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Research Article

Effect of Pyrimidine Nucleosides on Body Temperatures of Man and Rabbit in Relation to Pharmacokinetic Data[1]

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The effect of high-dose uridine on body temperatures of rabbits and man has been studied in relation to plasma concentrations of uridine and its catabolite uracil. Uridine induced fever in both rabbits and man. High-dose cytidine had no influence on body temperature in rabbits. Plasma concentrations of uridine were between 1 and 1.5 m*M* at 30 min after an iv bolus injection of 400 mg uridine/kg in rabbits and reached peak levels of 2 m*M* after a 1-hr infusion of 12 g uridine/m^2 in man. The plasma concentration of cytidine in rabbits was about 0.5 m*M* and that of uridine was 0.30 m*M* at 30 min after an iv bolus injection of 400 mg cytidine/kg. The mean residence time for uridine in patients and rabbits varied between 80 and 195 min. The area under the plasma concentration–time curve (AUC) for uridine in rabbits was 2.0 mmol · hr/liter, and that for cytidine was 0.6 mmol · hr/liter. A large AUC for uridine indicates a prolonged exposure of tissues to uridine, which might lead to extensive formation of degradation products. The administration of some of these catabolites, dihydrouracil (at 20–40 mg/kg), carbamyl-β-alanine (at 60 mg/kg), and β-alanine (at 300–400 mg/kg), resulted in a significant increase in body temperature. It is concluded that the change in body temperature associated with uridine administration was not due to bacterial pyrogens but that one of the degradation products might be involved in thermoregulation.

KEY WORDS: high-dose uridine; body temperature; uridine pharmacokinetics; uridine catabolites.

INTRODUCTION

The pyrimidine nucleoside uridine has been successfully used for the treatment of deficiency of orotate phosphoribosyltransferase for several decades (1). Uridine has also been demonstrated to prevent 5FU[4]-induced toxicity in mice without a loss of antitumor activity (2,3). Delayed administration of uridine after 5FU prevented severe myelosuppression. Different schedules with similar efficacy have been used. Klubes *et al.* (3) applied uridine as a subcutaneous continuous infusion, while Martin *et al.* (2) administered uridine as bolus injections at several time points after 5FU. Recently Martin (4) reported that cytidine was also able to "rescue" mice from 5FU toxicity. Cytidine can be a precursor for uridine by the action of cytidine deaminase and may act as a prodrug for uridine, thus providing a longer exposure of tissues to uridine at lower concentrations.

Martin *et al.* (2) postulated that a plasma concentration of 1 m*M* uridine would be sufficient to prevent 5FU toxicity. In a phase I trial we studied uridine administration as a 1-hr infusion (5). Peak plasma uridine concentrations between 1 and 2 m*M* were obtained at the highest doses (10 and 12 g/m^2), but uridine was eliminated rapidly. No rescue from 5FU toxicity was observed in two patients treated with 5FU and one or two delayed 1-hr infusions of uridine, possibly because this uridine treatment was insufficient. Therefore uridine was administered as a continuous infusion (6). However, these infusions had to be discontinued because of the development of fever (above 39°C). As the Limulus test was negative for formulated uridine, the increase in body temperature could not be attributed to bacterial pyrogens. However, uridine has also been tested for its effect on the body temperature in rabbits, a species with a high sensitivity to bacterial pyrogens. Formulated uridine appeared to be pyrogenic according to the USA Food & Drug Administration specifications (7). Since a discrepancy with the Limulus test was observed, the effect of uridine on body temperature was examined more extensively in rabbits.

In both rabbits and man fever was observed relatively late, suggesting that the formation of one or more metabolites of uridine, either anabolites or catabolites, is required in order to perturb thermoregulation. We measured the plasma concentrations of uridine and its catabolite uracil in

[1] Portions of this work have been presented in a preliminary form [G. J. Peters, *et al. Proc. Am. Assoc. Cancer Res.* 26:370 (abstr. 1462) (1985)].

