BIOEQUIVALENCE STUDIES OF DRUGS PRESCRIBED MAINLY FOR WOMEN

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ABSTRACT

The basic components of pharmacokinetics are absorption, distribution, metabolism, and excretion. During pregnancy there may be changes in one or many of these components. Early drug studies did not include a representative proportion of women, however, researchers as well as regulators agree that studies on the sex differences in the disposition of drugs are important, but at what stage in the clinical trial process? Except for drugs used only in women, such as those for estrogen-dependent breast cancer, caution prevails and the differences are usually studied at phase 3. Studies in pregnant women are much rarer but some do get done, e.g., with antivirals and antimalarials, where the positive risk-benefit of these agents is the likelihood that fetal transfer of these drugs might help protect the fetus. Women are being included in pharmacokinetic studies for new drug applications in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), U.S. Food and Drug Administration (FDA), and Health Canada (HC) guidances. A new look at bioequivalence studies, to compare results in men and women, would help determine if interactions of formulation and gender are a problem.

Key Words: *Pharmacokinetics, bioavailability, bioequivalence, pregnancy, sex differences*

Introduction

The basic components of pharmacokinetics are described under the acronym ADME: absorption, distribution, metabolism, and excretion. When we speak of bioavailability, we speak about what happens from the point of drug administration to its absorption, and whether there are differences in absorption between products (comparative bioavailability = bioequivalence). In pharmacokinetics, when we consider plasma levels—and sometimes urine levels—we also look at all of the processes that affect these levels, such as metabolism and protein binding, which show changes in pregnancy, are to some extent different between the sexes, and are also different among various drugs. In determining bioavailability/bioequivalence we have to tease out absorption from the other physiological processes that are not product dependent.

Regulatory Guidelines

In 1993, the U.S. Food and Drug Administration (FDA) issued a guideline for the study and evaluation of gender differences in the clinical evaluation of drugs in order to involve more women in clinical trials;[1] before then, drugs were not well studied in women. Since 2004, the FDA has had a draft guideline in place: Pharmacokinetics in Pregnancy – Study Design, Data Analysis, and Impact on Dosing and Labeling.[2] In Canada, the Drugs Directorate issued a policy in September 1996 for the inclusion of women in clinical trials during drug development.[3]

A number of papers have reviewed participation of women in clinical trials and differences between the sexes as regards pharmacokinetics. Yang *et al.*, in a study of the participation of women in clinical trials for new

drugs approved by the FDA between 2000 and 2002, reported "… overall participation by women and men was comparable, suggesting an improvement in including more women in clinical trials when compared with the previous FDA study evaluating women's participation from 1995 through 1999. As with the previous study, however, a significant underrepresentation of women in early phase trials and in certain areas, such as cardiovascular products, was observed and continues to be an issue of concern."[4] For new drugs, clinical pharmacology studies are reported according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Common Technical Documents (Section 2.7.2, with details in section 5).[5] Pharmacokinetics studies are often also reported under this category, with some analysis by gender; ICH Guideline E5 covers ethnic or special population data.

Reports on Pharmacokinetic Differences between the Sexes

In 2003, Schwartz published a paper on the influence of sex on pharmacokinetics.[6] He noted:

- Absorption was not significantly affected by sex, but that rates may be slightly slower in women.
- Bioavailability, for CYP3A substrates in particular, may be somewhat higher in women compared to men, resulting in greater exposure due to lower clearance.
- The role of sex on pharmacokinetics, when considered in conjunction with genetics, age, disease, and social habits is not yet known in the clinical setting and needs more study.

Beierle *et al.* reported that for the majority of investigated drugs in recent years, no, or only very minor, gender differences could be detected in pharmacokinetics or pharmacodynamics, and that their clinical significance seems very limited, i.e., seems rarely linked to treatment success or failure.[7] "Hence, it is undoubtedly necessary to include women in the clinical drug development process, but it seems questionable whether women of child-bearing capability should be exposed to potential risks in early phase I clinical trials."[7]

Soldin and colleagues, in their recent paper on sex differences in drug disposition, conclude: "Males and females may differ in specific drug pharmacokinetics and pharmacodynamics. It is, therefore, essential to understand those sex differences in drug disposition and response, as they may affect drug safety and effectiveness."[8]

Researchers as well as regulators agree that studies on the sex differences in the disposition of drugs are important, but at what stage in the clinical trial process? Except for drugs used only in women, such as those for estrogen-dependent breast cancer, caution prevails and the differences are usually studied at phase 3. Studies in pregnant women are much rarer but some do get done, e.g., with antivirals and antimalarials, as the positive risk-benefit with these agents is the likelihood that fetal transfer of these drugs might help protect the fetus.

