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ATTACHMENT H

Evidence for pre-systemic metabolism of anagrelide

This represents a summary of Shire data on file

Summary

The unavailability of a clinical intravenous formulation of anagrelide has precluded the generation of absolute oral bioavailability data on this compound. Nevertheless there is considerable indirect evidence to support the belief that anagrelide undergoes a significant first pass metabolism in the formation of its active metabolite (6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo[2,1-*b*]quinazolin-2-one) known as BCH24426. This comprises:-

- An estimate of the absolute oral bioavailability of the drug in the minipig, a species recently shown for several drugs to give reasonable estimates of human pharmacokinetic parameters, indicated this to be ~20%.
- Following oral administration of anagrelide to volunteers the superimposition of the early plasma concentration-time profiles for drug and active metabolite indicates formation of the metabolite by first pass.
- A study in subjects with moderate hepatic impairment showed an 8-fold increase in anagrelide AUC, a two fold increase in half-life and what appeared to be a four fold increase in V_d/F . While the latter could represent a real change in volume due to disease induced changes in plasma protein binding of the drug, it is possible that this change may be at least in part due to a significant increase in bioavailability. This would imply that bioavailability in the absence of hepatic impairment was low.
- A theoretical estimate of the extent first pass metabolism may be made from the equation:-

$$F = 1/(1 + (CL_b/F)/Q_H)$$

Where CL_b is blood clearance = $CL/F \times C/C_b$ (=1.196) and Q_H , is the liver blood flow (1.35L/min). The assumptions underlying this equation are that hepatic extraction is the sole cause of reduced bioavailability and that elimination occurs exclusively by hepatic metabolism.

- Evidence for the former assertion comes from two human radiolabelled studies which showed between 72-78% of the administered radiolabel was recovered in the urine implying near quantitative absorption. In the light of this, bioavailability is likely to reflect pre-systemic metabolism.

- Since there is no evidence for luminal degradation of the drug from biotransformation by gut flora or human intestinal microsomes it may be assumed that pre-systemic metabolism is due to hepatic biotransformation. There is no evidence of significant renal clearance of the drug itself.

Individual estimates of bioavailability from 69 volunteers, using Eq. 1, indicated a mean \pm SD of 43.4% \pm 9.8. Data from a smaller group of 16 essential thrombocythemia patients indicated a mean \pm SD bioavailability of 52.6% \pm 12.5. Thus between 47-57% of the drug is removed by hepatic extraction during the initial passage through the liver.

- Taken together, all these data suggest anagrelide undergoes significant first pass metabolism in the formation of its active metabolite, 3-hydroxy anagrelide.

1. Background

Anagrelide (imidazo [2,1-*b*] quinazolin- 2(3H)-one, 6,7-dichloro-1,5-dihydro, monohydrochloride), is extensively metabolised in man to two major metabolites; 3-hydroxy anagrelide (6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo[2,1-*b*]quinazolin-2-one, also known as SPD604, BCH24426 or 3-HA) and a subsequent biotransformation product RL603, 2-amino-5,6-dichloro-3,4-dihydroquinazoline. 3-hydroxy anagrelide is equipotent with the parent drug in its *in vitro* effects on megakaryocytopoiesis and therefore potentially platelet lowering but 40 times more potent as a PDEIII inhibitor and therefore as an inotrope, chronotrope and vasodilator. The further metabolite, RL603, is essentially inactive in these screens (Erusalimsky, Hong, and Franklin 2002)

It is believed that the primary active metabolite of anagrelide, 3-hydroxy anagrelide, is formed extensively during first pass through the liver although there are no absolute oral bioavailability data to confirm this assertion. Since essential thrombocythemia is a chronic condition marked by an elevation of blood platelets, a clinical intravenous formulation was not required and thus never developed. This has regrettably precluded the *direct* measurement of absolute oral bioavailability although other data provide a valuable insight into the likelihood of pre-systemic metabolism of anagrelide.

2. Non-clinical evidence for first pass metabolism

2.1 Oral bioavailability data in the minipig

Recently a number of reviews appear to suggest that the minipig may be a good model for man in terms of drug absorption and disposition. This has been attributed to the similarity of its cytochromes P450 and its gastrointestinal tract to those in man (Anzenbacher et al 1998).