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[4] Abbreviations used: TCA, trichloroacetic acid; GPT, 1-(2′-deoxy-β-D-glucopyranosyl)thymine; BAU, benzylacyclouridine; MRT, mean residence time; HPLC, high-performance liquid chromatography; AUC, area under the plasma concentration–time curve; V_D, volume of distribution; 5FU, 5-fluorouracil; GABA, γ-aminobutyric acid.

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order to obtain insight into the processes of metabolism and excretion of uridine and the possible relation between uridine catabolism and temperature. The initial catabolism of uridine to uracil is catalyzed by uridine phosphorylase. The catabolism of uracil to dihydrouracil (Fig. 1) is catalyzed by dihydrouracil dehydrogenase, which is present in all tissues and mostly in liver (8). Further catabolism to carbamyl-β-alanine and β-alanine proceeds mainly in liver. β-Alanine can be considered the end product of uracil degradation and is partly removed by oxidation via the citric acid cycle. However, β-alanine is also the precursor of carnosine (9), a small peptide that acts as a neurotransmitter. Furthermore, β-alanine is structurally related to GABA, which plays a role in thermoregulation, together with serotonin (10). Therefore, breakdown products of uracil were also tested for their effects on body temperature. We also tested whether inhibitors of uridine phosphorylase could prevent the effect of uridine on body temperature.

The aims of the present study were (a) to determine the extent and nature of side effects of uridine and cytidine and (b) to obtain insight into the influence of uridine metabolism on body temperature, which may lead to effective measures to minimize or prevent fever in patients treated with uridine in combination with 5FU.

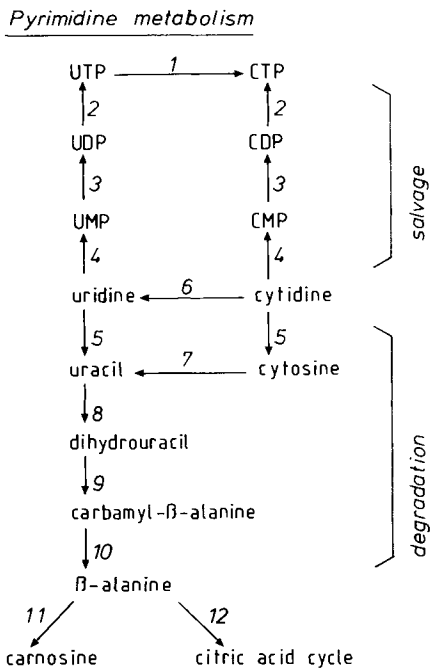

Fig. 1. Schematic outline of the metabolism of pyrimidine nucleosides. The enzymes catalyzing the reactions are as follows: 1, CTP synthetase; 2, nucleoside diphosphate kinase; 3, nucleoside monophosphate kinase; 4, uridine–cytidine kinase; 5, uridine phosphorylase; 6, cytidine deaminase; 7, cytosine deaminase; 8, dihydrouracil dehydrogenase; 9, dihydropyrimidase; 10, ureidopropionase; 11, carnosine synthetase; and 12, transamination yielding malonic semialdehyde, which will be converted to propionyl-SCoA and enter the citric acid cycle.

MATERIALS AND METHODS

Chemicals

Pyrimidine nucleotides, nucleosides, bases, dihydrouracil, carbamyl-β-alanine, and β-alanine were all obtained from Sigma, St. Louis, Mo. GPT (NSC 402666) was obtained from the Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, Bethesda, Md. The uridine solution for administration to patients and animals was formulated by the Pharmacy Department of the Free University Hospital as described previously (5). The other solutions were dissolved in 0.65% NaCl, adjusted to pH 7.0 if required and sterilized by passage through 0.22-μm Millipore filters. 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.) was obtained from Chrompack, Middelburg, The Netherlands. All other chemicals were of analytical-grade quality and obtained commercially.

Rabbits

Experiments were performed on healthy adult Dutch mixed-bred rabbits. They were kept in controlled areas and had access to food and water *ad libitum*. The rabbits received all compounds as bolus injections. They were injected either ip or iv via the ear vein. The volume of injection did not exceed 7 ml. The temperature was monitored rectally with a thermosensitive probe, before administration and at various intervals after treatment until the body temperature was normalized.

