

RATIONALIZATION OF TWO DOSAGE FORMS OF A MODEL COMPOUND ALPRAZOLAM, BASED ON PHARMACO- METABONOMIC CRITERION

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ABSTRACT

Differences in metabolic patterns as result of genotype variations or environmental factors have been known to alter drug efficacies and adverse reactions of drug molecules and attempts are being made to individualize drug dosages. Important amongst these are differences in drug effects due to pre-systemic metabolism arising out of different routes of administration particularly for those drugs which are subject to significant degree of first pass metabolism. For such compounds assessment of pre-systemic metabolism is difficult and subject to assumptions which are not always valid.(eg. approximate amounts of hepatic blood flow, 100% absorption, the entire amount of drug being metabolized in the liver etc). Here through pharmaco-metabonomic studies and comparison of responses we have rationalized doses and estimated the effect of differences in metabolism (effect of pre-systemic metabolism) on two dosage forms of a model compound alprazolam which to a significant degree is metabolized in the liver. We find that differences in metabolic pathways have significant effects on drug responses and conclude that rationalization of doses can be used to design more individualized, effective and safer dosage forms particularly in cases where genomic and metabolic factors show greater risk of adverse effects and for drugs with narrow therapeutic margins.

Keywords: Bio-equivalence, ADME, Efficacy, Clearance, Absorption, Dose response, First pass metabolism, Bio-availability, Sublingual

INTRODUCTION

Attempts are underway to personalize drug dosages based on individual genotypes[1,2] so that requirement based drug dosages may be administered to patients thereby improving efficacy and reducing the severity of adverse reactions[11]. Apart from genotypic characteristics phenotypic criteria like metabolism and therapeutic effects are influenced by environmental factors such as age, disease, drug interactions due to co-administration etc [3,4,11]. Based on these pharmaco-metabonomic phenotyping has been used in the individualization of drug dosages [5]and metabolic signatures have been used for prediction of efficacies and adverse effects and it has been shown in rats that individual responses to drugs may be determined by individual metabolic pattern of each animal. This being so it is thought that different dosage forms of a drug metabolized through different metabolic pathways due to different routes of administration would show large differences in response and its rationalization would play an important role in personalization of drug dosages particularly in situations where genotypic and phenotypic characteristics show greater risk of adverse reactions. There has however till date been no attempt to rationalize dosage forms of drugs where efficacies vary due to metabolism through different metabolic pathways. Also variation in pre-systemic metabolism cause varying responses to drugs and therefore if the same drug is administered through different routes where absorption and metabolic pathways differ the responses would be different. Here we determine requirement based dosages of two dosage forms of alprazolam (oral and sublingual) metabolized through different pathways by comparing their in vivo efficacies in animals and humans and quantifying the amount of drug required to produce the same effect. We administered alprazolam through different routes and observed huge differences in responses which cannot be explained on the basis of changes in oral bioavailability of alprazolam (88+/_ 16%)[13] alone. We attribute the difference to alteration of metabolic pathways and propose that personalization of medicine requires different dosage forms of drugs be standardized to a particular response depending on individual requirements and equivalence of different dosage forms be established.

MATERIALS AND METHODS

All animal experiments were carried out as per IAEC norms and with their approval. Rabbits used in the experiment were procured from Orissa University of Agricultural Technology Bhubaneswar and experiments performed in approved research labs CPCSEA Registration

no 1025/c/07/cpcsea dated 19.6.2007. Healthy rabbits of either sex , aged approximately 12 months weighing around 1.4 kg each were selected and housed individually in cages in a cool dark place free from outside noise. Alprazolam was administered in doses of 8 microgram /kg (corresponding to human dosages) or lower and sleep times recorded. Five doses were administered from which an oral log dose response was obtained. An interval of seven days was allowed before each dose for removal of residual effects of the previous dose (t 1/2 of alprazolam is 12+/_2 hours[8]). The post threshold portion of the curve where the response was linear to log dose (4 to 8 microgram /kg dose range) was chosen for the study. The animals were then dosed sublingually with alprazolam and permeation enhancers in the same dosage range and sleep times recorded again. Comparisons of these results were used to calculate dose equivalence ratios for oral and sublingual doses of alprazolam in rabbits.

On similar lines informed consent was obtained from two healthy female volunteers(age approximately 30 years body weight approximately 60 kgs) and they were administered alprazolam both orally and in dose equivalent amounts sublingually. Initially the dose equivalent ratios observed in rabbits were used for calculations of equivalent doses in humans (and it was later observed that the difference between the two was not very large). Responses for both human oral and sublingual doses were compared and dose equivalent ratios derived.

