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EFFECTS OF URIDINE DIPHOSPHOGLUCOSE (UDPG) INFUSION ON 5-PHOSPHORIBOSYL PYROPHOSPHATE (PRPP) LEVELS OF MOUSE TISSUES*

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Abstract—Uridine diphosphoglucose (UDPG) has been shown to have tissue-specific effects that have proved to be of clinical value in the treatment of some liver ailments. In an effort to determine something about the mechanism of action, we investigated the effect of UDPG on the levels of 5-phosphoribosyl pyrophosphate (PRPP) and PRPP synthetase in mouse liver, spleen and transplanted tumors. Three strains of mice were studied with and without tumors under various experimental conditions. Balb/c mice were infused with UDPG intraperitoneally at levels of 0.16 g/kg/day (0.28 mmole) to 1.6 g/kg/day (2.8 mmoles) for 5 days. At the low dose rate the PRPP level in the liver was found to increase 3-fold. A slight increase was noted in the activity of PRPP synthetase. However, when the UDPG was infused at a level of 2.8 mmoles/kg/day, the increases in both the synthetase and PRPP were inhibited. Both CRF_1 and CD_8 mice were less sensitive to the effects of UDPG *per se*. However, the high level of PRPP in the tumors they carried was greatly affected by the UDPG infusion. The tumor-specific inhibition of PRPP suggests that this action might prove to be useful combination therapy with inhibitors of purine and pyrimidine nucleotide synthesis in various rescue regimens. UDPG was found to enter cells intact before it was cleaved into glucose phosphate and UMP. The fact that UDPG was also found in the membrane fraction suggests that either there is a specific transport mechanism or UDPG exerts its action via interaction with the cell membrane.

5-Phosphoribosyl pyrophosphate (PRPP) is a necessary metabolic intermediate in the synthesis of purine and pyrimidine nucleotides as well as other important molecules. Perturbation of the intracellular level of PRPP has been found to be relevant to the origin of certain metabolic diseases [1, 2]. The inhibitory activity of several antimetabolites requires reaction with PRPP, and the therapeutic efficacy may depend on the level of PRPP in target cells relative to normal host tissues [3, 4].

PRPP is formed by the interaction of ATP and ribose-5-phosphate (R-5-P) catalyzed by the enzyme PRPP synthetase. R-5-P is related to glucose metabolism via the pentose phosphate shunt. Alteration in this, as seen in glycogen storage disease Type I, increases the PRPP level in affected cells [2]. Methylene blue stimulates the oxidative pentose phosphate pathway and concurrently increases PRPP availability in chick liver slices [5]. These findings seem to suggest that R-5-P concentrations are not saturating for intracellular PRPP production and that the availability of PRPP is directly related to purine synthesis. The present investigation is aimed at the determination of whether UDPG, a compound known to affect liver carbohydrate metabolism, exerts any effect on PRPP availability in specific cells either by action on cell membranes or by incorporation *per se* into the cytoplasm, and to assess any therapeutic potential of such effects.

EXPERIMENTAL

Infusion of UDPG. Mice of various strains, Balb/c, CD_8, and CRF with and without tumor implants, maintained on Purina rat chow and tap water, were infused intraperitoneally, as described previously [6], with a phosphate-buffered saline (PBS) solution of UDPG at a constant rate of 0.8 ml/day for 5 days. Two concentrations of UDPG were used: 8 and 80 mM. *At an average weight of 24 g/mouse, this amounts to 0.28 and 2.8 mmoles/kg body weight/day.* Control mice received only buffered saline solution.

After the 5-day infusion the mice were killed, and their tissues were excised and homogenized in cold PBS solution (1.5 ml/g tissue) to extract PRPP. The homogenates were centrifuged at 48,000 *g* for 10 min. Supernatant fractions thus prepared were assayed for PRPP and enzyme activity. Pellets were resuspended in the original volume of 10 mM Tris buffer (pH 7.4), 0.125 M sucrose, 1 mM dithiothreitol (DTT) and 2% Triton. The supernatant fractions from the second extraction were used for the assay of membrane-bound PRPP synthetase.