Data have been generated in a non-crossover design in two groups of three female Göttingen SPF minipigs. On the first dosing occasion each of three animals was given 2 x 0.5mg Agrylin® capsules with 20 mL water after an overnight fast. Fasting was continued for at least a further 4 hours. On a further dosing occasion an additional three minipigs were given a 1mg intravenous bolus of anagrelide in 2mL of propylene glycol, again after an overnight fast. In both cases heparinised blood samples were drawn at various times for assessment of plasma drug concentrations using a validated LC/MS-MS method.

Comparison of the oral AUC_{24h} (10.3ng.h/mL) with the intravenous AUC_{inf} estimate (50.7ng.h/mL) suggested an oral bioavailability for anagrelide in minipigs of about 20%. However this estimate should be treated with caution, since it is based on data from only two of the three animals given the intravenous dose (data for the third animal were lost) and due to a failure to be able to compare AUC over the same interval i.e., infinity. Nevertheless it does give an indication of a potentially low absolute bioavailability of anagrelide.

Attachment H Table 1: Pharmacokinetic parameters for anagrelide in Göttingen minipigs given 1mg of the drug orally or intravenously

Dosing	Animal No.	Tmax	Cmax	AUC24h
		(h)	(ng/mL)	(ng.h/mL)
oral	1	3.0	1.29	8.3
	2	8.0	1.09	10.4
	3	3.0	1.98	12.3
	Mean	4.7	1.45	10.3
	(CV%)	(62)	(32)	(20)
iv	4	0.0	83.5	42.7*
	5	0.0	90.6	58.7*
	Average	0.0	87.1	50.7*

* AUC_{inf}.

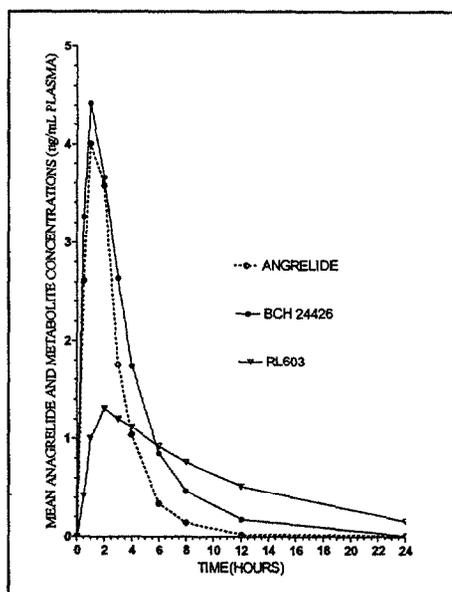
3. Clinical evidence for first pass metabolism

3.1 Superimposability of the rate of formation of the metabolite with absorption of the drug

Data from 38 healthy volunteers age (range 21-76 mean 52 yrs) who participated in three separate clinical pharmacokinetic studies showed that following a 1mg oral dose of the drug under fasting conditions the drug was rapidly absorbed (T_{max} of 1.3 hours) and eliminated (terminal half-life of 1.5 h).

Most importantly the rate of formation of the active metabolite 3-hydroxy anagrelide appeared to proceed in parallel with the absorption of the drug (Figure 1). The concentration-time profiles were superimposed suggesting the metabolite was formed during the first pass of anagrelide through the liver.

Attachment H Figure 1: Plasma concentration time profile for anagrelide and its active metabolite, 3-hydroxy anagrelide, in man following a single 1 mg oral dose of the drug under fasting conditions



3.2 Evidence from hepatic impairment PK study

A pharmacokinetic study in 10 subjects with moderate hepatic impairment (see Table 2) given a single 1mg oral dose of anagrelide revealed an 8-fold increase in total exposure (AUC) to anagrelide. Part of this increase may be related to the 2.2-fold increase in half-life. Surprisingly however, the apparent volume of distribution, (V_z/F), decreased by almost 4-fold. While this could be due to increased plasma protein binding (for example brought about by an increase in α_1 -acid glycoprotein in these hepatically compromised subjects, assuming this to be the binding protein), other mechanisms may play a role. For example, an increase in bioavailability may occur,

as is frequently the case for drugs exhibiting extensive first pass hepatic metabolism. This observation may also provide supporting evidence for first pass metabolism of anagrelide.

