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High Pressure Liquid Chromatographic Analysis of Metoclopramide in Serum

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Abstract: Metoclopramide has recently been approved at dose levels of 1 to 2 mg/kg for the treatment of nausea and vomiting resulting from cancer chemotherapeutic agents. A rapid, sensitive reverse phase HPLC quantitative procedure for metoclopramide in serum is described. The method involves a single-step extraction of metoclopramide and disopyramide (internal standard) from alkalinized serum into benzene and utilizes a reverse-phase C-8 system with a mobile phase of 11 : 22 : 66, methanol : acetonitrile: pH 3.7 acetate buffer, and UV detection at 268 nm. The method is highly reproducible and has a limit of sensitivity of 2.5 ng/ml from a 2.0 ml serum sample. The method has been successfully applied to clinical pharmacokinetic studies involving administration of IV oral metoclopramide to cancer patients receiving highly emetogenic cis-diamminedichloroplatinum.

Introduction

Metoclopramide is structurally related to procainamide and has been utilized clinically for more than 10 years as an antiemetic and for disorders of gastrointestinal motility. It has been administered parenterally as an aid to GI diagnostic procedures and orally for the symptomatic relief of diabetic gastroparesis (1). Typical dosage levels are in the 0.1 to 0.3 mg/kg range, and information on its pharmacokinetics following single doses has appeared in the literature (2–6). Recently, metoclopramide has received FDA approval for the control of nausea and vomiting associated with cancer chemotherapeutic drug administration. The drug has been reported to be highly effective at 2.0 mg/kg dosage levels, repeated at 2 h intervals for up to 8 h (7). Since CNS toxicity is apparent at these dosages (8), but about 25 to 30% of patients do not respond (9), pharmacokinetic and clinical pharmacologic studies are indicated in cancer patients receiving multiple doses of metoclopramide to allow dose optimization.

Although several quantitative analytical methods for metoclopramide in serum have appeared, none meets all of the requirements in terms of sensitivity, reproducibility, cost, routines, and the turn-around time for clinical studies. Published assays include thin-layer chromatography (9), gas chromatography with electron capture (10) and mass spectrometric detection (2), normal phase HPLC (3, 4), and reverse phase HPLC (12). In this paper, we report the details of a sensitive, yet rapid, reverse phase HPLC procedure that has enabled us to initiate pharmacokinetic studies of metoclopramide in patients receiving multiple dose intravenous and oral therapy.

Materials and Methods

Chemicals and Reagents

Purified, unformulated metoclopramide and disopyramide phosphate, the internal standard, were obtained from A. H. Robbins Co. (Richmond, VA) and G. D. Searle and Co. (Chicago, Ill), respectively, and were used as analytical standards as received. Benzene, methanol, and acetonitrile were all chromatography grade (Omnisolve, Matheson Coleman and Bell, Cincinnati, OH), and all other chemicals and solvents were analytical reagent grade. Distilled water was purified by passing through a reverse osmosis four filter system (Millipore, Bedford, MA). A stock standard solution of metoclopramide was prepared in methanol at a concentration of 0.1 mg/ml. An internal standard stock solution of disopyramide phosphate was prepared in purified water at a concentration of 5.18 mg/100 ml. These were refrigerated at 4° C and found to be stable over several months.

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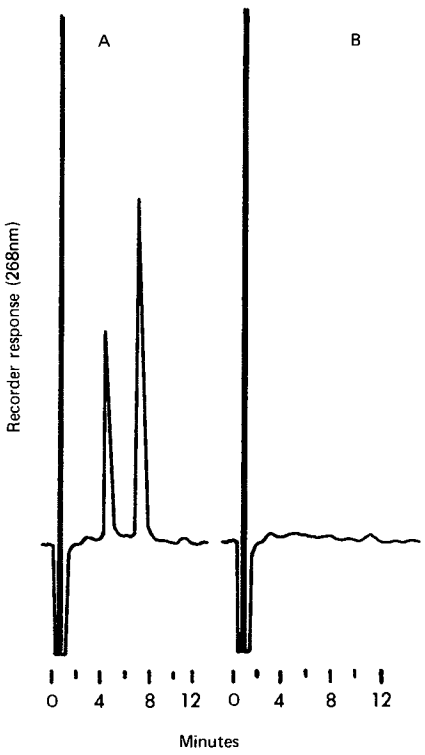

Fig. 1 High pressure liquid chromatograms of extracted patient sample (A) containing 120 ng/ml of metoclopramide and 5.1 μg/ml of disopyramide as the internal standard, and a patient blank (B) at an attenuation of 0.005 AUFS.

Chromatography and Instrumentation

A Waters Associate (Millford, MA) model 202 Liquid Chromatograph equipped with a model U6K injector and a Tracor (Austin, TX) model 970A variable wavelength UV detector was utilized for the analysis. Chromatography was performed on a 25 cm x 4.6 mm i.d., stainless steel column packed with 5μ, RP-8 (Ultrasphere, Altex, Berkeley, CA). The mobile phase consisted of 11:22:66 methanol: acetonitrile: pH 3.7, 0.75 M acetate buffer at a flow rate of 1.5 ml/min. The separation was run at ambient temperature at a detection wavelength of 268 nm.

Procedure

To 2.0 ml of a serum sample in a 15 ml conical centrifuge tube was added 200 μl of the internal standard stock solution, 200 μl of a 1:2 solution of 5 N sodium hydroxiede: saturated sodium chloride and 1.0 ml of benzene. The tube was vortexed for 1.0 min and then centrifuged for 10 min at 2700 × g. The upper (organic) layer was then transferred to a small conical centrifuge tube, evaporated under air to dryness and reconstituted with 50 μl of methanol. After vortexing for 10 sec, 10 μl was injected into the chromatograph.

