

“In vitro metabolism of ciclesonide in human lung and liver precision-cut slices”

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Objective

- To investigate the *in vitro* metabolism of ciclesonide in the human lung and liver using precision-cut slices

Methods

Three precision-cut human livers and three human lung slices were incubated with 10 μ M and 25 μ M ^{14} C-ciclesonide for 2, 6 and 24 hours. The metabolites formed and the remaining parent compound in the tissue samples were analyzed by using HPLC with ultraviolet and radio detection. Retention times of reference compounds or structure elucidation by mass spectrometry were used to attribute chromatographic peaks to a chemical structure.

Identification of the Formed Metabolites

The identity of the lipophilic metabolites (about 12-13 min) could be confirmed by the reference compound BYK204147 (oleate of B9207-021). BYK204147 is the major member metabolite of the M4-family in rats. A representative interexperiment sample of lung extracts (pool from 3 different human experiments: 25 mM, 24 h incubation) was prepared and assayed by LC-MS analysis. A m/z of 737 was detected as major metabolite of this group. As the mass m/z of ^{14} C-RM1 was 473, the difference in mass of 264 units indicated an oleate of RM1. Furthermore, the mass for the palmitate of RM1 (m/z 711) was clearly obtained in this sample (ion spectrum not shown). The LC-MS methodology was not validated for quantitative purpose, however, BYK204147 seemed to be the major member of the M4 family.

Results

All human organs used were highly viable as ascertained by ATP content and K^+ retention for the liver slices and protein synthesis and by ATP content for the lung slices.

The radioactivity increased over time in lungs and decreased in hepatic tissue. At the end of the experiment a 7.2 fold-higher radioactivity in the lung as compared to the liver was observed (Table 1). In lungs, ciclesonide converted to RM1 and RM1 further formed lipid (oleate) conjugates. In the liver, ciclesonide was metabolized to inactive polar compounds with di-hydroxylated RM1 being the major metabolite.

Table 1. Mean % formation of the parent compound and its metabolites in human precision-cut lung and liver slices incubated with 25 mc of radioactivity ciclesonide for 2h and 24h.

Study system: Human Lung slices		Time of incubation	Ciclesonide	RM1	RM1 fatty acid conjugates
			Mean % of formation		
Concentration (nM) : 10 nM	2 h		71.5	14.1	7.1
	6h		49.6	23.1	19.6
	24 h		14.7	29.1	50.4
Concentration (nM) : 25 nM	2 h		60.0	30.1	4.1
	6h		43.6	35.5	15.5
	24 h		20.7	29.2	45.7
Study system: Human Liver slices					
Concentration (nM) : 10 nM	2 h		2.0	45.6	6.3
	6h		1.0	30.1	5.1
	24 h		0.0	7.5	3.3
Concentration (nM) : 25 nM	2 h		2.2	53.4	4.5
	6h		0.6	39.9	5.3
	24 h		0.3	7.2	4.8

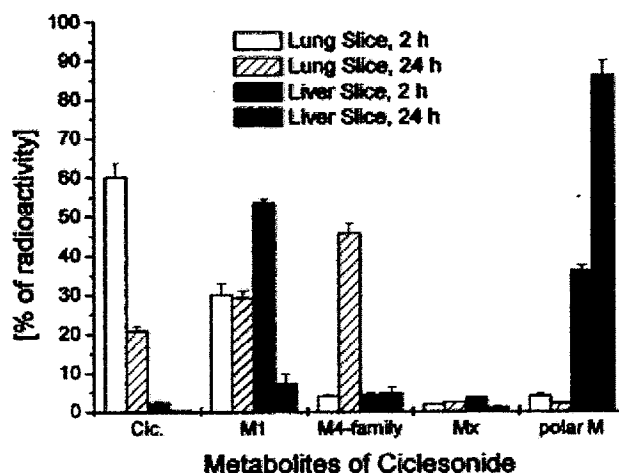


Figure 1. Mean (SEM) radioactivity counts (% of total) of the parent compound and its metabolite in human precision-cut lung and liver slices incubated with 25 mc of radioactivity ciclesonide for 2h and 24h (M4-family corresponds to the RM1 conjugates with fatty acids).

Conclusions. Lipid conjugates of RM1 are formed in lung tissue *in vitro*.

A two-phase incubation system was set up for the screening. Four tubes were prepared for the primary incubations without the addition of ciclesonide RM1 in Tubes 1 and 2 and with the addition of 300 nM of ciclesonide RM 1 in Tubes 3 and 4. Each tube contained microsomal mixtures at a protein concentration of 2 mg/mL (CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 for testosterone) or 1 mg/mL (CYP2A6 and 3A4 for midazolam). After equilibrating the four tubes aliquots of NADPH for Tubes 2 and 4, and water for Tubes 1 and 3 were added to the tubes. After pre-incubation, aliquots were transferred from each of the primary incubation tubes to the secondary incubation tubes aliquots of the solution which contained 50 mM potassium phosphate buffer, probe substrates and NADPH were added. The remaining activity of each enzyme after the secondary incubation step was determined by using the LC/MS/MS methods.

