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Transfer of dideoxyinosine across the human isolated placenta

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1 Dideoxyinosine (ddI) has recently been approved for the treatment of patients with HIV infection. As increasing numbers of such patients are pregnant, we wished to define the rate and mechanism(s) of ddI transfer by the placenta to the foetus. Using isolated single perfused human term placental cotyledons, the drug was shown to cross the placenta from mother to foetus at a rate of 25% that of a freely diffusible marker, antipyrine, and at about half the rate of zidovudine (AZT). The transfer of ddI was similar in both directions (maternal to foetal and the reverse), equal to that of L-glucose, a passively transported sugar, and was not inhibited by excess inosine or uric acid (structural analogues of ddI). ddI did not cross to the foetus against a concentration gradient. The transport process appeared to be passive and it was not altered by AZT.

2 ddI was not metabolized in the Krebs Ringer buffer/albumin perfusate, and placental homogenates converted only 4% of ddI to hypoxanthine over the 4 h incubation. However, when maternal term or cord blood was incubated with ddI for 3 h, 50% of the drug was converted to hypoxanthine in maternal blood and to hypoxanthine and uric acid in cord blood.

3 Thus, ddI metabolism in maternal blood should decrease its net transfer to the foetus *in vivo*. In the foetal circulation, ddI will be further metabolized by erythrocytes to hypoxanthine and possibly to uric acid. Hence, the fraction of administered ddI delivered to foetal tissues should be much lower than that of AZT.

Keywords dideoxyinosine ddI placenta

Introduction

An increasing number of women of child-bearing age are exposed to human immunodeficiency virus (HIV) infection [1]. In 1989, some 6000 HIV-infected women delivered live children [2] and it has been estimated that 30% of these will have acquired HIV infection [2]. It is evident, therefore, that the use of anti-HIV drugs in this patient population will increase.

We [3] and others [4, 5] have previously characterized the transport of azidothymidine (AZT) by the term normal human placenta and have shown this process to be a passive one, consistent with the lipophilicity of the drug. In the present study we characterized the placental transfer of a newer therapeutic nucleoside, 2',3'-dideoxyinosine (ddI) [6], using the isolated perfused single cotyledon technique [7]. Since ddI has been reported to be metabolized in human blood [8] we also assessed its stability on incubation with maternal and foetal blood, and placental homogenate. The possibility that concomitant placental transfer of AZT might affect transport of ddI was also investigated.

Methods

Placental perfusion

The single, isolated, perfused human placental cotyledon system utilizes normal fresh placenta at term, delivered vaginally or by Caesarian section. This method has been described extensively by others

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[7, 10] and often used by us [3, 11–13]. The placenta is perfused with heparinized Krebs-Ringer buffer immediately after delivery, and the foetal vessels supplying one cotyledon are cannulated with polyethylene catheters. After mounting in a perfusion chamber, the maternal compartment is perfused through needles placed in the intervillous space and the foetal compartment is perfused via the catheters. The system is oxygenated with room air delivered to each compartment, the pressure is maintained at 15–30 mm Hg, temperature kept at 37° C and the pH maintained at 7.4. The perfusate contains 2 g l^{-1} albumin in Krebs-Ringer buffer and is delivered to the maternal side at a rate of 10 ml min^{-1} and to the foetal side at a rate of 2–3 ml min^{-1}. Antipyrine, a freely diffusible marker, (−)-glucose (transferred passively), or (−)-leucine (transported actively), were used as reference markers for various studies. Validation of the integrity of the system has been described earlier [11, 13] and depends on the absence of fluid shift between the separate compartments, expected passive or active transport of appropriate agents, and lack of transfer of large water-soluble agents (i.e. inulin) implying an absence of non-specific leaks. The first two of these criteria were reaffirmed in the present studies. The study was approved by the Institutional Review Board.

