Medical Journal of the
Islamic Republic of Iran

Volume 11
Number 4
Winter 1376
February 1998

BILIARY EXCRETION AND BLOOD/PLASMA RATIO OF NOVEL 5-BROMO-6-ALKOXY-5,6-DIHYDRO PRODRUGS OF 5-ETHYL-2'-DEOXYURIDINE

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ABSTRACT

The biliary excretion and blood/plasma ratios of four novel 5-bromo-6-alkoxy-5,6-dihydro prodrugs to 5-ethyl-2'-deoxyuridine (EDU) including (-)-*trans*-(5S, 6S)-5-bromo-5-ethyl-6-methoxy-5, 6-dihydro-2'-deoxyuridine (BMEDU), (+)-*trans*-(5R, 6R)-5-bromo-5-ethyl-6-ethoxy-5, 6-deoxyuridine (BEEDU), (+)-*trans*-(5R, 6R)-5-bromo-5-ethyl-6-ethoxy-5, 6-dihydro-5'-O-valeryl-2'-deoxyuridine (VBEEDU) and (+)-*trans*-(5R, 6R)-5-bromo-5-ethyl-6-ethoxy-5, 6-dihydro-5, 6-dihydro-3', 5'-di-O-valeryl-2'-deoxyuridine (DVBEEDU) were determined using [4-^{14}C]-labelled compounds. Liver samples taken following iv injection of 126 kBq (3.4 μCi) of these [4-^{14}C]-labelled 5,6-dihydro prodrugs into the tail vein of male Balb-C mice showed a higher percentage of the injected radioactivity than blood samples. A substantial amount of radioactivity was present in the large intestine, small intestine and gallbladder. Intestinal samples collected at longer post injection times showed a higher percentage of the injected dose relative to earlier post injection times. Bile samples collected at 8 min post injection of [4-^{14}C]-labelled BMEDU, BEEDU, VBEEDU and DVBEEDU contained the highest radioactivity levels. Excretion of radioactivity in rat bile following a jugular vein injection showed a biexponential decline, but the radioactivity excretion rates in bile for all four compounds investigated were quantitatively similar. Accumulation of radioactivity in rat bile samples collected after injection of [4-^{14}C]-BEEDU was substantially higher than that for [4-^{14}C]-BMEDU, [4-^{14}C]-VBEEDU and [4-^{14}C]-DVBEEDU. The distribution of radioactivity in rat whole blood and plasma samples taken at the same post injection times were substantially different. It was postulated that the rate of conversion of the 5, 6-dihydro prodrug to EDU is the main determinant of the whole blood/plasma ratio.

Keywords: Biliary excretion; blood/plasma ratio; 5-ethyl-2'-deoxyuridine; 5, 6-dihydro prodrugs; herpes simplex virus.

MJIRI, Vol. 11, No. 4, 355-361, 1998.

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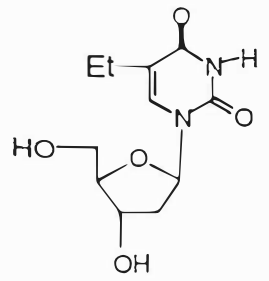
INTRODUCTION

5-Ethyl-2'deoxyuridine (EDU, Fig. 1) exhibits antiviral activity against herpes simplex virus type-1 (HSV-1), type-2 (HSV-2) and vaccinia virus.[1,2] EDU is also effective in the treatment of herpes keratitis in rabbits,[3] systemic herpetic infection in mice,[4] and cutaneous herpes infections in guinea pigs[5] and it increased the survival time of HSV-encephalitic mice.[6] One significant advantage of EDU is that it is not mutagenic. However, catabolic degradation of EDU by pyrimidine nucleoside phosphorylases results in cleavage of the glycoside bond to release 5-ethyluracil (EU), which is inactive against viruses and which undergoes *in vivo* biotransformation to 5-(1-hydroxyethyl) uracil.[7-9] It was recently reported that 5-bromo-6-alkoxy-5,6-dihydro prodrugs to EDU undergo metabolic and/or bioactivation reactions to form EDU.[8,9] In this study, the biliary excretion and blood/plasma ratios of some 5-bromo-6-alkoxy-5,6,-dihydro prodrugs to EDU (Fig. 1) were investigated.