Isolation of splenocytes and hepatic cells. The organs were gently teased apart with forceps over a fine tea strainer into PBS or minimum essential culture medium (MEM) containing 0.1 M potassium phosphate buffer, pH 7.4, with and without added effectors. The cells were incubated at 37° for 30 min and then washed once with PBS before they were lysed in PBS by freezing and thawing three times. The supernatant solutions were assayed for PRPP.

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Determination of PRPP concentration. The PRPP was determined through the reaction of [^{14}C]orotic acid with PRPP to form [^{14}C]carboxyl orotidine-5′-phosphate in the presence of orotate phosphoribosyltransferase followed by the liberation of $^{14}CO_2$ under the catalysis of OMP decarboxylase [7]. The reaction mixture (100 μl) contained 25 μl of 0.1 M Tris–HCl (pH 7.4), 25 μl of enzyme mix (Sigma, St. Louis, MO, containing 2–3 units/ml in Tris buffer), 25 μl of 0.5 mM [^{14}C]carboxyl orotic acid (5 mCi/mmole), 25 μl of 40 mM $MgCl_2$ solution and 25 μl of clarified tissue homogenate. The test tubes containing the reaction mixture were sealed with serum caps each of which had suspended from it a plastic well (Kontes, Vineland, NJ) containing 200 μl of protosol (New England Nuclear, Boston, MA). The tubes were incubated at 37° for 10 min before they were placed in an ice bath and 0.25 ml of 10% trichloroacetic acid (TCA) was injected through the serum cap. Tubes were returned to a 37° water bath and incubated for 1 hr. At the end of this time the tubes were uncapped, the outsides of the wells were wiped clean, and the suspended wells were cut. The wells were placed in scintillation vials, and 10 ml of counting solution was added. The vials were shaken vigorously, and their radioactivity was determined.

Enzyme assays. PRPP synthetase was assayed as described previously [8]. The assay is based on the coupling of the PRPP assay using [^{14}C]adenine with APRTase to form [^{14}C]AMP with the synthetase reaction (in the presence of ATP and R-5-P).

Protein determination. Protein concentrations were determined by the method of Lowry *et al.* [9].

Adenine incorporation studies. Isolated Balb/c mouse splenocytes and hepatocytes were incubated at 37° in MEM with 0.1 M KPB (pH 7.4) and 0.5 mM [^{14}C]adenine (sp. act. 5 Ci/mole) with and without 10 mM UDPG. After 30 min of incubation, cells were washed once with PBS before they were lysed in PBS by alternate freezing and thawing (3×). The ^{14}C-nucleotides thus formed were assayed by chromatography on DEAE-cellulose paper and subsequent assay of the radioactivity.

[^{3}H]*UDPG incorporation studies.* Isolated mouse hepatocytes were incubated at 37° in MEM with 0.21 mM [^{3}H]UDPG. After 30 min the cells were layered on top of 1.0 ml of dibenzylamine and centrifuged at 4° immediately to separate the cells from the incubation medium. The cells were lysed in 0.2 ml of KPB (pH 7.4) by vigorous vortexing. The solution was clarified by centrifugation at 30,000 *g* for 15 min, and 25 μl of 10% TCA was added to 0.1 ml of supernatant solution. The initial pellet (containing cell membranes) was solubilized in 2% Triton in 10 mM Tris (pH 7.4) buffer and deproteinized. Solubilized membrane and cytoplasm preparations were analyzed for ^{3}H-containing components by HPLC fractionation through a PXS-102SAX column with a linear gradient from 0.007 M KH_2PO_4 to 0.25 M KH_2PO_4, 0.5 M KCl (pH 4.5). The flow rate was 0.8 ml/min and fractions were 0.4 ml each. The u.v. absorbtion and the radioactivity profiles were compared to values of known standards. The radioactivities of the fractions were determined by liquid scintillation counting.

