THE GUT WALL METABOLISM OF ETHINYLOESTRADIOL AND ITS CONTRIBUTION TO THE PRE-SYSTEMIC METABOLISM OF ETHINYLOESTRADIOL IN HUMANS

D.J. BACK, A.M. BRECKENRIDGE, M. MacIVER, M. ORME,
H.S. PURBA, P.H. ROWE & I. TAYLOR*

Departments of Pharmacology and Therapeutics and Surgery, University of Liverpool, P.O. Box 147,
Liverpool L69 3BX

- 1 Five patients have been studied to determine the contribution of the gut wall to the pre-systemic metabolism of ethinyloestradiol. All patients had a catheter inserted into their hepatic portal vein as part of their surgical management.
- 2 After an oral dose of 50 μg (65 μCi) ethinyloestradiol, blood samples were taken from the hepatic portal vein and from a peripheral vein at intervals for 1 h.
- 3 In each patient the concentration of conjugated ethinyloestradiol in the portal vein was considerably higher than in the peripheral vein.
- 4 Although a number of assumptions have been made, calculations showed that the gut wall appeared to be twice as effective as the liver in conjugating ethinyloestradiol on the first pass.
- 5 In two patients there was no evidence of major uptake or metabolism of ethinyloestradiol in the lung.

Introduction

Ethinyloestradiol (EE) has been shown to be well absorbed in women but has a mean systemic bioavailability of only 42% (Back *et al.*, 1979). This reduced bioavailability could be due to pre-systemic metabolism in the gut wall and/or the liver. We have shown that EE is metabolised by human gut mucosa *in vitro* with the production of a sulphate conjugate (Back *et al.*, 1981). However, it is impossible for these studies to define the relative contribution of gut wall and liver metabolism to the low bioavailability of ethinyloestradiol.

This paper describes a study to quantitate the gut wall metabolism of ethinyloestradiol by sampling simultaneously from the portal vein and from the systemic circulation after oral dosing with tritiated ethinyloestradiol. A further study is described in which the possible role of the lung in metabolising ethinyloestradiol is examined.

Methods

Portal vein study

A group of five patients aged between 58 and 70

*Present address: Department of Surgery, Southampton University, Southampton.

years, three men and two women, were studied. Written informed consent was obtained from each patient, approval for the study was given by the Mersey Regional Ethics Committee, and permission to administer tritiated ethinyloestradiol was given by the radioisotope panel of the Department of Health and Social Security.

All the patients had recently undergone surgery for carcinoma of the colon and had then received treatment with 5-fluorouracil administered in the portal vein via a cannula inserted through the remnant of the umbilical vein. The patients were studied approximately 7 to 10 days after surgery.

After an overnight fast a single tablet of Eugynon 50 (Schering: 50 μg ethinyloestradiol, 500 μg norgestrel), incorporating 65 μCi tritiated ethinyloestradiol was given by mouth. Blood samples (10 ml) were taken simultaneously from the portal vein cannula and from a cannula inserted into a forearm vein, prior to dosing and at 10, 20, 30, 40, 50 and 60 min after dosing. The blood was centrifuged (1000 g) for 10 min and the plasma removed and analysed immediately or stored at -20°C until analysis.

Plasma (200 μl) was placed in duplicate tubes, scintillant (4 ml: NE 250; Nuclear Enterprises) added and total radioactivity counted. Duplicate plasma

samples (200 μ l) were also shaken with diethyl ether (3 ml: AnalaR grade; British Drug Houses Ltd) and the two phases fully separated by centrifugation (1000 g: 5 min). The aqueous phase was then frozen by immersion in a solid carbondioxide/methanol bath and the ether decanted. The ether extraction was repeated after thawing the aqueous fraction. Scintillant (4 ml: NE 260) was added direct to the aqueous fraction and also to the extract from the ether phase after removal of the ether in a vortex evaporator (Searle Analytic Inc.). Radioactivity in the two phases was then counted. In a previous study we have shown that hydrolysis of the aqueous phase (after ether extraction) with sulphatase and β -glucuronidase quantitatively yields ethinyloestradiol (Back *et al.*, 1981). The aqueous phase, therefore, contains conjugates of ethinyloestradiol. Extraction of plasma with ether (twice) removes more than 95% of ethinyloestradiol in the ether phase.

