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INHIBITION OF 6-PHOSPHOFRUCTO-2-KINASE ACTIVITY BY MERCAPTOPURINES

MARINA MOJENA,* LISARDO BOSCA,* MARK H. RIDER, GUY G. ROUSSEAU and LOUIS HUE†

Hormone and Metabolic Research Unit, Louvain University Medical School and International Institute of Cellular and Molecular Pathology, UCL 7529, avenue Hippocrate 75, B-1200 Brussels, Belgium

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Abstract—The activity of 6-phosphofructo-2-kinase (PFK-2), the enzyme that catalyses the synthesis of fructose 2,6-bisphosphate (Fru-2,6-P_2), was inhibited by mercaptopurines *in vitro*. Inhibition was observed with the purified enzyme from rat liver and bovine heart, and in extracts from rat lymphocytes and hepatoma cells, chick embryo fibroblasts, and human HeLa and lymphoblastoid cells. Half-maximal effect was obtained with 0.1–0.2 mM mercaptopurine and maximal inhibition ranged between 50 and 90% depending on the enzyme preparation. The inhibition resulted from a decrease in V_{max} with no change in K_m for ATP. The inhibition was relieved by treatment of the enzyme with thiol reducing agents, suggesting that it involves the formation of a mixed disulfide between mercaptopurine and thiol group(s) essential for enzyme activity. Incubation of intact lymphocytes or lymphoblastoid cells with 2- or 6-mercaptopurine resulted in a decrease in Fru-2,6-P_2 content and lactate release. A decrease in Fru-2,6-P_2 content but no change in lactate release was observed in HeLa cells and fibroblasts treated with 6-mercaptopurine but not with 2-mercaptopurine. Treatment of HeLa cells with 6-mercaptopurine resulted in a decreased PFK-2 activity which could be restored by treatment of the cell extract with dithiothreitol. In isolated rat hepatocytes and perfused rat hearts mercaptopurines had little or no effect on the Fru-2,6-P_2 content and lactate release. These results suggest that the effect of 6-mercaptopurine of arresting growth in lymphoid cells might involve the inhibition of glycolysis in addition to the known inhibition of *de novo* purine nucleotide synthesis.

Fructose 2,6-bisphosphate (Fru-2,6-P_2‡) is an intracellular signal that controls glycolysis in mammalian tissues. It is synthesized from fructose 6-phosphate and ATP by PFK-2 (EC 2.7.1.105), and hydrolysed to fructose 6-phosphate and Pi by FBPase-2 (EC 3.1.3.46). PFK-2 and FBPase-2 activities are catalysed by a single and thus bifunctional enzyme composed of two identical subunits, each of which bears the two catalytic sites. Experimental evidence supports the existence of several PFK-2/FBPase-2 isozymes which differ in kinetic properties, FBPase-2 content and phosphorylation by protein kinases (for reviews, see Refs 1–3). A detailed study of the PFK-2 domain of the liver bifunctional enzyme by Pilkis and colleagues [3–6] has shown that thiol groups are important for PFK-2 activity. Their results can be summarized as follows: (i) ascorbate/Fe^{3+} inactivates and thiols can reactivate PFK-2 [4]; (ii) *p*-mercuribenzoate stimulates PFK-2; (iii) whereas *N*-ethylmaleimide inactivates it [5]; (iv) iodoacetamide causes a 10-fold increase in its V_{max} but decreases 10–20-fold its affinity for fructose 6-phosphate [5]; (v) activation by iodoacetamide is associated with alkylation of two [5] to three [3, 6] cysteine residues. Therefore, although thiol groups are important for PFK-2 activity, it is difficult to predict on the basis of these studies the effect of a given thiol reagent on PFK-2 activity. This work was undertaken to study the effect of possible thiol reagents, such as mercaptopurines, on PFK-2 activity *in vivo* and *in vitro*.

