PHARMACOKINETICS

Increased drug delivery to the brain by P-glycoprotein inhibition

Background: **Although the antidiarrheal loperamide is a potent opiate, it does not produce opioid central nervous system effects at usual doses in patients. On the basis of in vitro studies demonstrating that loperamide is a substrate for the adenosine triphosphate–dependent efflux membrane transporter P-glycoprotein, we postulated that inhibition of P-glycoprotein with quinidine would increase entry of loperamide into the central nervous system with resultant respiratory depression.**

Methods: **To test this hypothesis, a 16-mg dose of loperamide was administered to eight healthy male volunteers in the presence of either 600 mg quinidine, a known inhibitor of P-glycoprotein, or placebo. Central nervous system effects were measured by evaluation of the respiratory response to carbon dioxide rebreathing as a measure of opiate-induced respiratory depression.**

Results: **Loperamide produced no respiratory depression when administered alone, but respiratory depression occurred when loperamide (16 mg) was given with quinidine at a dose of 600 mg ($P < .001$). These changes were not explained by increased plasma loperamide concentrations.**

Conclusion: **This study therefore demonstrates first the potential for important drug interactions to occur by a new mechanism, namely, inhibition of P-glycoprotein, and second that the lack of respiratory depression produced by loperamide, which allows it to be safely used therapeutically, can be reversed by a drug causing P-glycoprotein inhibition, resulting in serious toxic and abuse potential. (Clin Pharmacol Ther 2000;68:231-7.)**

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The ability of one drug to alter the effects of another, simultaneously administered drug is now a well-recognized mechanism for interindividual variability in drug response. The occurrence of such drug interactions may result in adverse drug effects.

Such drug interactions often involve one drug enhancing or impairing the other's elimination. Examples of such interactions are seen when drugs that are metabolized by the same cytochrome P450 are coadministered and interfere with one another's metabolism, resulting in elevated plasma drug concentrations and increased pharmacologic effect.[1-7]

Conversely, the increased metabolism of one drug produced by another, such as occurs after enzyme induction, may result in reduced drug concentration and effect.[8,9] Recently it has been recognized that resistance to some anticancer drugs develops as a result of the increased expression of the adenosine triphosphate–dependent efflux membrane transporter P-glycoprotein in cancer cells, resulting in multidrug resistance.

The P-glycoprotein pump is responsible for limiting absorption of a number of drugs from the gastrointestinal tract and entry of drugs into the central nervous system (CNS). Drugs excluded by P-glycoprotein include antineoplastic drugs, immunosuppressive drugs, and human immunodeficiency virus protease inhibitors.[10]

Loperamide is a potent opiate that reduces gut motility by its action at opiate receptors in the gut; however,

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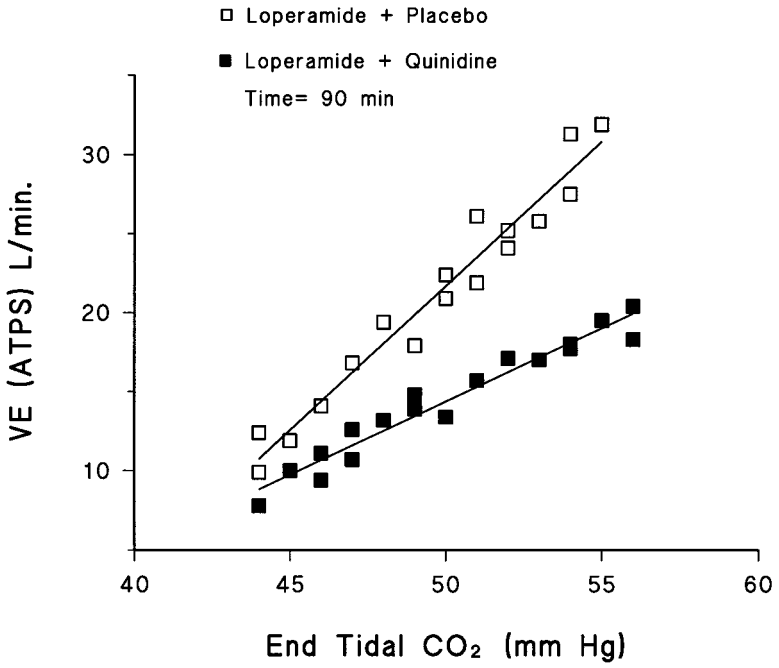

