Stimulatory as well as inhibitory effects of ethinyloestradiol on the metabolic clearances of propranolol in young women

T. WALLE¹, T. C. FAGAN², U. K. WALLE¹ & M. J. TOPMILLER²

¹Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, South Carolina and ²Departments of Medicine and Pharmacology, University of Arizona College of Medicine, Tucson, Arizona, USA

- 1 The metabolism of a single 80 mg oral dose of propranolol was determined in nine young women before and after administration of ethinyloestradiol alone (EE₂) or in combination with norethindrone (OC).
- 2 Whereas the total clearance of propranolol (2713 ± 404 ml min⁻¹ (mean $\pm$ s.e.mean)) was not significantly altered by either EE₂ (3365 ± 347 ml min⁻¹) or the combined OC (2905 ± 345 ml min⁻¹), significant changes in all three primary metabolic pathways were observed.
- 3 The clearance through side-chain oxidation decreased from 345 ± 55 ml min⁻¹ to 262 ± 33 ml min⁻¹ after EE₂ ($P < 0.05$). A similar reduction of cytochrome P450 metabolism by EE₂ has been observed for other drugs.
- 4 The clearance through glucuronidation increased from 364 ± 61 ml min⁻¹ to 625 ± 117 ml min⁻¹ after EE₂ ($P < 0.01$). Similar stimulation of glucuronic acid conjugation by EE₂ has also been observed for other drugs.
- 5 The clearance through ring oxidation increased from 697 ± 109 ml min⁻¹ to 1280 ± 162 ml min⁻¹ after EE₂ ($P < 0.01$). This observation appears to be a novel finding with EE₂ and cytochrome P450 metabolism.
- 6 The treatment with OC produced changes in propranolol's metabolic clearances which were qualitatively similar to those generated by EE₂.

Keywords ethinyloestradiol propranolol metabolism women oral contraceptives

Introduction

Although a marked sex difference in the metabolism of drugs has long been known to exist in the rat [1, 2], there are rather few drugs for which this has been shown to occur in humans [2]. These drugs include the β -adrenoceptor blocker propranolol, which has a 60–80% higher total clearance in young men compared with young women [3, 4]. This difference is metabolic pathway-specific, leading to higher clearance in men through both side-chain oxidation and glucuronidation but with no statistically significant gender difference in ring oxidation [3] (Figure 1). In further studies, focusing on the importance of sex steroid hormones, it was shown that plasma levels of testosterone were positively associated with the clearances through the gender-dependent pathways in young men; plasma levels of

oestradiol were not associated with differences in the partial metabolic clearances in young women [4, 5].

In the present study we have extended our studies in women to determine the influence of the synthetic hormone ethinyloestradiol (EE₂), alone and in combination with the progestin norethindrone, i.e. as a combined oral contraceptive (OC), on the metabolic clearances of propranolol in young women.

Methods

Subjects

Nine young women (26–41 years of age; 59.3 ± 2.5 kg) participated in the study. Seven subjects were white and

Figure 1 Primary metabolic pathways of propranolol in humans. NLA, α -naphthoxylactic acid; PG, propranolol glucuronide; 4HOP, 4'-hydroxypropranolol. Most of 4HOP is further metabolized to the glucuronide (4HOPG) and sulphate (4HOPS).

two were black. All subjects consumed a normal American diet prior to the study. They had not lost more than 10% of their body weight in the previous 3 months and their body weight was within 20% below and 40% above ideal body weight. All were healthy as determined by medical histories, physical examinations, selected blood chemistry tests, complete blood counts, urinalysis, ECGs and chest X-rays. All had undergone bilateral Fallopian tube ligation prior to the study. There was no evidence of renal disease in the study subjects as evidenced by normal blood urea nitrogen, serum creatinine, creatinine clearance, urinalysis and urine culture, nor of hepatic disease as judged from patient history and normal values on serum glutamic oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin and total serum proteins. No subject was smoking or taking any drugs. None had taken oral contraceptives within the previous 6 months. Urine pregnancy tests and drug screens were performed at the screening visit. The subjects were studied during the second week of the menstrual cycle. Written informed consent was obtained from each subject. The study was approved by the University of Arizona Institutional Review Board for Human Research.