Many tumour cells display a high glycolytic rate that is maintained even under aerobic conditions and might be controlled by Fru-2,6-P_2 [2, 7, 8]. In order to find inhibitors of tumour cell glycolysis, we searched for agents able to inhibit PFK-2 activity and to decrease the Fru-2,6-P_2 content of these cells. We show here that 2- and 6-mercaptopurines are potent inhibitors of Fru-2,6-P_2 synthesis *in vitro* and in certain cells *in vivo*. 6-Mercaptopurine is used in the therapy of acute lymphoblastic leukemia [9, 10]. The mechanism by which this drug causes cell death and the reason why it is more active on certain malignant cells than on normal tissues are not clear. However, it is generally believed that the therapeutic activity of 6-mercaptopurine is related to its structural analogy with natural purines and that mercaptopurines inhibit the synthesis and interconversion of purine nucleotides [9, 11]. The results presented here show that 6-mercaptopurine inhibits glycolysis in certain cells. In addition, the results suggest that this inhibition could be mediated by an inhibition of PFK-2 and could be involved in the therapeutic action of 6-mercaptopurine.

* Permanent address: Instituto de Bioquimica, Facultad de Farmacia, Universidad Complutense, E-28040 Madrid, Spain.

† Corresponding author: Prof. L. Hue, HORM unit, ICP-UCL 7529, avenue Hippocrate, 75, B-1200 Brussels, Belgium. Tel. (32) 2-764-75-32; FAX (32) 2-762-74-55.

‡ Abbreviations: Fru-2,6-P_2, Fructose 2,6-bisphosphate; PFK-2, 6-phosphofructo-2-kinase; FBPase-2, fructose-2, 6-bisphosphatase; PEG, polyethylene glycol 6000; HTC, rat hepatoma tissue culture; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

MATERIALS AND METHODS

Chemicals. Chemicals and PEG were from Merck (Darmstadt, F.R.G.). All biochemicals were purchased from Boehringer-Mannheim (Darmstadt, F.R.G.) or the Sigma Chemical Co. (St Louis, MO, U.S.A.). 2- and 6-Mercaptopurine and other purine analogues were from Janssen (Beerse, Belgium) or Sigma. Stock solutions of 2- and 6-mercaptopurine (20 mM) were made in 20 mM NaOH by heating for 15 min at 80°.

Cell cultures. HeLa (human adenocarcinoma) cells [12], secondary cultures of chick embryo fibroblasts [7], HTC cells [13], rat spleen lymphocytes [14] and human lymphoblastoid IM-9 cells [15] were grown or isolated as described. HeLa cells, fibroblasts and lymphocytes were incubated for the indicated periods of time in a medium containing 126 mM NaCl, 14 mM $NaHCO_3$, 38 mM KCl, 0.9 mM Na_2HPO_4, 0.6 mM KH_2PO_4, 0.6 mM $MgSO_4$, 0.3 mM $CaCl_2$, 6 mM glucose, 20 mM Hepes, pH 7.2, and supplemented with 1 mg/mL of bovine serum albumin. IM-9 cells and HTC cells were incubated for the indicated periods of time in phosphate-buffered saline containing 150 mM NaCl, 25 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4 at pH 7.4, 11 mM glucose and 1 mg/mL of bovine serum albumin. Hepatocytes were isolated from overnight-starved male rats and incubated as described previously [16]. Rat hearts were perfused as described previously [17].

Measurement of PFK-2 activity. Rat liver and bovine heart PFK-2 were purified as described [18]. Extracts from HeLa cells, IM-9 cells, HTC cells, fibroblasts or lymphocytes were prepared by homogenizing pellets of frozen cells in a buffer containing 100 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM dithiothreitol and 50 mM Hepes, pH 7.2. After centrifugation (100,000 *g* for 60 min) the supernatant was mixed with PEG (20% final concentration) and proteins were collected by centrifugation (10,000 *g* for 10 min). The pellet was resuspended in the homogenization buffer to reach a concentration of protein of about 1 mg/mL.

PFK-2 activity was measured after preincubating the enzyme for 5 min at 30° in 150 μL of homogenization buffer with or without mercaptopurines or analogues. PFK-2 assay reaction was started by the addition of 50 μL of the same buffer containing, except where otherwise stated, 16 mM MgATP, 8 mM fructose 6-phosphate and 24 mM glucose 6-phosphate. At 0, 5 and 10 min, 50 μL samples were mixed immediately with 50 μL of 100 mM NaOH and heated at 80° for 10 min to stop the reaction. Fru-2,6-P_2 was then measured as described previously [19]. One unit of enzyme activity corresponds to the formation of 1 μmol of product/min under the assay conditions.