DATA ANALYSIS

Results from incubation samples were expressed as mean of the triplicate samples. The CV of the triplicate samples was less than 19%.

RESULTS

Analytical method

The analytical runs in this study met the acceptance criteria in terms of accuracy. %Bias for all runs was less than 20%. No inter-day and intra-day precision, calibration curves, LOQ, etc. were provided.

Inhibition Studies

Positive controls were effective in decreasing the formation of marker metabolites under the experimental conditions used in this study (Table 1).

Table 1. Incubation conditions used in experiment for assessing direct inhibition of CYP450 enzymes in human liver microsomes (HLM)

CYP450 Isoform	Probe Substrate	Final Total Protein Conc. in HLM Solution (mg/mL)	Positive Control	Incubation Time (Min.)
CYP1A2	40 μ M Phenacetin	0.2	2 μ M Furofylline	30
CYP2A6	5 μ M Coumarin	0.1	200 μ M Pilocarpine	10
CYP2C9	50 μ M Tolbutamide	0.2	100 μ M Sulfaphenazole	30
CYP2C19	80 μ M S-(+)-Mephenytoin	0.2	100 μ M Tranylcypromine	30
CYP2D6	10 μ M Dextromethorphan	0.2	10 μ M Quinidine	30
CYP2E1	40 μ M Chlorzoxazone	0.2	100 μ M Diethyldithiocarbamate	30
CYP3A4	5 μ M Midazolam	0.1	1 μ M Ketoconazole	10
CYP3A4	80 μ M Testosterone	0.2	1 μ M Ketoconazole	10

The direct inhibition of RM1 to all the tested CYP450 enzymes at the compound concentrations up to 3 μM did not result in more than 25% decrease in enzyme activity (Table 2, Figure 1). Thus, the IC_{50} values for the direct inhibition of any of the tested CYP450 enzymes would be much greater than 3 μM . In the phase I clinical trial, the subjects receiving up to 3.6 mg/kg of ciclesonide via metered dose inhalers resulted in a peak plasma concentration of ciclesonide RM1 at 7.4 nM. Since the 3 μM concentrations of ciclesonide RM1 used in the in vitro enzyme inhibition study was about 400-fold higher than the peak plasma concentrations that is likely to occur in the clinic, this metabolite of ciclesonide are unlikely to significantly inhibit cytochrome CYP450 enzymes in patients receiving ciclesonide at therapeutic doses.

Table 2. Results of direct inhibition potential of RM1 metabolite of ciclesonide on CYP450 enzymes expressed as percent remaining enzyme activities in HLM

CYP450 Enzyme	RM1 Metabolite of Ciclesonide (Ciclesonide RM1)					Positive Control
	0.3 nM	3.0 nM	30 nM	300 nM	3000 nM	
CYP1A2	99	100	99	98	98	8.5
CYP2A6	101	88	88	97	87	22
CYP2C9	97	95	90	94	89	9.1
CYP2C19	101	97	95	90	80	56
CYP2D6	95	90	60*	98	98	1.3
CYP2E1	97	98	100	98	99	56
CYP3A4 (Midazolam)	99	97	76	86	86	<5.4
CYP3A4 (Testosterone)	94	97	88	76	89	6.4

*Outlier, not used for calculation.

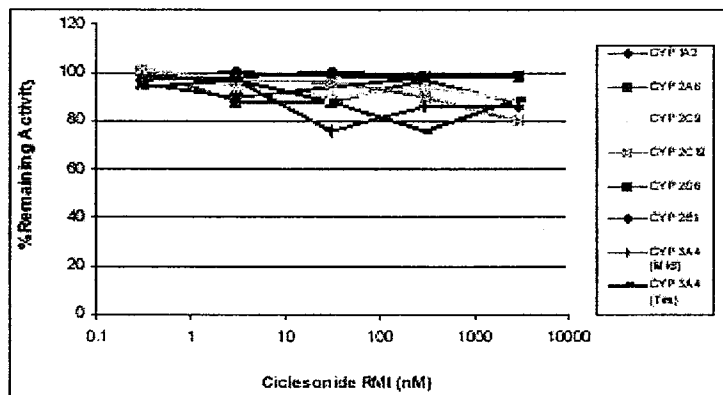


Figure 1. Effect of RM1 metabolite of ciclesonide (ciclesonide RM1) on remaining activities of various CYP450 isoforms.

In the metabolism-based inhibition screening, pre-incubation of ciclesonide RM1 with NADPH compared to that without NADPH did not result in more than 15% decrease in enzyme activity for all the tested CYP450 enzymes (Table 3). The data suggests that the potential for metabolism based inhibition was minimal, and therefore, the sponsor did not follow up with a

definitive study. The enzyme activity from control samples pre-incubated with NADPH in the absence of ciclesonide RM1 did not show a destabilizing effect in the incubation.