RESULTS

Changes in PRPP levels and PRPP synthetase of Balb/c mouse liver and spleen upon UDPG treatment. Table 1 shows that treatment with UDPG increased PRPP in the liver. Lower UDPG concentration, produced by injection of 0.5 ml of 3.5 mM UDPG per day (equals 0.07 mmole/kg per day) caused only a 13% increase of liver PRPP. At a slightly higher dose level produced by continuous infusion, a 3.3-fold increase in PRPP level was observed. Contrary to expectation, a 10-fold increase in UDPG gave only a 19% increase in PRPP. The changes in liver PRPP synthetase activity did not correspond with those of PRPP. Mouse spleen was shown to be less sensitive to the UDPG effect. No change in either PRPP or synthetase level was seen at low UDPG concentration. A slight (*ca.* 30%) increase in PRPP and synthetase in the cytosol was noted when an infusion of 2.8 mmoles of UDPG was given per day. UDPG had no effect on PRPP synthetase activity when it was added to the reaction mixture at concentrations up to 10 mM.

Effects of UDPG and G-6-P on splenocyte PRPP levels. Isolated splenocytes incubated in media containing various amounts of UDPG or G-6-P were observed to have an increased intracellular PRPP level (Table 2). However, the effect of G-6-P was not evident below a concentration of 5.5 mM, whereas

Table 1. Changes in PRPP level and PRPP synthetase after UDPG treatment

	Liver				Spleen			
	PRPP		Synthetase		PRPP		Synthetase	
	C	T	C	T	C	T	C	T
Inject (0.07 mmole/kg once per day)	206 ± 58	233 ± 20	4.7 ± 1.8	6.5 ± 3.5				
Infuse (low) (0.28 mmole/kg per day)	237 ± 95	772 ± 54*	4.6 ± 1.6	6.0 ± 1.6	242 ± 60	213 ± 32	7.7 ± 0.5	7.4 ± 3.7
Infuse (high) (2.8 mmoles/kg per day)	276 ± 12	328 ± 43*	4.5 ± 0.4	4.4 ± 0.6	228 ± 32	302 ± 27*	7.6 ± 0.8	9.9 ± 1.4*

Balb/c mice were used. C = control; T = treated animals. Controls received injections or infusions of PBS.
* Values of treated differed significantly from control ($P \leq 0.05$).

Table 2. Effects of UDPG and G-6-P on PRPP levels of isolated splenocytes

Conc of drugs (mM)	PRPP levels (pmoles/mg protein)
G-6-P	
0	981
1.1	969*
5.5	1418†‡
11	1510§
22	1998‡‖
UDPG	
0	981
0.7	1825‖
3.6	1937§‖
7.2	2135§‖
14.4	1508‡§

Cells were incubated *in vitro* in MEM containing the indicated compound. Assays were performed on the soluble fraction of homogenates. Values are means of triplicate experiments. SD was ≤15% of mean value. P values were calculated using Student's *t*-test in two-tailed analysis.

* Difference between value and control was not significant.

† Difference between value and control was significant at $P \leq 0.05$.

‡ Difference between value and next lower concentration was significant at $P \leq 0.05$.

§ Difference between value and next lower concentration was not significant.

‖ Difference between value and control was significant at $P \leq 0.01$.

UDPG effect was essentially maximal at 0.7 mM. The effect of G-6-P increased with concentration up to 22 mM, whereas at the highest concentration of UDPG used (14.4 mM) the effect of UDPG appeared to begin to decrease.

Effects of UDPG on tumor-bearing mice. CD_8 mice with mammary tumors and CRF mice with transplanted colon tumors were infused for 5 days with UDPG. At that time the tumors were excised, and their weight, level of PRPP and PRPP synthetase were determined. In one set of experiments, the average size of the mammary tumors was 50% of the controls. The decrease, although not statistically significant, was determined after only 5 days of treatment. Nevertheless, the effect is suggestive of possible specificity as is the decrease in tumor level of PRPP compared to the essentially unchanged levels in the host mice (Table 3). In a similar experiment (Table 4), PRPP levels of two other proliferating tissues, bone marrow and intestinal mucosa, were also assayed. Although there was a decrease in colonic and bone marrow cells, it was much less than that observed in the tumors. The low values seen in the small bowel may be related to the very high phosphatase level found in this tissue [10].