Alcohol Pharmacokinetics – An Example of Sex Differences in Drug Disposition

It has been known since antiquity that women are more susceptible than men to the effects of alcohol; and further, that fetal alcohol syndrome is a sad result of exposure. Some effects may be due to body mass, with higher blood levels more common in women. A small 1996 study found that "Dose-corrected values for AUC were on average 28% higher ($p < 0.0001$) in the women than in the men."[9] But the issue is more complex. One 2001 report noted "The gender difference in alcohol levels is due mainly to a smaller gastric metabolism in females (because of a significantly lesser activity of chi-ADH), rather than to differences in gastric emptying or in hepatic oxidation of ethanol."[10] Another review stated that "influences on alcohol elimination rate include gender, body composition and lean body mass, liver volume, food and food composition, ethnicity, and genetic polymorphisms in alcohol metabolizing enzymes."[11] More particularly, however, an "important determinant" was the allelic variants of the genes encoding the alcohol metabolizing enzymes, ADH and ALDH. Thus some women are less susceptible to the effects of alcohol, and even now we do not fully understand why.

Bioequivalence

Coming back to drug disposition, the main exposure metric of bioequivalence is absorption, which is affected by the formulation and in turn influences the plasma level and the area under the curve (AUC). However, it is metabolism that *primarily* influences the AUC. (See Table 1)

TABLE 1 Definitions

Bioavailability is a pharmacokinetic attribute. "It is defined as the rate and extent of absorption of a drug into the systemic circulation."[12] It is assessed by serial measurements of the drug in the systemic circulation, which provide a plasma concentration-time curve from which important pharmacokinetic parameters can be calculated, including the area-under-the-curve (AUC), the maximum observed concentration (C_{max}) and the time when C_{max} is reached (t_{max}).[12]

AUC provides an estimate of the amount of drug absorbed in the systemic circulation, while t_{max} reflects the rate of absorption. C_{max} is a more complex function, which, together with t_{max}, may reflect the rate of absorption."[12] AUC is a measure of total exposure; C_{max} is a measure of the rate of exposure.

Comparison of AUC values following oral vs. IV administration of the same active ingredient provides an estimate of the *absolute* bioavailability.[12]

Comparison of the test (T) and reference (R) product profiles of the drug provides an estimate of comparative bioavailability. T and R are said to be *bioequivalent* when the profiles are similar according to statistical assessment and by meeting stated standards.[12]

In Canada and the U.S., the general standard for AUC is that the 90% confidence interval (CI) of the geometric mean ratio (GMR) be within 80 and 125%. In the U.S., this is the same standard as for C_{max}. In Canada, the 90% CI of the GMR for C_{max} should be within 80 and 125%; however, for critical dose drugs (e.g., warfarin, phenytoin) the 90% CI of the GMR for AUC should be within 90 and 113% and the 90% CI for C_{max} should be within 80 and 125%. *[In July 2011, the FDA revisited bioequivalence of narrow therapeutic index (NTI) drugs and their advisory committee recommended tightening of the bioequivalence standards for these drugs.[13]]*

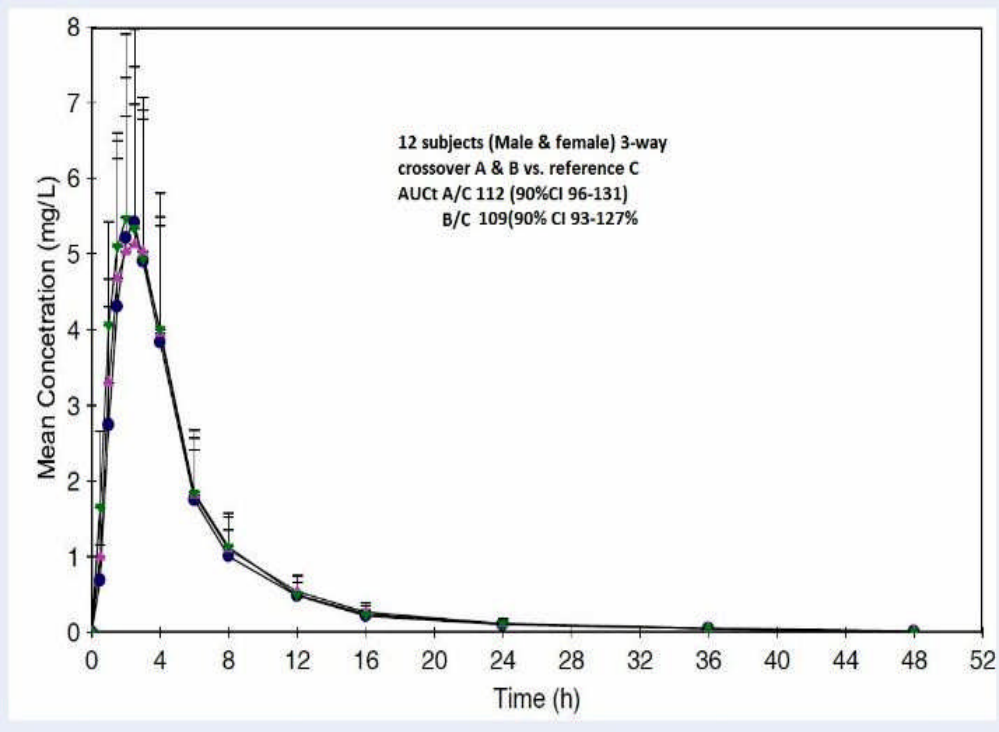
For oral drugs, the simplest absorption scenario is diffusion. This is dependent upon the environmental pH and the pKa of the molecule, but the process is typically much more complicated, often involving transporter-mediated absorption. Yet the main determinants of absorption remain the solubility of the drug released from the product and its pKa.

