DOES DOSE-STAGGERING REDUCE METABOLIC DRUG-DRUG INTERACTION BETWEEN CLOZAPINE AND FLUVOXAMINE IN DOG?

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

for the Degree of Masters in Pharmacy

in the Department of Pharmacy & Nutrition

University of Saskatchewan

By

Karen Elaine Mosier

July 2004

©Copyright Karen Elaine Mosier, 2004. All rights reserved.

In presenting this thesis in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the libraries of this University may make it freely available for inspection. Further, I agree that permission for copying of this thesis in any manner, in whole or part, for scholarly purposes may be granted by the professor or professors who supervised this thesis work or, in their absence, by the Head of the Department or the Dean of the College in which the thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that in any scholarly use which may be made of any material in this thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Dean

College of Pharmacy & Nutrition

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

S7N 5C9

ABSTRACT

Clozapine, an antipsychotic, and fluvoxamine, an antidepressant, are frequently coadministered in the treatment of schizophrenia. Oral coadministration of clozapine with fluvoxamine in humans results in profound increases in serum concentrations of clozapine due to reversible enzyme inhibition. Dose-staggering is a potential way to reduce drug-drug interactions with orally administered drugs. We hypothesized that staggering doses of clozapine and fluvoxamine twelve hours apart will decrease the extent of interaction between clozapine and its known inhibitor, fluvoxamine, in dog. We used the dog model due to similar metabolic profiles between humans and dogs, and for safety reasons, since doses that are well tolerated in schizophrenic patients may cause serious adverse side effects in healthy volunteers.

The principle focus of this research work was to validate a computer simulation model that may predict drug-drug interaction potential. An HPLC method was developed to quantify clozapine and its two major metabolites, clozapine N-oxide and N-desmethyl clozapine in dog plasma. The pharmacokinetics of clozapine was examined for a single dose (IV and oral) and multiple doses (oral) in dog. A two-phase, crossover design was used to determine if staggering multiple oral doses of clozapine and fluvoxamine twelve hours apart could decrease the extent of this drug-drug interaction.

Our HPLC method was fast, simple and demonstrated good recovery rates for clozapine and N-desmethyl clozapine. The average half-life, elimination rate constant, volume of distribution, and systemic clearance of clozapine in dog calculated from a single IV bolus dose (1 mg/kg) were 11.2 h ($\pm$2.9), 0.067 h^{-1} ($\pm$0.022), 115 L ($\pm$32), and

7.54 L/h (±2.21). The average half-life, elimination rate constant, and oral clearance of clozapine in dog calculated from a single oral dose (5 mg/kg) were 18.5 h (±14.7), 0.081 h^{-1} (±0.065), and 43.51 L/h (±3.06). The average AUC_0^{τ} value of clozapine between the simultaneous condition ($AUC_0^{\tau} = 757 \pm 181$ μg x h/L) and the staggered condition ($AUC_0^{\tau} = 573 \pm 72$ μg x h/L) approached significance ($p = 0.05$). Dose-staggering did not appear to decrease the extent of the drug-drug interaction between clozapine and fluvoxamine in dog. The prediction of the computer simulation model could not be confirmed.

Acknowledgements

I would like to express my sincere gratitude to Dr. Jim Fang, my supervisor, and to the members of my advisory committee: Dr. Jane Alcorn, Dr. John Tuchek, Dr. John Hubbard, and Dr. Fred Remillard for their generous support and guidance in completing this thesis. A special thank you to Dr. Dave Scott for his help with the statistical analyses. I would also like to thank Jiuxue Song, research technician, for her help, encouragement and friendship in the lab.

Dedication

In loving memory of my mother FRANCES ELIZABETH MOSIER who passed away on April 6, 2002 at the age of 58.

