Heterogeneity of CYP3A isoforms metabolizing erythromycin and cortisol

The *N*-demethylation of erythromycin and 6β-hydroxylation of cortisol are both functions of the glucocorticoid-inducible CYP3A in human liver microsomes. To determine whether 6β-hydroxylation and erythromycin *N*-demethylation are catalyzed by similar or distinct CYP3A isoforms, erythromycin *N*-demethylase activity, as reflected by the recently described ^{14}[C]-erythromycin breath test, was compared with urinary 6β-hydroxycortisol/cortisol ratios, a measure of cortisol 6β-hydroxylase activity, in nine patients. Erythromycin *N*-demethylation varied fourfold and 6β-hydroxycortisol/cortisol ratios varied sevenfold among the subjects; no correlation was found between these activities (r^2 = 0.065). New noninvasive tests of CYP3A strongly suggest cortisol 6β-hydroxylation and erythromycin *N*-demethylation are performed by distinct CYP3A isoforms. (CLIN PHARMACOL THER 1992;51:18-23.)

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The hepatic cytochromes P450 are a supergene family of microsomal hemoproteins that oxidize lipophilic compounds to polar metabolites for excretion into bile and urine. Characterizations of the human liver cytochromes P450 have revealed at least nine distinct gene families.[1] The glucocorticoid-inducible human hepatic CYP3A gene family consist of at least four closely related hemoproteins.[2-9] The hepatic CYP3A superfamily includes family members CYP3A3 (formerly known as HLp), CYP3A4 (hPCN1 or P450NF), CYP3A5 (hPCN3), and CYP3A6 (HLp2 or HFLa). The isoforms CYP3A3 and CYP3A4 metabolize clinically important drugs, including erythromycin, nifedipine, cyclosporine, midazolam, quinidine, troleandomycin, and clotrimazole.[1] Important endogenous substrates, such as testosterone and glucocorticoids, also undergo CYP3A-catalyzed metabolism to their respective 6β-hydroxylated derivatives in humans[4,5] and rats.[10-12]

The two major human liver CYP3A isoforms, CYP3A3 and CYP3A4, are encoded by distinct genes; however, these two genes and proteins are so similar that these forms cannot be reliably distinguished by oligonucleotide hybridization studies[13,14] or immunochemical detection.[15] By use of noninvasive tests of human hepatic CYP3A function, we sought to determine whether identical or distinct isoforms of the CYP3A gene family performed cortisol 6β-hydroxylation and erythromycin *N*-demethylation. These activities were examined by (1) urinary 6β-hydroxycortisol/cortisol ratios and (2) the [^{14}C]-erythromycin breath test, respectively. The urinary 6β-hydroxycortisol/cortisol ratio corrects for changes in glucocorticoid production rates and therefore more closely reflects rates of hepatic cortisol 6β-hydroxylation.[16,17] On the basis of the observation that erythromycin is a specific substrate for the hepatic CYP3A,[6,18] Watkins et al.[18] recently showed that measurement of exhaled $^{14}CO_2$ after administration of [^{14}C]-erythromycin provides a specific and reproducible noninvasive measurement of the amounts of the hepatic immunoreactive CYP3A and erythromycin *N*-demethylase activity in liver mi-

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Table I. Measurement of urinary 6β-hydroxycortisol/cortisol ratios and [^{14}C]-erythromycin metabolism at 60 minutes in subjects with hypertension

Patient No.	*Age (yr)*	*Gender*	*Race*	*Ideal body weight (%)*	*24-Hr urine 6β-OH cortisol (mg/day)*	*Ratio of urinary 6β-OH cortisol/cortisol*	*% Total ^{14}C-erythromycin metabolism (60 min)*	*Medications*
1	45	Female	Black	110	222.7	8.7	4.0	None
2	65	Female	Black	121	158.1	5.3	3.1	Ibuprofen, glyburide hydrochlorothiazide, chlorazepate dipotassium
3	57	Female	White	139	443.5	7.4	2.6	Atenolol, triamterene, hydrochlorothiazide, calcium
4	47	Female	Black	147	360.0	14.2	2.3	Gemfibrozil, atenolol
5	52	Female	Black	174	222.7	28.6	2.0	Propanolol, hydrochlorothiazide
6	50	Female	White	223	386.4	14.6	1.0	Chlorthalidone
7	72	Male	White	97	219.8	9.6	2.1	β-Blocker study drug
8	67	Male	White	115	363.6	6.6	1.9	β-Blocker study drug
9	50	Male	White	185	198.0	3.9	1.7	Diltiazem, atenolol, aspirin

crosomes. Moreover, because erythromycin decreases the clearance of methylprednisolone in humans,[19] it seemed reasonable to propose that cortisol 6β-hydroxylation and erythromycin *N*-demethylation may be performed by the same or similar CYP3A isoform(s). To examine this possibility, the erythromycin *N*-demethylation activity was measured by the [^{14}C]-erythromycin breath test in a convenience sample of nine patients with hypertension whose cortisol 6β-hydroxylase activity was previously characterized. Despite the abundant evidence for a common catalyst for these reactions, we conclude that erythromycin *N*-demethylation and cortisol 6β-hydroxylation proceed by distinct pathways in humans.