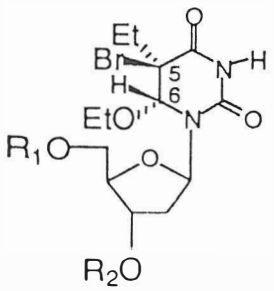
MATERIALS AND METHODS

(-)-*Trans*-(5S, 6S)-5-bromo-5-ethyl-6-methoxy-5,6-dihydro-2'-deoxyuridine (BMEDU), (+)-*trans*-(5R, 6R)-5-bromo-5-ethyl-6-ethoxy-5, 6-dihydro-2'-deoxyuridine (BEEDU), (+)-*trans*-(5R,6R)-5-bromo-5-ethyl-6-ethoxy-5,6-dihydro-5'-O-valeryl-2'-deoxyuridine (VBEEDU) and (+)-*trans*-(5R, 6R)-5-bromo-5-ethyl-6-ethoxy-5, 6-dihydro-3', 5'-di-O-valeryl-2'-deoxyuridine (DVBEEDU) (Fig. 1) were synthesized using methods described previously.[7-9] Male Balb-C mice (20-22g) and male Spraque Dawley rats (380-420g) were purchased from the University of Alberta Health Sciences Animal Services Facility. Three animals were used for each experiment. All animal studies were performed according to the Canadian Council on Animal Care guidelines. Male Balb-C mice were used for biodistribution studies of [4-^{14}C]-labelled BMEDU, BEEDU, VBEEDU and DVBEEDU. The biodistributions of the test compounds were determined after tail vein injection of a126 kBq (3.4 μCi) [specific activity= 2 GBq (54 mCi)/mmol, dissolved in 100 μL DMSO-water (50: 50 v/v)] dose. Each [4-^{14}C]-labelled test compound was mixed with 0.2 mmol/kg of non-radioactive compound. Animals were sacrificed by carbon dioxide asphyxiation. Samples, including blood, liver, gallbladder, large intestine (with its contents) and small intestine (with its contents) were collected at 8, 18, 30, 60, and 120 min post injection. The weights of samples collected for analysis from each tissue were limited to a maximum 180 mg of wet tissue or 100 μL of blood.

Biliary excretion and blood/plasma ratios for the 5,6-dihydro prodrugs BMEDU, BEEDU, VBEEDU and DVBEEDU were investigated in anesthetized rats having



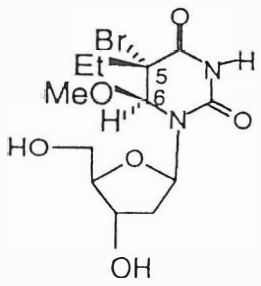
EDU



(5R,6R)-BEEBU; R_1=R_2=H

(5R,6R)-VBEEBU; R_1=Valeryl, R_2=H

(5R,6R)-DVBEEBU; R_1=R_2=Valeryl



(5S,6S)-BMEBU

Fig. 1. Structures of EDU and its 5,6-dihydro prodrugs BEEDU, VBEEDU, DVBEEDU and BMEDU.

catheters in the jugular vein for injection of the test compound and blood collection, and in the bile duct for bile collection. The ^{14}C-labelled test compounds [BMEDU, BEEDU, VBEEDU and DVBEEDU; 112 kBq (3.0 μCi), dissolved in 100 μL DMSO-water (50:50 v/v)] were injected into the jugular vein via the catheter. Each [4-^{14}C]-labelled test compound was mixed with 0.2 mmol/kg of non-radioactive compound prior to injection. The catheter was washed with 0.4 mL of heparinized saline after injection of the test compound and after collection of each blood sample. Blood samples (two of 0.1 mL) and bile samples were collected at 3,8,18,35,60,120,180, and 240 min post