Three types of perfusions were carried out. In one series of experiments (open/open), ddI was infused over 90 min at a constant rate into the maternal (or foetal) compartment and its concentration was measured in the opposite effluent at nine 10 min intervals. The clearance of ddI, calculated as described earlier [11–13] was compared with that of antipyrine or (−)-glucose measured at the same time. The concentrations of ddI used were 3 and 30 μM (7 μg ml^{-1}), that of antipyrine was 1.06 mM (200 μg ml^{-1}), and that of (−)-glucose was 80 nM. A concentration of 3 μM ddI is in the range of plasma drug concentrations seen in patients treated with ddI [14]. Specific activities of [^{3}H]-ddI, [^{14}C]-ddI, antipyrine, and (−)-glucose were 33 Ci $mmol^{-1}$, 59.7 mCi $mmol^{-1}$, 21.7 mCi $mmol^{-1}$ and 54.5 mCi $mmol^{-1}$, respectively. [^{3}H]-ddI was obtained from Moravek Biochemicals, Inc., Brea, CA, and [^{14}C]-ddI from Sigma Chemical Company, St Louis, MO. The radiochemical purity of these compounds was >98% as assessed by h.p.l.c. Unlabeled ddI was kindly provided by Bristol-Myers Squibb Company, Wallingford, CT. All substrates were measured by liquid scintillation spectrometry and clearance ratios of ddI/antipyrine and ddI/(−)-glucose in each direction were calculated. In some studies, 600 μM inosine, a structural analogue of ddI, was added to the perfusate. The perfusion fluid in both compartments and the perfused lobule (at the end of the study) were analyzed by h.p.l.c. for the presence of ddI metabolites.

In another set of studies, ddI (30 μM) was placed in the maternal or foetal compartment and the maternal and foetal perfusions were recirculated over 4 h (closed system). Antipyrine (100 μg ml^{-1}) was added to the same compartment as ddI and the rates of transfer of the two compounds were compared over the linear portion of their transfer and expressed as a ddI/antipyrine transfer ratio. Both compounds were usually asssayed by scintillation spectrometry, although in some instances (when other labeled substances were used) antipyrine was measured spectrophotometrically [15]. In some studies, inosine (600 μM) and uric acid (833 μM (140 μg ml^{-1})) (both structural analogues of ddI), or AZT (3 μM) (a concentration observed clinically [3]) were also added initially to the maternal reservoir. The effects of these compounds on ddI transfer were then determined. The presence of ddI metabolites in the perfusion fluids at various times and in the perfused lobule at the end of the study (4 h) was determined by comparison of isotopic and h.p.l.c. peaks in the extract, as described below. Use of scintillation spectrometry to assay ddI is only fully valid if lack of metabolism of the parent drug is established. The presence of a single isotopic peak, with an identical retention time to that of pure ddI by h.p.l.c., would imply lack of significant metabolism of ddI.

In the third group of studies, ddI (30 μM) was infused at a constant rate into the maternal compartment (open system) and the same concentration of the drug was placed in the foetal recirculating system. Transfer into the foetal system and accumulation of the drug in that compartment with a foetal/maternal concentration greater than 1.0 would imply transport against a concentration gradient. Leucine, known to be actively transferred against such a gradient, served as a positive control.

Assay of metabolites

Blood Maternal blood samples were taken by venepuncture into heparinized tubes from normal term pregnant women on admission to the University Hospital, San Antonio, Texas. Blood samples were collected from the cord of term placentas (foetal samples) upon delivery. The blood samples were preincubated at 37° C for 10 min. To 1 ml of heparinized whole blood was added [^{14}C]-ddI and this was incubated for 0.5, 1, 2 and 3 h at 37° C. At the end of the incubation, 2 ml of cold methanol was added with vortexing and the mixture was centrifuged at 3000 *g* for 10 min. The supernatant was decanted and evaporated to dryness under nitrogen. The samples were reconstituted in 0.5 ml double-distilled water, filtered and concentrated with a Centricon® 10 micro-concentrator by centrifugation (5000 *g* for 60 min; Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge). The percentage of recoveries of ddI, hypoxanthine, xanthine, and uric acid from the methanol extraction were found to be similar and essentially complete. Aliquots were analyzed by h.p.l.c. using an automated injector (Waters 712 WISP). Metabolites were identified by reference to the retention times of standards (uric acid, hypoxanthine, xanthine, and ddI) monitored by u.v. detection at 254 nm (Water Model 440 Absorbance Detector). Chromatography was by gradient elution on a 5μ Supelco Packing Supelcosil LC-18-S (25 cm long, 4.6 mm internal diameter) analytical column. The retention times of uric acid, hypoxanthine, xanthine, inosine, and ddI were 3.7, 7.0, 8.2, 19.1, 22.2 min, respectively. The mobile

phases were 0.1 M KH_2PO_4 (phase A) and 85:15 v/v 0.05 M KH_2PO_4:methanol (phase B) at a flow rate of 1.5 ml min^{-1}. Mobile phase A represented 100% of the eluent for the first 12 min of the gradient. After 12–15 min, phase A was replaced linearly by phase B for the next 10 min; at 25–28 min phase B was gradually replaced by phase A for the remaining run time. During the run, samples were collected in vials at 1 min intervals. Radioactivity was counted using a Beckman Ready-Protein scintillation cocktail and a Beckman LS6800 Liquid Scintillation Counter.