MATERIAL AND METHODS

[^{14}C]-*N*-Methyl-erythromycin and Aquasol scintillation cocktail were obtained from Du Pont Diagnostic Imaging Division (North Billerica, Mass.). Methylbenzethonium hydroxide and thymolphthalein were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Patient selection. A group of 28 patients (age range, 45 to 72 years) recently diagnosed with hypertension were prospectively enrolled in the study as paid volunteers. All patients had diastolic blood pressures between 90 and 110 mm Hg on at least two separate occasions. Patients who exhibited cardiac disease, pulmonary disease, or diabetes mellitus were excluded from the study. (All patients underwent determination of plasma renin and aldosterone to exclude primary hyperaldosteronism or hyperreninemic state before inclusion in the study.) Informed consent was obtained from each patient in accordance with a protocol approved by the Medical College of Virginia Committee for the Conduct of Human Research (Richmond, Va.). Each volunteer had a plasma free cortisol determination at 8 AM and a 24-hour urine collection for cortisol, 6β-hydroxycortisol, and creatinine determination. All antihypertensive medications were discontinued for at least 1 month before plasma and urine cortisol and 6β-hydroxycortisol collections. After successful completion of these tests, patients received antihypertensive medications as deemed appropriate by their private physician. The patients were subsequently asked to participate in the [^{14}C]-erythromycin breath test protocol. A convenience sample of nine of the 28 volunteers consented to participate in the [^{14}C]-erythromycin breath test protocol.

Measurement of urinary 6β-hydroxycortisol. Urinary free cortisol was measured at Hazelton Laboratory, Vienna, Va., by radioimmunoassay. Urinary 6β-hydroxycortisol values were determined by the laboratory of P. Saenger by use of a radioimmunoassay.[16] A single patient's urine (patient 9) underwent urinary 6β-hydroxycortisol and cortisol profiling by HPLC analysis, following the method of Kishida and Fukushima.[20]

[^{14}C]-Erythromycin breath test protocol. The [^{14}C]-*N*-methyl-erythromycin breath test protocol was performed in a manner identical to previously published methods.[18] Ideal body weight was determined by comparison with charts obtained from the Society

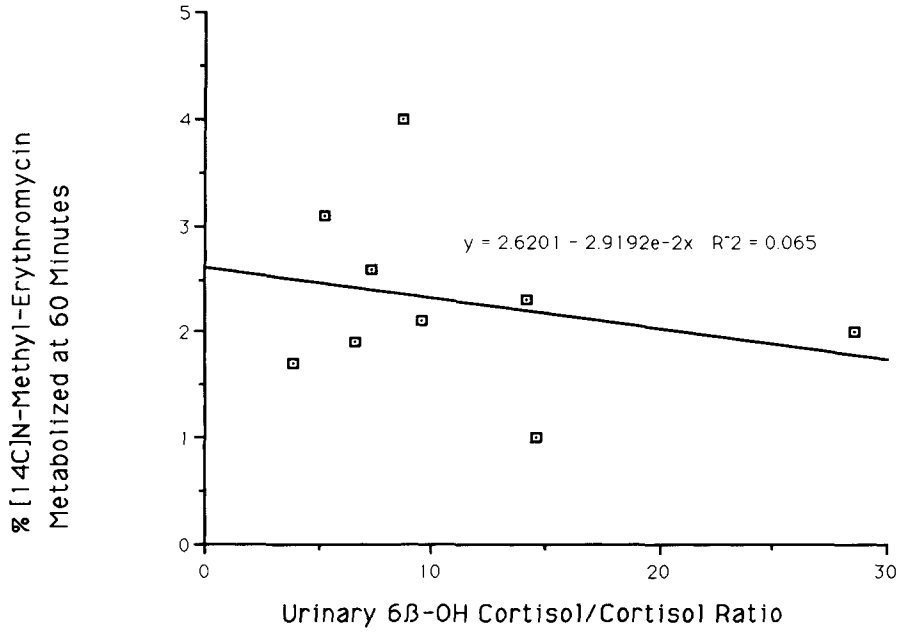

Fig. 1. Nine volunteers underwent a 24-hour urine collection for cortisol, 6β-hydroxycortisol, and creatinine determination. Urinary free cortisol was measured at Hazelton Laboratory, Vienna Va., by radioimmunoassay. Urinary 6β-hydroxycortisol values were determined by the laboratory of P. Saenger by radioimmunoassay.[18] Results were expressed as the ratio of urinary 6β-hydroxycortisol/cortisol. The [^{14}C]-*N*-methyl-erythromycin breath test protocol was performed as described previously.[20] Regression analyses were used to assess the relationship between 6β-hydroxycortisol/cortisol ratios and [^{14}C]-erythromycin clearance.

