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Short communication

Radioimmunoassay for zidovudine in rat placenta and fetus

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1. Introduction

Acquired immunodeficiency syndrome (AIDS) was first reported in 1981 [1,2] and the human immunodeficiency virus (HIV) was later found to be the causative agent of AIDS [3]. HIV infection in women of reproductive age has increased significantly over the past few years. In October 1993, the Centers for Disease Control reported 29 163 cases of HIV-infected female patients in the age range 20–39 years. The vertical transmission of HIV has also resulted in an increase in pediatric HIV infection.

Zidovudine (ZDV) was the first anti-HIV agent to be approved by the Food and Drug Administration for the treatment of AIDS [4]. ZDV is now widely used in the treatment of HIV-infected pregnant women. The nucleoside analogue seems to be well tolerated by both mothers and their newborn infants, although some minor transitory toxicity of ZDV on fetal hematopoiesis has been reported [5,6]. Several studies have examined the placental transfer of anti-HIV agents in pregnant women [7–9]; however, somewhat limited information can be obtained from clincial studies. Similarly, ex-vivo studies with human placental tissue [10–12] provide useful, but limited, results.

Animal models may provide mechanistic information that is clincially useful. Recent reports have utilized non-human primates as an animal model to investigate the placental transfer of ZDV [13,14]. While these studies have certainly contributed to the understanding of the maternal–fetal transfer of ZDV, much remains to be learned. Furthermore, studies in monkeys generally employ a small sample size and the monkey model may not be acceptable for many mechanistic investigations. Thus, the pregnant rat model is being developed to investigate basic mechanisms involved in the placental transfer of nucleoside analogues. The rat model has proven to be an effective animal model for the study of the disposition of nucleoside analoges [15–19]. This animal model has been used to explore mechanisms of antiviral disposition and to elucidate mechanisms of nucleoside transport. Furthermore, the hemochorial placenta and the hemodynamic changes in pregnant rats are similar to those in humans [20–22]. Thus the rat appears to be a suitable

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animal model for investigating the maternal–fetal transport of ZDV.

A sensitive and direct radioimmunoassay (RIA) for ZDV has been previously developed [23,24] and a ZDV ^{125}I RIA kit is commercially available. However, these methods are for the determination of ZDV in serum, plasma or semen. A sample preparation technique for rat placenta and fetus is required before using the ZDV kit for the analysis of these tissues. In this paper, the development and validation of an RIA methodology for ZDV in rat placenta and fetus is presented.

2. Experimental

2.1. Chemicals

Zidovudine (ZDV) was purchased from Sigma Chemical Co., St. Louis, MO. ZDV–Trac ^{125}I RIA® kits were purchased from Incstar Corp., Stillwater, MN. Methanol (HPLC grade) and all other chemicals (analytical grade) were obtained from J.T. Baker Inc., Phillipsburg, NJ.

2.2. Sample preparation

Tissues (placenta and fetus) were collected from pregnant Sprague–Dawley rats (Charles River, Wilmington, MA) on day 20 of gestation. Tissue samples were homogenized with a Tekmar homogenizer (Tekmar Co., Cincinnati, OH) in scintillation vials containing 2 vol (w/v) sodium phosphate buffer, pH 7.2. The tissue homogenate was diluted 1:50 with buffer to reduce non-specific binding and a 200 μl aliquot was transfered to a centrifuge tube. 100 μl of 2 M perchloric acid was added and tubes were vigorously mixed and centrifuged for 10 min at 2000g. The supernatant (230 μl) was taken and 280 μl 1 M potassium hydroxide was added to bring the pH to 6–8. After centrifugation for 10 min, the supernatant (440 μl) was applied to C_{18} Sep-Pak® Classic cartridges (Waters, Millipore Corp., Milford, MA) which were preconditioned with 5 ml methanol and 5 ml phosphate buffer, pH 7.2. The cartridges were washed with 1 ml phosphate buffer and twice with 1 ml 10% methanol. The ZDV was eluted by three 1 ml methanol washes. The eluant was dried under a stream of air at ambient temperature and reconstituted with 500 μl sample diluent from the Incstar ZDV RIA kit.