Perfused lobules and perfusate samples Perfusions were performed using both 2,3-[^{3}H]-ddI and 8-[^{14}C]-ddI. Maternal and foetal perfusate samples from [^{14}C]-ddI perfusions were taken sequentially during both recirculating and open perfusion studies, and were analyzed by h.p.l.c. using the mobile phase employed for the blood samples (see above). Fractions of h.p.l.c. effluent were collected and counted for radioactivity. Samples of perfused lobules at the end of the studies were homogenized and analyzed in the same fashion as the perfusate.

Lobule incubations Placental lobules which had been preperfused to remove blood were homogenized in phosphate buffered saline (PBS), pH 7.4. In four separate experiments, 1 ml of the crude homogenate was incubated with ddI (30 μM) for 4 h. The radiolabel used to detect the peaks was [^{14}C]-ddI. H.p.l.c. analysis, as above, was used to correlate peaks with radioactivity employing pure unlabeled ddI. As a positive control, in one study [^{14}C]-inosine was incubated similarly with normal and boiled placental homogenate [17].

Statistical analysis

Statistical analysis was by Student's *t*-test and the Mann-Whitney non-parametric U test with a *P* value of <0.05 (two-tailed) accepted as significant.

Results

As shown in Table 1, placental perfusion of ddI at a concentration of 3 μM over 90 min, gave maternal to foetal clearance ratios of 0.311 ± 0.126 (s.d.) and 1.147 ± 0.025 with respect to antipyrine and (−)-glucose. Similar results were obtained with 30 μM ddI ($P > 0.05$). Thus, over a 10-fold concentration range, the transport of ddI remained constant with respect to both antipyrine and (−)-glucose. Transfer of ddI was much slower than that of antipyrine and similar to that of (−)-glucose. Moreover, as shown in Table 1, ddI transfer in the opposite direction (foetal to maternal) was similar. As shown by the data in Table 2 from the closed (recirculation) studies, the mean maternal to foetal ddI/antipyrine transfer ratio at 30 min (the linear portion of antipyrine transfer) (see Figure 1) was 0.234 ± 0.054 s.d., a value similar to that observed in the open studies (Table 1). Moreover, the mean ddI/antipyrine transfer ratio in the opposite direction, foetal to maternal, at 30 min (Figure 2) was 0.295 ± 0.107 s.d. (Table 2), again consistent with data from the open studies and statistically indistinguishable from the value observed in the closed maternal to foetal studies ($P > 0.05$).

There was no inhibition of the clearance of ddI in the maternal to foetal direction on addition of 600 μM inosine (Table 1). Similarly, this 20-fold inosine excess did not alter the maternal to foetal ddI/antipyrine transfer ratio at 30 min in studies using the recirculating system ($P > 0.05$) (Table 2). Comparison of the data at 45 and 60 min of transfer likewise indicated no statistically significant differences. Uric acid, in 20-fold excess by weight, did not alter the ddI/antipyrine transfer ratio at 30 min (Table 2).

As shown in Figure 3, ddI did not cross the placenta from the maternal to the foetal side against a concentration gradient, while the positive control, leucine did so.

As shown in Table 2, there was no effect of 3 μM AZT on ddI transfer.

Table 1 ddI clearance by the human isolated perfused placenta

	ddI/Antipyrine	*95% CI*⁺ of the difference*	*ddI/(−)-Glucose*	*95% CI of the difference*
I. Maternal to foetal clearance ratios				
ddI alone (3 μM)	0.315 ± 0.126† (*n* = 3)		1.147 ± 0.025 (*n* = 3)	
ddI alone (30 μM)	0.279 ± 0.112‡ (*n* = 6) (*P* = 0.678)	−0.202 to 0.274	1.059 ± 0.106 (*n* = 6) (*P* = 0.212)	−0.070 to 0.245
ddI (30 μM) + inosine (600 μM)	0.243 ± 0.108* (*n* = 3) (*P* = 0.656)	−0.202 to 0.274	1.037 ± 0.051* (*n* = 4) (*P* = 0.709)	−0.122 to 0.166
II. Foetal to maternal clearance ratios				
ddI alone (30 μM)	0.246 ± 0.100** (*n* = 3) (*P* = 0.677)	−0.205 to 0.271	1.069 ± 0.037** (*n* = 3) (*P* = 0.877)	−0.147 to 0.168

