Metabolism of 2',3'-dideoxyinosine (ddI) in human blood

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- 1 2',3'-Dideoxyinosine (ddI) has potent activity against human immunodeficiency virus (HIV). It is converted within target cells to its active form dideoxyadenosine triphosphate (ddA-TP).
- 2 In addition to the intracellular formation of ddA-TP, ddI can be broken down to hypoxanthine, by purine nucleoside phosphorylase (PNP) and to uric acid, by xanthine oxidase. Since PNP is present in red blood cells we have examined the metabolism of [14 C]-ddI by human blood.
- 3 When incubated with whole blood at 37° C, ddI was extensively metabolised, principally to hypoxanthine ($50.4 \pm 12.5\%$ formed at 6 h; mean $\pm$ s.d.; $n = 16$). Small amounts of uric acid were formed ($3.8 \pm 2.4\%$).
- 4 ddI breakdown was temperature dependent, being virtually negligible at 4° C. Metabolism to hypoxanthine occurred within red blood cells.
- 5 The short half-life of ddI in patients is probably the result of both hepatic and erythrocytic metabolism.

Keywords dideoxyinosine metabolism blood

Introduction

The dideoxynucleoside 2',3'-dideoxyinosine (ddI) is an analogue of the naturally occurring purine nucleoside inosine, with potent anti-human immunodeficiency virus (HIV) activity (Hirsch, 1990; Mitsuya & Broder, 1986). ddI is converted within target cells to its active form ddA-triphosphate (Figure 1), which is thought to act as a chain terminator and inhibitor of reverse transcriptase (Ahluwalia *et al.*, 1987; Cooney *et al.*, 1987; Hirsch, 1990).

In culture, ddI appears to be less toxic to human blood cells than zidovudine (Du *et al.*, 1989; McGowan *et al.*, 1990; Mitsuya & Broder, 1986). Even when HIV infectivity is high, ddI can completely inhibit HIV replication at concentrations 10- to 20-fold lower than those that impair the growth and function of target T cells (Mitsuya & Broder, 1986). Du and co-workers (1989) compared the haematopoietic toxicity of ddI with that of zidovudine in murine and human primary progenitor cells and concluded that ddI was substantially less myelotoxic. Overall ddI appears to have a relatively high therapeutic/toxicity ratio *in vitro* and in preclinical studies.

In addition to the intracellular formation of ddA-TP, ddI can be broken down to hypoxanthine which can either re-enter the purine metabolic pool or be degraded further to uric acid, the enzymes involved being purine nucleoside phosphorylase (PNP) and xanthine oxidase,

respectively (Hartman *et al.*, 1990; Johnson *et al.*, 1988). In clinical studies ddI is rapidly metabolised with a plasma half-life of 38 min (Hartman *et al.*, 1990); 36% of the administered dose appears as ddI in the urine. There are reports of higher concentrations of serum uric acid in patients receiving high doses of ddI (Dolin *et al.*, 1990; Lambert *et al.*, 1990; Yarchoan *et al.*, 1990) and tentative identification of hypoxanthine in the plasma of a patient given ddI has been reported by Carpen *et al.* (1990). Since PNP is found in relatively high concentrations in human erythrocytes (Parks *et al.*, 1975; Simmonds *et al.*, 1988) we have investigated the metabolism of ddI in human blood. Appreciable metabolic breakdown in blood would have important implications for the interpretation of the results of pharmacokinetic studies.