Blood Sampling and Analysis

Blood samples from the rabbits were taken via the ear vein with a heparinized syringe before and after treatment. The rabbits were not anesthesized. Blood samples were centrifuged immediately, and plasma was pipetted off and stored at −20°C until analyses were carried out. All tested compounds are stable under these conditions. Plasma was deproteinized with TCA (final concentration, 5%, 5 g/100 ml H_2O), at +4°C for 20 min. Samples were neutralized with an alamine–Freon solution (11). Plasma levels of uridine, uracil, and the various uridine phosphorylase inhibitors were determined with an HPLC method described previously (11) using a LiChrosorb 10-RP-18 column.

Pharmacokinetics and Statistical Calculations

Pharmacokinetic calculations were generally performed according to Van Rossum and Van Ginneken (12). MRT and AUC were calculated using a programmed Hewlet-Packard TI-59 calculator. AUC calculations were based on the linear trapezoidal method. V_D, MRT, and AUC were calculated for the separate plasma concentration versus time plots. V_D was calculated by dividing the peak plasma concentration by the dose. Total clearance was calculated by dividing the dose by the AUC. Statistical analyses were performed using Student's *t* test for unpaired and for paired data.

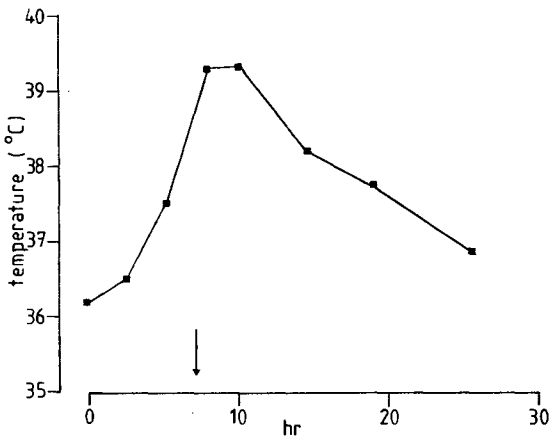

Fig. 2. Body temperature of patient B during and after continuous uridine infusion (2.5 g/m² per hr). Infusion was discontinued at the time indicated by the arrow.

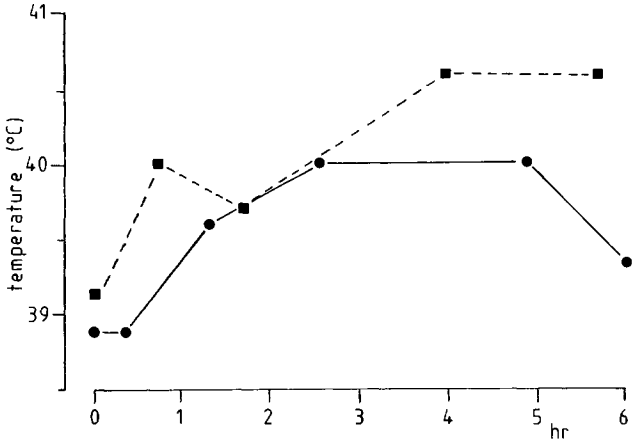

Fig. 3. Body temperature of two rabbits after an iv bolus injection of uridine (400 mg/kg). (●——●) rabbit A; (■- -■) rabbit B.

RESULTS

Effect of Pyrimidines on Body Temperature

In a previous paper (6) maximum temperatures were reported in patients after continuous infusions with uridine. Here we give the time course of the body temperature in one patient (Fig. 2) to allow better comparison with the rabbit. Fever was observed relatively late after the administration of uridine. The other patient (V.d.S.) received a lower dose (1 g/m² per hr). The elevation of body temperature (1.2°C) was observed after 10 hr. In order to prevent a further increase in temperature this infusion was discontinued.