Other determinations. To measure lactate [7], extracts were deproteinized by 1 M $HClO_4$ and neutralized with 3 M $KHCO_3$. For Fru-2,6-P_2 measurements, alkali extracts were prepared as follows: for fibroblasts, the incubation medium was removed from plastic Petri dishes (60 mm diameter), the cells were treated with 1 mL of 50 mM NaOH containing 1% (v/v) Triton X-100, the plates were scraped with a plastic spatula and the extracts were heated for 15 min at 80°. For other cells, collection was by centrifugation (3000 *g*, 1 min) and the pellet was treated immediately with 1 mL of 50 mM NaOH and heated for 15 min at 80°. Proteins were measured as described [20] with bovine serum albumin as a standard.

RESULTS

Mercaptopurines inhibit PFK-2 activity

In a systematic search for inhibitors of Fru-2,6-P_2 synthesis, we found that 2- and 6-mercaptopurines inhibit the activity of PFK-2. The inhibition was studied with purified rat liver PFK-2, but all PFK-2s tested, i.e. those purified from rat liver, bovine heart and the enzyme present in extracts from lymphocytes, HTC cells, chick embryo fibroblasts, HeLa, and IM-9 cells, were also inhibited by 2- and 6-mercaptopurines added *in vitro* to the enzyme preparation (Table 1). With 1 mM mercaptopurine, inhibition ranged from about 50 to more than 90% depending on the tissue (Table 1, Fig. 1). Half-maximal effect was obtained with less than 0.2 mM 2- or 6-mercaptopurine for the enzyme from rat liver

Table 1. Inhibition of PFK-2 activity by 2- and 6-mercaptopurines

Tissue	PFK-2 activity (mU/mg protein)	With 2-mercaptopurine (% of control value)	With 6-mercaptopurine (% of control value)
Rat liver*	3.7	16	9
Bovine heart*	2.6	51	33
Rat spleen lymphocytes	0.03	28	7
HTC cells	0.18	22	ND
Chick embryo fibroblasts	0.012	29	26
IM-9 cells	0.004	41	49
HeLa cells	0.07	9	19

The activity of PFK-2 present in extracts of tissues or cells was assayed after preincubation for 5–30 min in the presence of 1 mM mercaptopurine.

* Purified preparation; ND, not determined.

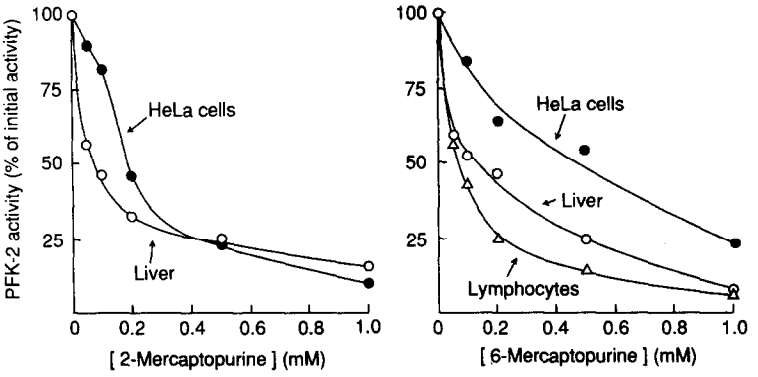

Fig. 1. Inhibition of PFK-2 activity by 2- and 6-mercaptopurine. PFK-2 activity was measured in a 20% PEG precipitate from HeLa cells (●) or rat spleen lymphocytes (△), or in a purified preparation from rat liver (○). The enzyme was incubated for 5 min at 30° with the indicated concentrations of 2- or 6-mercaptopurine before starting the reaction as described in Materials and Methods. The activity of PFK-2 was 0.08 (HeLa cells), 0.035 (lymphocytes) and 3.3 (liver) mU/mg of protein.

and from rat spleen lymphocytes; the enzyme from HeLa cells was about half as sensitive as the liver enzyme (Fig. 1). Despite the structural analogy between mercaptopurines and the purine moiety of ATP, the drugs did not affect the K_m for ATP and the inhibition resulted from a decrease in V_{max} (Fig. 2).