Fig 1. Change of ventilatory response with increasing end-tidal carbon dioxide after administration of loperamide with placebo *(open boxes)* and quinidine *(solid boxes)*. A representative measurement at a single time point (90 minutes) from one of the volunteers.

at usual[11] or even high clinical doses,[12,13] it is not associated with central opiate effects such as respiratory depression, although such effects have been seen in neonates and after overdose.[14,15] This apparent tissue selectivity reflects the fact that loperamide is a substrate for P-glycoprotein, so that normally it does not gain access to the CNS. In P-glycoprotein knockout mice, doses of loperamide that are normally without effect in wild type mice are lethal.[16]

A number of drugs that are currently in clinical use, including quinidine, verapamil, and ketoconazole, inhibit P-glycoprotein. Attempts have been made to use them therapeutically to increase intracellular concentrations of antineoplastic agents after the emergence of drug resistance as a result of induction of P-glycoprotein expression. Because these agents have low potency as P-glycoprotein inhibitors and have other effects such as inhibition of drug metabolism, efforts have been made to identify more potent and selective agents for the treatment of cancer.[17-20] However, use of such agents may also unexpectedly increase drug concentrations and drug effects in tissues other than the target tissue.

We postulated that inhibition of P-glycoprotein with quinidine would increase entry of loperamide into the CNS with resultant respiratory depression.[21] To evaluate such depression the respiratory response to CO_2 rebreathing was used.[21]

MATERIAL AND METHODS

After approval by the Vanderbilt University Committee for the Protection of Human Subjects, written informed consent was obtained from eight healthy white male volunteers aged 25 to 44 years (weight 200 ± 24.96 lbs, mean ± SD). Subjects had no clinically significant abnormality on routine history, physical examination, or routine laboratory tests of hepatic and renal function, and none were receiving regular medication. All subjects were instructed to abstain from medications including over-the-counter drugs, alcohol, and nicotine for at least a week before beginning and throughout the entire study period. The volunteers received a similar diet from the Vanderbilt Clinical Research Center for 3 days before each study and were acclimatized to the rebreathing equipment and all procedures used in this study before the actual study days.

Study design. Each subject was studied on 2 different days separated by at least 2 weeks. The study drugs were administered as identical-looking capsules. The subjects received either quinidine or placebo on each of the study days in a random double-blind fashion. The

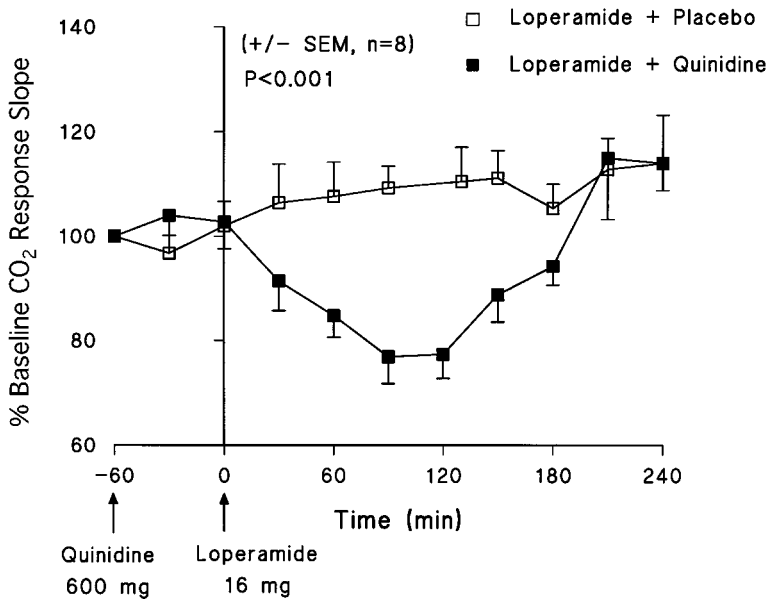

Fig 2. Effect of quinidine on slope of the carbon dioxide response curve after administration of loperamide after placebo *(open boxes)* or quinidine *(solid boxes)*.

blinding was maintained until the study had been completed and the recorded data were analyzed. On the morning of each study day, after an overnight fast and 3 days' abstention from caffeine-containing food and beverages, the subjects received either placebo or 600 mg quinidine sulfate in random order. One hour later, a single dose of 16 mg loperamide hydrochloride (4 capsules × 4 mg each) was administered. No food or drink was permitted from midnight until 6 PM on the study day. The subjects remained in the study room for the entire 12-hour study period on each study day. Standardized, caffeine-free meals were provided at the end of the study. An electrocardiogram was recorded every 30 minutes throughout the study and 1 to 2 hours after the study was completed before the subjects were allowed to leave. Loperamide concentrations were measured on blood samples obtained through an indwelling catheter before drug administration and hourly for 6 hours thereafter. Plasma was separated immediately and kept frozen at –20°C until analysis.

