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Uridine-induced hypothermia in mice and rats in relation to plasma and tissue levels of uridine and its metabolites

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Summary. Administration of high-dose uridine or cytidine (3500 mg/kg) resulted in severe hypothermia of 6–10° C in mice. This effect of uridine was observed in three different mouse strains, C57Bl/6, Balb/c, and Swiss. A high-dose of uridine also caused hypothermia in Wistar rats. Co-infusion of uridine with benzylacyclouridine, an inhibitor of uridine phosphorylase, partially prevented uridine-mediated hypothermia in mice. A low dose of uridine (100 mg/kg) resulted in a slight increase in temperature. Plasma pharmacokinetics of uridine (at 3500 mg/kg) were studied in two mouse strains, C57Bl/6 and Balb/c, and those of cytidine only in C57Bl/6 mice. Peak plasma concentrations of uridine in both strains after uridine administration were about 20 m*M* (at 30–60 min). The peak plasma concentration of cytidine in C57Bl/6 mice after cytidine administration was about 12 m*M* and that of uridine, 1.3 m*M*. The mean residence time for uridine was about 105 min. The area under the plasma concentration-time curve for uridine was about 50 mmol h/l, and that for cytidine, about 25 mmol h/l. In various tissues of C57Bl/6 mice the levels of uridine, uracil and total uracil and cytosine nucleotide pools were determined before and 2 h after uridine administration. Uridine levels increased about 53-fold in liver, about 70-fold in a colon tumor, and only about 7-fold in brain, while the corresponding uracil levels increased about 9-fold, 4-fold and 11-fold, respectively. Total uracil nucleotide pools increased about 8-fold, 3.2-fold and 1.6-fold, respectively. Cytosine nucleotide pools did not increase in the brain. In conclusion, high-dose uridine administration caused severe hypothermia. Plasma levels of uridine and uracil were enhanced to a considerably higher extent than the levels in the tissues. The hypothermia might be related to breakdown products of uridine, since inhibition of uridine breakdown partially prevented hypothermia and since in brain uracil nucleotide levels were only slightly increased after uridine administration, while those of uracil were more markedly increased than in other tissues.

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Abbreviations: TCA, trichloroacetic acid; GPT, 1-(2'-deoxy-β-*D*-glucopyranosyl) thymine; BAU, benzylacyclouridine; THU, tetrahydrouridine; MRT, mean residence time; AUC, area under the plasma concentration-time curve; V_D, volume of distribution; 5FU, 5-fluorouracil

Introduction

In preclinical studies it has been demonstrated that delayed administration of uridine might be used to prevent 5FU-induced myelosuppression [13, 21]. Both s.c. infusions [13] and repeated i.p. bolus injections [21] of uridine showed this protective effect. In these studies, no mention was made of any effect on body temperature. However, recently it was reported that uridine induced fever in rabbits [3, 34] and in human patients [34, 41]. Preliminary results demonstrated that high-dose uridine produced hypothermia in mice [30]. Recently, Martin [20] reported that in addition to uridine, cytidine was also able to 'rescue' mice from 5FU-induced toxicity, but no side-effects were reported. In an attempt to find out whether uridine-induced hypothermia is a general phenomenon, we determined the effects of uridine on body temperature in three mouse strains, C57Bl/6, Balb/c and Swiss, and in Wistar rats. The effect of cytidine was studied in C57Bl/6 mice only.

In both rabbits and humans the onset of fever after uridine administration was delayed [3, 34, 41], suggesting that the generation of one or more metabolities of uridine is required to affect thermoregulation. In addition to uridine we also measured the plasma concentrations of its catabolite uracil in C57Bl/6 and Balb/c mice in order to gain an insight in the processes of metabolism and excretion of uridine and the possible relationship between uridine metabolism and temperature. The initial catabolism of uridine to uracil is catalyzed by uridine phosphorylase. Therefore, we also tested whether inhibitors of uridine phosphorylase could prevent the effect of uridine on body temperature.

In brain and other tissues uridine will also be anabolized to nucleotides in a reaction catalyzed by uridine kinase [28]. Uridine appeared to be an essential factor for the maintenance of normal brain functions [8] and the predominant cofactor for synthesis of pyrimidine nucleotides in neural tissues [8, 10, 28] and for nucleotide sugars [8, 36]. Since body temperature is regulated in the hypothalamus [7] and since an aberration in pyrimidine nucleotide pools might affect brain function, we measured the concentration of uracil and cytosine nucleotides in murine brain after uridine administration, together with uridine and uracil concentrations. Furthermore, we determined uridine metabolism in liver, which is one of the major organs for the supply of pyrimidines [16]. For comparison we also measured uridine metabolites in a murine colon car-

cinoma, Colon 38. Some parts of this work have abready been presented in preliminary form [30].