The effect of several purine analogues and of thiol reducing agents was also studied (Table 2). The inhibition of PFK-2 activity was restricted to mercaptopurines, indicating that their effect is relatively specific. Furthermore, the 6-methyl-derivative of 6-mercaptopurine, which is formed *in vivo* by methylation of the thiol group [21], did not inhibit PFK-2, suggesting that the integrity of the thiol group of the purine analogues is essential for their inhibitory activity. In keeping with this interpretation, dithiothreitol, dithioerythritol or β-mercaptoethanol decreased the inhibition by mercaptopurines (Table 2). Furthermore, dialysis of rat liver PFK-2 pretreated with 2 mM 2-mercaptopurine could result in a 65 or 95% recovery of enzyme activity when 20 mM β-mercaptoethanol or 5 mM dithiothreitol was included in the dialysis medium. These results suggest that PFK-2 inhibition by mercaptopurines results from the formation of a mixed disulfide between the mercaptopurine and thiol group(s) essential for PFK-2 activity.

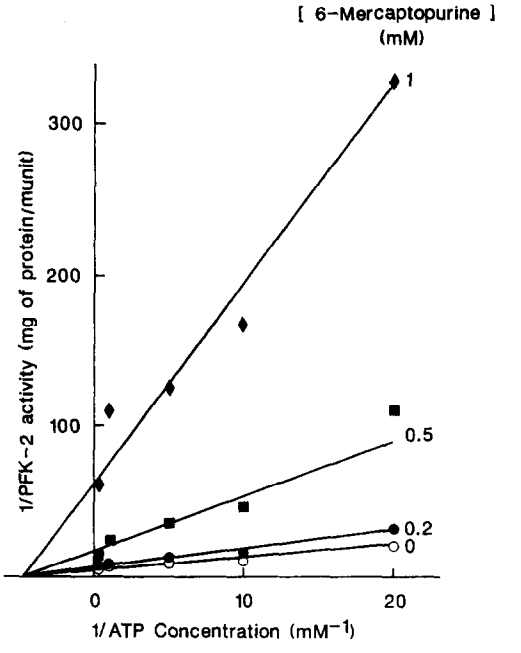

Fig. 2. Double reciprocal plot of rat liver PFK-2 activity at various concentrations of ATP and in the presence of the indicated concentrations of 6-mercaptopurine. Same experimental procedure as outlined in the legend to Fig. 1.

Effects of mercaptopurines on Fru-2,6-P_2 content and lactate release in intact cells

Since 2- and 6-mercaptopurine were found to inhibit PFK-2 *in vitro*, we studied their effect on the Fru-2,6-P_2 content of intact cells, first in isolated rat hepatocytes and in perfused rat hearts which were taken as models of two tissues with different PFK-2 isozymes [2]. In hepatocytes from overnight-starved rats incubated with various concentrations of glucose with or without 1 mM 6-mercaptopurine, a small decrease (less than 10%) in Fru-2,6-P_2 content and lactate release was observed (data not shown). Perfusion of rat hearts for 20 min with 1 mM 6-mercaptopurine did not affect heart Fru-2,6-P_2 content or lactate release (data not shown).

The poor sensitivity of rat hepatocytes and perfused hearts to 6-mercaptopurine prompted us to study tissues or cells, such as lymphocytes and lymphoblasts, which are known to be sensitive to mercaptopurine treatment [9, 11]. Incubation of rat spleen lymphocytes with 1 mM 2- or 6-

Table 2. Effect of purine analogues and thiol reducing agents on PFK-2 activity from HeLa cells and rat liver

Addition	PFK-2 activity (% of control value)	
	HeLa	Liver
None	100	100
2-Mercaptopurine	2 ± 1	17 ± 2
6-Mercaptopurine	11 ± 1	12 ± 1
6-Methylmercaptopurine riboside	112 ± 3	103 ± 5
2-Chloroadenosine	101 ± 6	100 ± 4
Caffeine	81 ± 3	78 ± 5
Theophylline	92 ± 4	99 ± 7
Adenosine	75 ± 5	77 ± 4
Dithiothreitol	ND	113 ± 7
Dithioerythritol	ND	86 ± 6
β-Mercaptoethanol	ND	87 ± 7
Dithiothreitol + 2-mercaptopurine	ND	80 ± 4
Dithioerythritol + 2-mercaptopurine	ND	101 ± 6
β-Mercaptoethanol + 2-mercaptopurine	ND	94 ± 17