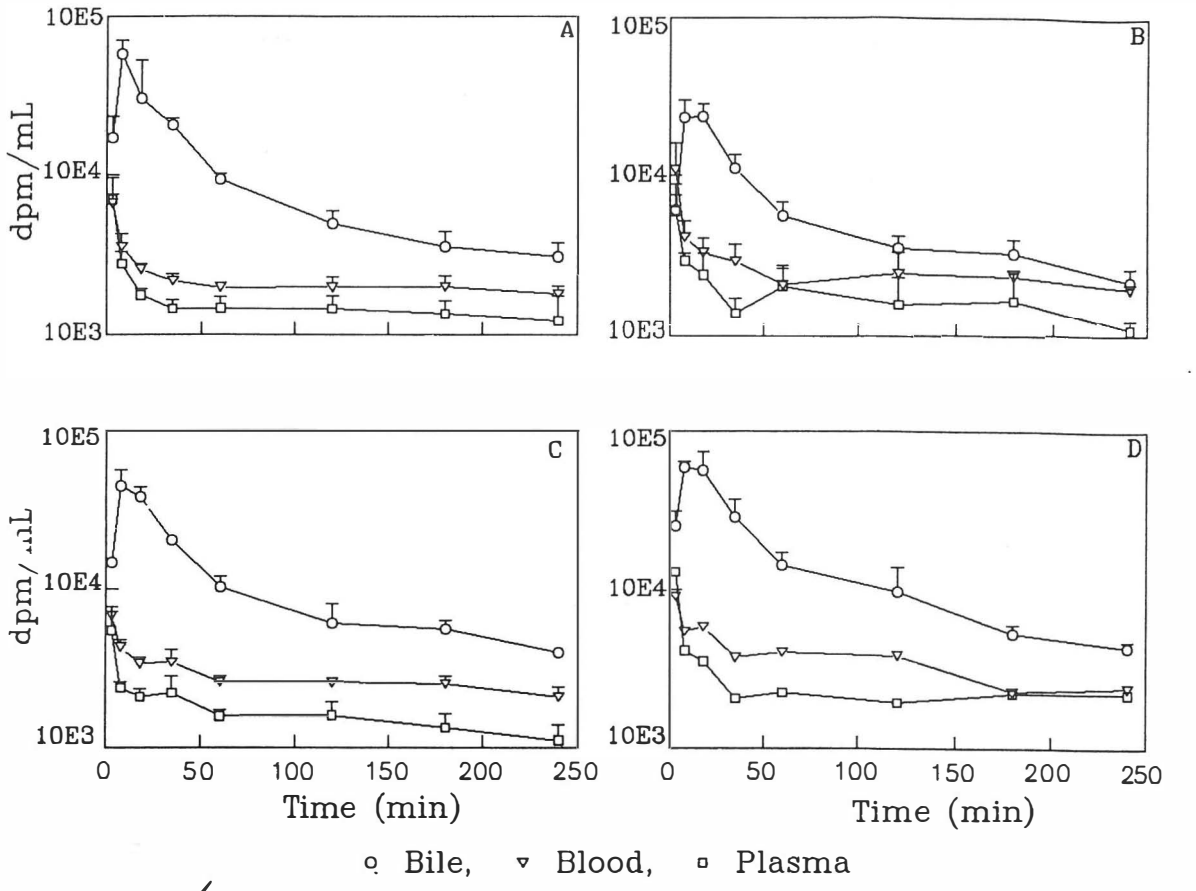


Fig. 2. Concentration of radioactivity in bile, whole blood and plasma after injection of [4-^{14}C]-labelled BEEDU, BMEDU, VBEEDU, and DVBEEDU into rats. Values are the mean ± SD (n= 3, except for DVBEEDU, where only one rat was used for measurement of radioactivity levels in whole blood and plasma samples).

injection. Blood samples were collected in heparinized tubes and one blood sample from each time period was centrifuged at 1500×g to separate the plasma from the blood cells.