Materials and methods

Chemicals. Pyrimidine nucleotides, nucleosides and bases were all obtained from Sigma, St Louis Mo, USA. GPT (NSC 402666) and THU were obtained from the Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, Bethesda, Md, USA, while BAU was kindly provided by Dr S. H. Chu, Brown University, Providence, RI, USA. The uridine solution was formulated by the Pharmacy Department of the Free University Hospital as described previously [19]. The other solutions were dissolved in 0.65% NaCl, adjusted to pH 7.0 if necessary, and sterilized by passage through 0.22-μm Millipore filters. The BAU solution had to be sonicated for solubilization. All solutions were tested for bacterial pyrogens with the Limulus test, which was negative for all compounds. A prepacked LIChrosorb 10-RP-18 column (150 × 4.6 mm, length × i.d.) and a prepacked Partisil-SAX column (250 × 4.6 mm, length × i.d.; particle size 10 μm) were obtained from Chrompack, Middelburg, The Netherlands. All other chemicals were of analytical grade quality and were obtained commercially.

Treatment of mice and rats. Experiments were performed on healthy adult animals, both females and males. They were kept in controlled areas and had access to food and water ad libitum. Three mouse strains, C57Bl/6, Balb/c and Swiss mice, and Wistar rats were used, all obtained from the REPGO-TNO animal breeding station at Zeist, The Netherlands. The murine colon carcinoma Colon 38 was obtained via Dr P. Lelieveld from the same Institute. Histology, growth characteristics and sensitivity to 5-FU have been described previously [2, 33, 42]. The mice and rats received all compounds as i.p. bolus injections. The volume of injection for mice did not exceed 0.4 ml, and that for rats did not exceed 3.5 ml. Temperature was monitored rectally with a thermosensitive probe before administration and at various intervals after treatment until body temperature was normalized.

Measurement of plasma uridine and uracil concentrations. Blood samples from the mice were taken before and after treatment via retro-orbital puncture under ether anesthesia with heparinized hematocrit capilaries. Blood samples were centrifuged immediately, and plasma was pipetted off and stored at −20° C until analysis. All the compounds tested in this study are stable under these conditions. Plasma was deproteinized with TCA (final concentration 5%; 5 g per 100 ml H_2O) at +4° C for 20 min. Samples were neutralized with an alamine-Freon (trioctylamine-1,1,2-trichlorotrifluoroethane) solution [29]. Plasma levels of uridine and uracil were determined with an HPLC method described previously [29], using a LiChrosorb 10-RP 18 column. Pharmacokinetic parameters were calculated as described previously [34], according to van Rossum and van Ginneken [43].

Tissue nucleotide measurements. In order to study the concentrations of nucleosides, bases, and nucleotides, mice received uridine or cytidine at 3500 mg/kg i.p. as a bolus injection. After 2 h mice were killed by cervical dislocation. Tissues were removed as soon as possible and were immediately frozen in and subsequently stored in liquid nitrogen. Initially tissues were obtained from mice which were completely frozen in liquid nitrogen immediately (less than 5 s) after cervical dislocation. This procedure diminishes degradation of nucleotides. Frozen tissues could be removed from these mice separately from the other tissues. In extracts prepared from these tissues the UTP and CTP pools were higher than in tissues obtained from mice that were not completely frozen, but the tissue pools of total pyrimidine nucleotides and of uridine and uracil were comparable for both methods (data not shown). Thus, during preparation of the tissues UTP and CTP were degraded to UMP and CMP, respectively, but not to uridine and cytidine.

For analysis the frozen pieces of tissues were weighed and subsequently pulverized using a micro-dismembrator [32]. The powder (still frozen) was extracted with a TCA solution (final concentration 5%) at 4° C for 20 min and neutralized with alamine-Freon. In this extract the concentrations of uridine and uracil were determined as described above with a LiChrosorb 10-RP-18 column. Analysis of all nucleotides was performed on a Partisil SAX column using gradient elution [31]. Analysis of the total pyrimidine nucleotide pool (UΣP and CΣP) was performed by heating the extracts in 1 *M* perchloric acid at 100° C for 14 min, which hydrolyzes UTP, UDP and UDP sugars to UMP, and CTP, CDP and CDP sugars to CMP [23, 38]. The hydrolyzed extract was neutralized with alamine-Freon. Analysis of UΣP and CΣP pools was performed on a Partisil-SAX column using isocratic elution [23] with 50 m*M* formic acid and 5 m*M* KH_2PO_4 (pH 4.1) at 1.5 ml/min. The retention time of CMP was 8.6 min and that of UMP, 13.2 min.