Table of Contents

List of Tables

List of Figures

List of Schemes

Abbreviations

AUC	Area under the plasma concentration versus time curve
C_{max}	Maximum plasma concentration
CN	Cyano
CSF	Cerebral spinal fluid
CYP	Cytochrome P450
Cl_{TB}	Total body clearance
Cl/F	Oral clearance
CPS	Cyano propyl silane
D_1	Dopamine receptor subtype 1
D_2	Dopamine receptor subtype 2
D_4	Dopamine receptor subtype 4
E_H	Hepatic extraction ratio
EPS	Extrapyramidal side effects
5-HT_{1C}	5-hydroxytryptamine subtype 1C
5-HT_2	5-hydroxytryptamine subtype 2
F	Bioavailability
FMO3	Flavin-containing monooxygenase 3
GC	Gas chromatography
GFR	Glomerular filtration rate
HCl	Hydrochloric acid
HIAA	Hydroxyindoleacetic acid
HLM	Human liver microsomes

HPLC	High performance liquid chromatography
HVA	Homovanillic acid
H_1	Histamine receptor subtype 1
IS	Internal standard
IV	Intravenous
K_i	Inhibition constant
λ_Z	Elimination rate constant
PAH	Polycyclic aromatic hydrocarbon
SD	Standard deviation from the mean
SEM	Standard error of the mean
SSRI	Selective serotonin reuptake inhibitor
$t_{½}$	Half-life
t_{max}	The time at which the maximum plasma concentration occurs
UV	Ultraviolet
V_d	Apparent volume of distribution

Chapter 1

Introduction

1.1 Drug Interactions

A pharmacokinetic drug interaction occurs when the level of a drug is altered by coadministration with another drug or with food (Brenner, 2000). Drug interactions may occur during absorption (e.g., reduction in gut motility, reduced dissolution, chelation, malabsorption, transporter interactions), tissue or protein binding, renal clearance, and metabolic clearance (e.g., induction, inhibition, changes in hepatic blood flow) (Stockley, 1994).

During absorption, an interaction can occur between a drug and polyvalent cations in the gastrointestinal tract. Fluoroquinolones form insoluble complexes with polyvalent cations by chelation and their absorption is greatly decreased (Stass et al., 2001). Food and drug interactions can also occur. For example, food greatly decreases isoniazid bioavailability (Self et al., 1999). Inhibition of transporters in the gastrointestinal tract can cause drug-drug interactions. For example, fruit juices can inhibit organic anion transporters, preventing the uptake of the drug from the gastrointestinal tract lumen, resulting in decreased bioavailability of fexofenadine (Dresser et al., 2002).

A drug displacement interaction occurs when one drug successfully competes with another drug and displaces it from plasma proteins. Fenofibrate potentiates the

effects of warfarin by displacing warfarin from plasma proteins, resulting in 2-3 fold increases in the international normalized ratio or INR (the ratio of the patient's clotting time to a particular lab's mean reference value) and an increased anticoagulant response (Kim & Mancano, 2003).

Renal clearance interactions occur via changes in urinary pH, changes in active kidney tubular excretion, or through changes in kidney blood flow. Coadministration of lithium and indomethacin results in increased plasma concentrations of lithium. Indomethacin inhibits the synthesis of renal vasodilatory prostaglandins partially involved in controlling blood flow through the kidney. This inhibition results in a decrease in the renal excretion of lithium, and as a result, lithium serum levels rise (Imbs et al., 1997).

One type of metabolic drug interaction is enzyme induction. Induction is a protective mechanism initiated by the body to protect cells from toxic compounds by rapidly increasing the rate of metabolic breakdown. Enzyme induction can modify the extent of a drug-drug interaction. Rifampin enhances the rate of elimination of mexiletine due to enzyme induction (Labbe & Turgeon, 1999). Induction can lead to decreased levels of therapeutic agents and/or an increase in active metabolites, which may lead to either diminished therapeutic effects or increased toxicity. Therefore, enzyme induction may have beneficial or negative effects (Lin & Lu, 1998; Okey et al., 1986).

Another type of metabolic drug interaction is enzyme inhibition. Drug-drug interactions due to reversible enzyme inhibition are the most common mechanism of drug interaction, particularly with CYP enzymes. Enzyme inhibition will alter the extent of a drug-drug interaction (Lin & Lu, 1998). Inhibition occurs when drugs are metabolized by

or inhibit the same enzyme (Shen, 1995). Inhibition can be either competitive (compete for the same active site) or non-competitive (does not enter the active site but binds elsewhere changing the shape of the enzyme so the active site no longer fits the substrate) and reversible or irreversible (Barry & Feely, 1990). Coadministration of ketoconazole with terfenadine impairs metabolism of terfenadine (the parent compound) leading to serious side effects (Honig et al., 1993). Autoinhibition, where the metabolites formed inhibit the metabolism of the parent drug, may also occur (Shargel & Yu, 1999). Clearance of valproic acid declines with increasing doses and is suggestive of autoinhibition (Bowdle et al., 1980).

