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Report

Effect of Uridine Diphosphoglucose on Levels of 5-Phosphoribosyl Pyrophosphate and Uridine Triphosphate in Murine Tissues

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The purpose of the present investigation was to determine whether a single bolus intravenous injection (2000 mg/kg) of uridine diphosphoglucose (UDPG) could affect levels of PRPP in a transplanted mammary adenocarcinoma and in liver of CD8FI mice. Six hours following a single intravenous injection of UDPG, 2000 mg/kg, tumor PRPP was lowered to 80 pmol/mg protein, a 53% decrease compared to saline control tumors. Liver was more sensitive than tumor to the 5-phosphoribosyl pyrophosphate (PRPP)-depleting effects of a single bolus intravenous injection of UDPG, since significantly lower levels of PRPP were found in liver, but not in tumor, at doses of 500–1000 mg/kg of UDPG. Maximal depression (30% of saline control) or PRPP occurred in liver 6 hr after intravenous UDPG at 1000–2000 mg/kg. Enhanced levels of UDPG in plasma (half-life less than 10 min) and tumor was detected at 30 min after intravenous UDPG at 2000 mg/kg. There was no detectable increase in endogenous levels of UDPG in liver at this time, probably as a result of rapid metabolism of UDPG by liver. At this same time, a twofold increase in uridine triphosphate (UTP) was measured in liver after intravenously administered UDPG. In contrast, the level of UTP was not increased significantly above control values in tumor. These data suggest the potential use of UDPG to elevate UTP pools in normal tissues in the delayed rescue of cancer chemotherapeutic drugs such as 5-fluorouracil which function as a uridine analogue in these tissues.

KEY WORDS: uridine diphosphoglucose; phosphoribosyl pyrophosphate; uridine triphosphate.

INTRODUCTION

Continuous intraperitoneal infusion of uridine diphosphoglucose (UDPG), a phosphorylated sugar derivative, at various dosages and schedules of administration has been shown to lower the levels of intracellular 5-phosphoribosyl pyrophosphate (PRPP) in CD8FI murine tumor and liver. Effects on the levels of PRPP depended on the dose rate of the infusion (1).

PRPP is a crucial metabolic intermediate in the de novo biosynthesis of purine and pyrimidine nucleotides as well as in salvage reactions. Any perturbation of intracellular levels of PRPP can greatly affect the activities and levels of incorporation of certain anticancer agents (2–4). The initial purpose of the present investigation was to determine whether a single bolus intravenous injection of UDPG could depress tissue levels of PRPP as was observed for continuous infusion (1). The data presented in this report indicate that 6 hr following a single intravenous injection of UDPG, elevated levels of UDPG were detectable by HPLC in both plasma and tumor. In addition, bolus injection of UDPG was found to lower the level of intracellular PRPP in CD8FI murine tumor and liver. It was further determined that intravenous administration of UDPG increased the level of intracellular uridine triphosphate (UTP) twofold above control levels of UTP in mouse liver but not in tumor.

METHODS

Animals, Tumors, and Treatment

CD8FI (BALB/C × DBA/8) mice with or without first-generation transplants of CD8FI mammary adenocarcinoma (5,6) were injected intravenously or intraperitoneally with a single bolus solution of UDPG in normal saline at various dosages (mg UDPG/kg body weight). Tissues then were harvested and quick-frozen in dry ice/acetone or liquid nitrogen at indicated times after UDPG injections. Control mice received only normal saline.

Tissue Processing for PRPP Levels

Tissue Extraction. Tumor and liver specimens which were excised and immediately quick-frozen in liquid nitrogen or dry ice/acetone and stored at −70°C were pulverized under liquid nitrogen with mortar and pestle. The fine powder was suspended in 4 vol of extraction media (0.2 Tris–HCl

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buffer, pH 7.4, 33 m*M* sodium fluoride, 0.5 m*M* 2-3 diphosphoglyceric acid, and 5 m*M* EDTA). After vigorous mixing, the suspension was frozen in dry ice–ethanol, allowed to thaw, centrifuged at 31,000*g* for 20 min, diluted 1:1 with 0.1 *M* potassium-phosphate buffer, pH 7.0, and filtered through Amicon CF25 filter cones (centrifuged at 1000*g* for 20 min). An aliquot of the homogenate was assayed for protein content by the Lowry procedure (9). The filtrates were stored at −70°C or immediately assayed for PRPP content.