Lung metabolism study

Two patients who were undergoing investigation for coronary artery disease were studied. Each gave written informed consent and the study was approved by the Regional Ethics Committee. As part of their investigation, cannulae were inserted under local anaesthesia, into the main pulmonary artery, into the left ventricle and into the inferior vena cava. A butterfly cannula was also placed in a forearm vein.

A sterile aqueous solution of ethinyloestradiol 50 μ g/ml was prepared by Wyeth Laboratories Ltd, and tested for sterility and absence of pyrogens. Polyethylene glycol (1 ml) was added to each 2 ml ampoule to maintain the drug in solution. Tritiated ethinyloestradiol (50 μ Ci), 6, 7, -[3 H], 58.3 ci/mmol; (New England Nuclear Corporation, W. Germany) was dissolved in the contents of the ampoule and diluted in 10 ml of sterile saline immediately prior to injection. The solution was administered through a millipore filter (Millex)—GS—Millipore S.A. via the forearm vein.

Blood samples were taken simultaneously from the left ventricle, the pulmonary artery and the inferior vena cava at 0, 1, 2, 3, 4, 5 and 8 min after injection. The plasma was removed and stored at -20°C until analysis.

The total radioactivity and non-ether extractable radioactivity was measured as described in the portal vein study. In addition, plasma (0.5 ml) from the 2, 3 and 4 min sample were extracted with diethylether (3 ml). The ether was evaporated to dryness, redissolved in methanol and run on thin layer chromatography (t.l.c.) plates, (Kodak Eastman: No. 13181) with an ethyl acetate: petroleum ether (3 : 2) solvent system to identify any phase I metabolites (Back *et al.*, 1981).

Calculations

The difference in the counts between plasma from the hepatic portal vein and the systemic vein was calculated for each sampling time for both total radioactivity and non-ether extractable radioactivity (i.e. conjugates).

Total radioactivity The rate of drug absorption across the gastrointestinal mucosa (dA_a/dt)

$$dA_a/dt = {}^0\text{HP} \cdot {}^c\text{HP} \cdot T - {}^0\text{HP} \cdot {}^c\text{A} \cdot T \quad (1)$$

where ${}^0\text{HP}$ is the blood flow in the hepatic portal vein; ${}^c\text{HP}$, T is the concentration of total radioactivity in the hepatic portal vein and ${}^c\text{A}$, T is the concentration of total radioactivity in the mesenteric artery. A number of assumptions are implicit in this equation;

1. That there is no enterohepatic re-cycling. Since the study only lasted 60 min it is unlikely that significant amounts of drug could be re-cycled in this time.
2. That blood flow through the gut is equal to hepatic portal blood flow.
3. That ${}^c\text{A}$, $T = {}^c\text{V}$, T where ${}^c\text{V}$, T is the concentration of total radioactivity in the peripheral vein. This assumption seems justified since in the lung studies we were able to show that counts both for total radioactivity and for the conjugate were very similar in the left ventricle compared to those in the inferior vena cava.

Therefore

$$dA_a = {}^0\text{HP} \cdot {}^c\text{HP} \cdot T^{dt} - {}^0\text{HP} \cdot {}^c\text{V} \cdot T^{dt} \quad (2)$$

We have also at this stage to make a further assumption.

4. That there is complete absorption of the drug across the gastrointestinal mucosa. There is some evidence that ethinyloestradiol is completely absorbed in humans (Back *et al.*, 1979).

Integrating equation 2 gives

Amount of total activity absorbed

$$(D) = {}^0\text{HP} (AUC_{\text{HP}, T} - AUC_{\text{V}, T}) \quad (3)$$

Ethinylloestradiol conjugate The rate of metabolism of ethinyloestradiol by the gut on the first pass = dA_m/dt

$$dA_m/dt = {}^0\text{HP} \cdot {}^c\text{HP} \cdot M - {}^0\text{HP} \cdot {}^c\text{A} \cdot M \quad (4)$$

where ${}^c\text{HP}$, M is the concentration of metabolite in the hepatic portal vein; and ${}^c\text{A}$, M is the concentration of ethinyloestradiol metabolite in the mesenteric artery.