Standard curves from spiked serum were prepared in the 0 to 2000 ng/ml range and subjected to linear regression analysis. Samples were quantitated from peak-height ratio measurements of drug: internal standard.

Clinical Studies

Cancer patients receiving cis-diamminedichloroplatinum therapy were administered metoclopramide intravenously and orally as indicated in the legends of Figures 2 and 3, according to an approved clinical protocol. Serial blood samples were drawn, allowed to clot, then the serum was harvested from the cells and frozen at −20° C prior to metoclopramide analysis.

Results and Discussion

Typical chromatograms from a patient sample and patient blank are shown in Figure 1. Under the analytical conditions described, retention times for metoclopramide and disopyramide were 4.5 and 6.9 min, respectively. No significant interferences with either drug or internal standard peaks were observed in a variety of extracted patient serum blanks or pools. Metoclopramide is used most frequently for control of nausea and vomiting following cis-diamminedichloroplatinum, adriamycin or cyclophosphamide chemotherapy. None of these agents elutes to produce interfering peaks under the conditions of the assay. Neither do any of the following commonly prescribed basic drugs: procainamide, lidocaine, quinidine, tricyclic antidepressants, benzodiazepines, morphine or codeine. Little is known concerning plasma metabolites of metoclopramide, and an assessment of possible interference could not be made. At low dose, however, metoclopramide is reportly execreted primarily unchanged or conjugated in the urine (11). Standard curves prepared from spiked human serum were linear over the range 10 to 2000 ng/ml and six consecutive runs produced mean values for slope, intercept and R^2 of 0.0014, 0.0074, and 0.999, respectively. Reproducibility between runs was excellent.

The precision of the assay was assessed by performing replicate analysis of aliquoted pools. The between run variability for a pool which averaged

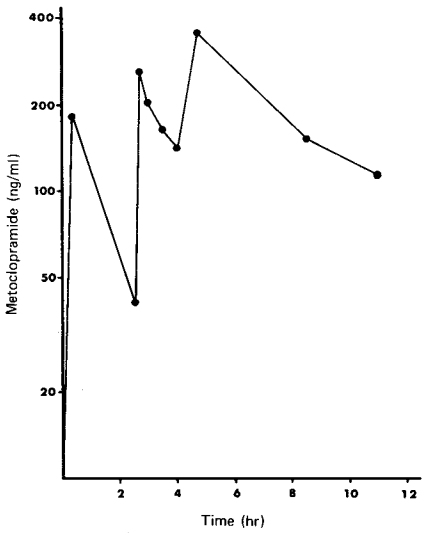

Fig. 2 Serum-time profile of metoclopramide in a patient receiving 2.0 mg/kg of metoclopramide intravenously at 0, 2, and 4 h. 100 mg/m^2 of cis-diamminedichloroplatinum was administered as a 30 min i.v. infusion 30 min after the initial metoclopramide dose.

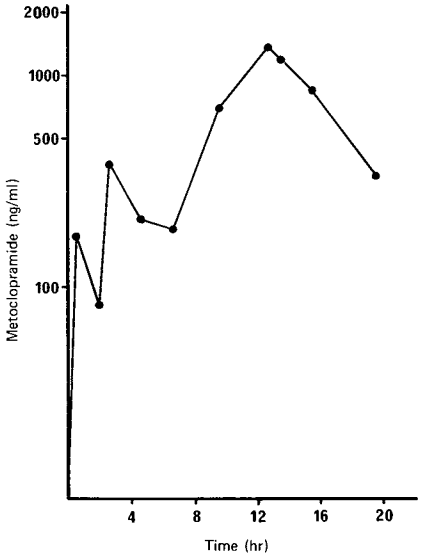

Fig. 3 Serum-time profile of metoclopramide in a patient receiving 1.0 mg/kg intravenously by i.v. infusion at times 0 and 2.0 h followed by 2.0 mg/kg oral doses at 3.5, 5.5, 8.0, and 11.0 h. 100 mg/m^2 of cis-diamminedichloroplatinum was administered as a 30 min i.v. infusion 30 min after the first metoclopramide dose.

404.2 ng/ml (n = 7) produced a coefficient of variation of 5.34 %. Within run variability was determined from pools of 431.5 (n = 5), 83.3 (n = 5), and 11.5 ng/ml (n = 6) for which coefficients of variation of 5.14 %, 2.6 %, and 4.0 % respectively, were calculated. The practical limit of sensitivity was determined by analyzing the smallest concentration which produced a 3 : 1 signal to baseline noise ratio and was found to be 2.5 ng/ml for a 2.0 ml serum sample.

The present procedure offers a distinct improvement over other published procedures in terms of time and ease of analysis and sensitivity. The only other reverse phase HPLC procedure (12) requires a 5 ml plasma sample and involves a complex double extraction to achieve a sensitivity of 10 ng/ml.

The analytical method was applied to the analysis of the pharmacokinetic behavior of metoclopramide administered as either multiple i.v. bolus doses or multiple oral doses to cancer patients receiving the highly emetogenic cis-diamminedichloroplatinum. Results from two patients are shown in Figures 2 and 3. Since these doses greatly exceed previous doses, no direct comparison of pharmacokinetic parameters reported here and elsewhere (2–5) is possible. As more patients are studied, important questions to be answered include whether the kinetics remain linear at very high dose and what level of accumulation occurs when frequent multiple doses are administered over the time frame when nausea and vomiting are most prevalent for cisplatinum therapy.

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