of Actuaries and Association of Life Insurance Medical Directors of America, 1979 Build Study.[21]

Statistical analyses. Regression analyses were used to assess the relationship between 6β-hydroxycortisol/cortisol ratios and [^{14}C]-erythromycin clearance and to evaluate the effect of the potential confounders of age, gender, race, and percentage of ideal body weight. Differences between group means were assessed with the Student *t* test.

RESULTS

Plasma free cortisol (8 AM) was normal in the nine patients with newly detected hypertension participating in this study (results not shown). From measurements of 6β-hydroxycortisol and of cortisol in 24-hour urine collections obtained in these patients, 6β-hydroxylase activity was estimated as the ratio of urinary 6β-hydroxycortisol and cortisol (6β-hydroxycortisol/cortisol). As described previously, this ratio corrects for changes in glucocorticoid production rates and therefore more closely reflects rates of cortisol 6β-hydroxylation.[16,17] The urinary 6β-hydroxycortisol/cortisol ratios ranged from 3.9 to 28.6 (sevenfold) among the patients (Table I). This range is similiar to previously measured control values in adults.[26] No significant association was found between age, gender, or race and the ratio of urinary 6β-hydroxycortisol/cortisol.

The patients then underwent [^{14}C]-erythromycin breath test analyses between 9 and 11 AM (within 2 hours of breakfast). [^{14}C]-Erythromycin *N*-demethylation curves revealed a fourfold difference in the $^{14}CO_2$ production among patients. Of the total administered dose of [^{14}C]-*N*-methyl-erythromycin, 1.0% to 4.0% was detected as $^{14}CO_2$ exhaled in the first hour (Table I). The amount of $^{14}CO_2$ exhaled after 2 hours was 2.0% to 4.9% of the total administered dose. This range of values is not significantly different from published results of [^{14}C]-erythromycin breath tests carried out on subjects without hypertension.[18] The [^{14}C]-erythromycin breath test results, expressed as a percentage of total dose of [^{14}C]-*N*-methyl-erythromycin metabolized at 60 minutes, and the urinary 6β-hydroxycortisol/cortisol ratio showed no statistical association (Fig. 1; $r^2 = 0.065$).

Of the medications being taken by the participants,

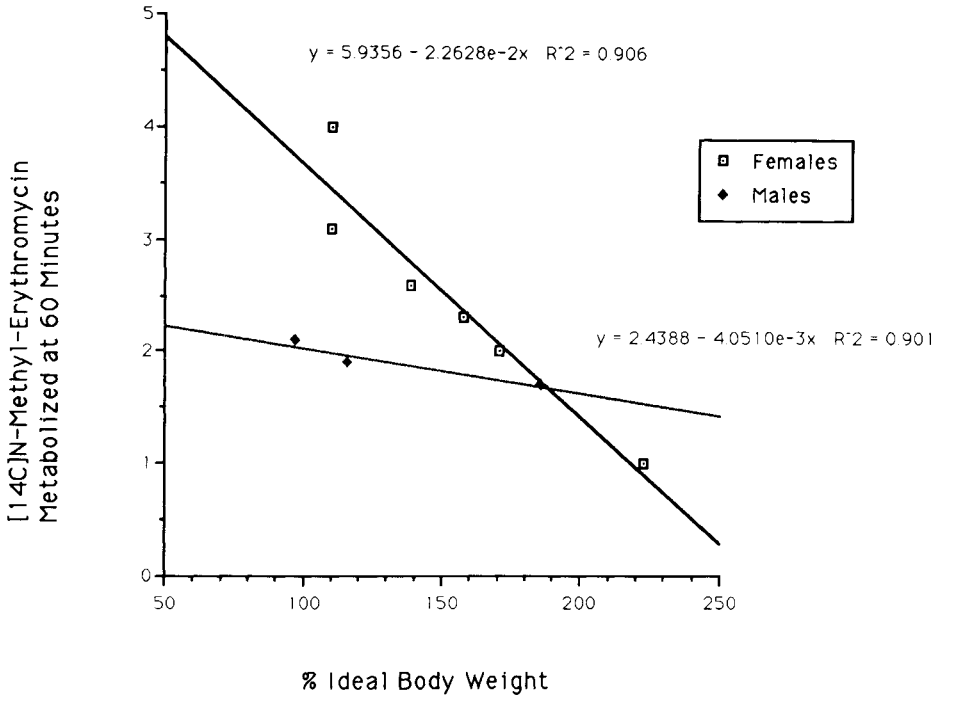

Fig. 2. The [^{14}C]-*N*-methyl-erythromycin breath test protocol was performed as described previously[20] in nine volunteers. Ideal body weight was determined by comparison with charts obtained from the Society of Actuaries and Association of Life Insurance Medical Directors of America, 1979 Build Study.[23] Regression analyses were used to assess the relationship between [^{14}C]-erythromycin *N*-demethylation and percentage of ideal body weight, when examined by gender.

