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INHIBITION AND REVERSAL OF THE DEGRADATION OF THE ANTIVIRAL DRUG (E)-5-(2-BROMOVINYL)-2'-DEOXYURIDINE IN VIVO

C. DESGRANGES[†], G. RAZAKA[†], H. BRICAUD[†] and E. DE CLERCQ[‡]

[†]Unité 8 de l'INSERM, Avenue du Haut-Lévêque, 33600 Pessac, France and
[‡]Rega Institute, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Following intraperitoneal (i.p.) administration (at 200 μmoles/kg) to rats, the highly potent and selective antiherpes drug (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdUrd) (1) attains a plasma concentration of 70 μM at 20 min, and is completely cleared from the bloodstream within 3 hours. Concomitantly with BVdUrd, its catabolite, (E)-5-(2-bromovinyl)uracil (BVUra), appears in the plasma, and the plasma levels achieved by BVUra (50–60 μM) are maintained for up to 7 hours. Even at 24 hours after the injection of BVdUrd, BVUra is still detectable in the plasma. This contrasts with several other 5-substituted uracils which, like their deoxynucleoside counterparts, are completely cleared from the bloodstream within 2–3 hours (2). The *in vivo* degradation of BVdUrd and the other dUrd analogues to the corresponding pyrimidine bases is probably mediated by pyrimidine nucleoside phosphorylases (PNPases) - i.e. dThd and uridine (Urd) phosphorylases -, since the dUrd analogues, and, particularly BVdUrd, are excellent substrates for these enzymes *in vitro* (3,4). The degradation of BVdUrd may affect its efficacy as an antiviral agent, and, therefore, attempts have been undertaken to prevent this degradation.

Figure 1

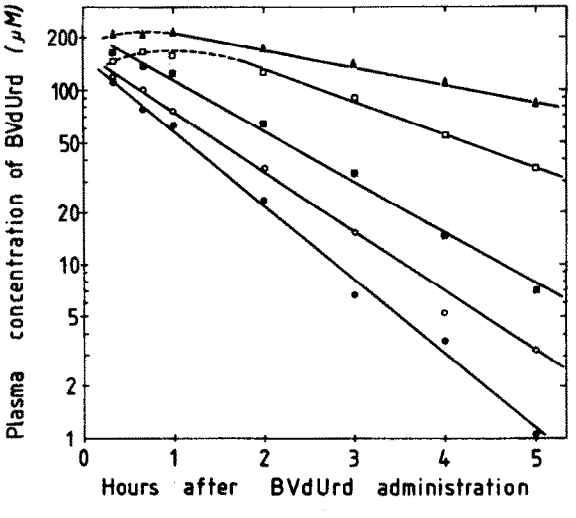

Figure 2

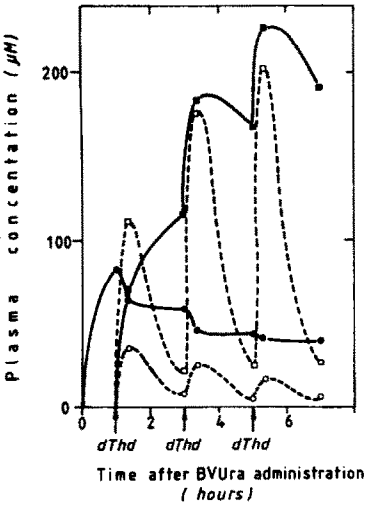

Figure 1. Effect of a previous administration of 6-amino-Thy on the plasma concentrations of BVdUrd in rats. All rats received an i.p. administration of 200 μmol/kg BVdUrd alone (●) or after a previous administration of 6-amino-Thy at 0.2 (○), 0.4 (■), 0.8 (□) or 1.6 (▲) mmol/kg. Blood was taken at different times after BVdUrd administration and the plasma concentrations of BVdUrd were determined by HPLC as previously described (2,3).

Figure 2. Repeated de novo formation of BVdUrd after a single BVUra administration. BVUra (200 μmol/kg) was administered i.p. to rats at time 0. One hour after BVUra administration, at a time when this base attained its maximal concentration, dThd (200 μmol/kg) was injected i.p.; this procedure was repeated 3 and 5 hours after BVUra administration. Plasma levels of BVUra (●—●), dThd (□--□), BVdUrd (○--○) and Thy (■—■) were determined at different times after BVUra administration.