Statistical analyses were performed using Student's *t*-test for unpaired and for paired data.

Results

Effect of uridine and cytidine on body temperature

Uridine given at a dose of 3500 mg/kg was able to prevent myeloid toxicity caused by 5FU in mice [20, 33]. This dose

Table 1. Body temperature in several mouse strains after treatment with uridine at 3500 mg/kg

Time (h-min)	Body temperature (° C)		
	C57Bl/6	Balb/c	Swiss
0-00	37.6	38.2	38.1
0-30	32.4***	33.4***	32.8**
1-00	31.2***	33.5**	–
2-00	31.9**	35.8°	31.8**
4-00	33.5°	37.0*	32.9*
6-00	36.5	35.9*	33.3*

Mean body temperature from 6–12 mice is given in each case. Temperature variation of mice treated with NaCl was less than 0.7° C. SD was less than 2° C. Significant different from the temperature before treatment as tested with the Student's test for paired data

*** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.02$; ° $0.02 < P < 0.05$

Other side effects: shivering, spasms, swollen eyelids, discolored fur, no activity, and no group formation

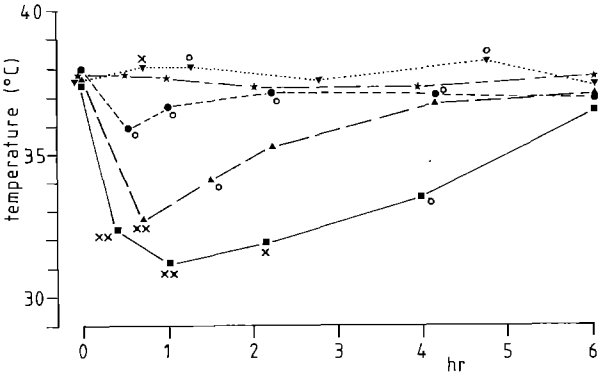


Fig. 1. Body temperature in C57Bl/6 mice after an i.p. bolus injection of uridine. Means from at least 6–12 mice are shown. SD did not exceed 2° C. ■———■, 3500 mg/kg; ▲– – –▲, 2000 mg/kg; *– – –*, 1000 mg/kg; ●--------●, 500 mg/kg; ▼.....▼, 100 mg/kg. Significant different from values before treatment according to Student's *t*-test for paired data; xx, $P < 0.001$; x, $0.001 < P < 0.01$; o, $0.01 < P < 0.05$

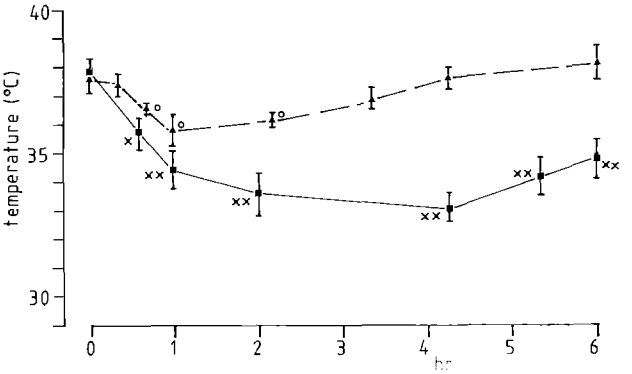


Fig. 2. Body temperature in Wistar rats after an i.p. bolus injection of uridine at 2000 mg/kg (▲– – –▲) and at 3500 mg/kg (■———■). Values are means ± SE from 3 and 7 rats, respectively. Significance indicated as in Fig. 1

reduced the body temperature of Swiss, C57Bl/6 and Balb/c mice significantly (Table 1). Furthermore, these mice showed spasms, shivering, swollen eyelids and discoloration of the fur. The lowest temperatures were observed in Swiss mice and were as low as 26° C in some animals. The lowest temperature found in C57Bl/6 mice was 28° C and the lowest in Balb/c mice, 27° C. When body temperature decreased to 26–28° C the mice required a longer time for recovery.

