

two in the management of esophageal and gastric varices.

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OMEPRAZOLE INDUCTION OF CYTOCHROME P-450IA2: THE IMPORTANCE OF SELECTING THE APPROPRIATE HUMAN MODEL

Rost KL, Brösicke H, Brockmöller J, Scheffler M, Helge H, Roots I. Increase of cytochrome P450IA2 activity by omeprazole: Evidence by the ^{13}C -[N-3-methyl]-caffeine breath test in poor and extensive metabolizers of S-mephenytoin

Omeprazole has been shown to induce cytochrome P450IA1 and P450IA2 activity *in vitro*. To reflect cytochrome P450IA2 (CYPIA2) activity *in vivo*, the ^{13}C -[N-3-methyl]-caffeine breath test was conducted in 18 volunteers: 12 extensive metabolizers, one intermediate metabolizer, and five poor metabolizers of S-mephenytoin. Breath tests were performed before treatment with an oral dose of 40 mg omeprazole, on the seventh day of treatment, and after a 7-day washout period. The mean percentage exhalation of the ^{13}C test dose, as determined by $^{13}\text{CO}_2$ in breath during 8 hours, was $23.0\% \pm 8.0\%$ ($n = 18$) before

treatment. The largest increases in exhalation rate of $^{13}\text{CO}_2$ were observed in the poor metabolizers and the intermediate metabolizers (range, 12.8% to 62.9%; median, 38.9%); median area under the plasma concentration-time curves (AUC) of omeprazole was four times higher than in the extensive metabolizers. The change after omeprazole treatment in extensive metabolizers ranged from -9.8% to +47.7% (median, 12.3%; $n = 12$) of pretreatment values. In both groups, exhalation rates of $^{13}\text{CO}_2$ returned to near pretreatment values within the 7-day washout period ($24.2\% \pm 7.8\%$; $n = 17$). Changes in the ^{13}C -caffeine breath test correlated well with both the pretreatment value $R = -0.67$, $p = 0.003$; $n = 18$) and the plasma AUC of omeprazole ($R = 0.61$, $p = 0.007$; $n = 18$). Therapeutic doses of omeprazole seem to induce CYP1A2 activity in poor metabolizers, whereas they exert minor inducing effects in extensive metabolizers of S-mephenytoin. *CLIN PHARMACOL THER* 1992;52:170-180.

COMMENTS

Omeprazole is a widely prescribed inhibitor of gastric acid secretion. Almost 3 yr ago, Diaz et al. (1) showed that omeprazole stimulates *de novo* synthesis and increases in the catalytic activity (induction) of a specific cytochrome P-450 enzyme, termed P-450IA2, in primary human hepatocytes maintained in culture. These investigators also performed liver biopsies on five patients before and after each had received therapeutic doses of omeprazole for 4 days (1). They reported that this treatment was associated with a fourfold - eightfold increase in induction in P-450IA2 protein and catalytic activity in the liver of each patient. The investigators concluded that therapeutic doses of omeprazole caused significant induction of liver cytochrome P-450IA2. This conclusion generated concern in some quarters because cytochrome P-450IA2 is the major aryl hydrocarbon hydroxylase in human liver. Aryl hydrocarbon hydroxylase activity has been associated with carcinogenesis; hence induction of cytochrome P-450IA2 could theoretically predispose patients treated with omeprazole to certain cancers. (The article by Diaz et al. has been reviewed in *Hepatology Elsewhere* [2]).

The clinical relevance of the studies by Diaz et al. were called into question on the basis of data recently reported by Andersson et al. (3). These investigators administered an oral dose of caffeine to 10 normal volunteers before and after each received 1 wk of treatment with omeprazole. After each caffeine dose, the investigators measured the production of specific caffeine metabolites in the subjects' urine and then calculated a metabolite ratio to reflect *in vivo* cytochrome P-450IA2 activity (4). They found that omeprazole treatment did not significantly alter this ratio in the volunteers. Andersson et al. therefore concluded that omeprazole treatment did not cause significant induction of hepatic cytochrome P-450IA2 activity in the volunteers.