Genetic polymorphisms can also modify a drug-drug interaction. Individual differences in the ability to biotransform a drug through a given metabolic pathway due to genetic variability is a result of genetic polymorphism (Benet et al., 1998). A genetic polymorphism is defined as genetic variations that may impact pharmacological response (Montgomery & Louie, 2001). This results in a subpopulation of people who are poor metabolizers for substrates of the affected enzyme. Genetic polymorphisms may increase the likelihood of a drug-drug interaction occurring at the metabolic level (Benet et al., 1998). The effects of genetic variations on individual response depend on the type of phenotype (e.g., slow metabolizer vs. extensive metabolizer), which pathway is affected, how important this metabolic pathway is to the elimination of the drug, and whether the drug has a wide or narrow therapeutic index (Cholerton et al., 1992). An extensive metabolizer can be converted to a poor metabolizer if an inhibitor is given with a drug. The severity of this interaction will depend on the affinity of the inhibitor for the enzyme, the dose of the inhibitor, and the length of treatment. Phenotyping (e.g., poor metabolizer,

extensive metabolizer, ultra-rapid metabolizer) or genotyping (e.g., normal vs. wild-type DNA sequence) of patients receiving narrow therapeutic drugs is recommended in order to prevent toxic side effects from occurring (Nemeroff et al., 1996).

Drug metabolic clearance depends on the relative contribution of each enzyme to the metabolism of the drug. If an enzyme involved in the breakdown of a particular drug is inhibited, drug clearance will not be affected if the enzyme has only a small contribution to the metabolism of the drug and the drug can be broken down via other pathways by unaffected enzymes. However, if the affected enzyme plays a major role in the breakdown of the drug, even though the drug may be metabolized via other pathways, drug clearance will be significantly reduced.

Because of the risk of genetic polymorphisms in drug metabolizing enzymes and drug-drug interaction through inhibition or induction, identification of CYP enzymes involved in the metabolism of specific drugs may reduce complications from drug-drug interactions (i.e., adverse side effects, inadequate response to treatment) (Pollock, 1994). The use of multiple techniques is recommended to determine the particular isoenzymes involved in the metabolism of certain drugs (Fang et al., 1998). In vitro systems are useful in identifying enzymes involved in the metabolism of a xenobiotic. They can also be used to predict the extent of drug-drug interactions due to enzyme inhibition (Obach et al., 2001). The in vitro inhibition constant of a drug (K_i) for a CYP enzyme can be used to estimate the magnitude of interactions likely to occur through CYP-mediated metabolism. Several factors can influence the accuracy of predicting the likelihood of in vivo inhibition. For example, the nature and design of in vitro experiments used to calculate K_i, the concentration of the inhibitor in the hepatic cytosol versus that in the

plasma, the extent of prehepatic metabolism, enzyme induction, and the presence of active metabolites can influence the extent of inhibition observed (Bertz & Granneman, 1997).

Certain characteristics of a drug enhance the likelihood for a serious interaction. Health Canada defines a "narrow therapeutic range" (NTR) drug as a drug with a ratio of the lowest concentration at which clinical toxicity occurs, to the median concentration providing a therapeutic effect is less than or equal to 2 (Therapeutic Products Directorate, 1997). A drug with a narrow therapeutic range is more likely to be involved in a clinically significant drug interaction due to the smaller window in which therapeutic effectiveness occurs. Drugs that inhibit the metabolism of other drugs through enzyme competition, and drugs that induce the metabolism of other drugs through enzyme induction, are more likely to be involved in drug interactions. If a drug has a wide therapeutic window, the addition of a new drug to a regimen will have little effect. If an inhibitor is added to a drug with a narrow therapeutic range, caution must be taken in order to avoid elevated plasma drug concentrations or diminished therapeutic effect (Devane, 1994).

Pharmacokinetic characteristics such as half-life, volume of distribution, hepatic extraction ratio, and extent of absorption may predispose a particular drug to a drug-drug interaction. A drug with a half-life of >24 hours may potentially cause a drug interaction due to drug accumulation with repeated doses, especially if administered with a known inhibitor. The degree of drug interaction will also depend on the concentration of each drug, and the dose and frequency of administration for each drug (DeVane, 1998). The size of the volume of distribution of the substrate and the inhibitor can have complex