C57Bl/6 mice were used more extensively to study the effect of uridine on body temperature (Fig. 1). Uridine was administered at 3500, 2000, 1000, 500, and 100 mg/kg. The temperature decrease observed at 2000 mg/kg was smaller than that with 3500 mg/kg and mice recovered more rapidly. The other side-effects, such as spasms, were also less pronounced. At 1000 mg/kg no temperature decrease was observed, but at 500 mg/kg a small but significant fall in body temperature was observed. These mice recovered within a few hours. At 100 mg/kg a small but significant rise in temperature was observed. Since uridine administration is intended for selective rescue from 5FU toxicity, without affecting antitumor activity it has been used in mice bearing the colon carcinoma Colon 38 [33]. Treatment of these tumor-bearing mice with uridine at 3500 mg/kg resulted in a comparable temperature decrease to that in mice without tumors.

Since the rat is a species closely related to mouse, we also determined the effect of uridine on body temperature of this species (Fig. 2). Uridine was tested at two doses which were injected i.p. The fall in body temperature in rats was comparable to that observed in mice. Rats treated with 3500 mg/kg recovered more slowly; after 24 h the body temperature was normalized.

Effect of GPT and BAU

In an attempt to prevent breakdown of uridine, two inhibitors of uridine phosphorylase, GPT and BAU, were injected simultaneously with uridine. These compounds are specific competitive inhibitors of uridine phosphorylase [15, 25, 26]. GPT and BAU did not affect the body temperature of mice. BAU was given at the same concentration as used by Darnowsky and Handschumacher [5]. GPT in combination with uridine significantly decreased body temperature (30.2 ± 0.9° C after 1 h; mean ± SE). This fall in body temperature was comparable to that with uridine alone (Table 1). In contrast, BAU in combination with uridine affected body temperature significantly less than uridine alone (34.5 ± 0.3° C after 0.5 h). Mice also recovered more quickly, body temperature being normal again after 6 h.

Effect of cytidine

Through deamination, cytidine can act as a precursor for uridine, with the same ability to rescue mice from 5FU toxicity [20]. Cytidine also significantly decreased the body temperature of mice at a dose of 3500 mg/kg (Fig. 3). THU, a potent inhibitor of cytidine deaminase, was administered at a dose reported to be capable of inhibiting cytidine deaminase in vivo [22]. However, 25 mg THU/kg did not prevent the temperature decrease caused by cytidine. At 50 mg THU/kg comparable results were found (data not shown). On closer examination it appeared that THU itself affected body temperature, leading to a temperature decrease at 25 mg/kg and an increase at 50 mg/kg.

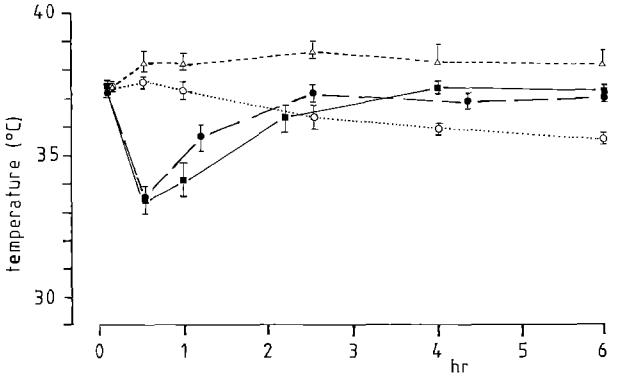


Fig. 3. Body temperature in C57Bl/6 mice after an i.p. bolus injection of cytidine at 3500 mg/kg (■———■), cytidine plus THU at 25 mg/kg (●– – –●), or THU alone at 25 mg/kg O....O) or at 50 mg/kg (Δ--------Δ). Values are means ± SE from 3–5 mice for THU alone, from 10 mice for cytidine, and from 6 mice for cytidine plus THU. Temperature decrease after 30 and 60 min was significant at $P < 0.001$ for cytidine and cytidine plus THU