only the diltiazem ingested by patient 9 could potentially interfere with this study by competitively inhibiting CYP3A and reducing $^{14}CO_2$ production.[23] Patient 9 displayed the lowest [^{14}C]-erythromycin metabolism of the men tested and the lowest urinary 6β-hydroxycortisol/cortisol ratio.

No significant gender-dependent difference in erythromycin *N*-demethylation was detected in this small patient population ($p = 0.36$). However, it was observed that percentage of ideal body weight was significantly negatively associated with the metabolism of [^{14}C]-*N*-methyl-erythromycin at 60 minutes in both men and women (Fig. 2; $r^2 = 0.91$ in women; $r^2 = 0.90$ in men). When the effect of percentage of ideal body weight was compared with the [^{14}C]-*N*-methyl-erythromycin metabolism at 60 minutes in the group as a whole, the correlation was less remarkable ($r^2 = 0.46$). Even though the single obese male patient was also receiving treatment with diltiazam, a calcium channel blocker metabolized by CYP3A,[23] exclusion of this subject from the analyses still left a negative correlation between [^{14}C]-erythromycin *N*-demethylation and percentage of ideal body weight ($r^2 = 0.42$). However, when urinary 6β-hydroxycortisol/cortisol ratios were compared with percentage of ideal body weight, this ratio was not found to show an association ($r^2 = 0.15$).

DISCUSSION

The most striking finding was the lack of correlation ($r^2 = 0.065$) between our estimates of 6β-hydroxylation and of erythromycin *N*-demethylation in each subject, even though these catalytic activities are supported by CYP3A-related isoforms in human liver microsomes.[5,6,18] This suggests that closely related but separate CYP3A isoforms in the liver mediate these two activities. Studies of an anhepatic man strongly support the conclusion that the [^{14}C]-erythromycin breath test largely reflects CYP3A4-catalyzed erythromycin *N*-demethylation in the liver.[24] However, extrahepatic CYP3A-related cytochromes have been detected immunohistochemically in human kidney, small intestine, polymorphonuclear leukocytes, pancreas, gall bladder, skin, testes, and ovaries[25] and could account for a significant amount of urinary excretion of 6β-hydroxycortisol.

The [^{14}C]-erythromycin breath test, performed in this small group of patients with hypertension exhibited similiar rates of [^{14}C]-*N*-methyl-erythromycin metabolism at 60 and 120 minutes to those obtained for a hospitalized population.[18] A fourfold range of values was seen in both patient groups. Patients with hypertension exhibited a range of values (sevenfold variation) in urinary 6β-hydroxycortisol/cortisol ratios similar to results from patients without hypertension.[26] Preliminary studies had revealed increased hepatic cortisol 6β-hydroxylase activity, as reflected by urinary 6β-hydroxycortisol/cortisol ratios,[16] in a subset of patients with essential hypertension, most of whom were black (Watlington CO, Wright JT, Grogan WM. Unpublished observations, July 1990). However, despite the suggestion that 6β-hydroxycortisol evokes hypertension,[27-29] the measured parameters of 6β-hydroxycortisol production did not support this hypothesis within this group of patients with hypertension. This may be attributable to the wide variety of etiologic factors that contribute to hypertension, in addition to 6β-hydroxycortisol, as well as a sampling artifact attributable to the small size of our patient population.

The [^{14}C]-erythromycin *N*-demethylation activity showed a strong negative association with percentage of ideal body weight but no association with 6β-hydroxylase activity. This finding suggests the intriguing possibility that specific CYP3A isoforms may decrease in obese subjects. It would be desirable to confirm this novel observation with additional techniques to exclude such alternative explanations as an expansion of the body's formaldehyde pool, resulting in a lower apparent rates of $^{14}CO_2$ production. Increases in total hepatic cytochrome P450 content, as well as the ethanol-inducible CYP2E1, have recently been described in a strain of obese rats.[30] The possibility that obesity resulted in the reciprocal repression of other isoforms, a commonly observed effect of induction, was not examined. The present approach of using noninvasive methods holds promise as a way to elucidate the roles that obesity and other previously unrecognized factors play in explaining interindividual variations in the expression of the human liver cytochromes P450.

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