Here again further assumptions are made

5. That ${}^c\text{A}$, M is equal to ${}^c\text{V}$, M . We have some evidence from the lung study that this is indeed true.
6. That there is no further metabolism of ethinyloestradiol by the gut wall when the drug is recirculated

in the blood stream. We have no evidence that this is true but the amount of drug re-circulated to the gut wall in the blood will be small compared with the amount of drug absorbed across the gut wall initially. Therefore

$$dAm = {}^0\text{HP} \cdot {}^c\text{HP} \cdot M^{\text{dt}} - {}^0\text{HP} \cdot {}^c\text{V} \cdot M^{\text{dt}} \quad (5)$$

and

$$A_M (\text{amount of conjugate absorbed}) = {}^0\text{HP} (AUC_{\text{HP},M} - AUC_{\text{V},M}) \quad (6)$$

Therefore, the fraction of drug metabolised in the gut wall on the first pass (fm_G)

$$fm_G = AM/D = \frac{(AUC_{\text{HP},M} - AUC_{\text{V},M})}{(AUC_{\text{HP},T} - AUC_{\text{V},T})} \quad (7)$$

The area under the plasma concentration versus time curve (AUC) for both total radioactivity and for the conjugate as calculated by the trapezoidal rule for both peripheral vein and hepatic portal vein samples.

The bioavailability of ethinyloestradiol into the splanchnic circulation (F_G)

$$F_G = 1 - fm_G \quad (8)$$

now

$$F = F_G \cdot F_H \quad (9)$$

where F = overall bioavailability of ethinyloestradiol and F_H is the fraction avoiding first-pass loss in the liver.

Therefore

$$F_H = F/F_G \quad (10)$$

We need to assume at this stage that the value for F in post-operative patients with cancer receiving 5 fluorouracil is the same as it is in normal young volunteers. An overall figure for F of 0.42 has been obtained for studies in young female volunteers (Back *et al.*, 1979) and is used in these calculations.

Statistical analysis was by Student's *t*-test for paired samples and linear regression analysis was carried out by the method of least squares.

Results

Portal vein study

Tables 1 and 2 show the levels of radioactivity in the plasma (d min^{-1}) for each subject for total (Table 1) and non-ether extractable (Table 2) fraction from both the portal vein and systemic vein. Figure 1 shows graphically the difference in the radioactivity between the portal and peripheral vein from one volunteer.

Table 3 shows the figures for the area under the plasma radioactivity: time curves for both the total radioactivity and non-ether extractable radioactivity.

Table 1 Total radioactivity ($\text{d min}^{-1} \text{ml}^{-1}$) in portal and systemic vein for each volunteer. Times are given in minutes after dosing.

Subject	Vessel	Time (min)					
		10	20	30	40	50	60
1	Portal	6212	7128	5726	5135	4796	—
	Systemic	319	4912	5362	5136	4715	—
2	Portal	589	2189	2908	4251	—	—
	Systemic	640	540	593	1578	—	—
3	Portal	220	4125	8950	8325	7635	8190
	Systemic	310	3250	6960	6860	6640	6305
4	Portal	5136	7224	11603	10119	9747	9354
	Systemic	1210	2274	5332	6101	7181	7694
5	Portal	675	1440	8367	14342	11457	10812
	Systemic	267	1158	3092	6422	9405	10337

Table 2 Non-ether extractable radioactivity ($\text{d min}^{-1} \text{ml}^{-1}$) in portal and systemic vein for each volunteer.

Subject	Vessel	Time (min)					
		10	20	30	40	50	60
1	Portal	2927	6371	5268	4921	4624	—
	Systemic	282	4304	5132	4705	4144	—
2	Portal	176	793	1147	1820	—	—
	Systemic	196	355	307	1353	—	—
3	Portal	310	2250	5960	6868	5640	6305
	Systemic	230	1105	4485	7025	6165	5930
4	Portal	1203	3375	7072	7503	8735	7869
	Systemic	316	1373	3832	5516	6616	7065
5	Portal	390	527	4620	9515	9875	9457
	Systemic	47	287	2627	5975	8795	9652

Figure 1 The difference in the radioactivity counts in the blood between the portal vein and the systemic vein in patient No. 1. ■—■ shows the difference for the total counts while □—□ shows the difference for the counts representing conjugates of ethinyloestradiol.