2.3. Preparation of standard curves

For preparing standard curves, 20 μl standard stock ZDV solution (5 ng ml^{-1}–5 μg ml^{-1}) was added to 180 μl blank tissue homogenate (1:50 tissue:buffer). ZDV standard concentrations were 0.5, 1.25, 2.5, 5, 12.5, 25, 50, 125, 250, and 500 ng ml^{-1}. After a 1 h equilibration period, the samples were prepared as described above. Standard curves for ZDV in placenta and fetus tissues were constructed by plotting the percent bound (B/B_0) vs. log ZDV concentration. A cubic polynomial function was fitted to the standard curves.

2.4. Radioimmunoassay

A commercially available ZDV RIA kit (ZDV–Trac ^{125}I RIA® kit) was used. In brief, placental and fetal tissue samples were prepared as described above. Plasma samples were diluted with the sample diluant provided with the ZDV RIA kit to yield concentrations within the range of the standard curve. Standard or unknown sample (100 μl) was added to a 12 × 75 mm^2 glass culture tube followed by 50 μl of ^{125}I–ZDV and 50 μl antiserum. Samples were mixed and incubated for 2 h at room temperature. Samples were then centrifuged for 20 min at 1000g and the pellet was counted for 2 min using a gamma counter (Gamma 5500, Beckman).

2.5. Recovery, accuracy and reproducibility studies

Relative and absolute recoveries of ZDV were assessed at drug concentrations of 0.5, 1.25, 2.5, 5, 12.5, 25, 50, 125, 250, and 500 ng ml^{-1}. For the determination of relative recovery, the assay was performed in tissue homogenate as described above and in 180 μl phosphate buffer. For absolute recovery studies, 20 μl standard stock solution was added directly to 480 μl sample diluent from the RIA assay kit. The relative recovery of ZDV

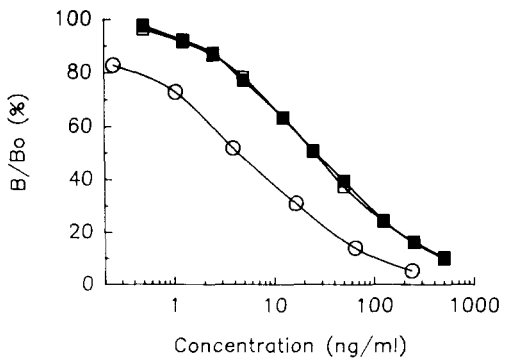

Fig. 1. Standard curves for ZDV kit (□), rat placenta (○) and fetus (■).

was calculated as $100\% \times cpm_{buffer}/cpm_{tissue}$ and absolute recovery as $100\% \times cpm_{direct}/cpm_{tissue}$.

The intra- and inter-day accuracy and precision of the RIA method were determined by analysis of six tissue samples containing 0.5, 1.25, 2.5, 5, 12.5, 25, 50, 125, 250, and 500 ng ml^{-1} concentrations of ZDV. To determine inter-day precision, the quality control samples were frozen at −20°C. Over a period of 6 days, samples were thawed and assayed. Assay precision was determined by calculating relative standard deviations (RSDs) for each drug concentration. The accuracy of the assay methodology, assessed by the deviation from expected values, was calculated as the measured ZDV concentrations minus the known concentration divided by the known ZDV concentration.

2.6. Animal studies

A single dose of 50 mg kg^{-1} of ZDV (dissolved in 0.1 N sodium hydroxide in normal saline) was administered intravenously to four pregnant Sprague–Dawley rats on day 20 of gestation. Blood (plasma), two fetuses and placenta were collected from each rat at selected times after ZDV administration. Plasma ZDV concentrations were determined directly by using the commercial RIA kit. Tissue samples were prepared and assayed as described above. Animal studies were approved by the University of Georgia Animal Care and Use Committee.