All blood and tissue samples were air dried at room temperature for at least three days to insure quantitative combustion using an OX-300 Harvey Biological Material Oxidizer.

The excretion rate of radioactivity via bile after injection of the [4-^{14}C]-labelled test compound was calculated using the $\Delta X_b/\Delta t$ equation, where ΔX_b is the amount of radioactivity excreted in bile divided by the time period Δt. These data were then plotted against the mid-point sampling time using a SigmaPlot program (Jandel Scientific).

RESULTS

The distribution of radioactivity after injection of 126 kBq (3.4 μCi) of [4-^{14}C]-BEEDU, [4-^{14}C]-BMEDU, [4-^{14}C]-VBEEDU or [4-^{14}C]-DVBEEDU in blood, liver, large intestine, small intestine and gallbladder of male Balb-C mice is summarized in Tables I and II. All of these 5-bromo-6-alkoxy-5,6-dihydro derivatives of EDU provided a higher percentage of the injected dose in liver than in blood. A substantial amount of radioactivity was also present in the large intestine, small intestine and the gallbladder. Intestine samples taken at longer times post injection of the test compound showed a higher percentage of the injected dose than those taken at shorter time intervals.

Biliary excretion and the difference between radioactivity levels present in whole blood and plasma after injection of the [4-^{14}C]-labelled 5-bromo-6-alkoxy-5,6-dihydro prodrugs to EDU are shown in Fig. 2 Bile samples that were collected 8 min post injection of these 5,6-dihydro prodrugs showed the highest radioactivity levels. Excretion of radioactivity in bile showed a biexponential decline. However, the excretion rates of radioactivity in bile after injection of all four 5-bromo-6-alkoxy-5,6-dihydro prodrugs to EDU were quite similar (Fig. 3A). Accumulation of radioactivity in bile samples collected after injection of [4-^{14}C]-BEEDU was substantially higher than after injection of [4-^{14}C]-BMEDU, [4-^{14}C]-VBEEDU and [4-^{14}C]-DVBEEDU (Fig. 3B).

Blood and plasma samples collected at the same post injection times showed substantially different levels of

Table I. Biodistribution of [4-^{14}C]-BMEDU and [4-^{14}C]-BEEDU at 8, 18, 30, 60, and 120 min post injection of 126 kBq (3.4 μCi) into the tail vein of Balb-C mice. Data are presented as dpm per g of wet tissue or mL of blood, as the mean±SEM (n=3).

BMEDU	8 min	18 min	30 min	60 min	120 min
Blood	4.8E5 ± 1.8E4	3.8E5 ± 3.1E4	2.9E5 ± 4.5E4	1.4E5 ± 6.2E4	2.5E5 ± 7.3E4
	6.3[a]	5.0	3.8	1.9	3.3
Liver	7.8E5 ± 6.7E4	4.0E5 ± 7.3E4	3.1E5 ± 7.6E4	1.6E5 ± 2.9E4	2.0E5 ± 7.7E4
	10.3	5.3	4.1	2.1	2.6
Large intestine[b]	ND[c]	ND	1.6E5 ± 4.1E4	2.5E5 ± 9.2E4	2.3E5 ± 3.6E4
			2.1	3.3	3.0
Small Intestine[b]	ND	ND	2.2E5 ± 5.9E4	1.3E5 ± 3.5E4	4.6E5 ± 1.8E5
			2.9	1.7	6.1
Gallbladder[d]	ND	ND	1.1E5 ± 2.7E4	9.6E4 ± 5.6E4	1.5E5 ± 5.7E4
			1.5	1.3	2.0
BEEDU					
Blood	4.4E5 ± 3.3E4	3.9E5 ± 5.3E4	2.3E5 ± 4.2E4	1.3E5 ± 3.4E4	1.0E5 ± 1.3E4
	5.8[a]	5.2	3.0	1.7	1.3
Liver	7.5E5 ± 2.1E5	4.1E5 ± 1.0E5	3.1E5 ± 4.4E4	1.5E5 ± 2.2E4	1.3E5 ± 1.2E4
	10.0	5.4	4.1	2.0	1.7
Large Intestine[b]	ND[c]	ND	2.1E5 ± 6.1E4	2.1E5 ± 3.5±E4	3.7E5 ± 1.8E4
			2.8	2.8	4.9
Small Intestine[b]	ND	ND	3.5E5 ± 1.1E5	1.7E5 ± 1.6±E4	3.2E5 ± 5.8E4
			4.6	2.2	4.2
Gallbladder[d]	ND	ND	1.8E5 ± 4.0E4	2.3E5 ± 5.6E4	1.4E5 ± 2.0E4
			2.4	3.0	1.9