Measurements of ventilatory response to carbon dioxide. The respiratory response to loperamide after administration of quinidine was evaluated by measurement of end-tidal carbon dioxide content and the ventilatory response to the increasing concentration of carbon dioxide produced by rebreathing into a closed circuit.[21] The subjects were studied after sitting comfortably for 10 to 15 minutes. They then breathed through a mouth piece (while wearing a nose clip) connected by way of a three-way valve to a balloon containing about 1.5 times their vital capacity of a 50% nitrogen/50% oxygen mixture. Standard breathing tubes connected the mouthpiece to a computerized exercise module (Med Graphics, St Paul, Minn) equipped with a pneumotachometer and an infrared CO_2 analyzer. The increasing concentration of carbon dioxide produced by rebreathing stimulated progressive hyperventilation. The tidal volume, ventilatory rate, and end-tidal carbon dioxide levels were measured continuously. Rebreathing continued for 3 to 4 minutes until the end-tidal carbon dioxide level reached approximately 55 mm Hg. Measurements were made every 30 minutes throughout the study period. The balloon was refilled with fresh gas, and the instrument was recalibrated before each carbon dioxide response curve was performed.

Measurement of loperamide and its metabolite in plasma. Loperamide and its main metabolite monodesmethyl loperamide were obtained from Janssen Pharmaceutical (Beers, Belgium). Loperamide and the metabolite were measured by liquid chromatography–tandem mass spectrometry using positive electrospray ionization (+ESI) as described elsewhere in detail.[22]

Data analysis. The minute ventilation measured every 10.0 seconds was plotted against the respective