Attachment H Table 2: Anagrelide PK parameters in hepatic impairment [arithmetic means (SD)]

Parameter	Hepatically Impaired	Healthy
C_{max} (ng/mL)	[N=10] 13.2 (8.12)	[N=10] 6.09 (6.59)
t_{max} (hr)	[N=10] 2.00 (0.50-2.00)	[N=10] 1.00 (1.00-3.00)
$AUC_{0-\infty}$ (ng·hr/mL)	[N=9] 83.8 (67.0)	[N=9] 10.8 (6.03)
$t_{1/2z}$ (hr)	[N=9] 3.30 (1.40-10.1)	[N=9] 1.53 (1.00-5.20)
V_z/F (L)	[N=9] 87.9 (32.4)	[N=9] 324 (250)
CL_T/F (L/hr)	[N=9] 26.0 (23.5)	[N=9] 119 (57.7)
CL_R (L/hr)	[N=0]	[N=5] 0.0094 (0.00531)

*except t_{max} which is median & range .

3.3 Equation derived estimates of bioavailability

In attempting to estimate the extent of first pass metabolism of anagrelide, the following equation was applied:-

$$F = 1/(1 + (CL_b/F)/QH) \text{ (Rowland and Tozer 1995)}$$

Where QH the liver blood flow (assumed to be 1.35L/min) The relationship between blood clearance and plasma clearance is given by $CL_b/F = CL/F \times C/C_b$ (the ratio $C/C_b = 1.196$.)

Use of this equation makes the following assumptions:-

- Hepatic extraction is the only cause of reduced bioavailability i.e. no issue of incomplete absorption or degradation of the drug in the gut lumen or wall.
- No extrahepatic elimination of the drug e.g. no renal clearance of the drug.

Evidence for hepatic extraction as the sole cause of reduced bioavailability

(i) Urinary recovery of orally administered radiolabelled drug showing good absorption. Two human radiolabelled studies showed extensive renal excretion of orally administered radioactivity. Assuming anagrelide is not degraded in the gut this

would suggest extensive absorption of anagrelide (Gaver et al 1981). The results of these studies are presented in Table 3.

Attachment H Table 3: Recovery of radioactivity in volunteers given a single oral dose of 1mg [¹⁴C]- anagrelide

Study number & subject number	% administered dose recovered in urine	% administered dose recovered in faeces	Grand totals
13,970-107A/1	80.3	15.4	95.7
13,970-107A /2	78.9	17.7	96.5
13,970-107A /3	78.7	23.3	102
13,970-107A /4	77.0	22.0	99.0
13,970-107A /5	76.8	22.7	99.5
Means ± SD	78.34 ± 1.5	20.2 ± 3.5	98.5 ± 2.5
3/1774/1	80.0	9.3	89.3
3/1774/2	71.2	2.9	74.1
3/1774/3	60.7	14.2	74.9
3/1774/4	73.3	17.7	91.0
3/1774/5	74.0	7.1	81.1
Means ± SD	71.8 ± 7.0	10.2 ± 5.8	82.1 ± 7.9
Overall mean ± SD	75.1 ± 5.9	15.2 ± 6.9	90.3 ± 10.3

13,970/017A study used a seven day collection period

3/1774 study (Gaver et al 1981) used a six-day collection period

Other volunteer studies showed rapid absorption from the Agrylin capsule formulation (see Figure 1). Across three separate volunteer studies involving the administration of 1mg of the drug to 38 fasted subjects, T_{max} was achieved with mean ± CV of 1.3h ± 53.8%. Since rapid absorption is usually associated with complete absorption these results suggest quantitative absorption of anagrelide.