Bioequivalence implies that the drug product can be expected to have the same systemic effects (both therapeutic and adverse) as the reference product when administered to patients under the conditions specified on the label. For over 30 years, the premise was, and remains, that crossover studies on healthy volunteers can support this assumption. Health Canada states, "Drugs with uncomplicated characteristics can usually be tested in normal, healthy volunteers. The investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study."[12]

The FDA states, "We recommend that if the drug product is intended for use in both sexes, the sponsor attempt to include similar proportions of males and females in the study."[14] In addition, the FDA recommends having a representative sample, e.g., if the drug is to be used in the elderly, then a large proportion of the group should be elderly volunteers.

The numbers of test subjects are also important when testing for bioequivalence, taking into consideration the intrasubject coefficient of variation (CV). With highly variable drugs, the crossover study CV can be greater than 30%. Where the CV is under 15%, then 15 to 20 subjects may be a sufficient number for testing; where the CV is 30%, perhaps 80-100 subjects, all falling within the GMR range of 80 to 125%, would be needed to meet the standard. Figure 1 presents an example of failed bioequivalence tests for AUC. Despite the mean AUCs looking almost matched, the variability is high.

FIG. 1 Failed AUC Standard



The crossover study reduces the variation (within-subject, rather than between-subject) compared to parallel studies in which each product is examined in different subjects (required for very long half-life drugs).

Another situation where there can be failed bioequivalence is in the case of formulation differences. For example, in the 1950s, it was found that the availability of poorly soluble griseofulvin was increased 50% by using a micronized formulation.

Women in Bioequivalence Studies

Chen *et al*., although noting that their "sample sizes for these studies were not chosen to examine the sex-related effects considered", reported that 26 bioequivalence studies performed between 1977 and 1995, with 20 or fewer subjects per study, found the AUC was higher 71% of the time and the C_{max} was higher more than 87% of the time in women.[15] Overall, female results were statistically higher for the reference product in 28% of the data sets. The frequency of statistically significant differences was lower when body weight was included in the statistical model, and the authors noted that women tended to have higher variability. "The results of this study support recommendations of the 1993 FDA gender guideline that women not be excluded from bioequivalence studies."[15] Statistical examination of the data from the products tested for positive, body-weight corrected, sex-by-formulation interaction, showed higher C_{max} values in women for two transdermal nitroglycerin patches, where rate of exposure can be variable and patch size can have an effect, and for a formulation of erythromycin. These results are based on small samples against which to make recommendations. Ideally, the FDA would repeat such a review of bioequivalence studies to glean more information on gender differences.

Interestingly, the FDA has individual drug bioequivalence guidances, with the website (in May, 2011) listing 805 draft and 153 final guidances.[16] Most individual guidances recommend that subjects be "healthy males and non-pregnant females, general population", but the instructions for determining if a woman is pregnant (or lactating) are not standardized. Furthermore, about 15% of the guidances do not

mention pregnancy checks, including phenytoin - surprising, as it has been associated with birth defects. Breast cancer drugs, (e.g., anastrazole), vaginal preparations, oral contraceptives (e.g., norethindrone, etc.) and some hormones require women-only as subjects in their guidances. The exemestane guidance lists post-menopausal women as subjects. Guidances for drugs for prostate cancer and erectile dysfunction require study in men only. For progesterone, the guidance recommends healthy males and post-menopausal females are suggested (possibly due to endogenous interference in pre-menopausal women). The tamoxifen guidance recommends both men and women, as it is used in both sexes. In general the subject inclusion "recommendations" are reasonable. However, it would be useful to examine bioequivalence variations in men *vs.* women subjects, now that the FDA has more data.

Gender-Related Pharmacokinetics

Before the mid-1990s, between-gender pharmacokinetic differences were infrequently studied, largely due to the lack of regulatory requirements. Since then, more women have been included in clinical trials, as well as in the determination of pharmacokinetics of new drugs. Diclectin (doxylamine succinate 10 mg and pyridoxine hydrochloride 10 mg, delayed-release tablet) is one of those drugs.