Table 3. Results of metabolism-based inhibition potential of RM1 metabolite of ciclesonide (ciclesonide RM1) on CYP450 enzyme expressed as percent remaining enzyme activities in HLM

CYP450 Enzyme	RM1 Metabolite of Ciclesonide (Ciclesonide RM1)	
	Pre-incubation w/o NADPH	Pre-incubation with NADPH
CYP1A2	100	86
CYP2A6	100	99
CYP2C9	100	100
CYP2C19	100	96
CYP2D6	100	80
CYP2E1	100	103
CYP3A4 (Midazolam)	100	114
CYP3A4 (Testosterone)	100	102

CONCLUSION

- At concentrations as high as 3 μM , RM1 failed to produce significant direct or metabolism-based inhibition of cytochrome CYP450s in pooled human liver microsomes.

Reviewer's Comments

- Optimal probe substrate and recommended concentrations were used for preferred substrate reactions in this study (in vitro report from inhibition working group internal guidance).
- The $[I]/K_i$ ratio ($.0074\mu\text{M}/3\mu\text{M}$) is 0.002 which is much lower than the 0.02 cut-off considered for likelihood of DDI.
- It was not specified if the racemic mixture of ciclesonide or only the R-ciclesonide was used in the conduct of this study. It is recommended that for racemic drugs, individual isomers be evaluated separately.
- The potential of ciclesonide to act as an inhibitor of CYP enzymes should be evaluated.
- No data on the validation of analytical methods used (intra, inter-day precision, calibration curves, etc.) was provided.

TREATMENT OF PRIMARY CULTURES OF HUMAN HEPATOCYTES

Upon receipt of human hepatocytes, the medium was replaced with 2 mL of fresh complete Hepato-STIM and cell morphology was evaluated with inverted phase contrast microscope (____). Cells were maintained at 37 ° C in a humidified incubator under an atmosphere with an air-to-CO₂ ratio of 95 : 5. After approximately 24 ±2 hours of adaptation period, cells were reexamined for normal morphology before initiation of the CYP450 enzyme induction studies. Hepatocytes were then treated daily for three consecutive days with 2 mL fresh medium containing the vehicle control, 0.5% acetonitrile; the negative drug control, 25 µM of probenecid ; the prototypical inducers, 25 µM of rifampin and 25 µM of omeprazole; and ciclesonide at 0.01 µM, 0.1 µM and 1 µM, respectively.

Acetonitrile was the solvent of choice for dissolving controls and ciclesonide resulting in a final concentration of 0.5 % in the medium. Approximately 24 hr after the last treatment, the hepatocytes were examined under microscope and photographed to document their morphology.

RESULTS

VIABILITY AND MORPHOLOGY OF THE CULTURED HEPATOCYTES

Hepatocyte cultures were observed by phase-contrast microscopy and judged to be morphologically normal with confluency adequate for initiating studies. At the completion of the final treatment, hepatocytes were examined to document their morphological integrity and any overt signs of toxicity of the test compounds. These observations indicated that human hepatocytes treated with vehicle (0.5% acetonitrile), drug negative control (25 µM probenecid), positive inducers (25 µM rifampin and 25 µM omeprazole), and with up to 1 µM of ciclesonide exhibited normal hepatocyte morphology. Representative photomicrographs from two donors were presented.

THE EFFECT OF CICLESONIDE ON CYP1A2 EXPRESSION

Determination of phenacetin *O*-deethylase activity

In human hepatocyte cultures the *O*-deethylation of phenacetin to paracetamol (4-acetamidophenol) is catalyzed by CYP1A2, which is highly inducible by omeprazole (5). Treatment of cultured human hepatocytes with omeprazole once daily for three consecutive days increased, on average, the *O*-deethylase activity by approximately 20-fold (348.69 pmol/mg/min) as compared to the vehicle control (17.00 pmol/mg/min). Treatment with probenecid, a negative drug inducer, showed activity of 32.50 pmol/mg/min, which was nearly two-fold the activity observed with the vehicle treatment. However, use of probenecid resulted in much less than 40% of the positive control activity and therefore probenecid was considered as a negative control. The effects of ciclesonide on CYP1A2 induction potential in human hepatocytes are shown in Table 1. Treatment of human hepatocytes daily for three days with up to 1 µM of ciclesonide did not affect the phenacetin *O*-deethylase activity.

Determination of CYP1A2 immunoreactive protein expression

Treatment of cultured human hepatocytes with omeprazole resulted in an increase, on average, of approximately 4.7-fold in immunoreactive CYP1A2 protein expression as compared to the vehicle treatment. Probenecid treatment showed negligible change in immunoreactive CYP1A2 protein expression as compared to the vehicle treatment. Ciclesonide treatment at concentrations up to 1 µM appeared to have a similar effect on CYP1A2 protein expression as did the negative control, probenecid. The results are summarized in Table 2.