PFK-2 activity was measured in a 20% PEG precipitate from HeLa cells or in a purified preparation from rat liver. PFK-2 was incubated with each addition (1 mM) for 5 min at 30° before starting the reaction as described in Materials and Methods. The concentration of β-mercaptoethanol was 5 mM. The activity of PFK-2 from controls was 0.08 (HeLa cells) and 3.3 (liver) mU/mg of protein. Results are means ± SEM for three different experiments. ND, not determined.

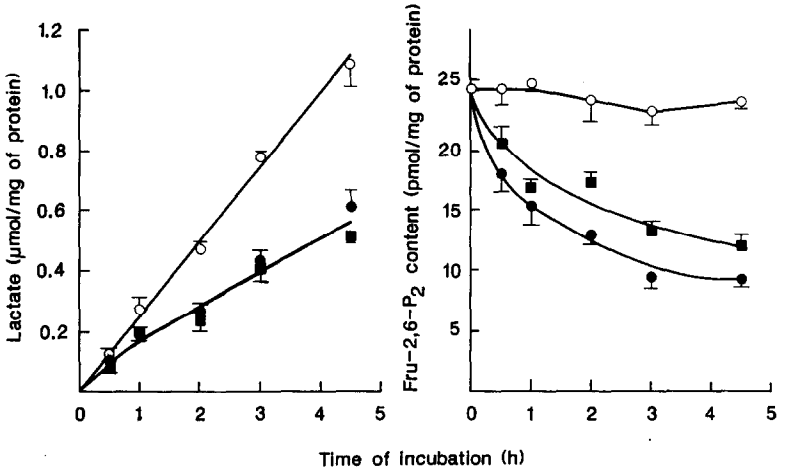

Fig. 3. Time-course of the effect of 2- and 6-mercaptopurine on lactate release and Fru-2,6-P_2 content in lymphocytes. Rat spleen lymphocytes were incubated for the indicated periods of time without (○) or with 1 mM 2-mercaptopurine (■) or 6-mercaptopurine (●). The Fru-2,6-P_2 content of control cells was 23 ± 1 pmol/mg of protein. The results are the means ± SEM for three different cell preparations.

mercaptopurine was indeed found to induce a time-dependent fall in the release of lactate and in the concentration of Fru-2,6-P_2 (Fig. 3). A 50–60% decrease in Fru-2,6-P_2 concentration and lactate release was obtained after 3 hr of incubation with 2- or 6-mercaptopurine. Although 6-mercaptopurine was more potent than 2-mercaptopurine in decreasing Fru-2,6-P_2, the inhibition of lactate production was similar (Fig. 3). The decrease in Fru-2,6-P_2 concentration caused by mercaptopurines was dose-dependent and, after 3 hr of incubation, half-maximal effect was observed with about 0.15 mM 2- or 6-mercaptopurine (Fig. 4). Incubation of lymphocytes for up to 5 hr in the presence of 1 mM mercaptopurine did not affect cell integrity which was measured by the release of lactate dehydrogenase in the incubation medium.

The effect of 1 mM 6-mercaptopurine on Fru-2,6-P_2 content and lactate release was also studied in IM-9 cells, a permanent cell line derived from human

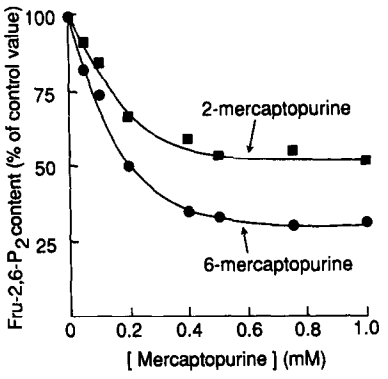

Fig. 4. Dose-dependent decrease in Fru-2,6-P_2 content of lymphocytes by 2- and 6-mercaptopurine. Rat spleen lymphocytes were incubated for 3 hr with the indicated concentrations of 2- (■) or 6-mercaptopurine (●). The values are the means for two different cell preparations.