(ii) Evidence for lack of degradation in gastrointestinal tract and gut wall

An investigation of the possible *in vitro* biotransformation of [¹⁴C] anagrelide by human gut microflora was conducted using a human faecal preparation (bacterial cell suspension). After incubation of this preparation with a 6 µM solution of the radiolabelled anagrelide for up to 24 hours under anaerobic conditions, subsequent radio-HPLC of the incubates revealed no convincing evidence of bioconversion. Less than 10% of the drug had apparently disappeared, but this was not significantly different from the control incubates, conducted in the absence of bacterial cell suspension. On the basis of these results it was concluded that there was no evidence for gut luminal metabolism of anagrelide.

An investigation of the potential for metabolism of anagrelide by the gut wall was undertaken using human intestinal microsomes. Following a 45 minute incubation period the positive control compound, midazolam, was near completely metabolised but 86.4% of anagrelide was still remaining comparable to the NADPH deficient control incubations (see Table 4).

Attachment H Table 4: Investigation of *in vitro* metabolism of anagrelide by use of human intestinal microsomes

Compound ID	% Parent Compound Remaining					Minus NADPH control
	0 min	5 min	15 min	30 min	45 min	
Anagrelide	100.0	98.3	92.7	83.1	86.4	79.3
Midazolam	100.0	66.1	27.9	5.9	2.1	100.7

(The apparent small reduction in substrate concentrations was attributed to non-specific binding of anagrelide)

It was therefore concluded that anagrelide was not significantly metabolised by human intestinal microsomes.

Direct evidence of hepatic metabolism

Direct evidence for the hepatic metabolism of anagrelide comes from work with human hepatocytes. [¹⁴C]-anagrelide (2µM) was incubated with cryopreserved human hepatocytes in Williams' Medium E (supplemented with HEPES (10 mM), foetal calf serum (10%, v/v), dexamethasone (1 µM) and SPITE (1%, v/v) for 3h. Samples were periodically removed and submitted to radio-HPLC for quantification of disappearance of drug and appearance of metabolites. The results showed a time dependent decrease in the anagrelide concentrations in the incubates and a concomitant increase in the amount of the two metabolites BCH24426 and RL603.

Subsequent work with expressed cytochromes (Supersomes[®]) showed that amongst the isoforms evaluated (3A4, 2C9, 2C19, 2D6, and 1A2) only CYP1A2 appeared to metabolise anagrelide. Since this enzyme is known to be present in the liver but largely absent in the gut wall this provides further support for the assertion of hepatic metabolism of anagrelide.

Evidence for lack of renal clearance of anagrelide

No significant amount of unchanged anagrelide has been found in the urine of any of the volunteers involved in the human radiolabelled study (estimated <1%, Gaver et al 1981). A more formal study in which a quantitative bioanalytical method was applied to the analysis of urine from the ten healthy volunteers age, matched for age, weight,

and sex in a renal impairment study confirmed this initial indication of an essential absence of unchanged drug in the urine (see Table 5).

Attachment H Table 5: Amount of anagrelide excreted in the urine (Ae) in ten volunteers (healthy matched controls from renal impairment study, SPD422-103) after a single 1mg oral dose

Subject number	Ae 0-4h ng	Ae 4-8h ng	Ae 8-12h ng	Ae 12-24h ng	Grand totals	% administered dose
1	183.2	27.75	<0.05	<0.05	210.9	0.021
2	44.8	<0.05	<0.05	<0.05	44.8	0.005
3	75.15	<0.05	<0.05	<0.05	75.15	0.008
4	139.1	<0.05	<0.05	<0.05	139.1	0.014
5	48.96	24.40	<0.05	<0.05	73.36	0.007
6	38.74	<0.05	<0.05	<0.05	38.74	0.004
7	91.8	9.86	<0.05	<0.05	101.7	0.010
8	286.65	19.4	<0.05	<0.05	306.0	0.031
9	161.7	23.7	<0.05	<0.05	185.4	0.019
10	70.0	19.04	<0.05	<0.05	89.04	0.009
Means ± sd	114 ± 79	12.4 ± 11.6	<0.05	<0.05	126.4 ± 84.8	0.013 ± 0.01

3.4 Derivation of estimates of oral bioavailability of the drug

Having satisfied the criteria for use of the equation, $F = 1/(1 + (CL_b/F)/QH)$ this was applied to estimating the bioavailability in 69 volunteers used in four clinical PK studies. These volunteers were the “control” subjects i.e. age, weight and sex matched healthy comparators for the renal and hepatic studies while for the food and aspirin interaction studies these data were derived from volunteers who were fasted or who took the drug without aspirin respectively.