†Mean ± s.d. of separate perfusions, open/open.
‡NS ($P > 0.05$) *vs* 3 μM ddI transfer data.
*Not statistically significantly different from 30 μM ddI alone ($P > 0.05$).
**Not statistically significantly different from maternal to foetal transfer ($P > 0.05$) of 30 μM ddI.
*⁺CI refers to confidence limits.

Table 2 ddI/antipyrine transfer across the human isolated perfused placenta

	ddI/Antipyrine	*95% CI of the difference**⁺
I. Maternal to foetal transfer†		
A. ddI alone	0.234 ± 0.054‡ (n = 5)	
B. ddI with inosine	0.179 ± 0.061* (P = 0.173) (n = 5)	−0.078 to 0.187
C. ddI with uric acid	0.291 ± 0.059* (P = 0.173) (n = 4)	−0.197 to 0.083
D. ddI with AZT	0.305 ± 0.040* (P = 0.101) (n = 4)	−0.233 to 0.082
II. Foetal to maternal transfer†		
A. ddI alone	0.295 ± 0.107** (P = 0.296) (n = 4)	−0.201 to 0.079

†All data obtained at 30 min of linear antipyrine transfer in a recirculating system. Estimates of ddI/antipyrine transfer ratio at 15, 30, 45 and 60 min were similar ($P > 0.05$).
‡Mean ± s.d. of data from separate perfusions, as indicated in brackets.
*Not statistically significantly different from ddI alone ($P > 0.05$).
**Not statistically significantly different from maternal to foetal transfer.
*⁺CI refers to confident limits.
ddI = 30 μM; inosine = 600 μM (7 μg ml^{-1}); uric acid = 833 μM (140 μg ml^{-1}); AZT = 3 μM.

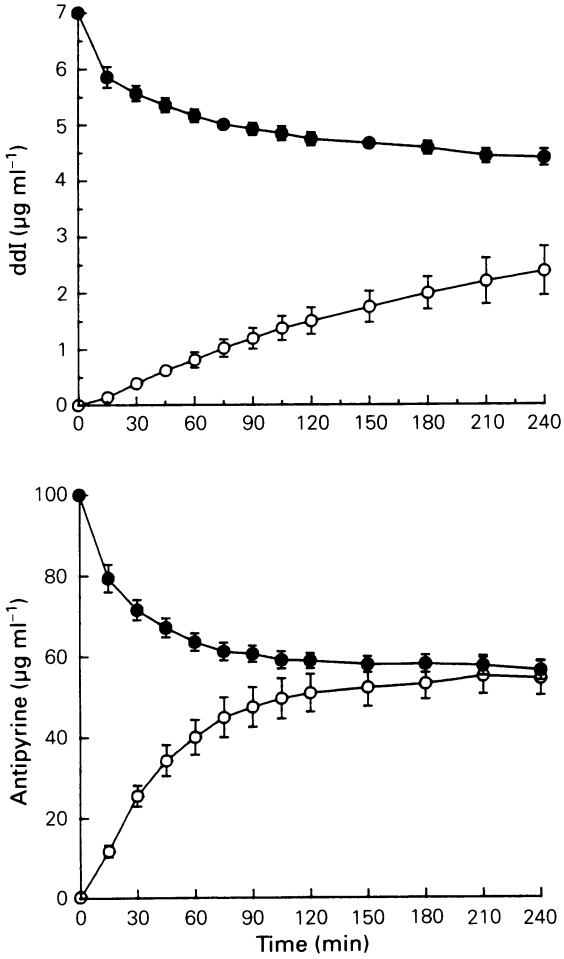

Figure 1 Mean (± s.e mean) maternal (●) and foetal (○) perfusate concentrations of ddI and antipyrine after administration of the drug into the maternal reservoir of the human isolated perfused placenta preparation perfused in a recirculation mode (n = 5 preparations). The ddI/antipyrine transfer ratio (Table 2) was calculated at 30 min, the linear portion of antipyrine appearance in the foetal perfusate.

