaptopurine riboside and 6-mercaptopurine were present in the tissue. The mucosal concentration of 6-mercaptopurine was greater in perfusions with luminal riboside, suggesting a substantial rate of phosphorolysis. The slow basolateral exit of 6-mercaptopurine riboside limits its usefulness in chemotherapy, but this problem can be effectively circumvented by the increased phosphorolysis of the riboside in the mucosa, which releases free 6-mercaptopurine. In addition, 6-thioguanine was present in the mucosa after perfusion with either substrate suggesting that a proportion of the 6-mercaptopurine that occurs as a result of 6-mercaptopurine riboside phosphorolysis may go through the interconversion pathway mentioned previously. The extent to which this interconversion occurs could in fact limit the bioavailability of the oral 6-mercaptopurine or 6-mercaptopurine riboside in the absence of elevated phosphate, since 6-thioguanine is not released into the vascular effluent. It is also noteworthy that the higher level of luminal phosphate increased the retention of 6-mercaptopurine in the mucosal tissue. For the perfusions with 6-mercaptopurine this resulted in a decrease in the rate of transepithelial transfer, presumably because of the reduction in the inwardly directed concentration gradient. In the perfusions with 6-mercaptopurine riboside the disadvantage of the increased retention of 6-mercaptopurine was compensated for by the large increase in phosphorolysis of the nucleoside, which resulted in higher mucosal concentrations of 6-mercaptopurine and, thus, in higher rates of vascular appearance of 6-mercaptopurine from luminally perfused 6-mercaptopurine riboside.

Increased rates of vascular appearance of 6-mer captopurine from 6-mercaptopurine riboside in the presence of 10 mmol phosphate/l occurred over the concentration range from 5 to 20 mmol/l. At 10 mmol phosphate/l and 20 mmol luminal 6-mercaptopurine riboside/l the combined rate of 6-mercaptopurine and 6-mercaptopurine riboside appearance [0.167 ± 0.011 μmol min^{-1} (g dry wt.)$^{-1}$] was not different from

the rate of 6-mercaptopurine appearance [0.162 ± 0.013 μmol min^{-1} (g dry wt.)$^{-1}$] observed when 5 mmol 6-mercaptopurine riboside/l was luminally perfused at 10 mmol phosphate/l. These results indicate that at 10 mmol luminal phosphate/l the maximal rates of 6-mercaptopurine delivery can be achieved with 5 mmol 6-mercaptopurine riboside/l.

It was also very interesting that at 10 mmol luminal phosphate/l no other thiopurine metabolite could be detected in the mucosa when the lower concentrations of 6-mercaptopurine or 6-mercaptopurine riboside were used and that the mucosal thioguanine concentration was significantly reduced in the presence of the higher concentration of 6-mercaptopurine riboside. The reduced levels of 6-thioguanine may be indicative of a shift in direction of the interconversion pathway of 6-mercaptopurine to 6-thioguanine, which is effected by the high phosphate levels, and this also acts to increase the mucosal levels of 6-mercaptopurine. Increasing the luminal phosphate concentration from 2 to 10 mmol/l did not result in the intestinal transport of phosphate.

The results presented in this paper suggest that with a small increase in luminal phosphate concentrations, 6-mercaptopurine riboside can be a more effective substrate than the free drug for the oral delivery of 6-mercaptopurine.

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