Dose equivalences of alprazolam in different species also served to compare the ADME effects of different species for alprazolam (species allometry). Using this data the oral doses of alprazolam in rabbits corresponding to that of human doses (see results and discussion) were calculated and administered to rabbits and their sleep times recorded. Subsequently the same animals were administered alprazolam sublingually in amounts corresponding to rabbit dose equivalence ratios and sleep times recorded again. Both doses elicited a similar response as per expectations.

RESULTS AND DISCUSSIONS

Alprazolam was chosen as the experimental compound because of its high therapeutic index enabling larger than normal doses to be given to animals and humans without any appreciable risk and also because its response can easily be measured by measurement of sleeping time. The drug once taken sublingually would pass directly into the blood stream via the reticulated vein [6] and bypass the first pass metabolism unlike its oral dosage form which would be predominantly metabolized in the liver by the first pass route.

Although a portion of the sublingual dose would reach the liver through the second pass the amount reaching would be much less thereby increasing systemic bioavailability and reducing hepatotoxicity in the long term. Differences in drug absorption routes and metabolic pathway cause differences in systemic availability and drug response profiles. Higher peak plasma level and shorter time to reach peak for sublingually administered alprazolam compared to oral alprazolam have been reported [7].

In order to determine the dose equivalences – defined as relative quantities (amounts in a single dose) of drugs in different dosage forms having equivalent effect- we have compared the responses of oral and sublingual dosage forms in animals and humans (table -1). In rabbits the doses ranged from 2.86 microgram/kg body weight to 8 microgram/kg bodyweight (8 microgram/kg corresponding to the human dose of 500 micrograms per person). A sigmoidal response was obtained which showed a linear graded post threshold dosage range of 4microgram/kg to 8 microgram/kg the threshold value being approximately 4 microgram/kg (see table-1 for details). Subsequently the same animals were dosed sublingually with doses within the same linear range. (See materials and methods section for details)

The log dose responses of sublingual dosing (table-1) were calculated on a 4 point assay format along with oral log dose response values for comparison of efficacies . The sublingual doses were chosen so that they fell within the linear range (5 microgram/kg and 7 microgram/kg). From the calculations dose equivalence ratio of oral to sublingual dose of alprazolam 1:0.5 (approximately) in rabbits was derived and from the equation $\text{Dosing rate} = C_p \times Cl / F$ [9] (where C_p = steady state plasma conc. Cl = clearance and F = Calculated bio-availability) we derive an increase in Calculated bio-availability (at constant values of C_p and Cl) of 1:2

in rabbits due to sublingual dosing.(Substituting 0.5 for dose in the equation)

The oral doses administered to human volunteers (see experimental section and table-2) were in the same dosage range, 7.08 micrograms/kg and 7.9 micrograms/kg (the normal human dosage range being 4 micrograms/kg and 8 micrograms/kg corresponding to 250 and 500 microgram of drug per person) and the corresponding sublingual doses were those that corresponded to the post threshold dose equivalent oral doses. The log dose response values for humans (both oral and sublingual) were calculated on a 4 point assay format and dose equivalence and calculated bio-availability calculated. The values obtained were 1: 0.55 (approximately) for oral to sublingual dose equivalence and 1:1.80 for increase in calculated bio-availability (at constant values of C_p and Cl) .(Substituting 0.55 for dose in the equation.)

Since oral bio-availability of alprazolam is more or less constant (88 +/- 16)% compared to iv absorption [13]) and clearance is also constant, if the effect of a sublingual dose is 1.8 times that of an oral dose the value of C_p would have to increase by a factor of 1.8 in order to provide for the increase in available dose on sublingual administration (Substituting 1.8 for dose in the equation). We therefore conclude that different dosage forms be evaluated and standardized to a fixed response. Since the maximum increase in available dose possible due to increased bioavailability is approximately 15% (1/0.88) the balance increase in C_p is attributed to altered systemic availability due to alteration of metabolic pathways especially when possibilities of different metabolic routes exist. An oral dose of 8.2 micrograms per kg (15% increase over the lower oral dose) would elicit a response of 251 min equivalent to a sublingual dose of 4.25 micrograms per kg and not the dose of 7.1 micrograms per kg which is an increase in available dose of 67% (7.1/4.2).

Table 1: Oral and sublingual log dose, onset time and duration of action (sleep time) of alprazolam in rabbits.