Determination of CYP1A2 mRNA Expression

Treatment of hepatocytes with omeprazole resulted in an average 23.4-fold increase of mRNA expression. Probenecid treatment showed no significant effect. Ciclesonide treatment at concentrations up to 1 μ M appeared to have no significant effect on CYP1A2 mRNA expression. The results are summarized in Table 3.

THE EFFECT OF CICLESONIDE ON CYP2C9 EXPRESSION

Determination of diclofenac 4'-hydroxylase activity

In human hepatocyte cultures the 4'-hydroxylation of diclofenac is catalyzed by CYP2C9, which is moderately inducible by rifampin. Treatment of cultured human hepatocytes with rifampin once daily for three consecutive days increased, on average, the diclofenac 4'-hydroxylase activity by approximately 5.4-fold (72.94 pmol/mg/min) as compared to the vehicle control (13.42 pmol/mg/min). The results are summarized in Table 4. Probenecid treatment resulted in no significant effect. The effects of ciclesonide on CYP2C9 induction potential in human hepatocytes are shown in Table 1. Treatment of human hepatocytes daily for three days with up to 1 μ M of ciclesonide produced no effect on diclofenac 4'-hydroxylase activity.

Determination of CYP2C9 immunoreactive protein expression

Treatment of cultured human hepatocytes with rifampin resulted in a slight increase, on average, of approximately 1.6 fold in immunoreactive CYP2C9 protein expression as compared to the vehicle treatment. Ciclesonide treatment at concentrations up to 1 μ M appeared to have no effect on CYP2C9 immunoreactive protein expression. The results are summarized in Table 2.

Determination of CYP2C9 mRNA Expression

Treatment of hepatocytes with rifampin resulted in an average 2.5-fold increase in mRNA expression. Probenecid produced no effect on CYP2C9 mRNA expression as compared to the vehicle treatment. Ciclesonide treatment at concentrations up to 1 μ M appeared to have no effect on CYP2C9 mRNA expression. The results are summarized in Table 3

THE EFFECT OF CICLESONIDE ON CYP2C19 EXPRESSION

Determination of S(+)-mephenytoin 4'-hydroxylase activity

In human hepatocyte cultures the S(+)-mephenytoin 4'-hydroxylation is catalyzed by CYP2C19, which is moderately inducible by rifampin. Treatment of cultured human hepatocytes with rifampin once daily for three consecutive days increased, on average, the S(+)-mephenytoin 4'-hydroxylase activity by approximately 3.6-fold (5.04 pmol/mg/min) as compared to the vehicle control (1.40 pmol/mg/min). Probenecid treatment resulted in similar activity as the vehicle treatment. The results are summarized in Table 1. Treatment of human hepatocytes daily for three days with up to 1 μ M of ciclesonide showed no significant effect on S(+)- mephenytoin 4'-hydroxylase activity.

Determination of CYP2C19 immunoreactive protein expression

Treatment of cultured human hepatocytes with rifampin produced little effect on immunoreactive CYP2C19 protein expression as compared to the vehicle treatment. Ciclesonide treatment at concentrations up to 1 μ M appeared to have no effect on CYP2C19 immunoreactive protein expression. The results are summarized in Table 2.

Determination of CYP2C19 mRNA Expression

Treatment of hepatocytes with rifampin or probenecid resulted in no significant change of mRNA expression as compared to the vehicle treatment. Ciclesonide treatment at concentrations up to 1 μ M appeared to have no effect on CYP2C9 mRNA expression. The results are summarized in Table 3.

THE EFFECT OF CICLESONIDE ON CYP3A4 EXPRESSION

Determination of testosterone 6 β -hydroxylase activity

In human hepatocyte cultures the 6 β -hydroxylation of testosterone is catalyzed by CYP3A4/5, which is highly inducible by rifampin. Treatment of cultured human hepatocytes with rifampin once daily for three consecutive days increased, on average, the testosterone 6 β -hydroxylase activity by approximately 8.1-fold (1156.18 pmol/mg/min) as compared to the vehicle control (141.87 pmol/mg/min). Treatment with probenecid, a negative control, produced a 1.3-fold increase in the activity as compared to the vehicle treatment. However, when compared to the positive control, the increase was negligible. Treatment of human hepatocytes daily for three days with up to 1 μ M of ciclesonide resulted in no change in testosterone 6 β -hydroxylase activity as compared to the vehicle treatment. Ciclesonide had no effect on CYP3A4/5 enzyme activity. The results are summarized in Table 1.

Determination of CYP3A4 immunoreactive protein expression

Treatment of cultured human hepatocytes with rifampin resulted in an increase, on average, of approximately 4.0-fold in immunoreactive CYP3A4 protein expression as compared to the vehicle treatment. Probenecid treatment produced only a negligible effect on CYP3A4 protein expression. Ciclesonide treatment at concentrations up to 1 μ M appeared to have no effect on CYP3A4 immunoreactive protein expression. The results are summarized in Table 2.