Nine rabbits were treated with uridine. With ip injections several uridine doses (180, 300–700 mg/kg) were tested. At 180 mg/kg no significant increase in body temperature was observed, while no significant difference in the temperature increase was observed in the dose range of 300–700 mg/kg. For this reason, data obtained with these doses have been pooled (Table I). Because of the limited solubility of uracil, dihydrouracil, and carbamyl-β-alanine, higher doses than those indicated could not be administered. With dihydrouracil and carbamyl-β-alanine a rise in temperature was observed, comparable to that found with β-alanine (Table I). The maximal temperature rise occurred relatively late. The injection of isotonic saline at the largest volume applied for the other injections (7 ml) did not cause a significant change in temperature. This variation in body temperature was comparable to that observed in untreated rabbits.

Uridine, carbamyl-β-alanine, and β-alanine were also injected iv (Table I). Uridine was injected at 400 mg/kg since this dose is comparable to that given to patients and since uridine was tested extensively at this concentration by others (7). The maximum temperature rise (Fig. 3) was comparable to that found with ip injections (Table I). The effect of carbamyl-β-alanine was less.

Effect of Uridine Phosphorylase Inhibitors

In an attempt to prevent breakdown of uridine, inhibitors of uridine phosphorylase were injected intravenously simultaneously with uridine. All compounds employed are specific competitive inhibitors of uridine phosphorylase (13–15). Intravenous injections of thymine and GPT did not affect body temperature. Because of its low solubility thymine could not be used at a higher dose. At 15 mg/kg no

Table I. Effects of Uridine and Its Catabolites on the Body Temperature of Rabbits[a]

Compound	Dose (mg/kg)	Max. temp. rise (°C)	Time (hr)	N
Uridine	300–700, ip	0.830 ± 0.058** (**)	4	6
Uracil	5.3, ip	0.3	4	2
Dihydrouracil	20–40, ip	0.690 ± 0.064** (*)	4	4
Carbamyl-β-alanine	60, ip	0.825 ± 0.103* (*)	5–6	4
β-Alanine	300–400, ip	0.713 ± 0.072* (*)	5–6	4
NaCl	ip/iv	0.3	2–7	2/2
Uridine	400, iv	1.042 ± 0.156* (**)	2–4	6
Carbamyl-β-alanine	40, iv	0.475 ± 0.063* (ns)	0.5–2	4
β-Alanine	300, iv	0.3–0.7	0.5	2

[a] Maximum temperature rise is given as mean ± SE. The significance of the difference from pretreatment temperature is at the following level: *, $0.001 < P < 0.01$; **, $P < 0.001$ (Student's t test for paired data). The daily variation in body temperature was 0.300 ± 0.047°C (maximum temperature rise, mean ± SE from five rabbits). Within parentheses the significance of the difference between this value and those of the treated rabbits is given: *, $0.001 < P < 0.01$; **, $P < 0.001$ (Student's t test for unpaired data).

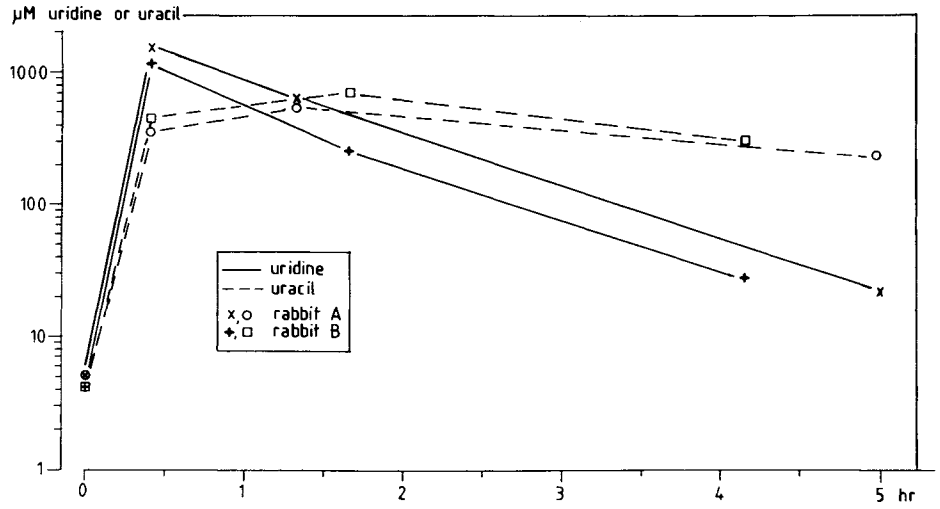

Fig. 4. Time versus concentration curves of uridine and uracil in plasma of two rabbits after an iv bolus injection of uridine at 400 mg/kg.