^{14}C-Orotic Acid Assay for PRPP. The assay is based on the conversion of ^{14}C-orotic acid to uridine monophosphate with release of $^{14}CO_2$ by orotidine-5-phosphate-pyrophosphorylase + orotidine-5-phosphate decarboxylase (7,8). The 200-μl incubation mixture contained 1.0 m*M* dithiothreitol, 5.0 m*M* magnesium sulfate, 0.312 mg enzyme freshly dissolved in 0.1 ml Tris–HCl buffer, 0.43 μCi ^{14}C-orotic acid, 2.5 m*M* Tris–HCl buffer, pH 7.4. The incubation was carried out in scintillation vials. Absorbent paper (3 M, 2.3-cm-diameter circle) saturated with NCS tissue solubilizer (Amersham) was placed in a the cap before closing. After 1 hr of incubation at 37°C in a shaking water bath, 50 μl if 59% perchloric acid was added and the incubation was continued at 37°C for 40 min. At the end of the second incubation, the filters were placed in 8.5 ml hydrofluor (National Diagnostics) plus 1.0 ml water and 0.5 ml NCS tissue solubilizer in order to remove the counts from the surface of the filter into the scintillation fluid. The ^{14}C was then counted. The results are expressed as picomoles of PRPP per milligram of protein.

Processing of Tumor and Liver Samples for UDPG and UTP Content. Frozen tumor or liver specimens were homogenized in ice-cold 1.2 *N* perchloric acid. The acid-insoluble fraction was removed by centrifugation (7000 rpm for 15 min). The acid-soluble fraction was neutralized by extraction with a mixture of freon and tri-*N*-octylamine (2:1). The extract was then filtered through a 0.22-μm Millipore membrane filter prior to HPLC analysis. UDPG and UTP contents in tumor and liver were normalized to the protein content of the acid-insoluble fraction.

HPLC Measurement of Plasma Levels of UDPG. Plasma was collected in heparinized capillary tubes. The plasma was then briefly centrifuged to remove red blood cells. The volume of plasma was measured, and the plasma then transferred to clean 12 × 75-mm glass tubes and deproteinized with an equal volume of 20% TCA. The samples were kept on ice for 15 min and recentrifuged. The supernatant was then neutralized with 3 vol Freon:tri-*N*-octylamine (2:1). HPLC measurement of UDPG in plasma extracts was performed isocratically on a Waters 840 HPLC system with a WISP automatic sampler using a SAX Column (Waters) equilibrated with a 5% 0.5 *M* $NH_4H_2PO_2$ buffer, pH 5.0. The run time for 200 μl of each sample was approximately 22 min. Plasma UDPG levels are expressed as picomoles of UDPG per milliliter.

HPLC Measurement of UDPG and UTP Levels in Tumor and Liver. HPLC analysis in tissue was also performed on a Waters 840 HPLC system with a WISP automatic sampler. UDPG and UTP levels were analyzed by ion-exchange gradient chromatography using a Waters SAX column starting with 3 m*M* $NH_4H_2PO_4$, pH 3.5, proceeding in two steps to 70% 0.5 *M* $NH_4H_2PO_4$, pH 5.0, plus 30% starting buffer.

Table IA. Effect of a Single Injection of UDPG on PRPP Levels in a Mammary Tumor of CD8FI Mice[a]

Treatment (mg/kg)	PRPP (pmol/mg protein)	Ratio: treated/control	*P* value
1. Saline control	167 ± 25	—	—
2. $UDPG_{500}$	113 ± 13	0.68	NS
3. $UDPG_{1000}$	119 ± 19	0.71	NS
4. $UDPG_{2000}$	80 ± 10	0.53	<0.02

Table IB. Effect of a Single Injection of UDPG on PRPP Levels in Liver

Treatment (mg/kg)	PRPP (pmol/mg protein)	Ratio: treated/control	*P* value
1. Saline control	166 ± 27	—	—
2. $UDPG_{500}$	91 ± 13	0.55	<0.05
3. $UDPG_{1000}$	54 ± 7	0.33	<0.01
4. $UDPG_{2000}$	57 ± 9	0.34	<0.01

[a] Tumor-bearing CD8FI female mice received the indicated dose of UDPG intravenously. Subscripts indicate dose as mg/kg. Six hours after drug administration, the animals were sacrificed and PRPP was determined in the liver. Data are the mean ± SE for 15 mice per treatment group. These data were pooled from three separate experiments.

