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INHIBITION OF THE DEGRADATION OF THYMINE AND 5-SUBSTITUTED URACIL ANALOGUES BY (E)-5-(2-BROMOVINYL)URACIL IN VIVO

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Thymine (Thy), uracil (Ura) and several 5-substituted Ura analogues are eliminated *in vivo* either by renal clearance or by saturable metabolic processes. At high plasma concentrations, when metabolism may be saturated, urinary clearance would become the predominant route of pyrimidine elimination, whereas, at low plasma concentrations, metabolic processes, particularly catabolic pathways, must be taken into account. In mammals, the main catabolism of pyrimidines proceeds *via* a reductive pathway (1); the first step in this degradation is the reduction of the 5:6 double bond of the pyrimidine ring by a NADPH-requiring enzyme, dihydrothymine dehydrogenase (E.C.1.3.1.2.), following the reaction (2) :

$$5\text{-XUra} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{NADP}^+ + 5\text{-XH}_2\text{Ura}$$

where X = -H, $-CH_3$, -F, -I or some other substituents (2-5). This reaction is known to be the limiting step of the degradative pathway of pyrimidines (6-8). Then follows an oxidative cleavage of the 3:4 bond to give β-ureido acids, from which β-amino acids are formed with the release of carbon dioxide and ammonia. The degradation of 5-halogenated uracils, particularly 5-fluorouracil (FUra), may restrict their therapeutic potentials, and inhibitors of this degradation may be of therapeutic usefulness, i.e. for the potentiation of the antineoplastic activity of FUra (9-11).

When Thy, FUra, 5-iodouracil (IUra) or 5-trifluorothymine (F_3Thy) are administered i.p. to rats (at 200 μmol/kg), they promptly appear in the plasma but they are completely cleared from the bloodstream 3 to 4 hours after injection (Fig. 1, dashed lines). In contrast, when (E)-5-(2-bromovinyl)-uracil (BVUra) is administered i.p. to rats, it persists for a long time in the bloodstream, achieving a plasma concentration of 50-60 μM for up to 7 hours; but even at 24 hours after injection, BVUra gives a plasma concentration of 10-20 μM. BVUra plasma concentrations following administration of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdUrd) follow the same pattern as those obtained upon injection of BVUra (12).

The peculiar behavior of BVUra *in vivo* has lead us to evaluate its *in vitro* catabolism in comparison with Thy or FUra. To this end we used liver extracts, since the liver appears to be the major site for pyrimidine catabolism *in vivo* (6,13). At the saturating concentration of 20 μM (2), Thy and FUra are degraded with initial velocities of 36 and 70 nmol/hr/mg protein respectively, whereas BVUra is not significantly catabolized. Moreover, when BVUra is preincubated with the liver extract, it inhibits the degradation of Thy and FUra with an ID_{50} of about 2-3 μM. Thus, BVUra is not a substrate but an inhibitor of pyrimidine degradation.

Consequently, we determined the influence of BVUra on the pyrimidine degradation *in vivo*. When Thy, FUra, IUra or F_3Thy were administered i.p. to rats 1 hour after an i.p. injection of BVUra (200 μmol/kg), the half-life of these bases increased considerably : 8-fold for Thy, 6-fold for FUra, 4-fold for IUra and 5-fold for F_3Thy (Fig. 1). Thus, all the pyrimidine bases are protected by BVUra against degradation *in vivo*. When the corresponding nucleosides (2'-deoxyribosides), i.e. dThd, FdUrd, IdUrd and F_3dUrd were given i.p. to rats, the nucleosides were rapidly transformed to their

respective bases, and both nucleosides and bases were eliminated from the plasma within 3 to 4 hours. If BVUra was administered before these nucleosides, the half-life times of the pyrimidine bases increased considerably, whereas the nucleoside half-times were not affected.

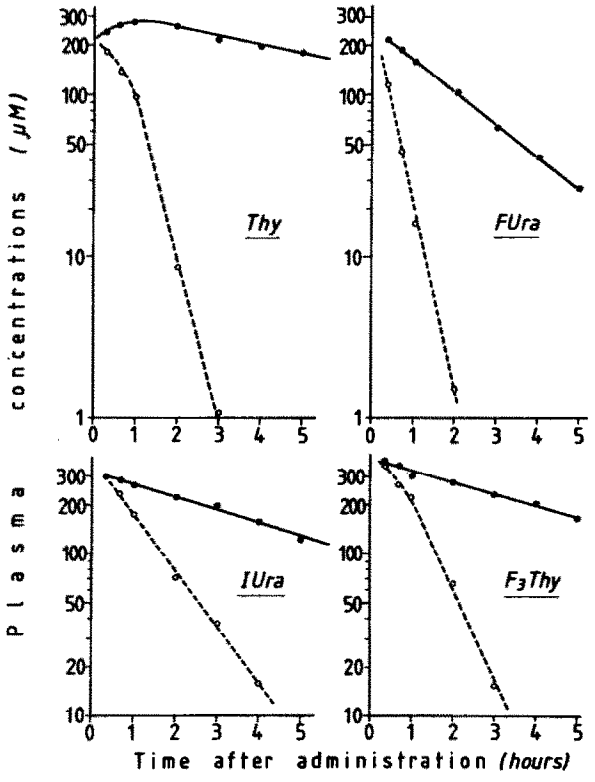

Figure 1. Influence of preadministration of BVUra on the plasma concentrations of some pyrimidines. Thy, FUra, IUra and F3Thy were administered i.p. to rats at 200 μmol/kg and their plasma concentrations were determined by HPLC as previously described [12,14]. Pyrimidine concentrations with (•—•) or without (o--o) a previous administration of BVUra.

Our findings clearly demonstrate that BVUra is slowly cleared in vivo because it is not degraded by the usual pyrimidine catabolic pathway and that BVUra acts in vitro and in vivo as an inhibitor of the degradation of other pyrimidines. BVUra appears to be a useful agent for increasing the half-life of pyrimidines, like FUra, and thereby potentiating their therapeutic effects, i.e. antitumor activity.

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