No ddI metabolites were detected in either maternal or foetal perfusates up to 4 h. Moreover, almost all of the isotope found in the perfused lobules at the end of 4 h of recirculation was in the form of ddI. In the *in vitro* homogenate studies, only 3.7 ± 1.4% of the label could be accounted for in the form of a metabolite, most likely hypoxanthine, by h.p.l.c. retention time. By contrast, 75% of added [^{14}C]-inosine was rapidly metabolized, consistent with the finding of others [17]. There was no degradation of inosine in boiled placental homogenate.

As shown in Figure 4, hypoxanthine was readily generated from [^{14}C]-ddI in maternal and cord blood. Uric acid was also apparently produced in the latter system.

Discussion

The results show that ddI crosses the perfused human term placenta from mother to foetus at a rate of 25% that of antipyrine, a freely diffusible marker, and half that of AZT [3]. Others have reported similar rates of placental transfer of ddI at term [5, 9].

The transfer of ddI across the placenta appeared to be a passive process as indicated by 1) a clearance equivalent to that of (−)-glucose, a passively transferred marker, 2) equal transfer in both directions, 3) lack of inhibition of transfer by excess amounts of the structural analogues, inosine and uric acid, and 4) absence of transfer against a placental ddI concentration gradient. Similar conclusions were reached by others using the perfused human placenta [5, 9], as well as near term chronically catheterized pregnant macaques [16]. The lower rate of ddI transfer relative to AZT may be explained by its greater polarity [9].

Significant amounts of ddI metabolites were not detected in the present studies, either in the perfusions or with *in vitro* placental incubates. This contrasts with the finding of Dancis *et al.* [9], using a non-recirculating system, where substantial metabolism of ddI was detected within 1 h of perfusion. Recalculation of the transfer rate of ddI on the basis of such metabolism would lower it by about 50% [9]. This also contrasts with data published recently by another group which measured ddI by h.p.l.c. [55]. We have no explanation for the differences between our findings with regard to metabolism of ddI and those of Dancis *et al.* [9]. In a recent report, a 20 min incubation of ddI with human placental homogenate also failed to reveal ddI metabolites [17].

The conversion of ddI to hypoxanthine in blood (Figure 4) apparently is catalyzed by purine nucleo-

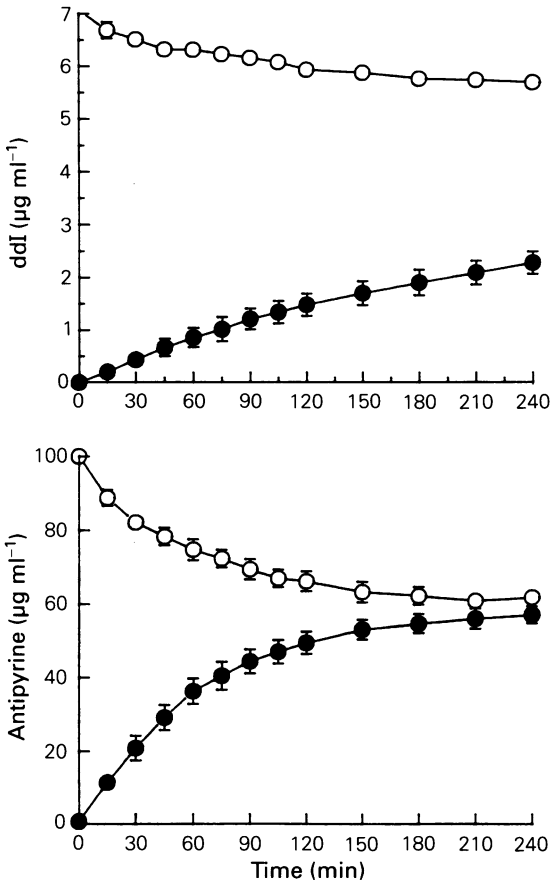

Figure 2 Mean (± s.e mean) maternal (●) and foetal (○) perfusate concentrations of ddI and antipyrine after administration of the drug into the foetal reservoir of the human isolated perfused placenta preparation perfused in a recirculation mode (n = 4 preparations). The ddI/antipyrine transfer ratio (Table 2) was calculated at 30 min, the linear portion of antipyrine appearance in the foetal perfusate.