[a]% of injected dose per g or mL.
[b]including its contents.
[c]not determined.
[d]dpm/100 mg.

radioactivity (Fig. 2). Radioactivity levels in blood samples taken at 8 min or longer post injection of [4-^{14}C]-VBEEDU showed significantly (p<0.05) higher radioactivity levels than in the corresponding plasma samples. Although blood samples collected 18 min post injection of [4-^{14}C]-BEEDU showed significantly (p<0.05) higher radioactivity levels than those for the corresponding plasma samples, radioactivity level in blood samples collected at time intervals shorter than 35 min post injection of [4-^{14}C]-BMEDU did not show significant difference compared to plasma samples.

DISCUSSION

Several novel 5-bromo-6-alkoxy-5,6-dihydro prodrugs to EDU were recently developed. The biotransformation and biodistribution of these prodrugs were studied in mice and rats.[7-9] The increased molecular weights of these prodrugs in conjugation with the presence of polar groups in their structures may act as driving forces for their excretion into bile. It has been reported that compounds with molecular weights between 300 and 500 are usually excreted in both urine and bile. In addition to a higher molecular weight, drugs that are excreted into bile usually possess a strongly polar group (s).[10,11]

Among the four 5,6-dihydro derivatives of EDU investigated, BMEDU showed the lowest overall radioactivity level in intestinal samples obtained after injection of the [4-^{14}C]-labelled prodrugs into mice (Table II). Although the radioactivity excretion rates in bile after injection of the four [4-^{14}C]-labelled prodrugs in rats were quantitatively similar (Fig. 3), BEEDU showed a slightly higher excretion rate into bile relative to BMEDU, VBEEDU and DVBEEDU. In addition [4-^{14}C]-BEEDU also provided a substantially higher cumulative radioactivity level in bile samples collected up to 4 hrs post injection relative to the other three [4-^{14}C]-labelled compounds in rats. There is precedence that conjugated derivatives comprise the major type of products excreted in bile.[10,11] Therefore, the enhanced excretion of BEEDU in bile compared to VBEEDU and DVBEEDU, which have higher molecular weights, could be due, at least in part, to the fact that BEEDU has two free hydroxyl groups in the sugar moiety (Fig. 1). These hydroxyl groups are readily available for glucuronide conjugation which would afford highly hydrophilic metabolites having

Table II. Biodistribution of [4-^{14}C]-VBEEDU and [4-^{14}C]-DVBEEDU at 8, 18, 30, 60, and 120 min post injection of 126 kBq (3.4 μCi) into the tail vein of Balb-C mice. Data are presented as dpm per g of wet tissue or mL of blood, as the mean±SEM (n=3).