effect of thymine on the rise in body temperature caused by uridine (400 mg/kg) was observed. The peak concentration of thymine was 17 μ*M* at 2 hr after injection, which is too low to inhibit uridine phosphorolysis. The effect on body temperature of GPT in combination with uridine was also not significantly different from that of uridine alone. Peak plasma concentrations of GPT were about 300 μ*M* after 1.5 hr, which is higher than the K_i value for uridine phosphorylase (13). Although the K_i of BAU for uridine phosphorylase is lower than that of thymine or GPT, BAU could not be used in rabbits because of its limited availability and solubility.

Effect of Cytidine on Body Temperature

Since cytidine can act as a precursor for uridine, it was also tested for ability to rescue mice from 5FU toxicity (4). An equal effectiveness was observed. Cytidine might be less toxic and serve as a slow releasing prodrug for uridine. To examine this possibility we determined the effect of cytidine on the body temperature of rabbits. At a dose of 400 mg/kg cytidine did not affect the body temperature of rabbits.

Plasma Concentrations of Uridine, Uracil, and Cytidine

From the two rabbits presented in Fig. 3, plasma samples were taken and uridine and uracil concentrations were measured. Uridine concentrations reached a peak value of 1–2 m*M* (Fig. 4) after 30 min. Uridine catabolism to uracil proceeded rapidly, and after 1.5 hr uracil concentrations exceeded those of uridine. In the presence of GPT or thymine, plasma concentrations of uridine and uracil were comparable to those observed in the absence of thymine or GPT (data not shown). After reaching its peak value, GPT disappeared rapidly from plasma.

Cytidine plasma concentrations in rabbits were determined after iv administration of 400 mg cytidine/kg. Peak plasma levels of cytidine (±0.6 m*M*) were reached after 30 min (Fig. 5). Cytidine was rapidly converted to uridine and uracil. Uridine levels reached a plateau of about 200 μ*M*

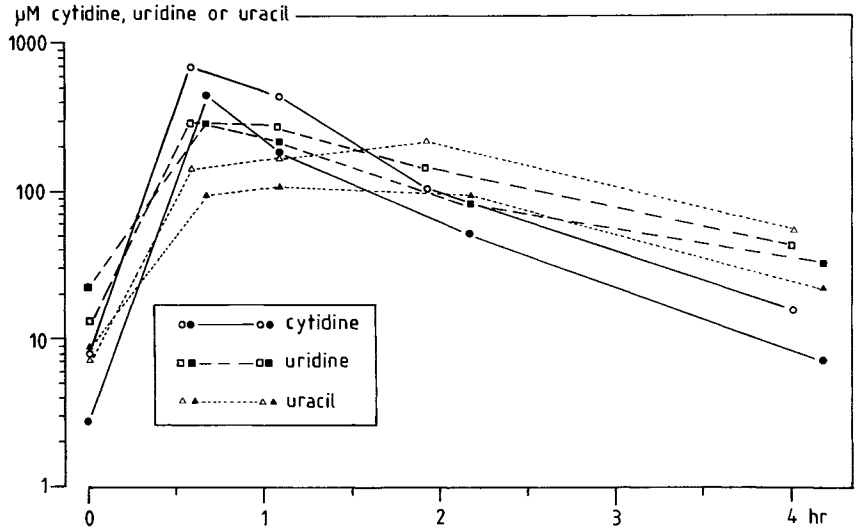

Fig. 5. Time versus plasma concentration curves of cytidine, uridine, and uracil of two rabbits after an iv bolus injection of cytidine at 400 mg/kg.