All subjects were studied in a Clinical Research Unit and were maintained on an isocaloric diet (15% protein, 50% carbohydrate, 35% fat) for 2 days prior to and for 24 h after each drug administration. The subjects were studied with no hormone pretreatment (control), after 21 days of pretreatment with 50 $\mu\text{g}/\text{day}$ of EE_2 (Estinyl) and after 21 days of pretreatment with an oral contraceptive (OC), a combination of EE_2 (50 $\mu\text{g}/\text{day}$) and norethindrone acetate (1 mg/day) (Norlestrin 1/50), with the last dose of hormone taken at the same time as the propranolol dose. Single oral 80 mg doses of ($\pm$)-propranolol hydrochloride (Inderal) were administered in the morning (about 07.00 h) after an overnight fast. The subjects did not eat breakfast. Lunch was allowed at 3 h after drug administration. Blood samples were drawn before and 0.5, 1, 1.5, 2, 3, 5, 8 and 12 h

after the dose through separate venepuncture (predose sample) or through an indwelling catheter (subsequent blood samples). Heparinized blood samples were centrifuged and the plasma was kept frozen until analysis. An aliquot of the complete 24-h urine (collected refrigerated with sodium bisulfite) was also kept frozen [3].

One of the nine subjects studied did not complete the EE_2 study phase. Another subject's OC data were excluded due to a subsequently discovered inflammatory condition with markedly elevated α_1 -acid glycoprotein levels with ensuing abnormally high plasma propranolol binding [6].

Analytical methods

Plasma propranolol was measured by gas chromatography/mass spectrometry (GC/MS) as previously described [3]. The main metabolites of propranolol (Figure 1) measured in urine were propranolol glucuronide (PG), α -naphthoxylactic acid (NLA), which is an accurate index of total side-chain oxidation [3, 7], and the glucuronic acid and sulphate conjugates of 4'-hydroxypropranolol (4HOP), which together are accurate markers of total ring oxidation [3, 7]. These metabolites were measured by previously established GC/MS or h.p.l.c. methodology [3].

To minimize inter-assay variability, all samples from each subject were analysed simultaneously. All urine analyses were done in duplicate with a mean between-sample variability of 6%. Standard curves with spiked urine samples were prepared with each batch of patient samples. The correlation coefficients were always 0.990 or better. Similarly, in all plasma analyses aliquots of blank plasma spiked with known amounts of propranolol and d_6 -propranolol were analysed with each batch of samples. The correlation coefficients were 0.995 or better. All concentrations measured both in urine and in plasma exceeded the minimal detectable concentrations by at least 10-fold.

Pharmacokinetic calculations

The area under the plasma concentration-time curve (AUC) for propranolol was calculated by the trapezoidal rule and extrapolated to infinity. The oral clearance (CL_{oral}) was calculated as $\text{Dose}_{\text{oral}}/\text{AUC}_{\text{oral}}$. The partial oral clearances through the primary metabolic pathways (CL_m) were determined as $CL_m = CL_{\text{oral}} \times F_m$, where F_m is the fraction of the dose excreted as metabolite(s) in urine. The use of partial oral clearances in describing the metabolism of propranolol *in vivo* has been discussed previously [3, 7].

Statistical analysis

Due to the non-normal distribution of clearance values, nonparametric methods were employed for data analysis. The Wilcoxon signed-rank test for paired data was used to test for a significant effect of EE₂ and OC administration on the total and partial metabolic clearance values. In a paired comparison, where a 50% change in propranolol clearance by hormone treatment compared with control would be regarded as clinically relevant and the mean difference is estimated to be equal to the standard deviation of the difference, a total number of eight subjects is required for a power index of 2.8 ($\alpha = 0.05$; $\beta = 0.20$) [8]. With the number of subjects employed in this study, there is thus a 95% probability that the observed differences in partial metabolic clearances are truly different and an 80% probability that a 50% change in total clearance would have been detected.

Results

The mean total clearance of propranolol (2713 ml min^{-1} ; $41 \text{ ml min}^{-1} \text{ kg}^{-1}$) (Table 1) in the nine women studied was quite similar to a previous report [4]. The administration of EE₂ led to a 25% increase in the total clearance, which did not quite reach statistical significance ($P = 0.056$). With the progestin added to the EE₂ in the OC pretreatment there was no effect on total propranolol clearance.

When examining the individual partial metabolic clearances of propranolol, EE₂ administration produced highly significant but opposing effects on the metabolic pathways of propranolol (Table 1). Whereas propranolol clearance through glucuronidation (PG) increased by 71% ($P < 0.01$) and through ring oxidation (4HOP sulphate + glucuronide) by 84% ($P < 0.01$), the clearance through side-chain oxidation (NLA) decreased by 24% ($P < 0.05$). After OC administration the clearance through glucuronidation increased by 32% ($P < 0.05$) and through ring oxidation by 65% ($P < 0.05$), while the clearance through side-chain oxidation decreased by 37% ($P < 0.05$). The OC administration had a small, statistically insignificant, inhibitory effect on all clearances compared with the administration of EE₂ alone.