3. Results and discussion

3.1. Assay validation

Typical standard curves for ZDV in placenta and fetus tissue, as well as from the commercially available ZDV kit, are shown in Fig. 1. Cubic polynomial regression analysis showed very good correlation ($R^2 > 0.95$) between B/B_0 and ZDV concentration. From Fig. 1 it is evident that the

Table 1
Mean absolute and relative (%) recoveries (± SD) of ZDV from rat fetus and placenta tissues

Conc. (ng ml^{-1})	Fetus		Placenta	
	Absolute recovery[a]	Relative recovery[a]	Absolute recovery[b]	Relative recovery[c]
0.5	111.3 ± 10.0	101.7 ± 1.7	111.2 ± 8.1	103.2 ± 3.5
1.25	108.8 ± 8.7	100.8 ± 1.8	110.4 ± 4.2	104.6 ± 4.3
2.5	103.0 ± 7.5	103.0 ± 2.2	102.9 ± 5.9	103.1 ± 1.4
5	97.8 ± 6.8	101.8 ± 4.5	94.4 ± 6.5	101.0 ± 1.5
12.5	88.5 ± 3.7	100.3 ± 1.3	87.5 ± 3.8	100.8 ± 5.1
25	82.7 ± 3.4	99.9 ± 1.5	83.4 ± 3.6	103.1 ± 1.9
50	80.6 ± 4.6	101.3 ± 1.5	81.2 ± 4.8	102.6 ± 0.8
125	84.7 ± 2.9	106.0 ± 2.5	79.7 ± 3.0	101.6 ± 7.3
250	86.6 ± 3.5	103.4 ± 2.4	79.2 ± 3.5	102.9 ± 1.2
500	82.1 ± 5.0	106.1 ± 3.8	85.1 ± 2.8	97.0 ± 2.9

[a] $n = 4$.
[b] $n = 8$.
[c] $n = 5$.

ZDV concentrations for the placenta and fetus tissue extracts had higher B/B_0 values than those obtained from the commercially available ZDV kit standard curve. Therefore, it was necessary to generate standard curves using placenta and fetus for determining ZDV concentrations in these tissues.

The absolute and relative analytical recoveries of ZDV are shown in Table 1. The absolute recoveries of ZDV for rat placental and fetal tissues were 79–111% and 80–111% respectively. Some loss of ZDV occurred during the tissue sample preparation. To minimize possible contamination from the pellet after the centrifugation steps, not all of the supernatant was collected. However, precise volumes of supernatant were transferred to maintain accuracy and precision. Slight loss of ZDV during placental and fetal tissue preparation resulted in somewhat less than complete absolute recoveries. Even so, the absolute recoveries of ZDV from rat placenta and fetus are acceptable. The loss of ZDV during the tissue sample preparation resulted in slightly decreased ZDV concentrations at the final step of reconstitution with 500 μl sample diluent from the Incstar ZDV RIA kit. Thus, higher B/B_0 values for placenta and fetus, compared to those for the RIA kit (Fig. 1), were observed. The use of blank placental and fetal tissues for generating standard curves accounted for the loss of ZDV during tissue sample preparation and yielded a practical method for quantitating the nucleoside in these tissues.

Relative recoveries of ZDV for placenta and fetus were all around 100% (Table 1). Relative recoveries of ZDV were 97–104% and 99–106% for placenta and fetus tissues respectively. The approximately 100% relative recovery demonstrates the lack of endogenous interferences in rat placenta and fetus tissues with ZDV in the RIA assay.

The intra-assay and inter-assay accuracy and precision of the RIA assay for placenta and fetus tissues are given in Tables 2 and 3 respectively. The assay accuracy and precision were very good over the ZDV concentration range 2.5–500 ng ml^{-1}. Generally, the deviation in measured ZDV concentrations to known drug concentrations were less than 10%, demonstrating the high degree of accuracy of the analytical methodology. In addition, the intra-assay and inter-assay relative standard deviations were less than 12%. Thus, the assay accuracy and precision were acceptable over the ZDV concentration range 2.5–500 ng ml^{-1}. However, due to high variation at 0.5 ng ml^{-1} (Tables 2 and 3), sample concentrations determined to be less than 1.25 ng ml^{-1} ZDV should be interpreted with caution.