The run time for each 100 μl of extracted sample was 60 min. Tumor and liver UDPG and UTP levels are expressed as micrograms of UDPG or UTP per milligram of protein.

Statistical Evaluation

Students *t* test was used for evaluation of differences between treatment groups. A *P* value of 0.05 or less was considered to be significant.

RESULTS

In three separate experiments, PRPP levels were measured in CD8FI breast tumors at 6 hr after a single intravenous injection of UDPG. As can be seen from the pooled results in Table IA, the administration of UDPG resulted in a lowering of the level of PRPP, but only the highest dose of UDPG employed (2000 mg/kg) produced a statistically significant depression of PRPP level (53% of saline control tumor) ($P < 0.02$).

Data demonstrating the effect of UDPG in lowering PRPP 6 hr after a single intravenous injection of UDPG in liver of tumor-bearing CD8FI mice are shown in Table IB. UDPG significantly lowered the level of PRPP in liver at the three doses employed, producing a depression of PRPP levels to approximately 30% of saline control liver at the doses of 1000 and 2000 mg/kg. Thus, only UDPG at 2000 mg/kg effected a statistically significant depression of the levels of PRPP in both tumor and liver of CD8FI mice.

The pharmacokinetics of UDPG in plasma of CD8FI mice after intravenous or intraperitoneal administration of UDPG are shown in Fig. 1. As is seen when UDPG (1200 mg/kg) is injected intravenously, the plasma levels of UDPG

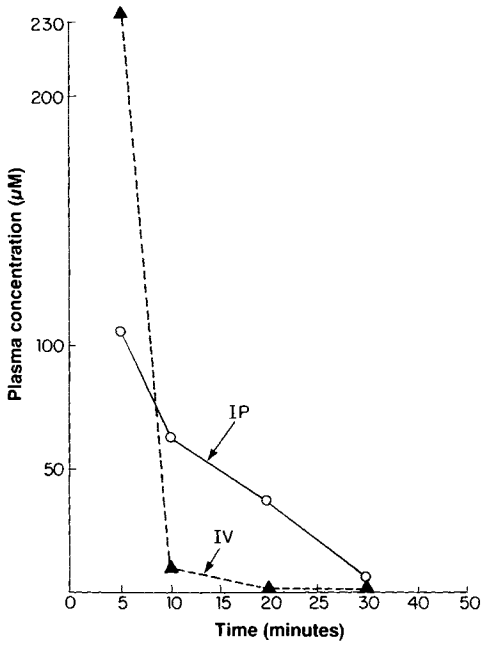

Fig. 1. Plasma UDPG levels in normal CD8FI male mice following a single intraperitoneal (IP) or intravenous (IV) injection of UDPG, 1200 mg/kg.

reach a maximum of approximately 240 μ*M* with a half-life of less than 10 min. An intravenous injection of UDPG is essentially cleared from the plasma within 10 min. In contrast, following an intraperitoneal injection, UDPG in plasma of mice reached a maximum of approximately 100 μ*M*, which declined steadily with time and was cleared from the plasma only after 30 min.

In Table II are given HPLC measurements of UDPG in tumor and liver of CD8FI mice after a single intravenous injection of UDPG. The data indicate that UDPG (2000 mg/kg) when injected intravenously produced elevated levels in the tumor after 30 min. This increase was approximately twofold compared with the endogenous level of UDPG in saline control tumor. In contrast, the level of UDPG in the liver of the same mice was not significantly increased above the endogenous level in the saline control liver.

HPLC measurements of UTP levels in both tumor and liver at various times following a single intravenous injection of UDPG (2000 mg/kg) are presented in Table III. As can be seen at 30, 60, and 120 min after injection of UDPG the levels of UTP are not significantly increased above control levels in tumor. In contrast, the levels of UTP in liver are increased twofold or higher starting at 30 min after injection of UDPG, and elevated levels persisted for at least 2 hr.