Table II. Pharmacokinetic Parameters of Uridine and Cytidine[a]

Parameter	Patients (uridine)		Rabbits	
	1-hr infusion	Continuous (B; VdS)	Uridine	Cytidine
Peak concentration (m*M*)	2.27	1.15	1.59	0.44
	2.24	0.50	1.56	0.71
MRT (min)	130	—	111	72
	195	—	80	71
AUC (mmol · hr/liter)	4.18	5.98	2.5	0.46
	5.35	4.54	1.5	0.83
V_D (ml/kg)	499	—	1032	3704
	651	—	1418	2310
Total clearance	5.04	—	0.657	3.57
(ml/kg · min)	3.95	—	1.086	1.97

[a] Parameters for rabbits have been calculated from the data presented in Figs. 4 and 5. Data from man have been calculated from those presented by Leyva *et al.* (5) from the 12-g/m^2 dose and by Van Groeningen *et al.* (6). Peak concentrations for B and VdS are those at the end of infuson, and the AUC for B from 0 hr to 8 hr 5 min and that for VdS from 0 to 12 hr.

after 30 min and decreased slowly. After 2 hr uridine concentrations exceeded those of cytidine. Uracil concentration increased slowly up to 2 hr after cytidine administration and reached peak values of 100–200 μ*M*. Uridine and uracil levels remained much lower than those that were observed after uridine administration. No cytosine could be detected.

From the individual rabbits pharmacokinetic parameters were calculated for uridine and compared with those reported earlier (5,6) for uridine administered as 1-hr infusions and as continuous infusions to patients (Table II). Some differences between the rabbits and the patients could be observed, such as the higher peak plasma concentrations and the higher AUC and MRT values in patients. The latter was also observed at the lower doses (data not shown). The V_D was lower in patients, but the clearance was higher.

Some marked differences between uridine and cytidine existed. The AUC for cytidine was lower than that for uridine, while the V_D and the total clearance appeared to be higher.

Table III lists some relevant pharmacokinetic data which were calculated for the metabolites of uridine and cytidine. Peak plasma concentrations and AUC for uracil were lower than for uridine in both patients and rabbits. In rabbits these parameters were also lower for uridine and uracil in comparison to cytidine. The AUC for uridine formed from cytidine is considerably lower than that of uridine after uridine administration itself. The same holds for uracil.

DISCUSSION

This is the first study to compare the effect of the pyrimidine nucleosides uridine and cytidine and their catabolites on body temperature in relation to pharmacokinetic data. The data show that various pyrimidines can affect body temperature, resulting in hyperthermia in man and rabbits. To date, there has been only one report (7) showing the induction of fever by uridine administration. In contrast, in mice it was observed that high doses (up to 3500 mg/kg) of

Table III. Relevant Pharmacokinetic Parameters for Metabolites from Uridine and Cytidine[a]

Species	Peak concentration (m*M*)	MRT (min)	AUC (mmol · hr/liter)
Uracil			
(from uridine)			
Rabbit	0.56; 0.70	153; 123	2.0; 2.0
Man			
1-hr	0.42; 0.22	245; 257	2.37; 1.24
Continuous			
(B; VdS)	0.39; 0.08	—	1.30; 0.49
Uridine			
(from cytidine)			
Rabbit	0.29; 0.30	92; 97	0.50; 0.62
Uracil			
(from cytidine)			
Rabbit	0.11; 0.23	116; 121	0.31; 0.62

[a] Parameters have been calculated as described in Materials and Methods and in Table II, footnote *a*.

uridine caused a sharp decrease in body temperature (16). This was probably due to a dose-dependent relationship since a low dose of uridine (100 mg/kg) resulted in a small but significant temperature increase (unpublished data).

Rabbits developed fever even when uridine was extensively purified using various procedures (7); it was shown *in vitro* that uridine can release endogenous pyrogen from leukocytes. However, since the Limulus test was negative and the time span required to develop fever is inconsistent with an endotoxin effect (17), uridine itself or some metabolite might be responsible for the effect on body temperature. The effect of uracil did not support this theory. In a study on uracil administration to dogs, no effect of uracil on body temperature was mentioned (18); the same was true in a study with long-term oral administration to rats (19). The failure to provoke fever may have been related to the low doses administered. However, the other degradation products of uridine (Fig. 1) could be administered in higher doses. All compounds induced a significant rise in body temperature, both compared to the initial body temperature and compared to the variation in body temperature of untreated rabbits. These data indicate that a degradation product of uridine might be involved in the regulation of body temperature.