Table 2
Intra-assay accuracy and precision of the RIA assay for rat placenta and fetus tissues ($n = 6$)

Known conc. (ng ml^{-1})	Fetus			Placenta		
	Measured conc. (ng ml^{-1})	Deviation (%)	RSD (%)	Measured conc. (ng ml^{-1})	Deviation (%)	RSD (%)
0.5	0.56	11.0	35.9	0.61[a]	22.5	47.3
1.25	1.30	4.20	23.0	1.31[a]	4.80	18.0
2.5	2.90	15.9	15.4	2.23	−10.8	4.59
5	5.29	5.8	12.5	4.73	−5.42	10.3
12.5	11.0	−11.9	1.40	12.2	−2.01	14.7
25	22.8	−8.98	4.03	23.7	−5.35	7.07
50	50.6	1.12	7.31	53.1	6.15	8.88
125	137	9.59	6.35	131	5.02	11.7
250	263	5.10	2.56	253	1.28	9.40
500	455	−9.00	2.64	485	−2.95	6.61

[a] $n = 3$.

Table 3
Inter-assay accuracy and precision of the RIA assay for rat placenta and fetus tissues ($n = 6$)

Known conc. (ng ml^{-1})	Fetus			Placenta		
	Measured conc. (ng ml^{-1})	Deviation (%)	RSD (%)	Measured conc. (ng ml^{-1})	Deviation (%)	RSD (%)
0.5	0.37	−25.7	8.81	0.31	−37.2	14.0
1.25	1.48	18.5	6.25	1.40	11.7	5.82
2.5	2.40	−4.00	1.67	2.67	6.80	8.94
5	5.01	0.20	2.95	4.79	−4.18	4.48
12.5	12.1	−3.55	0.67	12.0	−3.89	2.55
25	24.7	−1.16	2.97	25.0	−0.04	3.44
50	50.3	0.50	1.84	51.3	2.68	3.92
125	126	0.71	3.30	129	3.07	3.67
250	263	5.37	3.64	242	−3.25	1.41
500	470	−5.97	2.59	500	0.02	3.70

3.2. ZDV distribution in pregnant rats

Concentrations of ZDV is plasma, placenta and fetus tissue after intravenous administration of 50 mg kg^{-1} ZDV to pregnant rats are shown in Fig. 2. Maximum concentrations of ZDV in placenta and fetus are achieved within 1 h of ZDV administration. ZDV concentrations in placenta and fetus tissue are similar, suggesting a rapid distribution of the nucleoside between placenta and fetus. The concentrations of ZDV in both the placenta and fetus declined in parallel with plasma ZDV concentrations of the pregnant female rat. Hence, the half-lifes of ZDV in these three tissues are the same.

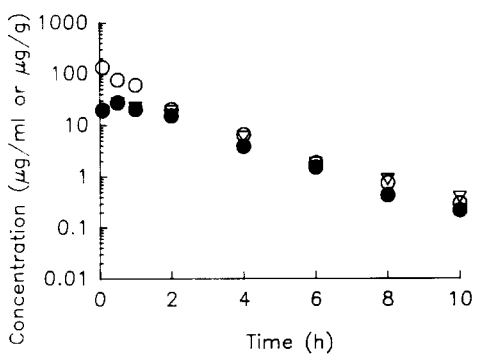

Fig. 2. Zidovudine plasma (○), placenta (▽) and fetus (●) concentrations vs. time profiles after intravenous administration of 50 mg kg^{-1} ZDV to pregnant rats.

In conclusion, the assay methodology presented provides an accurate, sensitive, and reproducible analysis of zidovudine in rat placenta and fetus samples.

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