DISCUSSION

Uridine diphosphoglucose, when administered as a bolus intravenous injection (500–2000 mg/kg body weight), decreased the PRPP content in both tumor and liver of CD8FI mice. The depression of PRPP induced by UDPG is dose related and mouse liver was more sensitive than the CD8FI tumor. The results presented in this report (bolus intravenous administration of UDPG at a dosage of 2000 mg/kg body weight) are consistent with published findings (1) for continuous infusion of UDPG (2.8 mmol/kg body weight/day of UDPG), which lowered PRPP levels in both tumor and liver in CD8FI mice. The values for the concentration of PRPP in the liver of CD8FI saline control mice in this report are comparable to liver PRPP data previously published, in which values ranged from 206 to 276 ± 58 pmol PRPP for liver. The depression of PRPP by infusional UDPG was correlated with corresponding decreases in PRPP synthetase (1).

UDPG, when administered intravenously, can be detected in plasma with a short half-life (less than 10 min) before clearance. Although most phosphorylated sugars do not traverse mammalian cell membranes, UDPG has been shown to enter intact before cleavage of the molecule into glucose-1-phosphate and UMP (1,10). We report here additional data confirming the fact that bolus intravenous administration of UDPG results in an increase in endogenous levels of UDPG in a solid tumor. The values for the concentration of UDPG in the saline control liver of CD8FI mice in this report are comparable to published values (11) for liver UDPG content (approximately 0.32 ± 0.04 μmol/mg protein).

Of major interest in this report is the differential increase in UTP content in liver, but not in tumor, due to the bolus intravenous administration of UDPG (Table III). Although the administration of UDPG resulted in a twofold increase in the UTP content of liver, it did not elevate endogenous levels of UDPG in this tissue. This result suggests the presence of enzymes in the liver that may rapidly metabolize UDPG, cleaving it to UDP, which can serve as a source of UTP. These provocative results suggest further

Table II. Levels of UDPG in Mammary Tumor and in Liver of CD8FI Mice After a Single Bolus Intravenous Injection of UDPG[a]

Treatment (mg/kg)	Tissue	Time (mins)	UDPG (μg/mg protein)	Ratio: treated/control	*P* value
1. Saline control	Tumor	30	1.90 ± 0.3	—	—
2. $UDPG_{2000}$	Tumor	30	3.7 ± 0.4	2.0	0.05
1. Saline control	Liver	30	0.53 ± 0.04	—	—
2. $UDPG_{2000}$	Liver	30	0.61 ± 0.1	1.2	NS

[a] Tumor-bearing female mice received UDPG at a dose of 2000 mg/kg. At 30 min following administration of UDPG, the animals were sacrificed and UDPG was measured by HPLC. Data are mean ± SE for four mice per treatment group. These data were pooled from three separate experiments.

Table III. Differential Effect of $UDPG_{2000}$ in Liver and Tumor on UTP Levels[a]

Treatment (mg/kg)	Tissue	Time (min)	UTP (μg/mg protein)	Fold increase: ratio, treated/control	*P* value
1. Saline control	Tumor	—	2.4 ± 0.3	—	—
2. $UDPG_{2000}$	Tumor	30	3.2 ± 0.2	1.1×	NS
		60	2.6 ± 0.3	0.9×	NS
		120	2.5 ± 0.3	0.8×	NS
3. Saline control	Liver	—	0.7 ± 0.1	—	—
4. $UDPG_{2000}$	Liver	30	1.9 ± 0.3	2.4×	0.05
		60	2.0 ± 0.1	2.5×	0.01
		120	1.5 ± 0.03	1.9×	0.01

[a] Measured by high-pressure liquid chromatography. CD8FI first-generation mammary tumors in female CD8FI mice received UDPG, i.v., at a dose of 2000 mg/kg. At 30, 60, and 120 min following administration of UDPG, the animals were sacrificed and UTP measured. Data are means ± SE for four mice per treatment group in two separate experiments.

studies using UDPG in cancer chemotherapy as a delayed rescue agent to restore or elevate UTP pools following the administration of certain antipyrimidine drugs (e.g., 5-fluorouracil) (12,13).

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