Inhibition of PNPases may decrease the degradation of BVdUrd. Several inhibitors are known to inhibit both dThd and Urd phosphorylases, or specifically uridine phosphorylase (see ref. 5 for review). 6-Amino-Thy is one of the most potent inhibitors of mammalian dThd phosphorylase (6-8), but also inhibits the activity of Urd phosphorylase (9). Moreover, 6-amino-Thy inhibits BVdUrd degradation by either purified dThd phosphorylase or intact cells (3), and does not interfere with the transmembrane transport of nucleosides as do some other inhibitors. For these reasons, we have studied the influence of 6-amino-Thy on the BVdUrd clearance in rats. When BVdUrd is administered i.p. at 200 μmol/kg to rats, its plasma half-life is about 42 min. When 6-amino-Thy is administered 20 min before BVdUrd, its clearance is significantly depressed : the half-life of BVdUrd is increased 1.25-, 1.5-, 2.4- and 4.4-fold if the rats receive a previous injection of 0.2, 0.4, 0.8 or 1.6 mmol/kg of 6-amino-Thy respectively (Fig. 1). The inhibition of BVdUrd degradation is correlated to the plasma concentrations of 6-amino-Thy (data not shown). That BVUra concentrations are decreased in presence of 6-amino-Thy further demonstrates the role of PNPases in the degradation process of BVdUrd.

Thus, the half-life of BVdUrd and, consequently its therapeutic efficacy could be increased by inhibition of PNPase activities; however, this procedure requires the use of large amounts of inhibitor. In addition to the inhibition of BVdUrd degradation, we have developed a novel procedure to reverse its degradation in vivo (2). The latter is based upon the finding that BVUra, the first catabolic product of BVdUrd, persists for such a long time in the blood stream. This prompted us to attempt to reverse the phosphorolytic cleavage of BVdUrd and to regenerate BVdUrd from BVUra by a pentosyl transfer, according to the general reaction :

$$\text{BVUra} + \text{5-XdUrd} \rightleftharpoons \text{BVdUrd} + \text{5-XUra}$$

where X = -H, $-CH_3$, $-C_2H_5$, -F, -I, or $-CF_3$.

Indeed, i.p. administration of dThd (200 μmol/kg) at 3 hours after the injection of BVdUrd, at a time when BVdUrd has almost completely disappeared from the plasma, results in the re-partition of BVdUrd in the plasma. BVdUrd attains a peak plasma level of 40 μM at 20 min after the injection of dThd. In this procedure, dThd may be replaced by dUrd, FdUrd, IdUrd, CF_3dUrd, ethyldUrd and, probably, other 5-substituted dUrd analogues that are substrates for PNPases. BVdUrd could even be generated directly in vivo from BVUra if the dUrd analogues are injected at 3 hours after BVUra. It is likely that the deoxyribosyl transfer from the dUrd analogues to BVUra is catalyzed by PNPases in vivo, since (i) such a transfer occurs readily in vitro upon the action of dThd phosphorylase; (ii) the plasma level of BVUra promptly decreases when BVdUrd re-appears in the bloodstream (Fig. 2); (iii) 6-amino-Thy inhibits the regeneration of BVdUrd from BVUra and dUrd analogues (2). Thus, the regeneration or the de novo formation of BVdUrd from BVUra seems to be a more attractive procedure for prolonging the efficacy of BVdUrd in vivo than merely inhibiting BVdUrd degradation by PNPases, since the pentosyl transfer can be repeated several times, i.e. upon repeated injection of dThd (Fig. 2) or any other dUrd analogue, until BVUra has completely disappeared from the plasma; the exchange is still possible 24 hours after BVUra administration, giving rise to a plasma BVdUrd level of 5 μM. This concentration exceeds by far the minimum antiviral concentration of the compound (1,10).

REFERENCES

1. E. De Clercq, J. Descamps, P. De Somer, P.J. Barr, A.S. Jones and R.T. Walker, Proc. natn. Acad. Sci. U.S.A. 76, 2947 (1979).
2. C. Desgranges, G. Razaka, F. Drouillet, H. Bricaud, P. Herdewijn and E. De Clercq, Nucleic Acids Res. 12, 2081 (1984).
3. C. Desgranges, G. Razaka, M. Rabaud, H. Bricaud, J. Balzarini and E. De Clercq, Biochem. Pharmacol. 32, 3583 (1983).
4. B. Liermann and G. Herrmann, Biomed. Biochim. Acta 42, K35 (1983).
5. J.G. Niedzwicki, M.H. El Kouni, S.H. Chu and S. Cha, Biochem. Pharmacol. 32, 399 (1983).
6. P. Langen, G. Etzold, D. Bärwolff and B. Preussel, Biochem. Pharmacol. 16, 1833 (1967).
7. B.R. Baker and J. Kelley, J. Med. Chem. 14, 812 (1971).
8. C. Desgranges, G. Razaka, M. Rabaud, P. Picard, F. Dupuch and H. Bricaud, Biochem. Pharmacol. 31, 2755 (1982).
9. P.W. Woodman, A.M. Sarrif and C. Heidelberger, Biochem. Pharmacol. 29, 1059 (1980).
10. E. De Clercq, J. Descamps, P. De Somer, P.J. Barr, A.S. Jones and R.T. Walker, Antimicrob. Agents Chemother. 16, 234 (1979).