Methods

Blood samples were obtained by venepuncture into lithium heparin tubes from volunteers in the Department of Pharmacology and Therapeutics. In Study 1, incubations contained [14 C]-ddI (0.1 μ Ci; 2 μ M; Bristol-Myers Squibb, Syracuse, USA) and whole blood (1 ml) in 5 ml glass tubes. Tubes were incubated for 0.5, 1, 3 and 6 h at 37° C. At the end of the incubation methanol (2 ml)

Figure 1 Metabolic pathways of 2',3'-dideoxyinosine (ddI). PNP, purine nucleoside phosphorylase; ddIMP, 2',3'-dideoxyinosine-5'-monophosphate; ddAMP, 2',3'-dideoxyadenosine-5'-monophosphate; ddADP, 2',3'-dideoxyadenosine-5'-diphosphate; ddATP, 2',3'-dideoxyadenosine 5'-triphosphate.

was added with vortexing followed by centrifugation (2000 rev min⁻¹ for 10 min). The supernatant was decanted and evaporated to dryness. Samples were subsequently reconstituted in double-distilled water and an aliquot was analysed by high performance liquid chromatography. Separations were performed on an Ultratech ODS-5 column (25 cm × 0.46 cm) protected by an in-line guard column. The mobile phase was methanol: water (12:88 v/v) and a flow rate of 1 ml min⁻¹. Metabolites were identified according to the retention times of the authentic standards (hypoxanthine, xanthine, uric acid, obtained from Sigma) monitored by u.v. detection at 254 nm. ddI and metabolites were measured by on-line radiometric detection (FLO-ONE/Beta series A200, Radiomatic, Packard). Retention times of ddI, hypoxanthine, xanthine and uric acid were 11.5, 4.5, 5 and 3 min, respectively.

In Study 2, blood samples were incubated with [¹⁴C]-ddI for 15, 30, 45 or 60 min at either 4° C, room temperature (20° C), 37° C or 58° C and metabolite profiles were determined as above.

In Study 3, polymorphonuclear leucocytes (neutrophils) and mononuclear leucocytes were prepared from heparinized venous blood by centrifugation (2000 rev min⁻¹ for 20 min) through Mono-Poly Resolving Medium (Flow Laboratories). The cell bands were removed and diluted with 3 volumes of RPMI 1640 medium (Hepes buffered; Flow laboratories). After centrifugation at 2000 rev min⁻¹ for 5 min, contaminating erythrocytes were lysed by adding 9 ml of double-distilled water and shaking vigorously for 20 s; tonicity was then restored by adding 1 ml 0.9% (w/v) NaCl. Purified neutrophils and monocytes were washed, resuspended in RPMI and counted in a haemocytometer. Incubations

of cells, including packed red cells, with radiolabelled ddI proceeded for 1 h as described above.

Results

Chromatographic separation of authentic standards of hypoxanthine, xanthine and uric acid from ddI was achieved. Resolution of hypoxanthine and xanthine was not always to baseline and hence the peak at R_T 5 min is designated as hypoxanthine. Representative high performance liquid chromatograms from 0.5 and 3 h incubations of [¹⁴C]-ddI with whole blood (at 37° C) are shown in Figure 2 and indicate extensive metabolism, principally to hypoxanthine. Mean data from 16 subjects indicated that 45.8% (95% confidence intervals 38.2–53.5%) of added ddI was metabolised in 6 h (Figure 3), mostly to hypoxanthine (50.4 ± 12.5% mean ± s.d.) with small amounts of uric acid (3.8 ± 2.4%). Within 30 min of incubation more than 15% of the ddI had been metabolised (Figure 3). The breakdown of ddI was temperature dependent (Figure 4); virtually no metabolites were detected at 4° C and only 5% at room temperature. Breakdown occurred in red blood cells (Table 1). Only traces of metabolites were detected after incubation with up to 1 × 10⁷ neutrophils or mononuclear cells, although the low turnover may be a reflection of the number of cells rather than absence of enzyme.

Discussion

Using inosine as substrate Parks *et al.* (1975) reported the PNP activity of packed erythrocytes to be 13 μmol

Figure 2 High performance liquid radiochromatograms showing the formation of hypoxanthine and uric acid on incubation of human blood with [^{14}C]-ddI (for 0, 0.5 or 3 h at 37° C).