Determination of CYP3A4 mRNA Expression

Treatment of hepatocytes with rifampin, a known CYP3A4 inducer, resulted in a marked increase averaging 42.5-fold in mRNA expression. Probenecid treatment produced an approximately 2-fold induction which was much less than 40% of the induction of the positive control and was considered a negligible effect. Ciclesonide treatment at concentrations up to 1 μ M appeared to have a similar effect to that of the probenecid treatment on CYP3A4 mRNA expression, and ciclesonide was not considered to be an inducer. The results are summarized in Table 3.

Table 1. Effects of treating cultured human hepatocytes with ciclesonide, probenecid or prototypical inducers on the expression of cytochrome P450 enzyme activities

Treatment	Enzyme Activity (pmol/mg/min) ^a			
	Phenacetin O-deethylation (CYP1A2)	Diclofenac 4'-hydroxylation (CYP2C9)	Mephenytoin 4'-hydroxylation (CYP2C19)	Testosterone 6 β -hydroxylation (CYP3A4/5)
Vehicle 0.5 % ACN	17	13.42	1.40	141.87
	1.0	1.0	1.0	1.0
Omeprazole 25 μ M	348.69	-	-	-
	20.5	-	-	-
Rifampin 25 μ M	-	72.94	5.04	1156.18
	-	5.4	3.6	8.1
Probenecid 25 μ M	32.5	14.96	1.41	185.45
	1.9	1.1	1.0	1.3
Ciclesonide 0.01 μ M	15.82	13.93	1.26	123.08
	0.9	1.0	0.9	0.9
Ciclesonide 0.1 μ M	14.64	15.24	1.33	143.94
	0.9	1.1	0.9	1.0
Ciclesonide 1 μ M	16.38	17.55	1.56	200.19
	1.0	1.3	1.1	1.4

^a values are the average of two human hepatocyte preparations: HH128 and HH129

Values in parenthesis are the values obtained from individual liver

Bold value refer to the fold induction of the mean value relative to the vehicle treatment

b(4)

Table 2. Effects of treating cultured human hepatocytes with ciclesonide, probenecid or prototypical inducers on the expression of each cytochrome P450 detected by immunoblot method

Treatment	P450 content normalized with protein loading in the immunoblot analysis (fmol/ μ g) ^a			
	CYP1A2	CYP2C9	(CYP2C19)	CYP3A4
Vehicle 0.5 % ACN	1.5	1.4	1.8	1.0
	1	1	1	1
Omeprazole 25 μ M	7	-	-	-
	4.7	-	-	-
Rifampin 25 μ M	-	23	2.3	120
	-	1.6	1.3	4.0
Probenecid 25 μ M	2	15	1.3	40
	1.3	1.1	0.7	1.3
Ciclesonide 0.01 μ M	2	15	1.8	40
	1.3	1.1	1	1.3
Ciclesonide 0.1 μ M	2	15	1.3	30
	1.3	1.1	0.7	1
Ciclesonide 1 μ M	1.5	14	1.8	30
	1.0	1	1	1

^a values are the average of two human hepatocyte preparations: HH128 and HH129

Values in parenthesis are the values obtained from individual liver

Bold value refer to the fold induction of the mean value relative to the vehicle treatment

b(4)

Table 3. Effects of treating cultured human hepatocytes with ciclesonide, probenecid or prototypical inducers on the expression of cytochrome P450 mRNA

Treatment	mRNA levels (arbitrary unit) ^a			
	CYP1A2	CYP2C9	CYP2C19	CYP3A4
Vehicle 0.5 % ACN	1.66	3.02	2.83	0.13
	1.0	1.0	1.0	1.0
Omeprazole 25 µM	38.70	-	-	-
	23.4	-	-	-
Rifampin 25 µM	-	7.47	3.00	5.42
	-	2.6	1.1	41.7
Probenecid 25 µM	2.36	3.71	2.33	0.26
	1.4	1.2	0.8	2.0
Ciclesonide 0.01 µM	1.34	3.14	3.23	0.15
	0.8	1.0	1.1	1.1
Ciclesonide 0.1 µM	1.36	3.38	3.19	0.14
	0.8	1.1	1.1	1.1
Ciclesonide 1µM	1.50	4.03	2.83	0.33
	0.9	1.3	1.0	2.6

b(4)

SUMMARY OF FINDINGS

- The treatment of hepatocytes with up to 1 µM of ciclesonide showed no cytotoxicity, and the cell viability was comparable to that resulting from the vehicle treatment.
- The test system of the two human hepatocyte cultures was functional for CYP1A2, CYP2C9, CYP 2C19 and CYP 3A4.
- Under the *in vitro* experimental conditions in primary cultures of human hepatocytes, the results indicated that the ciclesonide treatment for three days at the concentration up to 1 µM (approximately 100x Cmax) showed no cytotoxicity, and did not induce enzyme activities, mRNA and protein expressions of the four CYP isoforms.