The study under review provides a new twist to the story. Rost et al. designed their study on the basis of the

In this article, P-450IA2, P-450IIC and P-450IIIA correspond to the proteins encoded by the CYP1A2, CYP2C and CYP3A genes, respectively (12).

observation that omeprazole is chiefly metabolized by an enzyme in the cytochrome P-450II gene family, mephenytoin hydroxylase (5). Mephenytoin is an anticonvulsant administered to patients as a racemic mixture of enantiomers. It has been shown that the ability to hydroxylate *S*-mephenytoin (in the 4-position) is greatly diminished in approximately 5% of whites and in 20% of Asians (6). Although the specific gene corresponding to mephenytoin hydroxylase has not yet been identified, the "poor metabolizer" phenotype is inherited as an autosomal recessive trait, suggesting a single genetic locus for the trait. To determine mephenytoin metabolizer phenotype, a patient is given a small oral dose of racemic mephenytoin, and the ratio of R and S enantiomers of the parent drug is measured in urine (7).

When the mephenytoin poor metabolizer takes a therapeutic dose of omeprazole, the subsequent blood concentrations of the parent drug are significantly higher than those observed in extensive metabolizers (5). Rost et al. logically speculated that poor metabolizers are particularly susceptible to cytochrome P-450IA2 induction by omeprazole. Thus they determined the mephenytoin metabolizer phenotype of all subjects before enrolling them in the study and intentionally included five poor metabolizers in the group. They again used caffeine as a probe to determine cytochrome P-450IA2 activity in the patients before and after treatment with omeprazole (40 mg/day for 1 wk). They administered an oral dose of [¹³C N-3-methyl] caffeine and then measured the production of ¹³CO₂ in the breath. The *in vivo* reaction detected by this breath test (3-demethylation) has been shown to be catalyzed mainly by cytochrome P-450IA2 in the liver (8). The results of this breath test should therefore reflect liver cytochrome P-450IA2 activity. The investigators found that omeprazole treatment did not cause a significant increase in the mean breath excretion in the extensive mephenytoin metabolizers. However, a significant increase was observed among the poor metabolizers. As expected, the poor metabolizers had higher omeprazole blood levels than did the extensive metabolizers, and the magnitude of increase in the breath test results was correlated with the mean omeprazole blood levels observed during the study. Rost et al. concluded that significant induction of cytochrome P-450IA2 activity occurs in mephenytoin poor metabolizers.

This conclusion is not at odds with the data of Andersson et al. (3). Blood levels of omeprazole were measured in the subjects studied by Andersson et al.; they indicate that no mephenytoin poor metabolizers were present in the group. In addition, the daily dose of omeprazole used by Andersson et al. was 20 mg/day, half that used by Rost et al. These two facts probably account for why no induction was observed by Andersson et al. The human hepatocyte experiments of Diaz et al. (1) may also be explained by the fact that the concentrations of omeprazole in the culture media were high relative to those attained *in vivo*. However, the observations made by Diaz et al. in the five patients who underwent serial liver biopsies remain unexplained. These patients re-

ceived 20 mg omeprazole/day for 4 days and, although their mephenytoin metabolizer phenotypes were not assessed, it is statistically unlikely that all five were poor metabolizers. One possibility is that omeprazole produces induction of cytochrome P-450IA2 in all patients but the induction is of magnitude sufficient to be detected by *in vivo* caffeine assays only in poor metabolizers. Consistent with this idea is the fact that the baseline caffeine urinary ratios and caffeine breath test results varied less than fourfold and sixfold, respectively, among the volunteers in the two studies. It is believed that hepatic cytochrome P-450IA2 concentrations vary up to 60-fold in patient populations (9). The relatively narrow range of caffeine assay results suggests that the *in vivo* caffeine measurements may not be very sensitive. It is therefore possible that fourfold - eightfold induction in liver cytochrome P-450IA2 activity, as observed by Diaz et al., occurs in the extensive metabolizers but is undetectable by the caffeine assays.

Rost et al. suggested that physicians consider performing mephenytoin phenotyping on patients receiving therapy with omeprazole. A means of performing mephenytoin phenotyping is not currently available to most physicians prescribing omeprazole, making the point moot for the moment. It should also be noted that the health risks associated with cytochrome P-450IA2 induction are still theoretical and controversial (2); therefore the need for such tests is unclear at this time. On the other hand, it is unlikely that questions surrounding the health risks of cytochrome P-450IA2 induction will be resolved to everyone's satisfaction any time soon. Now that the Rost study has appeared, long-term liability issues, particularly in mephenytoin poor metabolizers who receive omeprazole therapy outside FDA recommendations (such as the long-term treatment of patients with severe esophageal reflux disease) may become important. In addition, there should be no "down side" to dose reduction in poor metabolizers once they are identified. At standard doses, the mephenytoin poor metabolizer is probably getting more omeprazole than is necessary to suppress stomach acid production.