Table 3 Area under plasma radioactivity ν time curve for the difference between portal and peripheral venous samples for non-ether extractable (conjugates) and total radioactivity. The third column shows the fraction of drug metabolised in the gut wall on the first pass (fm_G).

Subject	$AUC_{HP,M}$ $-AUC_{V,M}$	$AUC_{HP,T}$ $-AUC_{V,T}$	Fraction of drug metabolised in the gut wall (fm_G)
	($d\ min^{-1}\ ml^{-1} \times min$)	($d\ min^{-1}\ ml^{-1} \times min$)	
1	53,040	85,125	0.62
2	14,915	52,815	0.28
3	22,055	61,775	0.40
4	106,370	225,610	0.47
5	70,985	161,745	0.44
		mean	0.44
		$\pm$ s.e.	0.05

This table also shows the ratio of these two areas for each individual and gives the fraction of drug metabolised in the gut wall on the first pass (fm_G equation 7) for which the mean figure for the group was 0.44 ± 0.05 ($\pm$ s.e. mean).

From equation (8)

$$F_G = 1 - fm_G = 1 - 0.44 = 0.56$$

from equation (10)

$$F_H = F/F_G$$

since F from previous data (Back *et al.*, 1979) is 0.42

$$F_H = 0.42/0.56$$

$$= 0.75$$

Therefore fm_H the fraction of ethinyloestradiol which is conjugated in the liver.

$$\text{Therefore } fm_H = 1 - 0.75 = 0.25$$

$$\text{Thus } fm_G = 0.44$$

$$\text{and } fm_H = 0.25$$

The radioactivity in the portal vein in four of the volunteers peaked between 20 and 40 min. In one patient sampling only occurred for 40 min for technical reasons and it is impossible to say whether this is a true peak level for this subject.

Figure 2 shows, plotted on a linear scale, the amount of drug conjugated against the amount of drug absorbed at each time point for all the individuals. There is a significant linear relationship with a correlation coefficient of 0.92 ($P < 0.001$), a gradient of ± 0.46 , and an intercept on the y axis of -13.8 .

Lung metabolism study

Table 4 shows the total and non-ether extractable radioactivity in the pulmonary artery and left ventricle for the two patients. The levels of non-ether extractable radioactivity are essentially similar suggesting that the lung does not conjugate this steroid. In addition, t.l.c. of the ether extractable radioactivity showed no evidence of phase I metabolites. As the levels of total radioactivity are similar, the lung does not appear to take up the steroid, to any considerable extent.

Figure 3 shows the levels of conjugated metabolites present in each plasma sample from the three sampling sites in one volunteer. By 5 min the level of conjugates in the inferior vena cava are the same as the levels in the left ventricle. This is important for assumption 5 in the calculations section.

Figure 2 Plot of the amount of ethinyloestradiol conjugated (in $d\ min^{-1}\ ml^{-1}$) (on y axis) against the total amount of ethinyloestradiol absorbed ($d\ min^{-1}\ ml^{-1}$) (x axis) in all patients studied, ($r = 0.92$, $P < 0.001$).

Table 4 Total and non-ether extractable radioactivity ($\text{d min}^{-1} \text{ ml}^{-1}$) from pulmonary artery (PA) and left ventricle (LV) from each patient

Patient 1 Time (min)	Total radioactivity		Non-ether extract	
	PA	LV	PA	LV
1	1814	5117	206	374
2	984	784	178	197
3	640	1204	174	110
4	390	413	209	226
5	484	467	209	219
8	438	491	234	339

Patient 2 Time (min)	Total radioactivity		Non-ether extract	
	PA	LV	PA	LV
1	6184	9770	543	593
2	2024	2341	304	261
3	1324	1541	170	182
4	1281	1253	191	159
5	1153	1183	199	149
8	1053	1061	329	394

Figure 3 Plasma concentrations of conjugated ethinyloestradiol (as $\text{d min}^{-1} \text{ ml}^{-1}$) in the left ventricle (■), pulmonary artery (Δ) and inferior vena cava (●) for the first 5 min after i.v. injection of ethinyloestradiol.