Table 1 Effect of ethinyloestradiol (EE₂) alone or in combination with norethindrone (OC) on the total and partial metabolic clearances of propranolol in young women. Mean values $\pm$ s.e.mean [95% confidence interval for the difference between EE₂ or OC and control] are shown

	Control (<i>n</i> = 9)	EE ₂ (<i>n</i> = 8)	OC (<i>n</i> = 8)
		(ml min ⁻¹)	
Total clearance	2713 $\pm$ 404	3365 $\pm$ 347 [22 to 1100] <i>P</i> = 0.056	2905 $\pm$ 345 [−706 to 1442] NS
Clearance through			
PG	364 $\pm$ 61	625 $\pm$ 117 [97 to 388] <i>P</i> < 0.01	481 $\pm$ 64 [5 to 278] <i>P</i> < 0.05
NLA	345 $\pm$ 55	262 $\pm$ 33 [15 to 176] <i>P</i> < 0.05	218 $\pm$ 31 [1 to 245] <i>P</i> < 0.05
4HOPS + 4HOPG	697 $\pm$ 109	1280 $\pm$ 162 [288 to 829] <i>P</i> < 0.01	1152 $\pm$ 142 [128 to 880] <i>P</i> < 0.05

PG, propranolol glucuronide; NLA, α -naphthoxylactic acid; 4HOPS, 4'-hydroxypropranolol sulphate; 4HOPG, 4'-hydroxypropranolol glucuronide.

Discussion

Whereas plasma levels of the natural oestrogen hormone oestradiol do not appear to be associated with differences in metabolic clearance of propranolol in women [4, 5], the synthetic hormone EE₂ had profound but complex effects on the individual metabolic pathways. Thus, whereas one of the cytochrome P450 pathways (side-chain oxidation) was inhibited by EE₂, the other cytochrome P450 pathway (ring oxidation) was markedly stimulated, as was glucuronidation.

The decreased clearance of propranolol through side-chain oxidation by EE₂ is similar to previous observations with other drugs, which are eliminated by oxidative metabolism, including certain benzodiazepines [9], theophylline [10], caffeine [11], antipyrine [12] and cyclosporine [13]. The gender- and testosterone-sensitive side-chain oxidation of propranolol is mediated by at least two distinct cytochrome P450 (CYP) isoforms, including CYP1A1/1A2 [14–17] and CYP2C19 [18]. These observations, together with our knowledge of the CYP isoforms involved in the metabolism of several of the other drugs mentioned above, suggest that EE₂ has the potential to inhibit not only CYP3A isoforms, the main catalysts of EE₂ metabolism [19], but also multiple other CYP isoforms.

The increased clearance of propranolol through glucuronidation by EE₂ pretreatment is consistent with previous findings for other drugs, which are eliminated by glucuronidation. These drugs include several benzodiazepines [9], paracetamol (acetaminophen) [20], clofibric acid [21] and morphine [22]. The mechanism of this interesting stimulatory effect is not understood.

The increased clearance of propranolol through ring oxidation caused by EE₂ appears to be a novel

observation in humans. To our knowledge there is no example in man where ethinyloestradiol has been shown to stimulate CYP-mediated oxidative metabolism. Although CYP2D6 is involved in the ring oxidation of propranolol [18, 23, 24], other CYP isoforms likely are involved as well. There is, however, precedence for CYP induction by EE₂ in the rat [25]. In that study a very large dose of EE₂ increased the activity of one of the metabolic pathways of cyclosporine two- to fivefold. Immunoblot analysis showed reduction in CYP3A1/3A2 but increase in CYP2C6 content by EE₂. However, cyclosporine was not a substrate for CYP2C6. The levels or activities of several other CYP isoforms such as 2B1/2, 2C11, 2E1 and 2A2 were not affected. Thus, based on our findings in humans, it may be possible that CYP2D1 (rat) and CYP2D6 (human) are inducible by EE₂.

Although the effects observed after OC pretreatment were qualitatively the same as after EE₂ alone, the total as well as the partial metabolic clearances were 10–20% lower. This observation suggests that the progestin present in the OC, i.e. norethindrone, has some general inhibitory effect on propranolol metabolism. Inhibitory effects of norethindrone on drug metabolism have previously been seen both in animals [26] and humans [27].

The therapeutic significance of the present findings with propranolol is modest, as the biochemical effects observed are opposing each other with respect to the clearance of the parent drug. The results with other drugs could, however, be substantial, either inhibitory, as substantiated with the examples above, or stimulatory. The apparently novel finding in this study of a marked stimulatory effect of EE₂ on a CYP-mediated oxidative reaction should deserve further attention, both from biochemical and therapeutic points of view.

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