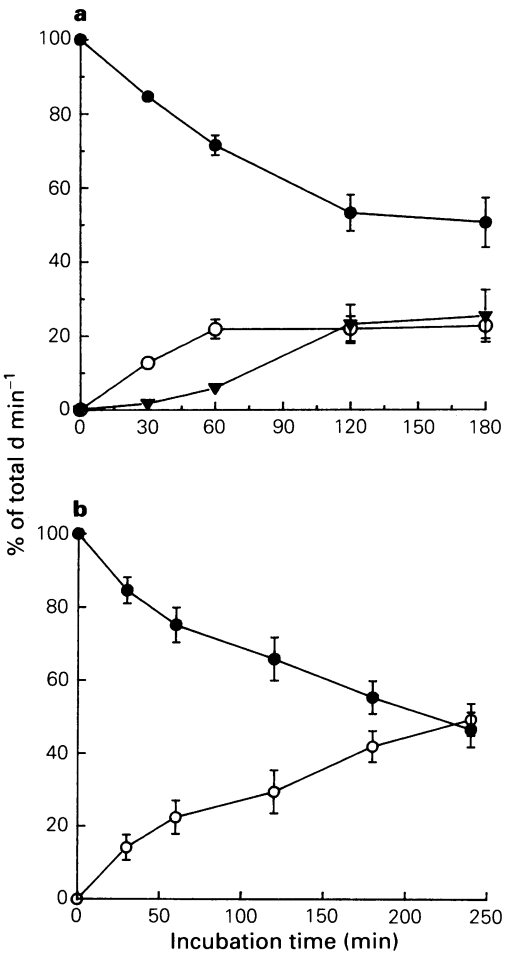

Figure 4 Formation of hypoxanthine (○) and uric acid (▼) from ddI (●) (30 μM) incubated with a) cord (n = 4) and b) maternal (n = 3) blood (mean ± s.e. mean).

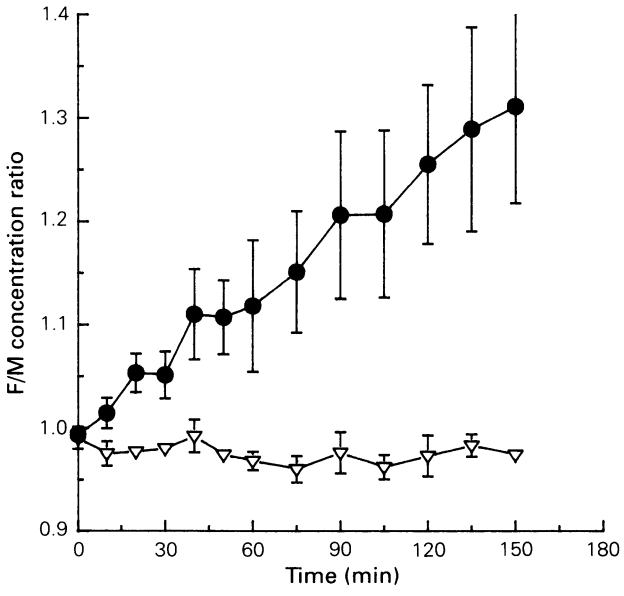

Figure 3 Mean (± s.e mean) foetal to maternal perfusate concentration ratios of ddI (▽) and leucine (●) when the same concentrations of the compounds were placed in the foetal (recirculating) perfusate of a human isolated perfused placenta preparation perfused on the maternal side in an open (constant) infusion mode (n = 3 preparations).

side phosphorylase and that to uric acid by xanthine oxidase [8]. This is consistent with detection of metabolites with use of [^{14}C]-ddI labeled in the purine ring. Thus, although in the blood-free *in vitro* placental preparation, ddI was essentially not metabolized, significant metabolism of ddI should occur *in vivo* in maternal blood thereby reducing net transfer of the drug to the foetus [9]. Moreover, in foetal blood, the half-life of ddI is likely to be decreased by metabolism of the drug. It seems, therefore, that based on significant metabolism of ddI in blood, the transfer of AZT to the foetus may be greater for a given dose than that of ddI. Indeed, foetal blood concentrations of ddI are only 18% of those in maternal blood [18]. Our data also indicate that AZT, given together with ddI, should not affect the placental transfer of the latter. A similar conclusion was reached from studies in the near-term pregnant macaque [19].

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