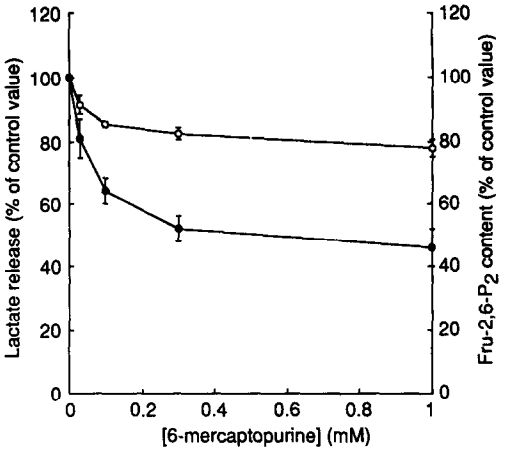

Fig. 5. Dose-dependent decrease in the Fru-2,6-P_2 content of and lactate release in lymphoblastoid IM-9 cells by 6-mercaptopurine. IM-9 cells were incubated for 6 hr with the indicated concentrations of 6-mercaptopurine. Fru-2,6-P_2 content (●) and lactate release (○) were measured and are expressed as % of the corresponding control value. Fru-2,6-P_2 content of control cells was 16.2 ± 5.3 pmol/10^6 cells; lactate release from control cells was 2.5 ± 0.6 μmol of lactate/6 hr and per 10^6 cells. The values are the means ± SEM for six different preparations.

lymphocytes [15] (Fig. 5). The results indicate that 6-mercaptopurine decreased Fru-2,6-P_2 content and inhibited lactate release in IM-9 cells, although to a smaller extent than in rat spleen lymphocytes. Half-maximal decrease in Fru-2,6-P_2 content in IM-9 cells was obtained with about 0.2 mM 6-mercaptopurine (Fig. 5).

In suspensions of HeLa cells (Fig. 6A) and in chicken embryo fibroblasts (Fig. 6B), 6-mercaptopurine but not 2-mercaptopurine caused a time-dependent decrease in Fru-2,6-P_2 concentration. However, the time-course of the phenomenon differed, the maximal effect being reached within 20 min in HeLa cells, but requiring 5–10 hr in fibroblasts. A similar difference in the time-course between the two cell types had already been observed in the study of the effect of glutamine on lactate release and Fru-2,6-P_2 content [12]. The reason for the difference is not clear and could be related to the low metabolic activity of fibroblasts compared to HeLa cells [12]. In HeLa cells, the half-maximal effect on Fru-2,6-P_2 concentration was obtained with 0.1–0.2 mM 6-mercaptopurine (not shown). Despite the fall in Fru-2,6-P_2, no change in lactate production was observed in HeLa cells and in fibroblasts (not shown).

To determine whether the effect of 6-mer-

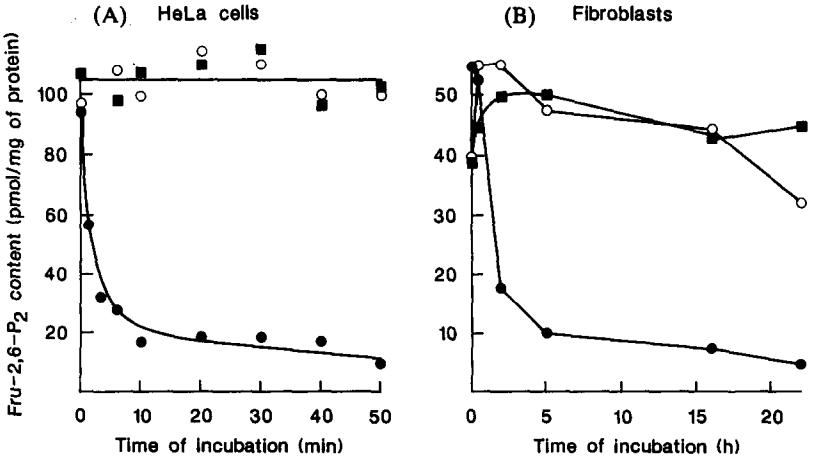

Fig. 6. Time-course of the effects of 2- and 6-mercaptopurine on the Fru-2,6-P_2 content of (A) HeLa cells and (B) chick embryo fibroblasts. The cells were incubated for the indicated periods of time without (○) or with 1 mM 2-mercaptopurine (■) or 6-mercaptopurine (●).