For the reasons outlined above, many physicians would probably consider determining mephenytoin phenotype in their patients receiving omeprazole if a convenient, safe, inexpensive means of doing this were available. This could be done in several ways. Simple monitoring of omeprazole blood levels at the start of therapy, perhaps after the initial single oral dose, should identify the poor metabolizer. This would, in effect, be using omeprazole as the "probe" for the mephenytoin hydroxylase and would save the patient exposure to a second drug. However, omeprazole blood assays are not widely available. For the future, the most attractive tests are genetic. For example, it is now possible to identify patients with the poor metabolizer phenotype of another cytochrome P-450 (P-450IID6) with genotyping assays of routine venous blood samples (11). It should be possible to design similar tests for mephenytoin hydroxylase when the relevant gene defects are identified. It

seems likely that such tests would be useful in the use of drugs other than omeprazole. Some commonly used medications are already known to be metabolized by the mephenytoin hydroxylase (10), and it seems likely that many others exist. The presence of the poor metabolizer genotype, which should not change over time, could therefore alert the physician to enhanced susceptibility to toxicity by other drugs. This technology may therefore reduce the need for blood level monitoring (and hence specific assays) for such drugs. Many investigators believe that noninvasive tests of specific cytochromes P-450, and probably of other important metabolic pathways, will become a routine part of clinical practice in the future.

The manuscript by Rost et al. sends an important message to the pharmaceutical industry. Many pharmaceutical companies are developing the technology to identify the major liver cytochromes P-450 responsible for metabolizing drugs they have in development. This knowledge appears to be useful in the design of pre-clinical studies. For example, rabbits and rats appear to have liver cytochrome P-450III levels comparable to those observed in the average human being. Hence the rabbit may be the more appropriate preclinical model for the study of a drug determined to be chiefly metabolized by cytochrome P-450III. The study by Rost et al. points out an additional important reason why knowledge of which cytochromes P-450 metabolize specific drugs should be important in drug development. This knowledge should help identify the subpopulations, or the appropriate "human model," to test potential toxic responses to drugs. As Rost et al. point out, even in a large clinical study an induction effect on cytochrome P-450IA2 could be missed if the susceptible subpopulation was not recognized and intentionally included in the study.

In summary, Rost et al. provide good evidence that omeprazole is capable of inducing hepatic cytochrome P-450IA2 activity, at least in an identifiable subset of patients. The work reminds us that, although we would all prefer patients to be homogeneous in terms of drug response, they are not. The characterization of individual cytochromes P-450 and of other metabolic pathways and the development of safe, inexpensive and noninvasive means of assessing the activity of these enzymes in patients should improve the drug development process and the rational delivery of medication therapy to patients.

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BIDIRECTIONAL TRANSPORT OF GLUTATHIONE IN HEPATOCYTE MEMBRANES: DOES GLUTATHIONE EFFLUX STIMULATE ORGANIC ANION UPTAKE?

Garcia-Ruiz C, Fernández-Checa J.C., Kaplowitz N. Bidirectional mechanism of plasma membrane transport of reduced glutathione in intact rat hepatocytes and membrane vesicles

ABSTRACT

We determined the *trans* effects of extracellular reduced glutathione (GSH) on the rate of efflux of endogenous labeled GSH from freshly isolated rat hepatocytes. The presence of GSH (10 mM) in the medium significantly stimulated the fractional rate of efflux of [³⁵S]GSH from 5.2 to 12.6%/15 min ($p < 0.01$). This effect was concentration-dependent, had sigmoid type of kinetics (D_{50} of 0.32 mM), and was reversible upon removal of external GSH. *trans*-Stimulation (counter-transport) was also observed with 5 mM oxidized glutathione (GSSG) and ophthalmic acid (fractional [³⁵S]GSH efflux: 13.4% ± 4.1 and 8.8% ± 2.3 in 15 min, respectively, compared with control: 4.7 ± 2.5/15 min). Bromosulphthalein-glutathione (BSP-GSH, 5 mM) in Krebs buffer inhibited the fractional [³⁵S]GSH efflux (1.1%/15 min), whereas in Cl⁻-free buffer, GSH efflux was stimulated (14.2%/15 min) compared with control. *trans*-Stimulation was independent of chloride. BSP-GSH *cis*-inhibited and *trans*-stimulated the initial rate of GSH transport in basolateral-enriched membrane vesicles (bLPM) but not in canalicular-enriched membrane vesicles (cLPM). γ -Glutamyl compounds also *cis*-inhibited and