VBEEDU	8 min	18 min	30 min	60 min	120 min
Blood	2.5E5 ± 2.7E4	2.7E5 ± 1.3E4	1.8E5 ± 5.1E4	1.8E5 ± 7.8E3	6.9E4 ± 7.4E3
	3.3[a]	3.6	2.4	2.4	0.9
Liver	5.3E5 ± 8.0E4	5.5E5 ± 7.2E4	3.5E5 ± 1.0E4	2.9E5 ± 3.5E4	1.2E5 ± 3.6E3
	7.0	7.3	4.6	3.8	1.6
Large intestine[b]	ND[c]	1.3E5 ± 2.3E4	1.8E5 ± 3.1E4	1.6E5 ± 1.8E4	1.7E5 ± 7.5E4
		1.7	2.4	2.1	2.2
Small Intestine[b]	ND	1.4E5 ± 4.3E4	5.9E5 ± 5.4E4	5.7E5 ± 1.5E5	1.5E5 ± 1.9E4
		1.9	7.8	7.5	2.0
Gallbladder[d]	ND	8.7E4 ± 1.6E4	4.5E5 ± 3.3E5	9.2E5 ± 3.1E5	5.6E4 ± 4.4E3
		1.2	6.0	6.9	0.7
DVBEEDU					
Blood	3.0E5 ± 2.5E4	3.7E5 ± 4.0E4	3.3E5 ± 2.8E4	1.8E5 ± 1.9E4	7.8E4 ± 1.5E4
	4.0[a]	4.9	4.4	2.4	1.0
Liver	7.3E5 ± 1.1E5	7.6E5 ± 8.1E4	5.6E5 ± 7.7E4	3.1E5 ± 1.9E4	1.3E5 ± 7.7E3
	9.6	10.1	7.4	4.1	1.7
Large Intestine[b]	ND[c]	ND	1.3E5 ± 1.3E4	3.5E5 ± 6.1E3	4.1E5 ± 7.6E4
			1.7	4.6	5.4
Small Intestine[b]	ND	ND	2.8E5 ± 1.5E4	2.3E5 ± 4.3E4	2.1E5 ± 1.7E4
			3.7	3.0	2.8
Gallbladder[d]	ND	ND	5.8E4 ± 2.0E4	1.1E5 ± 2.2E4	3.0E4 ± 1.3E4
			0.8	1.5	0.4

[a]% of injected dose per g or mL.
[b]including its contents.
[c]not determined.
[d]dpm/100 mg.

a higher molecular weight. Such O-glucuronide metabolites would be very good candidates for biliary excretion.[10,11] Glucoronides are the major metabolites of some antiviral nucleosides such as AZT.[12]

Biliary radioactivity data following injection of [4-^{14}C]-labelled 5,6-dihydro prodrugs of EDU into rats are summarized in Table III. [4-^{14}C]-BEEDU showed the highest amount of radioactivity excreted in bile compared to the other prodrugs investigated in this study. However, in contrast to the results observed in mice, total radioactivity excreted in rat bile for all of these 5,6-dihydro prodrugs is negligible. It is very unlikely that this low amount of the prodrug in bile would contribute significantly to the pharmacokinetic parameters of these prodrugs in rats.

The 5,6-dihydro prodrugs investigated in this study showed substantially different concentrations in whole blood and plasma (Fig. 2). Although the transport of BMEDU, BEEDU, VBEEDU and DVBEEDU were not investigated, these compounds are lipophilic prodrugs to EDU which could easily diffuse into blood cells. It is possible that the differences between the radioactivity levels present in blood samples compared to those of plasma samples collected after injection of the [4-^{14}C]-labelled prodrugs BMEDU, BEEDU, VBEEDU and DVBEEDU is dependent upon the lipophilicity of these prodrugs. Blood samples collected 18 min and longer post injection of [4-^{14}C]-BEEDU showed significantly (p<0.05) higher radioactivity levels relative to the corresponding plasma samples. In contrast, injection of [4-^{14}C]-BMEDU did not provide significantly higher radioactivity levels in blood samples collected up to times of 35 min post injection, compared to those for the corresponding plasma samples. It would be expected that lipophilic compounds, which do not undergo intracellular metabolism in blood cells, should give rise to a constant equilibrium between the inside and

Table III. Total radioactivity excreted in bile during the 0-4 hr period following injection of [4-^{14}C]-labelled 5,6-dihydro prodrugs of EDU into rats. Values are the means±SD (n=3).