Table 4. Effect of UDPG on PRPP levels of tissues of tumor-bearing mice

Tissue	PRPP (nmoles/g protein)	
	Control	Treated
Tumor	1704	385
Colon	230	112
Jejunum	20	49
Bone marrow	131	82

Mice were CD_8 carrying second generation mammary tumors. They were infused with 2.8 mmoles of UDPG/kg body weight/day. Values are means of triplicate experiments. SD was ≤15% of mean value. P values were calculated using Student's *t*-test in two-tailed analysis. Differences between treated and control were significant at $P \leq 0.05$ for jejunum and bone marrow and at $P \leq 0.01$ for tumor and colon.

Studies on the incorporation in vitro *of* [^{14}C]*adenine into splenocytes.* Incorporation of adenine into isolated splenocytes was stimulated by the presence of 10 mM UDPG in the incubation medium. This result is consistent with the observation (Table 2) that UDPG increased the level of PRPP. The PRPP participates in the retention of purine bases by phosphoribosylation of the aglycone [11].

Table 3. Effects of UDPG on tumor-bearing mice

	Mammary carcinoma in CD_8 mice			Colon tumor in CRF mice		
	PRPP (nmoles/g protein)	Synthetase (I.U. in lysate)	Synthetase (I.U. in membrane)	PRPP (nmoles/g protein)	Synthetase (I.U. in lysate)	Synthetase (I.U. in membrane)
Liver						
Control	153	4.3	1.54	213	4.8	1.32
Treated	119	3.8	1.19	218	4.58	1.37
Spleen						
Control	100	1.3	2.15	176	8.4	4.6
Treated	127	1.17	2.12	197	6.7	4.2
Tumor						
Control	1322	2.3	1.56	1117	6.8	2.25
Treated	319	1.56	1.22	626	5.8	2.51

Treated: mice were infused with 2.8 mmoles/kg body weight/day of UDPG. Control: saline-infused controls. I.U. (International Unit) = 1 μmole of product/min/mg protein.

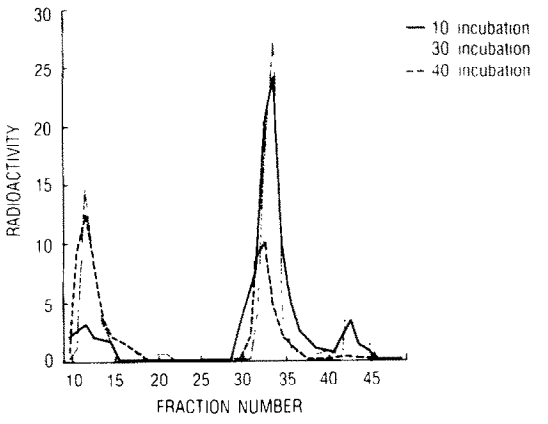

Fig. 1. UDPG uptake: Distribution of intracellular label. Cells were incubated in PBS containing 0.5 mM UDPG. Cell lysates were analyzed by HPLC on calibrated columns. Fractions 11–15 are glucose, fractions 32–36 are glucose monophosphate, and fractions 41–46 are UDPG.

Studies on the incorporation in vitro *of UDPG into hepatocytes.* We studied the transport of tritiated UDPG at 10, 30 and 40 min. The cells were prepared as single cell suspensions under conditions that left them viable in terms of several biochemical parameters and vital dye exclusion. The plot (Fig. 1) shows the fraction of the material in each of the three compounds that were derived from the glucose moiety of the UDPG.