CONCLUSION

- Ciclesonide at therapeutic serum concentrations is not likely to induce CYP1A2, CYP2C9, and CYP 3A4.
- The lack of effect of ciclesonide on CYP 2C19 reported in this study is questionable since lower concentration than those recommended for rifampin were used.

Reviewer's Comments

- Optimal inducers and recommended concentrations were used in this study except for the effect on CYP2C19 (in vitro report from induction working group internal guidance). The recommended rifampin concentration in this case is 100 µM and the sponsor used 25 µM.
- The concentrations of probe substrates used in this study are much higher (more than 2-fold higher than those recommended by the wording group).

- The potential of RM1 (the major metabolite of ciclesonide) to act as an inducer of CYP enzymes was not assessed in this study.
- It was not specify if the racemic mixture of ciclesonide or only the R-ciclesonide was used in the conduct of this study. It is recommended that for racemic drugs, individual isomers be evaluated separately.
- The lack of effect of ciclesonide on CYP 2C19 reported in this study is questionable since lower concentrations than those recommended for rifampin and probe substrate were used. The mechanism of CYP3A5 induction appears to be different than from CYP3A4 induction. Recently it has been shown that glucocorticoid can induce CYP3A5.
- It was not specify if the racemic mixture of ciclesonide or only the R-ciclesonide was used in the conduct of this study. It is recommended that for racemic drugs, individual isomers be evaluated separately.
- The potential of RM1 to act as an inducer of CYP enzymes should be evaluated.
- No data on the validation of analytical methods used (intra, inter-day precision, calibration curves, etc.) was provided.

APPEARS THIS WAY ON ORIGINAL

Clinical Study Report no.: 246/2001
Study Report Date: Feb 18, 2002

- To investigate the serum protein binding of ciclesonide in rats, dogs and humans

Ciclesonide (batch No. L.J.15-17, stock No. 623, stated specific activity 39.41 μ Ci/mg) was

Serum samples were obtained after centrifugation of the blood samples from Sprague-Dawley rats, Beagle dogs and healthy volunteers. Serum protein binding of ciclesonide was determined in both animal species and human serum at 10, 50, 100, 1000, 10000 ng/ml. Free and bound drug was separated by equilibrium dialysis and quantitated by liquid scintillation counting.

PTFE (Teflon) cells with 1 ml cell volume separated by cellulose membranes (Diachema dialysis membranes) were used to determine the serum protein binding. The cells were subjected to rotation in a water bath for 5 hours. One side of the dialysis cell is filled with serum containing the appropriate drug amount (after determination of the total concentration), the other side is filled with physiological phosphate buffer (20 mM, pH 7.4). 100 μ L of the spiked serum were used to determine the total radioactivity. Free and bound radioactivity were quantitated by taking 500 μ L aliquots from the dialysis chambers and mixed with 10 ml scintillant in a scintillation vial. All samples were then counted in a $\text{scintillation counter}$. The non-specific binding of ciclesonide to the membrane was determined in the same way using spiked buffer samples.

Data analysis was performed using the following equations:

$$A = (\text{CO}-\text{CO}')/\text{CO}$$

A is non-specific binding

CO is the drug concentration in the deproteinated plasma

CO' is the drug concentration in the filtrate.

$$B = [C_1(0) - 2C_1(t)] / C_1(0)$$

B is the percent protein binding (%)

$Cl(o)$ is the total drug concentration (bound and unbound)

Cl(t) is the concentration of the free drug

RESULTS AND DISCUSSION

Analytical Method

No data was provided

Binding

The non-specific binding of ciclesonide on membranes used in equilibrium dialysis was determined with spiked buffer samples and ranged from 0.21% to 0.65% (Table 1). No raw data was provided.

The in vitro serum protein binding results of ciclesonide in different species, expressed as the mean percentage of drug bound to serum proteins, are summarized in Table 1. No raw data was provided. In addition, no data was submitted on equilibrium dialysis. In general, the serum protein binding of ciclesonide was high and was similar among species with slightly lower protein binding in the rat. The extent of protein binding was in the following order: rat (98.6-99.9%) <human (98.9-99.4%) <dog (99.5-99.6%). The sponsor claims that no apparent saturation of protein binding was observed in all species in the concentration range of 10 to 10000 ng/ml of ciclesonide; however, no data was provided to support this statement.

Table 1. Non-specific and in vitro serum protein binding (%) of ciclesonide in different species

Ciclesonide concentration (ng/ml)	Species					
	Rat		Dog		Human	
	Serum	Membrane	Serum	Membrane	Serum	Membrane
10	98.6	0.33	99.5	0.52	99.4	0.28
50	98.7	0.51	99.5	0.28	99.4	0.28
100	98.7	0.25	99.6	0.36	99.0	0.43
1000	98.7	0.31	99.5	0.44	98.9	0.26
10000	98.9	0.29	99.6	0.35	98.9	0.23

GENERAL COMMENTS

- Data on analytical method validation was not provided.
- The sponsor did not provided raw data or study protocols for any of the analysis presented in this report.