Table 1 Metabolism of [^{14}C]-ddI (0.1 μCi ; 2 μM) by red blood cells (RBC), polymorphonuclear leucocytes (PMNs) and mononuclear cells. Incubation was for 1 h at 37° C

Cell type	Hypoxanthine/Uric acid*	ddI
RBC 10 μl	2.8 $\pm$ 0.8	97.2 $\pm$ 0.8
25 μl	4.8 $\pm$ 1.6	95.2 $\pm$ 1.6
50 μl	7.8 $\pm$ 2.3	92.2 $\pm$ 2.3
100 μl	12.6 $\pm$ 3.9	87.4 $\pm$ 3.9
250 μl	19.0 $\pm$ 5.2	81.0 $\pm$ 5.2
PMN 1×10^5	1.6 $\pm$ 0.2	98.4 $\pm$ 0.2
1×10^6	1.7 $\pm$ 0.4	98.3 $\pm$ 0.4
1×10^7	1.8 $\pm$ 0.6	98.2 $\pm$ 0.6
Mononuclear cells		
1×10^5	1.4 $\pm$ 0.3	98.6 $\pm$ 0.3
1×10^6	1.8 $\pm$ 0.3	98.2 $\pm$ 0.3
1×10^7	1.5 $\pm$ 0.4	98.5 $\pm$ 0.4

Cell types were prepared as described in the methods. Taking a mean value of 5.5×10^{10} RBCs/10 ml whole blood, approximately 1.4×10^9 RBCs were present in 100 μl of the pellet used. Values are mean $\pm$ s.d. of determinations on cells from 6 individuals.

*Sum of hypoxanthine and uric acid.

Figure 3 Disappearance of ddI (●) and appearance of hypoxanthine (□) and uric acid (×) in incubations of whole blood with [^{14}C]-ddI. At zero time, 100% = 2 μM ddI. Results are expressed as mean $\pm$ s.d. with data from blood samples obtained from 16 individuals. Incubation was for 0.5, 1, 3 or 6 h at 37° C.

Figure 4 Metabolism of [^{14}C]-ddI by blood obtained from six volunteers. Incubation was for 15, 30, 45 or 60 min at 4° C (×), room temperature (20° C, ◇), 37° C (△) or 58° C (■). Results (mean $\pm$ s.d.) are expressed as the percentage of ddI remaining. At zero time, 100% = 2 μM ddI.

$\text{min}^{-1} \text{ml}^{-1}$. The K_m for inosine has been reported to be 30 μM (Agarwal *et al.*, 1975) and 48 μM (Stoeckler *et al.*, 1978) and for deoxyinosine to be 66 μM (Stoeckler *et al.*, 1978). In the present study we have demonstrated the ability of human blood to metabolise the analogue of inosine, ddI, with formation of both hypoxanthine and uric acid. The concentration of ddI we used (2 μM) is

equivalent to the peak plasma concentration obtained after an oral dose of 0.4 mg kg^{-1} ddI (Hartman *et al.*, 1990) and is probably well below the K_m value for erythrocytic PNP. Since xanthine oxidase is normally absent or only present in very low amounts in the serum of healthy people (Heinz & Reckel, 1983), the principle metabolite seen is hypoxanthine. The formation of hypoxanthine increased with time, was temperature dependent and occurred in the red cells. Results may be different in patients with liver disease since there is an increase in serum xanthine oxidase activity (Heinz & Reckel, 1983).

What are the implications of this study? The reported short elimination half-life of ddI of 38 min (Hartman *et al.*, 1990) could result not only from hepatic metabolism

but also from erythrocytic breakdown *in vivo*. The possibility of additional red cell metabolism occurring after blood sampling has to be considered. Therefore, it will be important in all pharmacokinetic investigations to have consistent procedures for blood collection and storage. If blood samples are taken and left at room temperature (or higher) for any length of time concentrations of ddI will be underestimated because of its continued breakdown in the blood. When possible, blood samples should be centrifuged immediately and the plasma separated to minimise breakdown by PNP.

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