In an attempt to study possible correlations with the effect on body temperature we also analyzed plasma levels of uridine and uracil and calculated pharmacokinetic data. The value of peak concentrations of uridine in relation to fever is not clear, since the peak concentrations of uridine after 1-hr infusions (at 12 g/m^2) in patients are higher than those in rabbits although no fever was observed. However, transient shivering was noted, which might be a sign of onset of fever. At lower doses of uridine (<10 g/m^2) no side effects were noted. It has been postulated that the extent of exposure, that is, the AUC, appears to be of more importance in relation to drug toxicity (20). This also seems to hold for toxicity in the form of fever. At a relatively low AUC for uridine as in rabbits for uridine formed from cytidine, no fever was observed, while in patients, only shivering was observed with 1-hr infusions. However, fever was observed during continuous infusions where the AUC for uridine continued to increase during infusion. At the time of onset of fever the AUC was similar in both patients, indicating the importance of prolonged exposure to uridine.

A direct relationship between the AUC of uridine and the change in body temperature appears to be valid if uridine itself is responsible for the observed effects. An increased AUC for uridine might be related to the effect on body temperature. This might lead to the accumulation of a degradation product, which is consistent with the observation that the onset of change in body temperature is not direct. It has been shown (8) that ip administered uridine is extensively catabolized to β-alanine; further, considerable amounts of degradation products of uridine could be demonstrated in rat brain (21). These breakdown products, the secondary amino acids, carbamyl-β alanine and β-alanine, closely resemble GABA (Fig. 6), which plays an important role in the regulation of body temperature (10,22). Regulation of body temperature takes place in the central nervous system in the hypothalamus and is affected by neurotransmitters, such as prostaglandins, GABA, and serotonin (10,22,23). Depending

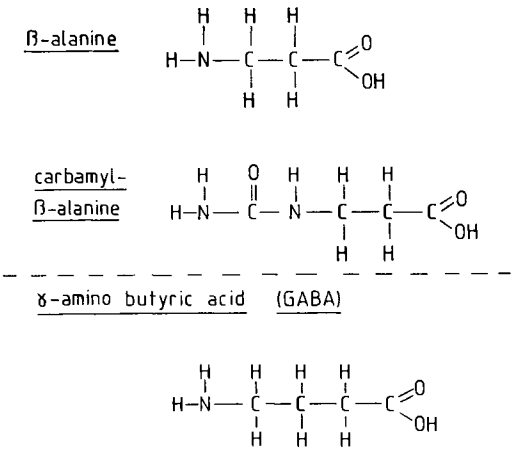

Fig. 6. Structural formulas of carbamyl-β-alanine, β-alanine, and γ-aminobutyric acid.

on the concentration and the site of administration of GABA, both a hypothermia and a hyperthermia have been reported (10,23) in dogs and rabbits. GABA-induced hypothermia in rats after systemic administration involved a serotonergic mechanism (24); a high GABA concentration might lead to serotonin depletion (25). It has been shown that β-alanine can inhibit presynaptic GABA binding in brain, leading to increased GABA levels (9). Such an interaction of uridine catabolites with GABA might be responsible for the effects on body temperature, both hyperthermia in man and rabbits and hypothermia in mice (16). Variations in the dose of uridine might also affect the thermoregulation.

Fever caused by uridine precludes the treatment of patients with this pyrimidine given by continuous infusion in order to rescue patients from 5FU toxicity. In order to prevent fever we developed an intermittent administration schedule in which a 3-hr infusion period was alternated with 3-hr treatment free periods (6) for 72 hr. At a dose of 2 g/m^2 per hr uridine could be administered without a significant influence on body temperature. Prevention of uridine-induced fever is important in future studies on uridine rescue of 5FU toxicity. Different ways of administration or inhibition of uridine phosphorylase might be useful in the regulation of body temperature.

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REFERENCES

1. H. A. Simmonds, D. R. Webster, D. M. O. Becroft, and C. F. Potter. *Eur. J. Clin. Invest.* 10:333–339 (1980).
2. D. S. Martin, R. L. Stolfi, R. C. Sawyer, S. Spiegelman, and C. W. Young. *Cancer Res.* 42:3964–3970 (1982).