CONCLUSION

- The protein binding of ciclesonide appears to be (98.9 to 99.8%) in various species including humans.

"PROTEIN BINDING IN PLASMA OF VARIOUS SPECIES AND IN ISOLATED HUMAN PLASMA PROTEINS"

Clinical Study Report no.: DMPK 2003-0001
Study Initiation Date: Feb 04, 2003
Study Director: _____
Study Leader: _____
Coordinator- Bioanalysis: _____

b(4)

Objectives

- to determine the in vitro protein binding of RPR 257970 (RM1) in the plasma of various animal species and humans, and in isolated human plasma proteins at nominal concentrations of 0.5, 5.0, 25, 100, and 500 ng/mL using equilibrium dialysis.

MATERIALS AND METHODS

Test Article Information:

RPR 257970 (Batch ID: GU03/165A, Molecular Weight: 556.7 g/mol), RM1 metabolite of ciclesonide. All analytical reagents were of at least standard laboratory reagent grade.

Test Systems:

Matrices of mouse, rat, rabbit, dog and human plasma, and isolated human plasma proteins (albumin, α 1-acid glycoprotein and transcortin) and nominal concentrations of 0.5, 5.0, 25, 100, and 500 ng/mL were selected per study objectives and the observed RPR 257970 plasma concentrations in Drug Safety and clinical studies.

Plasma from mouse, rat, dog (adult beagle), rabbit (New Zealand White), and humans were obtained from _____. All isolated human plasma proteins: albumin, α 1-acid glycoprotein and transcortin, were purchased from _____. All three isolated human plasma proteins were studied at their respective physiological concentrations, i.e., albumin, 40 g/L (40 mg/mL, 580 μ M); α 1-acid glycoprotein: 1 g/L (1 mg/mL, ~25 μ M); transcortin, 0.037 g/L (37 μ g/mL, 0.71 μ M).

b(4)

Fortification of Study Matrices:

Fortified study matrices at concentrations of 500-, 100-, 25-, 5-, and 0.5 ng/mL were prepared and stirred for approximately 15 min. The fortified matrices were then incubated at 37 °C for at least 15 min prior to introduction into the dialysis cells.

Free and bound fractions of RPR 257970 were determined by dialysis of RPR 257970 fortified mouse, rat, rabbit, dog, and human plasma and RPR 257970 fortified isolated human plasma protein solutions against phosphate buffer (pH 7.4) at 37 °C. The extent of binding of RPR 257970 was determined at nominal concentrations of 0.5, 5.0, 25, 100, and 500 ng/mL.

Establishing Equilibrium Time:

The incubation time necessary for equilibration of RPR 257970 in the dialysis system were determined by sampling four replicate cells after 4, 6, and 8 h of dialysis. Only human plasma at 500 ng/mL was dialyzed to determine the equilibration time. Measurements of plasma

and buffer weights in each cell half were obtained to evaluate volume shift and recovery of RPR 257970 from the dialysis system.

Equilibrium Dialysis:

The plasma protein binding studies were carried out using a Spectrum Dialysis System. Plasma was dialyzed against phosphate buffer, pH 7.4, at 37 °C using membrane discs (MWCO 2, 12,000–14,000 molecular weight cut-off (MWCO). Approximately 1 mL of RPR 257970 fortified matrices was added to each donor

b(4)

Sample Collection and Analysis:

After the equilibrium period, the contents of each cell half (matrices/buffer) were recovered and placed in a labeled plastic vial. A single aliquot (800 µL) of plasma and albumin solution samples was collected directly. To prevent the non-specific binding of RPR 257970, a single aliquot (800 µL) of all dialysate samples, as well as α1-acid glycoprotein and transcortin solution samples was collected into sample vials containing 200 µL of bovine serum albumin solution (200 mg/mL in dialysis buffer),

The samples were analyzed by LC/MS/MS to determine the equilibrium concentrations of RPR 257970. Concentration values were reported without correction for human α1-acid glycoprotein and transcortin solution samples and all dialysate samples. The concentration values for dialysate, human α1-acid glycoprotein and transcortin solution samples were corrected for BSA dilution during sample collection before calculations.

Calculations of Binding:

Percent unbound (free) = $[C_{\text{buffer}}/C_{\text{plasma}}] \times 100$

Percent bound = 100 - percent unbound

RESULTS AND DISCUSSION

Analytical Method

Pre-study validation

Stability and % of Recovery

Table A. Assay performance for RPR 257970

	RPR 257970
Linearity	Satisfactory: Standard curve range from _____
Accuracy	Satisfactory: % Bias: _____
Inter-day Precision	Satisfactory: % CV: _____
Intra-day Precision	Satisfactory: % CV: _____
Specificity	Satisfactory: sample chromatograms submitted

b(4)

Equilibrium Time:

Free concentrations of RPR 257970 following dialysis of fortified (500 ng/mL) human plasma increased slightly between 4 and 6 h and remained constant from 6 to 8 h, indicating equilibrium was achieved after 6 h of dialysis. The results are presented in Figure 1. Therefore a dialysis time of 6 h was selected as the equilibrium time for the current equilibrium dialysis of RPR 257970.