Route of Administration	Log dose	Onset of action(mins)	Sleep time (mins)
Oral	0.456	37 +/- 4	5 +/- 2
	0.574	40 +/- 6	25 +/- 3
	0.602**	50 +/- 4	177 +/- 4
	0.778	70 +/- 4	214 +/- 8
	0.903	67 +/- 3	236 +/- 17
Sublingual	0.699	22 +/- 3	274 +/- 11
	0.845	17 +/- 3	320 +/- 5

* +/- figures indicate deviation around mean, ** indicates threshold values, Test for parallelism of lines F(1,4) at 95%=5.502 (limit 7.71) [12] See supplementary information.

Table 2: Oral and sublingual log dose, onset time and duration of action (sleep time) of alprazolam in humans.

Route of Administration	Log dose	Onset of action(mins)*	Sleep time (mins)*
Oral	0.849	54 +/- 1	203 +/- 8
	0.899	51 +/- 3	240 +/-
Sublingual	0.560	20 +/- 1	188 +/- 8
	0.599	25 +/- 2	224 +/- 9

* +/- figures indicate deviation around mean, Test for parallelism of lines F(1,4) at 95%=5.98 (limit 7.71) [12] see supplementary information

Table 3: Oral dose and sleep time and corresponding sublingual dose and sleep time of alprazolam

Route of Administration	Dose (micrograms/kg)	Sleep time (mins)*
Oral	6.5	206 +/- 12
Sublingual	3.2	200 +/- 6

* +/- figures indicate deviation around mean

Current literature shows a faster onset and higher plasma peak concentration on sublingual administration of alprazolam compared to oral administration [7] although their AUC values are the same. Efficacy studies show that onset is faster with sublingual alprazolam and having equal AUC values do not translate into equal efficacies for the two dosage forms, which is evident from the data in tables 1 and 2. This also substantiates observations elsewhere [10] that comparative blood level AUC studies are inadequate to assess bio-equivalence of two different

dosage forms. We therefore propose comparative efficacy studies with measurement of onset times and threshold doses for establishing bio-equivalence. Attainment time and peak plasma concentration does not per se' indicate attainment of therapeutically effective concentration neither does it show the time duration of effect or quantify increase or decrease in efficacy. In this instance the sublingual dose would be more effective than the oral dose (table-2) in humans by 1:1.80 times. Although as stated the AUC's would be the same.

Since dose equivalences are inversely proportional to calculated bio-availabilities (the values are retained for the purpose of calculations) they can be used to quantify ADME factors following oral dosing in closely related species and thereby predict equiactive dose in one species from knowledge of the corresponding dose in another species and dose equivalence values. Accordingly it may be concluded that $(\text{Rabbit oral dose}/\text{Human oral dose}) = (\text{Rabbit dose equivalence ratio}/\text{Human dose equivalence ratio}) = (\text{Calculated human bio-availability}/\text{Calculated rabbit bio-availability})$. (Calculated bio-availabilities have been retained for comparison purposes). A comparison of the responses of humans and rabbits at the two oral dose levels (table-1 and table-2) yields a value of 0.98 (value based on averages) where as the ratio of dose equivalences and inverse ratio of calculated bio-availabilities of rabbits to humans yields a value of 0.910. This ratio would therefore enable us to quantify species variation in ADME factors (species allometry).

Using the above formula we calculate rabbit oral doses corresponding to human oral doses of 7.943 micrograms/kg and 7.079 micrograms/kg. We choose a dose in between the two calculated doses 6.5 micrograms/kg approximately. The sleep time recorded for this dose in rabbits (table-3) is (206 ± 17) min. The corresponding human dose ie; 7.21 microgram/kg shows a calculated sleep time of 208 min (calculated from table-1) confirming the above relation. Moreover the corresponding rabbit sublingual dose of 3.2 microgram/kg calculated as per rabbit dose equivalence ratio has a sleep time of (200 ± 11) min which is very near to the sleep time of the rabbit oral dose. (Normally an oral dose of 3.2 microgram/kg would give a sleep time of 25min approximately as seen from table-1).

CONCLUSION

Personalization of health care must take into account metabolic profiles of drugs in humans and variations in metabolic profiles (whether arising from genetic epigenetic environmental factors or due to different routes of administration) are therefore of great interest. It may be mentioned in this context that metabolic profiles include and represent the combined effect of genetic epigenetic environmental and route of administration related factors. For the future once the responses to different dosage forms are standardized one can expect customization of different dosage forms to a standardized response depending on individual requirements. The necessity of such customization of dosage forms cannot be over emphasized particularly for potent drugs with low therapeutic margins and also for patients where genotypic and metabolic variations cause a greater risk of adverse reactions. Such studies would also enable us to rationalize dosage forms so that drug adverse reactions and toxicity would be reduced and efficacy

improved. This is particularly true for Alprazolam which is hepatotoxic on long term use. Sublingual administration on a reduced dose would minimize hepatotoxicity while retaining efficacy.

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