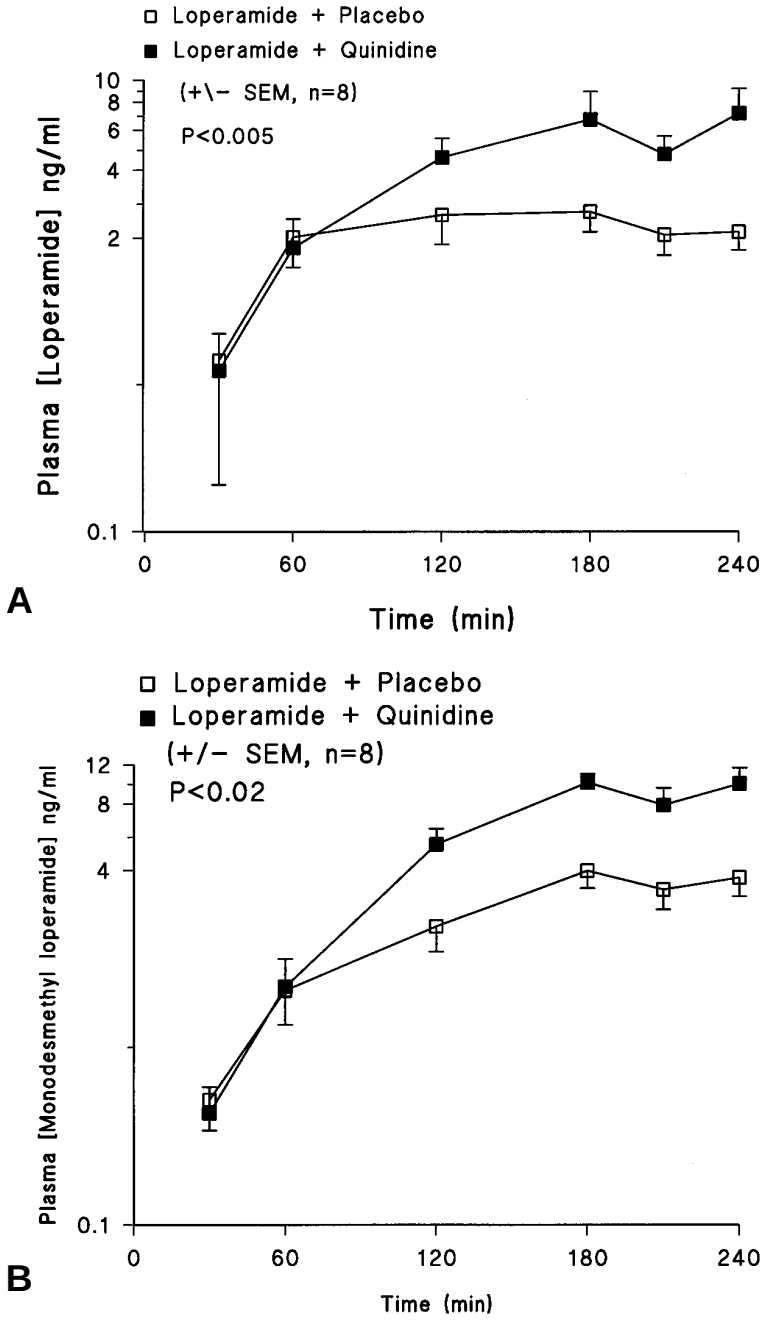

Fig 3. Concentration of loperamide **(A)** and monodesmethyl loperamide **(B)** with placebo *(open boxes)* or quinidine *(solid boxes)*. At the 30-minute time point, plasma loperamide n = 4 and monodesmethyl loperamide n = 3, because only these were greater than the lower limit of detection at this time point.

end-tidal carbon dioxide tension, and the slope was calculated by least-square linear regression analysis. The area under the pharmacodynamic effect/time curve was measured for the first 240 minutes after the administration of loperamide [AUC(0-240 min)] by the trapezoidal rule. The first 240 minutes was chosen because all patients had returned to baseline respiratory function by then. Plasma concentrations of loperamide and

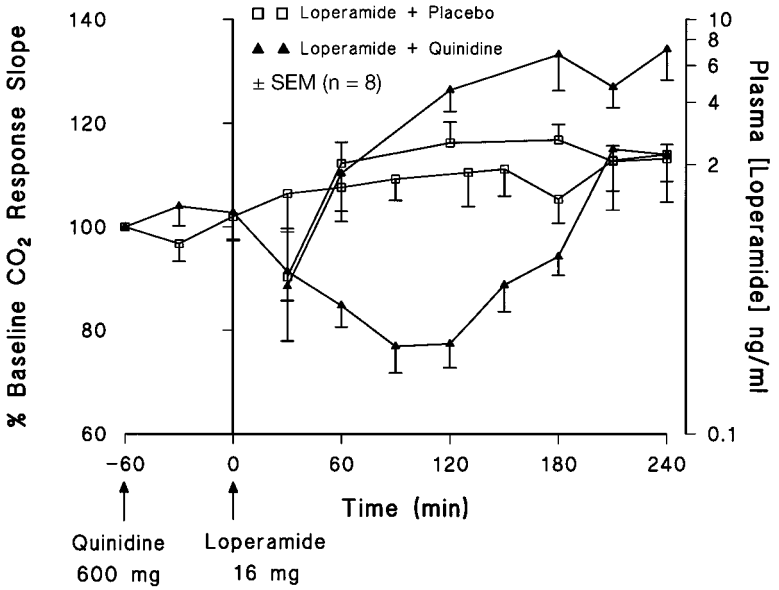

Fig 4. Comparison of pharmacodynamics and pharmacokinetics of loperamide after administration of placebo *(open boxes)* or quinidine *(solid triangles)*.

its metabolite were plotted semilogarithmically against time, and the area under the concentration-time curves was calculated by the log trapezoidal rule [AUC(0-240 min)]. Statistical comparisons were performed by two-tailed paired *t* testing.

RESULTS

Effect of quinidine on carbon dioxide response slope. Loperamide was administered 1 hour after quinidine administration to all eight subjects. When loperamide was administered after placebo in the absence of quinidine, there was no evidence of impairment of the CO_2 response. The effects of loperamide on the ventilatory response to increasing concentrations of CO_2 after administration of both placebo and quinidine are presented in a representative subject in Fig 1. It is apparent that the coadministration of quinidine along with loperamide impaired the respiratory response to CO_2 compared with loperamide alone. The slope of the ventilatory/CO_2 response slope plotted over time in all subjects (Fig 2) showed impairment of the response to CO_2 ($P < .001$), which occurred within 30 minutes of loperamide administration. This respiratory depression after administration of quinidine lasted for more than 2 hours.