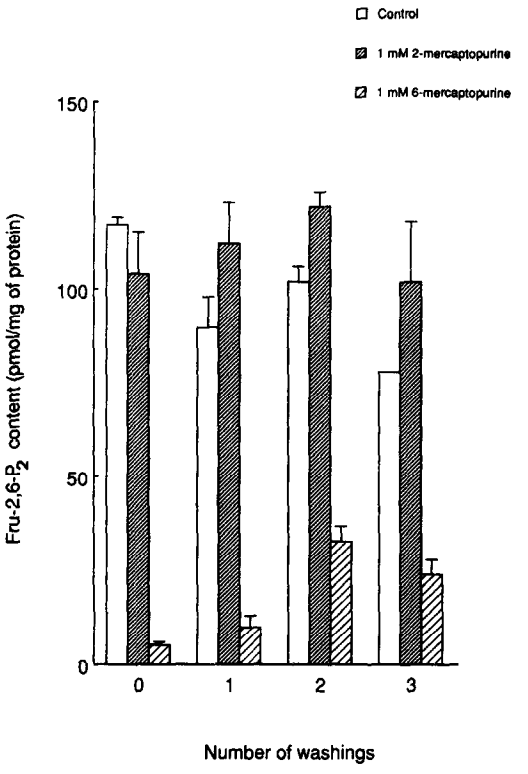

Fig. 7. Fru-2,6-P_2 content of HeLa cells pre-treated with 1 mM mercaptopurine and washed in mercaptopurine-free medium. Cells were incubated for 20 min with 1 mM 2- or 6-mercaptopurine and were then collected by centrifugation, resuspended in control medium without mercaptopurine and incubated for 10 min. The washing procedure was repeated as indicated. The results are the means ± SEM for three different experiments.

captopurine on Fru-2,6-P_2 concentration in HeLa cells was reversible, cells pre-treated with mercaptopurines were washed and resuspended in control medium containing glucose but without mercaptopurine, and samples were taken to measure Fru-2,6-P_2 after 10 min of incubation. The washing procedure was repeated twice. The results presented in Fig. 7 show that Fru-2,6-P_2 concentration remained low (25–30% of the control values) in cells pre-treated with 6-mercaptopurine even after three washings. This suggests a persistent and stable inhibition of PFK-2. Moreover, in extracts of fibroblasts the activity of PFK-2, which was decreased to 28 ± 4% of the control values after 16 hr of treatment with 6-mercaptopurine, could be restored to 91 ± 10% of the control values by addition of 5 mM dithiothreitol to the extracts. This is consistent with the formation of a mixed disulfide between mercaptopurine and thiol group(s) of PFK-2.

DISCUSSION

This paper shows that mercaptopurines inhibit PFK-2 activity *in vitro*. The data suggest that this results from the formation of a mixed disulfide between the drug and reactive thiol group(s) essential for PFK-2 activity. These thiol groups could correspond to one or several of the cysteine residues that are required for the maintenance of the integrity of the substrate binding site in the kinase domain of PFK-2 [5, 6]. It is interesting to note that *p*-fluorosulfonylbenzoyladenosine, an ATP analogue, inhibits PFK-2 by reacting with these thiol groups [6]. Such a mechanism involving the formation of mixed disulfide at the catalytic site had been proposed for the inhibition of inosine 5′-monophosphate dehydrogenase by mercaptopurines [22]. The results also suggest that inhibition of PFK-2 by these drugs may take place in intact cells. This would explain the decrease in Fru-2,6-P_2 content observed for lymphocytes, IM-9 cells, HeLa cells and fibroblasts treated with 6-mercaptopurine. Indeed, the dose–response curves for the *in vitro* inhibition of PFK-2 activity were comparable with those for the *in vivo* decrease of Fru-2,6-P_2 content in HeLa cells and rat spleen lymphocytes. The hypothesis is strengthened by the observation of a decreased activity of PFK-2 in extracts of treated cells. Inhibition of PFK-2 activity is not the only mechanism that could decrease the Fru-2,6-P_2 content of intact cells. Such a decrease could also result from an increased FBPase-2 activity, a decreased fructose 6-phosphate concentration and/or changes in the concentration of PFK-2/FBPase-2 regulatory ligands. An increased FBPase-2 activity resulting from mercaptopurine treatment is unlikely if one assumes that mercaptopurines react with the same thiols and display the same effect as *p*-fluorosulphonylbenzoyladenosine, namely an inhibition of both PFK-2 and FBPase-2 [6].