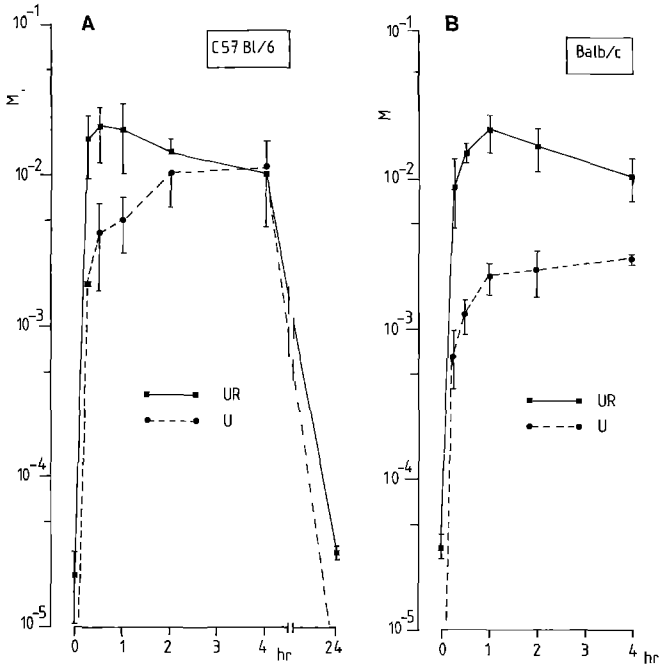


Fig. 4A, B. Time-versus-plasma concentration curves for uridine (UR) and uracil (U) in C57Bl/6 **(A)** and Balb/c **(B)** mice. Values are means ± SEM from 4 animals. Blood samples were obtained from the same animal before and after uridine administration

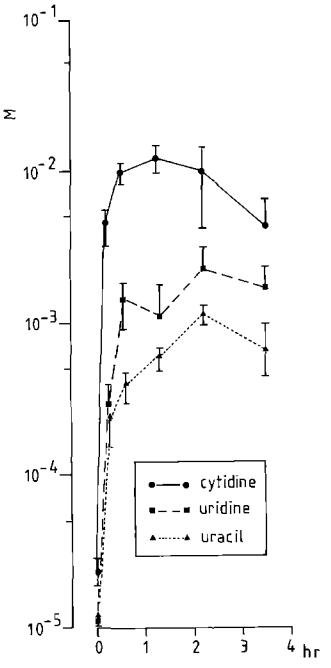


Fig. 5. Time-versus-plasma concentration curves for cytidine, uridine and uracil in C57Bl/6 mice after an i.p. bolus injection of cytidine at 3500 mg/kg. Blood samples were obtained from the same animal before and after cytidine administration. Values are means ± SE from 4 animals

Plasma concentrations of uridine, uracil and cytidine

In C57Bl/6 and Balb/c mice the plasma concentrations of uridine and uracil were measured after administration of 3500 mg uridine/kg. Blood samples were obtained from the same mice at 0, 15, 30, 60, 120, and 240 min and at 24 h after uridine administration. For all mice comparable concentration-time curves were found. The mean values are presented in Fig. 4. In C57Bl/6 mice (Fig. 4A) the peak uridine concentration was reached after 30 min and amounted to about 20 m*M*. In Balb/c mice (Fig. 4B) the peak concentration was reached after 60 min and amounted to about 20 m*M*. Thereafter, the uridine concentration decreased relatively slowly. In both mouse strains uridine was rapidly broken down to uracil. After 4 h uracil levels were higher than those of uridine in C57Bl/6 mice. In Balb/c mice uracil levels remained lower than those of uridine. Peak levels were observed after 2–4 h.

In C57Bl/6 mice we measured cytidine, uridine, and uracil concentrations after administration of cytidine at 3500 mg/kg (Fig. 5). Cytidine reached a peak concentration of about 10 m*M* after 1 h. Cytidine was deaminated to uridine, but uridine levels did not exceed those of cytidine. Uridine levels remained relatively constant between 1 and 1.5 m*M* for 3 h. Uracil reached a peak plasma concentration of 1 m*M* after 2 h. No cytosine was detected.

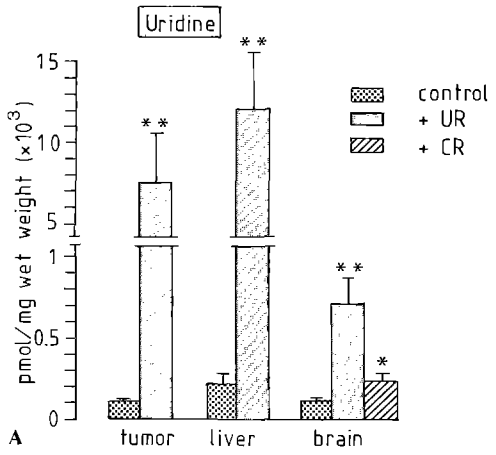
In general, pharmacokinetic parameters of uridine for both mouse strains are comparable (Table 2). The total uridine clearance is slightly lower in Balb-c mice. The AUC for uracil is slightly lower in Balb/c mice, although the difference is smaller than for the peak concentration.