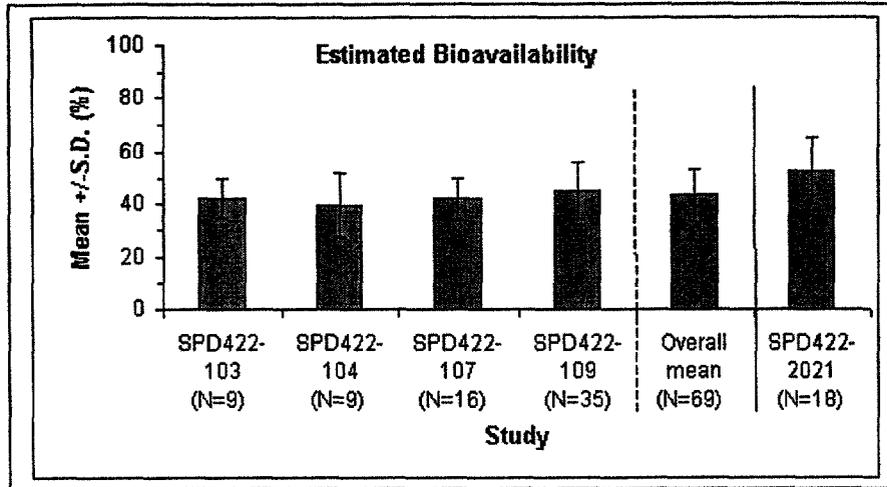
The data for the patient study were drawn from a paediatric vs adult comparison with the latter group (n=18) providing the data used here. The results of these analyses are shown in the Table 6 and Figure 2.

Attachment H Table 6: Estimated oral bioavailability in healthy volunteers and ET/MPD patients

Study	Mean %	S.D.	N
renal impairment study	42.4	7.6	9
hepatic impairment study	39.7	12.4	9
aspirin interaction study	42.1	7.8	16
food interaction study	45.1	10.4	35
Overall mean	43.4	9.8	69
ET patient study ¹	52.6	12.5	18

¹ after repeat administration; normalised to 70kg bodyweight

Attachment H Figure 2: Comparison of estimates of anagrelide's bioavailability in various clinical pharmacokinetic studies in volunteers and patients



These estimates of bioavailability - and hence first pass metabolism - are remarkably consistent between all the healthy volunteers with a mean \pm S.D. 43.4 % \pm 9.8. Those data from patients are not dramatically different showing only a slightly higher bioavailability (i.e. lower first pass) but in either case the extent of pre-systemic metabolism ranged from 47-57%.

4. Conclusion

On the basis of all the data presented here it would seem that anagrelide undergoes a major first pass effect of approximately 50% giving rise to its primary active metabolite, BCH24426.

5. References

Anzenbacher P, Soucek P, Anzenbacherova E, Gut I, Hruby K, Svoboda Z, Kvetina J. Presence and activity of cytochrome P450 isoforms in minipig liver microsomes. Comparison with human liver samples. *Drug Metab Dispos.* 1998 Jan;26(1):56-9.

Erusalimsky JD, Hong Y & Franklin R. Is the platelet lowering activity of anagrelide mediated by its major metabolite 2-amino-5,6-dichloro-3,4-dihydroquinazoline (RL603)?
Exp Hematol. 2002 Jul;30(7):625-6;

Gaver RC, Deeb G, Pittman KA, Smyth RD. Disposition of anagrelide, an inhibitor of platelet aggregation. *Clin Pharmacol Ther.* 1981 Mar;29(3):381-6

Roland M & Tozer TN in "Clinical Pharmacokinetics - Concepts and Applications" published by Lippincott Williams & Wilkins 1995.