The administration of quinidine increased the plasma concentrations of loperamide and its metabolite compared with placebo (Fig 3), resulting in increased areas under the loperamide and metabolite plasma concentration time curves (AUC) of 99.55 ± 20.31 versus 247.0 ± 45.25 ng/mL · hr ($P < .005$) for loperamide and 149.15 ± 39.30 versus 289.55 ± 49.39 ng/mL · hr ($P < .02$) for metabolite, after administration of placebo and quinidine, respectively.

To determine whether the impaired respiratory response to CO_2 could be explained simply by the increase in plasma loperamide concentrations, the change in the CO_2 response and the plasma drug concentrations was superimposed (Fig 4). It is apparent that up to at least 60 minutes, when the plasma loperamide concentrations after administration of quinidine and placebo were identical, there was substantial impairment of ventilatory response to CO_2 after quinidine but not placebo, thus demonstrating that coadministration of quinidine with loperamide produces respiratory depression independent of changes in plasma concentrations.

DISCUSSION

We found that the inhibition of P-glycoprotein by quinidine resulted in respiratory depression after administration of loperamide that was not seen after administration of loperamide alone. Loperamide is a potent opiate that is widely available without prescription as an antidiarrheal. Absence of analgesic efficacy, respiratory depression, or impairment of CO_2 response reflects its normal lack of CNS effects, which appears to be due to the fact that it is a P-glycoprotein substrate that is so efficiently removed from the CNS by the P-glycoprotein efflux pump that pharmacologically effective concentrations are not normally achieved in the CNS.[16] In

wild-type mice that express P-glycoprotein normally, loperamide does not enter the brain in sufficient concentrations to produce characteristic opiate effects; however, in P-glycoprotein knockout mice, which do not express P-glycoprotein, loperamide administration results in classic opiate effects that may be lethal.[16]

P-glycoprotein is expressed in multiple tissues, including the small intestine, where it limits drug absorption. Inhibition of P-glycoprotein in the intestine would therefore be expected to increase drug absorption and plasma drug concentrations. Such an increase in plasma drug concentrations was seen in our study. To exclude increased plasma drug concentrations as the explanation for loperamide's effects after administration of quinidine, we examined the relationship between plasma drug concentration and effect (Fig 4). It was clear from that examination that the respiratory depressant effects of loperamide occurred at times when plasma loperamide concentrations were not increased, demonstrating that a simple increase in plasma drug concentrations could not explain the effect. It is unlikely that inhibition of loperamide's metabolism alone explains the increased plasma concentrations after administration of quinidine. In vitro studies have demonstrated that loperamide is principally metabolized to monodesmethyl loperamide by CYP3A so that inhibition of CYP3A would be expected to decrease this metabolite concentration. In contrast, the concentration of metabolite was increased by quinidine. Second, quinidine is a relatively selective inhibitor of CYP2D6, only inhibiting CYP3A in vitro at much higher concentrations than are likely to be achieved in vivo.[23] Finally we have previously shown that administration of quinidine alone does not impair respiration.[24]

The demonstration of potentially dangerous CNS effects of such a widely used drug because of a novel drug interaction mechanism is of great interest because it raises both safety concerns and demonstrates a novel strategy to overcome the blood-brain barrier to increase therapeutic drug delivery to the brain. Quinidine has previously been shown to be a major cause of drug interactions with digoxin.[25] The explanation for this interaction that results in substantial increase in plasma digoxin concentrations was, in the past, unclear. However, the recent recognition that digoxin is a P-glycoprotein substrate and that coadministration of quinidine increases plasma digoxin concentration in mice[25] demonstrates that P-glycoprotein inhibition is the likely explanation for these digoxin interactions. Other P-glycoprotein inhibitors such as verapamil also increase digoxin concentrations in patients presumably by the same mechanism.

Thus in conclusion this study has demonstrated that it is possible to increase drug penetration into the brain by P-glycoprotein inhibition, resulting in central effects from the opiate, loperamide, whose effects are normally restricted to the gut. Undoubtedly further such interactions will be recognized in the future, with both potential toxic and therapeutic results.

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