Both peak concentration and AUC are lower for cytidine in comparison to uridine. Considerable differences were also observed in the V_D and the total clearance, which are both higher for cytidine. The AUC for uridine and uracil formed from cytidine are comparable. However, the AUC for uridine formed from cytidine is considerably lower than that of uridine after uridine administration itself. The same holds for uracil.

Table 2. Pharmacokinetic parameters of uridine and cytidine and their metabolites

Compound species	Peak concentration (m*M*)	MRT (min)	AUC (mmol h/l)	V_D (ml/kg)	Total clearance (ml/kg·min)
Uridine					
C57Bl/6	21.5 ±6.3	96±11	47 ±6	773±115	0.319±0.035
Balb/c	20.8 ±3.2	119± 2	59 ±6	689± 89	0.249±0.030
Uracil (from uridine)					
C57Bl/6	10.62±5.91	118±15	11.9 ±1.1	–	–
Balb/c	2.83±0.10	136± 1	8.7 ±0.9	–	–
Cytidine	11.6 ±2.2	112± 9	25 ±4	1265±347	0.841±0.119
Uridine (from cytidine)	1.32±0.43	–	3.11±0.56	–	–
Uracil (from cytidine)	1.10±0.16	–	2.57±0.29	–	–

Parameters are calculated from data obtained in separate animals. The values are calculated from the time of injection up to 4 h after injection. Values are means ± SE. Cytidine values are from C57Bl/6 mice



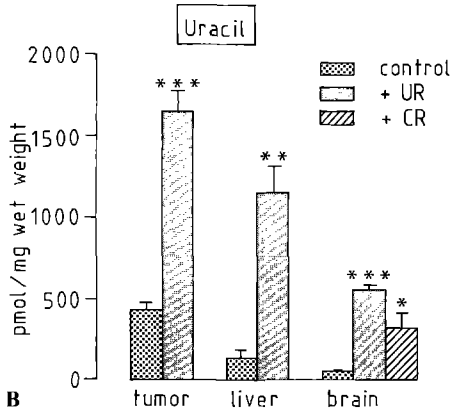
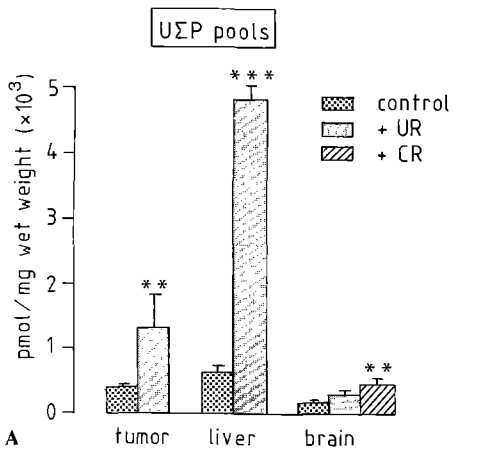


Fig. 6A, B. Tissue levels of uridine **(A)** and uracil **(B)** at 2 h after administration of uridine (*UR*) at 3500 mg/kg or of cytidine (*CR*) at 3500 mg/kg. Control data are from 9 tumors, 4 livers and 3 brains; data from uridine-treated mice are from 4 tumors, 3 livers and 4 brains; data from cytidine-treated mice are from 5 brains. Values are means; *bars*, SEM. Values from treated mice were significantly different from control mice: *** $P < 0.001$; **$0.001 < P < 0.01$; *$0.01 < P < 0.02$



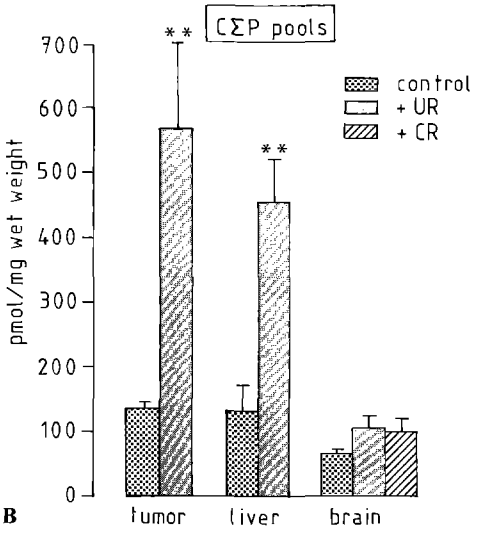


Fig. 7A, B. Tissue levels of **(A)** total uracil nucleotide pool (UΣP) and **(B)** cytosine nucleotide pool (CΣP) **(B)** at 2 h after administration of uridine and cytidine. Further details are given in the legend to Fig. 6