Discussion

This study has shown that gut wall metabolism is an important factor in the low systemic bioavailability of ethinyloestradiol. The results demonstrate higher levels of conjugated metabolites in the portal vein than in the systemic vein. The calculations made show that the fraction of ethinyloestradiol conjugated in the gut wall (fm_G) is 0.44 while the fraction of ethinyloestradiol conjugated in the liver (fm_H) is only 0.25. This therefore appears to show that the gut is almost twice as effective as the liver in conjugating ethinyloestradiol.

There are however a number of assumptions inherent in this work and these are referred to in the calculations section. In particular the amount of conjugate appearing in the hepatic portal vein is overestimated if significant biliary recycling takes place. However, it seems unlikely that over the time scale of the study (1 h) there would be any significant biliary recycling. Studies of ethinyloestradiol and conjugate concentrations in the lung study show that the lung is not a major organ of uptake for ethinyloestradiol although we cannot exclude a minor degree of lung uptake for ethinyloestradiol. This study did however show that arterial and venous concentrations of total radioactivity and conjugate are very similar.

We have also assumed that all the ethinyloestradiol conjugate appearing in the hepatic portal vein has been derived from absorbed ethinyloestradiol. This is unlikely to be the whole truth. In the early minutes of the study this assumption will be correct but during the later stages of the study ethinyloestradiol delivered to the gut wall in the arterial blood supply may be conjugated in the gut wall. It is extremely difficult in man to differentiate between this pre-absorptive and post-absorptive metabolism in humans (Routledge & Shand, 1979). We have some evidence that levels of sulphate conjugate of ethinyloestradiol in peripheral blood are 6 to 10 times higher after an oral dose than after an equivalent intravenous dose (Back, Breckenridge, Orme & Rowe personal communication). It seems likely that most of the sulphate conjugate of ethinyloestradiol is produced after direct mucosal exposure. The calculations may have also overestimated the gut wall metabolism of ethinyloestradiol by ignoring the volume of distribution of the sulphate conjugate. It seems likely that this polar metabolite will have a smaller volume of distribution than ethinyloestradiol. The plasma concentration of sulphate conjugate in the hepatic portal vein, may be high because steady state has not been achieved and this may lead to a slight overestimate of the gut wall metabolism.

In contrast to these possible overestimates, the study was performed in patients, who were recovering from cancer surgery, who had recently received 5-fluorouracil and who were older than the

female volunteers in whom an F value of 0.42 was obtained (Back *et al.*, 1979). These factors would be expected to reduce gut wall metabolism rather than to enhance it and thus lead to an underestimation of the role of the gut.

The absence of a plateau when the amount of drug conjugated is plotted against total amount of drug absorbed (Figure 2) suggests that the metabolism is a first order kinetic process, there being no saturation of the enzyme system. Levy & Matsuzawa (1967) have shown that the sulphating mechanism of the gut wall is easily saturable mainly because 'active sulphate' (3' phosphoadenosine-5' phosphosulphate) is rapidly depleted. If larger amounts of drug had been given in this study, saturation might have been demonstrated.

Previous *in vitro* studies have demonstrated metabolism of oestrogens by the gut wall (Diczfalusy *et al.*, 1961, 1962). Oestriol and oestradiol were metabolised to oestrone and oestradiol glucuronide demonstrating both phase I and phase II metabolism. Knapstein *et al.* (1967a,b) have demonstrated, on the other hand, sulphate conjugation of androgens by the

gut wall, and Mahon *et al.* (1977) directly demonstrated phase I metabolism of flurazepam by the small intestine. More recently, procillaridin has been shown to undergo first-pass inactivation in the gut wall (Anderson *et al.*, 1977).

The study has shown that about two thirds of the first pass effect of ethinyloestradiol appeared to be due to sulphate conjugation in the gut wall. This is of particular importance since sulphate conjugation is saturable (Levy & Matsuzawa, 1967) and suggests that drugs like salicylamide or ascorbic acid may enhance the bioavailability of ethinyloestradiol with potentially adverse effects.

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