Compound	Total radioactivity (% injected dose)
[4-^{14}C]-BEEDU	3.9 ± 0.1
[4-^{14}C]-DVBEEDU	3.2 ± 0.8
[4-^{14}C]-BMEDU	2.9 ± 0.8
[4-^{14}C]-VBEEDU	2.9 ± 0.1

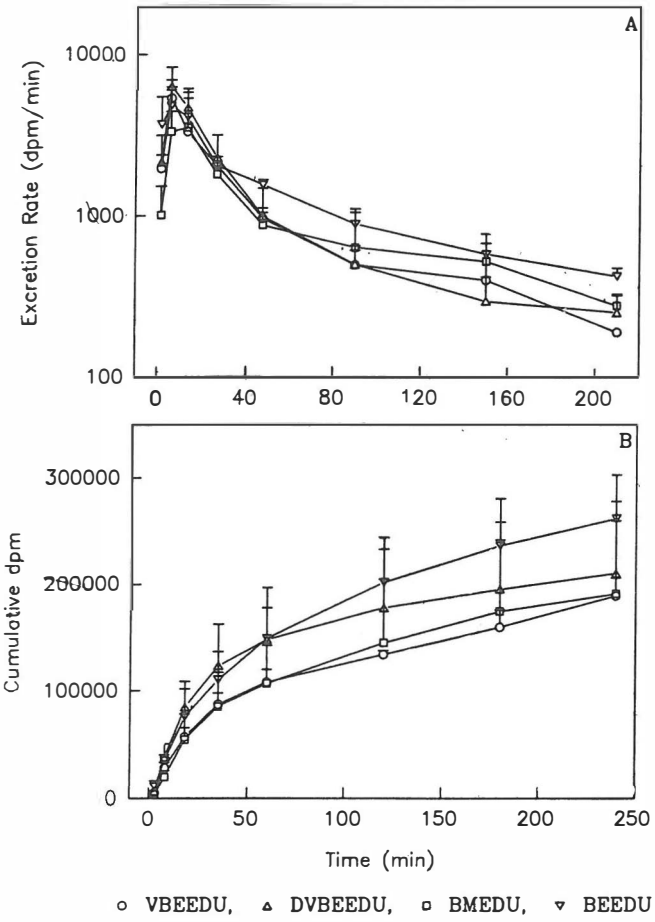


Fig. 3. Excretion rates (A) and cumulative excretion of radioactivity in bile samples (B) after injection of [4-^{14}C]-(5S, 6S)-BMEDU, [4-^{14}C]-BEEDU, [4-^{14}C]-VBEEDU and [4-^{14}C]-DVBEEDU into rats. Values are the means ± SD (n=3).

outside of blood cells. However, it has been observed that blood cells are capable of transforming BEEDU to EDU by regeneration of the 5,6-olefinic bond present in EDU.[8] EDU is a more hydrophilic compound than the prodrug and its further biotransformation in blood cells may result in trapping of radioactivity inside the blood cells.