Tissue levels of uridine, uracil cytidine and nucleotides

It was reported earlier that there are wide differences in uridine and uracil levels between plasma and various tissues of mice [5]. Modulation of plasma levels depends on the activities of uridine phosphorylase and uridine kinase in tissues, since it has been shown that erythrocytes themselves have a very low capacity for either phosphorylation or phosphorolysis of uridine [40]. Therefore, we measured uridine and uracil levels (Fig. 6) in the liver, as a major organ responsible for uridine breakdown in Colon 38, and in brain because of the effect of uridine on temperature. After 2 h both uridine and uracil were at or almost at plateau levels in the plasma (Fig. 4), and body temperature was still low (Figs. 1, 3). Therefore tissues were removed after 2 h. In both the tumor and the liver uridine concentrations were increased considerably, about 70- and 53-fold, respectively, while in brain the uridine concentration was only increased 7-fold (Fig. 6). Marked differences were observed in uracil levels in tumor, liver, and brain. Although the absolute uracil levels were lower in brain tissue than in liver or tumor tissue, the relative increase in uracil levels was higher in brain (11-fold) than in liver (8.8-fold) or tumor (3.8-fold).

The effect of cytidine was studied only in brain. The cytidine level in brains of untreated mice was 42 ± 9 pmol/mg wet weight, increasing after treatment to 90 ± 32 (means $\pm$ SE from 3 and 5 mice, respectively). After cytidine administration uridine levels in brain increased about 2.1-fold, but uracil levels increased about 6.8-fold.

Striking differences were observed in the ability of uridine to modulate total uracil and cytosine nucleotide pools in tumor, liver and brain (Fig. 7). In the Colon 38 tumor, uridine increased the UΣP pools only 3.2-fold, but CΣP pools increased 4.1-fold. In liver, UΣP pools increased more than 7.8-fold, while CΣP increased only 3.5-fold. In brain, administration of uridine did not significantly affect UΣP or CΣP pool. Although cytidine is a direct precursor for cytosine nucleotides, no increase in CΣP pools was observed in brain, but a small but significant increase was seen in UΣP pools.

Discussion

In this study we observed that systemic administration of uridine can result in a severe hypothermia in mice and rats. Furthermore, a comparable dose of cytidine also decreased body temperature in mice, although to a lesser extent than uridine. In previous studies on high-dose uridine

and cytidine no mention was made of an effect on temperature [13, 14, 21]. In contrast, uridine induced fever in rabbits [3, 34] and in human patients [41]. However, the dose which was administered was lower than that used in the experiments with mice and rats. Interestingly, at the lowest dose of uridine (100 mg/kg) a slight but significant temperature increase was observed.

In a previous study we found evidence that uridine-induced hyperthermia in rabbits was caused by a catabolite of uridine [34]. The data obtained in this study indicate that the hypothermia in mice might also be cuased by a catabolite of uridine. Inhibition of uridine catabolism to uracil by BAU partially prevented the uridine-induced hypothermia, indicating that uracil or further catabolites, such as carbamyl-β-alanine or β-alanine, interfere with thermoregulation [34]. It might also be possible that the uridine-induced hyperthermia is a shock-like effect due to a sudden load of salts. However, a comparable injection with saline did not lead to hypothermia. Furthermore, injection of the same doses of cytidine resulted in less severe hypothermia. This effect of cytidine is probably due to its deamination to uridine or that of cytosine to uracil. Unfortunately, THU, a potent inhibitor of cytidine deaminase [22], affected body temperature. It might be that an effect on body temperature is an inherent property of pyrimidine ribonucleosides. No effect of pyrimidine deoxynucleosides has been reported, although thymidine has been administered at very high doses (80 g/m^2) both to patients [18, 47] and to mice (4 g/kg [9, 46, 47]. Up to now, deoxycytidine has only been administered at low doses to patients suffering from purine nucleoside phosphorylase deficiency [39, 44].

A hypothermic effect has been described for several purines, such as adenosine and analogues [1, 12]. The effect was observed at rather low doses of the compounds, much lower than those of the pyrimidine nucleosides used in this study. Therefore, this effect of adenosine is probably mediated by a different mechanism [1] via the adenosine receptors, leading to an increase of cAMP levels [4]. Since adenosine can be considered as a local hormone [1] the hypothermic effects of adenosine are most probably peripheral in nature [12], although interaction with the hypothalamic temperature center cannot be excluded.