3. P. Klubes, I. Cerna, and M. A. Meldon. *Cancer Chemother. Pharmacol.* 8:17–21 (1982).
4. D. S. Martin. In Proceedings, 8th Bristol-Myers Symposium on Cancer Research; New Avenues in Developmental Cancer Chemotherapy, 1986.
5. A. Leyva, C. J. van Groeningen, I. Kraal, H. Gall, G. J. Peters, J. Lankelma, and H. M. Pinedo. *Cancer Res.* 44:5928–5933 (1984).
6. C. J. van Groeningen, A. Leyva, I. Kraal, G. J. Peters, and H. M. Pinedo. *Cancer Treat. Rep.* 70:745–750 (1986).
7. J. C. Cradock, B. R. Vishnuvajjala, T. F. Chin, H. D. Hochstein, and T. K. Ackerman. *J. Pharm. Pharmacol.* 38:226–229 (1986).
8. T. Yngner, C. Engelbrecht, L. Lewan, and J. E. Annerfeldt. *Biochem. J.* 178:1–8 (1979).
9. C. R. Scriver, W. Nutzenadel, and T. L. Terry. In G. B. Stanbury, J. B. Wijngaarden, and D. S. Fredrickson (Eds.), *The Metabolic Basis of Inherited Diseases,* McGraw-Hill, New York, 1978, pp. 528–542.
10. V. R. Dhumal, O. D. Gulati, P. R. Raghunath, and N. Sivaramakrishna. *Br. J. Pharmacol.* 50:513–524 (1974).
11. G. J. Peters, I. Kraal, E. Laurensse, A. Leyva, and H. M. Pinedo. *J. Chromatogr.* 307:464–468 (1984).
12. J. M. Van Rossum and C. A. M. Van Ginneken. In E. Gladtke and G. Heimann (eds.), *Pharmacokinetics,* Gustav Fischer Verlag, Stuttgart, 1980, pp. 53–73.
13. J. G. Niedzwicki, S. H. Chi, M. H. El Kouni, E. C. Rowe, and S. H. Cha. *Biochem. Pharmacol.* 31:1857–1861 (1982).
14. P. Langen and G. Etzold. *Biochem. Z.* 339:190–197 (1963).
15. J. G. Niedzwicki, S. H. Chi, M. H. El Kouni, E. C. Rowe, and S. H. Cha. *Biochem. Pharmacol.* 31:1857–1861 (1982).
16. G. J. Peters, C. J. van Groeningen, A. Leyva, E. Laurensse, J. Lankelma, and H. M. Pinedo. *Proc. Am. Assoc. Cancer Res.* 26:370 (abstr. 1462) (1985).
17. S. E. Greisman and R. B. Hornick. *Proc. Soc. Exp. Biol. Med.* 131:1154–1158 (1969).
18. E. J. F. Spicer, W. R. Richter, and K. Morita. *J. Appl. Toxicol.* 5:333–338 (1985).
19. T. Shirai, E. Ikawa, S. Fukushima, T. Masui, and N. Ito. *Cancer Res.* 46:2062–2067 (1986).
20. J. M. Collins, D. S. Zaharko, R. L. Dedrick, and B. A. Chabner. *Cancer Treat. Rep.* 70:73–80 (1986).
21. A. F. Hogans, G. Guroff, and S. Udenfriend. *J. Neurochem.* 18:1699–1710 (1971).
22. W. Feldberg and R. D. Myers. *Nature* 200:1325 (1963).
23. G. P. Sgaragli, V. Carla, M. Manani, and A. Giotti. *Arch. Pharmacol.* 305:155–158 (1978).
24. J. S. Serrano, F. J. Minnano, and M. Sancibrian. *Gen. Pharmacol.* 17:327–332 (1986).
25. M. F. Belin, D. Nanopoulos, M. Didier, M. Aguera, H. Steinbusch, A. Verhofstad, M. Maitre, and J. F. Pujol. *Brain Res.* 275:329–339 (1983).