A putative mechanism which may cause differences between concentration of the 5,6-dihydro prodrugs in whole blood and plasma is shown in Fig. 4. Based on this mechanism, the 5,6-dihydro prodrugs diffuse into (k_1) and out of (k_{-1}) blood cells based on their lipophilicity. Regeneration of the 5,6-olefinic bond results in production of EDU both inside the cell (k_2) and in plasma (k_4). However, it was previously observed that the conversion of BEEDU into EDU upon *in vitro* incubation with whole blood was more extensive than with plasma ($k_2 >> k_4$). It was also previously reported that most of the 5-substituted derivatives of 2'-deoxyuridine equilibrate inside and outside the red blood cells by a facilitated diffusion mechanism.[13] Since it is known that red blood cells are not capable of

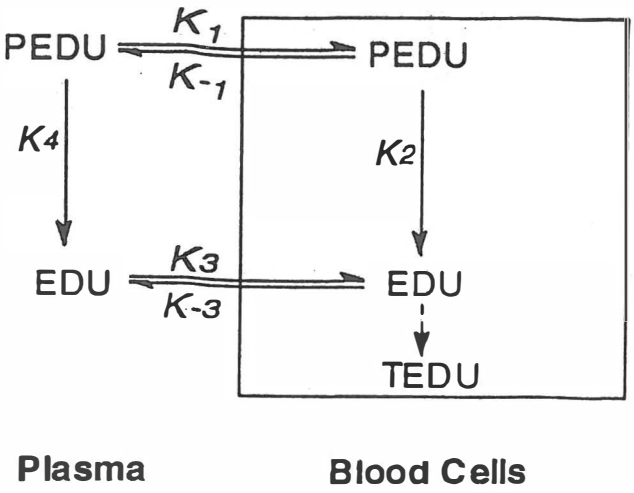


Fig. 4. Putative mechanism responsible for differences between radioactivity levels of 5,6-dihydro prodrugs to EDU(PEDU) in whole blood and plasma. TEDU is trapped EDU.

biotransforming the nucleosides, the differences between radioactivity levels show that perhaps other blood components play a crucial role in regeneration of the 5,6-olefinic bond of pyrimidine ring and further bioconversion of these 5-bromo-6-alkoxy-5,6-dihydro prodrugs to EDU. The importance of other blood components rather than red blood cells such a s leucocytes in uptaking some drugs such as chloroquine and hydroxychloroquine was reported previously.[14] However, it has been postulated that the structurally similar 5,6-dihydro prodrugs of AZT may also bind to red blood cells and therefore cause a difference between the concentrations of the 5,6-dihydro prodrugs in whole blood and plasma.[15]

However, regardless of the exact mechanism of trapping of EDU in blood cells, this phenomenon causes trapped EDU to be excluded from the central compartment, thereby precluding its availability to localize in viral infected cells. The stability of the 5-bromo-6-alkoxy-5,6-dihydro prodrugs, and their rate of conversion to EDU, may play a crucial role in the trapping of EDU, which arises from the prodrug, in blood cells *in vivo*. It was previously reported that BMEDU is more stable than BEEDU after iv injection into rats, since BMEDU undergoes slower conversion than BEEDU to EDU.[8] Therefore it was expected that the trapping of EDU, formed after regeneration of the 5,6-olefinic bond of BEEDU, would be less pronounced at shorter post injection times, relative to BMEDU. In contrast to BMEDU, blood samples collected 18 min post injection of [4-^{14}C]-BEEDU showed significantly higher radioactivity levels than those of the corresponding plasma samples.

In conclusion, the results of this study show that the 5-bromo-6-alkoxy-5,6-dihydro prodrugs to EDU studied in this investigation (BMEDU, BEEDU, VBEEDU and DVBEEDU) undergo substantial excretion in bile after iv administration to mice and rats. This phenomenon may be

responsible for the evaluated blood concentration of these compounds after high or multiple doses. These 5,6-dihydro prodrugs also produced significantly higher radioactivity levels in whole blood samples compared to those of plasma samples after administration of the [4-^{14}C]-labelled prodrugs to rats. The difference between the concentration of the prodrug in whole blood and plasma would therefore be an important factor when calculating kinetic parameters depending on whether plasma or whole blood were used.

ACKNOWLEDGEMENTS

We are grateful to the Medical Research Council of Canada for financial support of this research and to the Iranian Ministry of Health and Medical Education for a Ph.D. studentship to the first author.

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