Although 2-mercaptopurine was as active as 6-mercaptopurine in inhibiting PFK-2 activity *in vitro*, it did not cause a decrease in Fru-2,6-P_2 in fibroblasts and HeLa cells, whereas 6-mercaptopurine did. Such discrepancies could result from a difference in transport and/or intracellular metabolism of these purine analogues. Different metabolic fates have indeed been reported which seem to depend on, among other factors, the clonal origin of the lymphocytes, the duration of exposure to 6-mercaptopurine and inter-individual genetic differences [23, 24]. On the other hand, the Fru-2,6-P_2 content did not always decrease in cells whose PFK-2 was sensitive to *in vitro* inhibition by mercaptopurines. Since the anti-PFK-2 effect of mercaptopurines seems to require the integrity of their thiol groups, any mechanism interfering with these thiol groups, such as oxidation or methylation, should influence mercaptopurine reactivity [25]. For example, the cell capacity to keep glutathione in the reduced state would be expected to prevent mercaptopurines from reacting with PFK-2. This is probably the case for the liver in which glutathione is relatively abundant (about 5 mM) and maintained in the reduced form [26], and in which Fru-2,6-P_2 was indeed almost insensitive to 6-mercaptopurine. In lymphoid cells, glutathione concentrations are much lower (0.2–0.5 mM) than in liver cells and glutathione is rapidly oxidized during proliferation as, for example, after lectin stimulation [25, 27]. Furthermore, the addition of β-mercaptoethanol to suspensions of lymphocytes is required to maintain

viability and to enable the appropriate response to specific stimulators to be observed [25, 27]. Regarding the methylation of thiol groups, large differences in mercaptopurine methylation and metabolism have been observed not only between species but also within the same species [21, 28, 29]. We have no explanation for the fact that 6-mercaptopurine decreased lactate production only in lymphocytes and in lymphoblastoid cells. This cell-specific inhibition corresponds to the specific cytotoxic action of 6-mercaptopurine which is indeed restricted to lymphoid cells [11, 30].

It is unlikely that the anti-glycolytic effect of mercaptopurines described here is a consequence of their anti-proliferative effect since it could be demonstrated in resting lymphocytes. On the other hand, one might speculate that the inhibition of glycolysis exerted by mercaptopurines in cells of lymphoid origin participates in the cytotoxicity of these drugs. Indeed, the metabolic importance of carbohydrate consumption and hence of glycolysis in the proliferative response is well known and is illustrated by the fact that activated lymphocytes consume several times more glucose than resting cells [31, 32]. Consequently, it is conceivable that in these cells the inhibition of glucose consumption and of energy provision, as it results from 6-mercaptopurine treatment, affects cell growth and proliferation.

Finally one may wonder whether the anti-glycolytic effect of 6-mercaptopurine is solely due to the fall in Fru-2,6-P_2 concentration and whether other glycolytic enzymes could also be inhibited by the drug. Among these, glyceraldehyde-3-phosphate dehydrogenase is a possible target for mercaptopurine since thiol groups are essential for its activity [33, 34]. Our preliminary results indicate that glyceraldehyde-3-phosphate dehydrogenase can indeed be inhibited by mercaptopurine *in vitro*. Whether such an inhibition occurs in intact cells and whether other glycolytic enzymes could be inhibited as well remains to be established. In any case, the possibility exists that, thanks to their ability to react with thiol groups, mercaptopurines inhibit glycolysis in a concerted manner by inactivating several key enzymes. Our results therefore offer insight into the mechanism of action of mercaptopurines and offer new perspectives for the design of anti-leukemic drugs.

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