A multiple dose pharmacokinetic study in 18 non-pregnant female subjects was sponsored by Duchesnay Inc.[17] An oral dose of 2 Diclectin tablets was given at 10 PM on Days 1 and 2, followed by multiple oral doses on Days 3 to 18, according to the following schedule: 1 Diclectin tablet at 9 AM and 4 PM, and 2 tablets at 10 PM, under fasted conditions (at least 2 hours after eating). This is the maximum dose of 4 tablets daily as recommended in the product monograph.

This new study determined the pharmacokinetic parameters when Diclectin was administered to 18 healthy non-pregnant women in the recommended maximum dose regimen of doxylamine 40 mg/pyridoxine 40 mg per day, compared to a single 10/10 mg dose. Comparison of the first dose AUC with the final AUC, from time of dosing (0 h) to 24 hours post dose on day 18, provided an accumulation index (AI): AI = $AUC_{0\text{-}24,\ day18} \div AUC_{0\text{-}24,\ day1}$. The AI calculated from the study findings suggests an approximately 3-fold accumulation of doxylamine after multiple doses.

Pyridoxine is more difficult to research, due to its more complex metabolism. It is primarily metabolized in the liver, with the main active metabolite being pyridoxal 5'-phosphate (PLP). Other metabolites are pyridoxal (PYL), pyridoxamine (PYM), and pyridoxamine 5'-phosphate (PMP). The new data demonstrate that doxylamine and pyridoxine metabolites show clear dose accumulation after a total dose of 40 mg daily for 18 days. Some metabolites displayed 7-fold accumulation (see Table 2), along with increases in elimination half-life. The complex metabolism of pyridoxine, including reversible metabolism, presents difficulties in interpretation. The concern is the potential impact on the safety of patients, in view of anecdotal reports of patients taking off-label doses of Diclectin of up to 60 mg daily.

TABLE 2 Pyridoxine Kinetics[16]

Metabolite	**$t_{1/2el(h)}$ Mean ± SD**	**AI AUC_{24} Day 18/Day 1**
Pyridoxine (parent drug)	0.37 ± 0.16	1.59
PYL	2.14 ± 2.2	6.09
PLP	81.6 ± 42.1	3.98
PYM	3.1 ± 2.54	6.17
PMP	66.5 ± 51.3	6.67

For this presentation, there is not time to show other recent studies and to review doxylamine and pyridoxine bioequivalence information. However, women tended to be more variable (intrasubject CV%) than men and there appeared to be a gender difference in the effect of food; yet there were insufficient data to indicate a formulation interaction by gender. Nonetheless, the accumulation information from the first multiple dose study of this drug in women suggests such information is of concern, especially if higher doses are being used off-label.

CONCLUSIONS

Women are being included in pharmacokinetic studies for new drug applications in accordance with ICH, FDA, and HC guidances. Older drugs have been less studied and there are few studies available in pregnant women, other than for antivirals and antimalarials. A new look at bioequivalence studies, to compare results in men and women, would help determine if interactions between formulation and gender are a problem. It should be cautioned that body weight corrections do not remove all clearance differences.

Except for drugs used entirely in one gender, bioequivalence studies are supposed to include "representative numbers" of men and women. This may present a problem when bioequivalence studies are outsourced to offshore clinical research organizations, where cultural differences can affect gender participation in research studies.

We need to understand that questions remain about the effects of pregnancy, menarche, and menopause on pharmacokinetics, including bioavailability.

In the opinion of this speaker, bioequivalence is of less concern than are pharmacokinetics and the related drug effects. Furthermore, bioequivalence studies for drugs to be used exclusively in one gender are best studied in that gender only.

[It is interesting to note that the Health Canada Scientific Advisory Panel on Bioequivalence Requirements for Gender-Specific Drug Products (SAP-GSDP) noted in June 2011: "For the specific case of doxylamine succinate 10 mg and pyridoxine hydrochloride 10 mg, the panel recommended that the current practice of Health Canada to accept bioequivalence studies in only males, males and females or only females is acceptable."[18] As doxylamine succinate 10 mg with pyridoxine hydrochloride 10 mg is only indicated for prescribing to women, this recommendation is perhaps not in line with later remarks: "Panel members stated that cases certainly exist where bioequivalence studies do not require gender-specific samples, however, because of the nature of certain drugs; gender-specific samples are used (e.g., oral contraceptives). The members agreed that from a pragmatic standpoint, bioequivalence studies are occasionally done in gender-specific samples; the members acknowledged that Health Canada's current bioequivalence guidance already allows flexibility to accommodate these cases." [18] The final conclusion was: "while the panel does not view the issue as closed, presently there is no compelling scientific evidence to warrant gender-specific bioequivalence studies."[18]]

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