Uptake of intact UDPG was maximal at 30 min. By 40 min the cells had begun to die in this medium, and the UDPG had broken down. At 30 min about 1/6 of the label was as UDPG. A major amount of the UDPG entered the cells intact. The data further show that the initial product was glucose phosphate which, in time, was dephosphorylated to glucose.

It is obvious that the UDPG did not break down before entering the cell, both from its presence intact and from the fact that the breakdown products did not cause the same metabolic response that UDPG did.

Other preliminary studies showed the presence of some intact UDPG in the cell membranes. This suggests that there may be an active transport mechanism responsible for the entry of UDPG into the hepatic cell.

DISCUSSION

UDPG is known to have multiple effects on intrahepatic bilirubin metabolism which, in turn, are related to the glycogen synthesis occurring in the liver [12]. The precise mechanism of these interactions is not clearly defined, but it is believed that UDPG is involved in the induction of various enzymes including some active in carbohydrate metabolism [13]. PRPP is required in purine and pyrimidine metabolism. Perturbation of the level of PRPP in cells has been found to be related to many severe metabolic disturbances including glycogen storage disease Type I [1, 2].

The results presented here indicate that the intracellular level of PRPP in animal tissues is greatly affected by extracellular UDPG. The alteration of PRPP level by UDPG is not linearly dose-related. The changes in PRPP that were induced by UDPG were also tissue-specific; mouse liver was more sensitive than was the spleen. This latter specificity may well be related to the therapeutically beneficial effects of UDPG.

Our data on the permeability of cell membranes to UDPG indicate that a significant amount (*ca.* 20%) of the UDPG, even though it is a highly polar compound, does pass through the membrane unchanged. A large fraction of the UDPG added to incubation media, however, was found in the cell as glucose phosphate, indicating cleavage after penetration of the cell. Eventually, all the UDPG that entered the cells was degraded to glucose phosphate and glucose. The *in vitro* studies show that G-6-P is not the active form, and several tests show that uridine does not lead to changes like those seen with UDPG [14–16]. Thus, the most likely mechanism of action involves the membrane. The effects of UDPG on cell metabolism do not appear to be limited to enzyme induction. Others have reported that UDPG can have effects under conditions that bar enzyme induction [17].

Since PRPP is involved in many metabolic pathways, changes in intracellular PRPP levels are often not accompanied by obvious changes in the synthetase, which has been shown in many respects to be a self-regulating enzyme [18]. Its activity can be controlled by further aggregation or dissociation of molecular aggregates [18, 19]. This phenomenon may explain some of the observations reported here and also suggests that the effect of UDPG on PRPP levels is most likely not due to the induction of additional synthesis of the enzyme, but to alteration of the conformation of the enzyme. Under the assay conditions employed, it is quite possible that these differences are masked. This line of reasoning is consistent with the rapid increase in PRPP seen in splenocytes with incubation in medium containing UDPG.

Glucose-6-phosphate can easily be converted intracellularly into ribose-5-phosphate and thus activate the production of PRPP. However, the different effects that we have observed between the G-6-P and UDPG incubation indicate that the UDPG action is not due solely to its increases in intracellular glucose concentration. Among other things, the product of UDPG cleavage, glucose phosphate, inhibits the UDPG pyrophosphorylase [20].

Contrary to the effect of UDPG on normal cells, UDPG was seen to decrease PRPP concentration in tumor cells. Since with tumor cells only one very high UDPG concentration was studied, it is difficult to assert that the reverse effect is due to the specific sensitivity of the tumor, as was noted between liver and spleen, or to the nature of the tumor cell membrane. Nevertheless, other rapidly proliferating tissues were also affected, albeit less so. Others have reported different changes in PRPP pool sizes in tumors and gut [21]. These observations plus the fact that the weights of the mammary tumor were actually decreased after only 5 days of exposure to UDPG

are sufficiently provocative to suggest further studies of UDPG as a therapeutic agent, especially in combination with inhibitors of the PRPP utilizing steps in purine and pyrimidine synthesis.

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