The plasma uridine concentrations reported by Martin et al. [21] after an i.p. injection of 3500 mg/kg are comparable to our values. Although no pharmacokinetic data have been reported, the AUC estimated from their data appears to be smaller. Recently, Klubes et al. [14] reported a study on the bioavailability of uridine in mice following s.c. and oral administration. For s.c. administration these authors reported an AUC value for uridine from 0 to 2 h of 5890 μmol.h/l, which is considerably lower than we could calculate for i.p. uridine after 2 h in our two mouse strains. This variance in data might be due to either the different routes of administration or to the use of different mouse strains.

Plasma uridine and uracil levels were studied together with tissue levels of uridine, uracil and pyrimidine nucleotides after uridine administration, in order to correlate these levels with the effect on body temperature. Uridine concentrations increased much more markedly in plasma than in the tissues examined, including brain. Although the absolute uracil concentration in brain was lower than in the tumor or liver, the relative increase was higher. If the lack of effect of uridine on total brain uracil and cytosine nucleotide pools is also true for different functional subregions of the brain, and if the hypothermic effects are not peripheral in nature, then the effect of uridine on body temperature may be mediated by uracil or another catabolite rather than by a nucleotide. This is supported by the data obtained following cytidine administration. Cytidine not only enhanced uridine levels, but the relative increase in uracil levels was much higher. Also uracil nucleotide pools were increased. Santos et al. [37] reported that cytidine in tracer amounts was a better substrate for labeling brain pyrimidine nucleotides than uridine. It also appears to be a better substrate than uridine for labeling of liver pyrimidine nucleotides [17, 24]. This could be related to the longer exposure of tissues to uridine, due to gradual metabolism of cytidine to uridine. Studies on differences in nucleotides in the various anatomical sections of the brain were beyond the scope of the study.

Our data on physiological concentration of uracil nucleotide pools in liver and the tumor Colon 38 are comparable to data reported by Darnowsky and Handschumacher [5], although in their report no data on levels in brain were included. No expansion of the uracil nucleotide pools was observed by these investigators after administration of BAU with uridine at 250 mg/kg, probably because their dose of uridine was considerably lower than ours. An interesting difference was observed concerning the concentration of uridine and uracil in plasma and tissues. While the physiological plasma concentration of uridine is between 5 and 10 μ*M*, concentrations in brain and tumor were about 100 μ*M* and those in liver, as much as 200 μ*M* (Fig. 6). Even wider differences were observed for uracil. This means that concentrations of nucleosides in tissues are not equal to those in plasma; the higher concentrations in tissues are probably due to an active transport system for uridine [35] and to differences in uridine metabolism between blood cells and tissues [6, 27, 28]. Comparable differences have been observed by Darnowski and Handschumacher [6]. These authors postulated that a concentrative transport mechanism is responsible for the large tissue uridine pools, which might form a reservoir for pyrimidines. The differences in uridine pools and in the effect of uridine on its nucleotides might play an important role in the selective uridine 'rescue' from 5FU toxicity. The low uridine pool in Colon 38 and the relatively minor effect of uridine on uridine nucleotide pools in Colon 38 might be linked with the sensitivity of this tumor to 5FU even with delayed administration of uridine [33]. Our data support the theory that rescue is mediated by the competition of UTP with 5FU nucleotides for incorporation into RNA in non-malignant tissues [21, 38]. Toxicity caused by 5FU is attributed to its incorporation into RNA [11, 38, 45].

This difference in uridine pools in tissues and plasma might be related to a selective effect of uridine on the action of 5FU. Assuming similar concentrations of uridine in plasma and bone marrow, the large increase in uridine concentration might selectively influence the pyrimidine and fluoropyrimidine metabolism in bone marrow cells. Since the uridine increase in the tumor is less pronounced, the effect on (fluoro)pyrimidine metabolism in tumor and other tissues might also be less marked. Up to now, only reversal of myelosuppression has been observed in patients [30], but reversal of gastrointestinal toxicity has not yet been demonstrated.

In conclusion, this paper is the first report on a temperature-decreasing effect of the pyrimidine nucleosides uridine and cytidine. The effects are probably an inherent property of these pyrimidines and might be mediated by breakdown products. Evidence has been obtained from studies with the uridine phosphorylase inhibitor BAU and from measurements of tissue uridine, uracil, and uracil nucleotide pools. The increase in uracil level was relatively highest in brain, supporting the relevance of uracil or further breakdown products for thermoregulation. It might be possible to regulate the effects on body temperature by using an appropriate combination of BAU and uridine. The marked differences in uridine and uracil levels between the various tissues are relevant for the optimization of more specific therapy with 5FU and uridine.

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