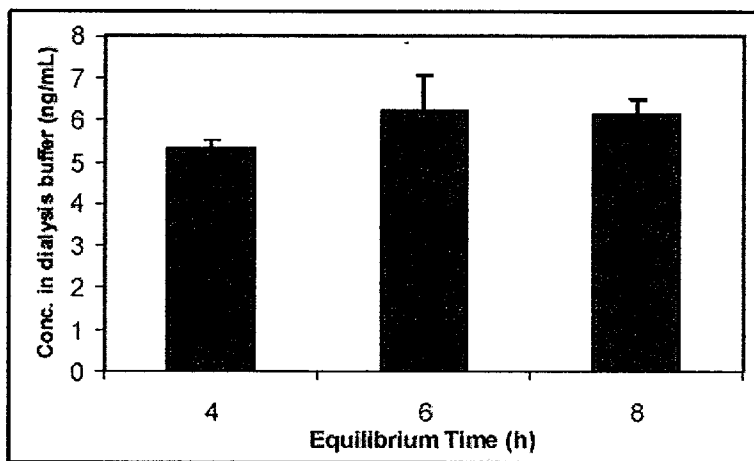


Figure 1. Free RPR 257970 Concentrations in human plasma at a Nominal Concentration of 500 ng/mL during Determination of Equilibrium Time (n=4 Replicates)

In Vitro Protein Binding of RPR 257970:

The mean percentages of bound RPR 257970 in mouse, rat, rabbit and dog plasma are summarized in Table 1. The mean percentages of bound RPR 257970 in human plasma, human albumin solution and human α 1-acid glycoprotein solution are summarized in Table 2. The total compound recovery from the equilibrium dialysis system following 6 hr dialysis was not complete, indicating non-specific binding to the dialysis apparatus and the membrane. This is consistent with the high non-specific binding observed in the previous protein binding study using ultrafiltration¹. Most recoveries were in the range of 80 – 90%, some over 90% and some just ~70%. Therefore, the incomplete recovery of RPR 257970 from the equilibrium dialysis system suggests the reported protein binding data from this study should be interpreted with caution.

The total compound recoveries with human transcortin solution were very low, mostly below 10% (Table 3), thus the percent binding of RPR 257970 could not be determined for human transcortin solution in the present study.

Table 1. Summary of *In Vitro* Protein Binding of RPR 257970 in Mouse, Rat, Rabbit and Dog Plasma

Matrix	Nominal Conc.	Conc. In Matrix	Conc. In Dialysate*	bound
	(ng/mL)	(pg/mL)	(pg/mL)	%
Mouse Plasma	0.5	352 ± 28.7	BLQ	ND
	5	4982 ± 947	47.5 ± 5.14	99.0 ± 0.20
	25	24573 ± 1107	213 ± 8.86	99.1 ± 0.05
	100	83543 ± 1874	890 ± 11.1	98.9 ± 0.01
	500	389175 ± 10843	4225 ± 130	98.9 ± 0.02
Rat Plasma	0.5	471 ± 17.3	BLQ	ND
	5	4630 ± 82.2	114 ± 16.3	97.5 ± 0.38
	25	28500 ± 1133	541 ± 24.5	98.0 ± 0.17
	100	84453 ± 4871	2078 ± 17.6	97.5 ± 0.14
	500	445050 ± 27346	10460 ± 793	97.7 ± 0.09
Rabbit Plasma	0.5	247 ± 5.46	BLQ	ND
	5	4369 ± 128	38.0 ± 0.94	99.1 ± 0.01
	25	23465 ± 1533	185 ± 2.85	99.2 ± 0.05
	100	84178 ± 3158	738 ± 33.6	99.1 ± 0.05
	500	443225 ± 12261	3811 ± 80.2	99.1 ± 0.04
Dog Plasma	0.5	410 ± 14.7	BLQ	ND
	5	3701 ± 117	76.7 ± 2.78	97.9 ± 0.06
	25	18143 ± 1082	377 ± 29.6	97.9 ± 0.24
	100	74968 ± 2729	1486 ± 56.7	98.0 ± 0.14
	500	358275 ± 17434	7390 ± 165	97.9 ± 0.06

*concentrations in dialysate were corrected for dilution during BSA sample collection. Bioanalytical reported concentrations without correction for BSA dilution.

BLQ, below the limit of quantitation of 12.5 pg/mL in dialysate (10 pg/mL for BSA collected in dialysate samples)

ND, not determinable, concentration in dialysate below the limit of quantitation.

Table 2. Summary of *In Vitro* PB of RM1 in Human Plasma and Isolated Human Plasma Protein Solutions

Matrix	Nominal Conc.	Conc. In Matrix*	Conc. In Dialysate*	bound
	(ng/mL)	(pg/mL)	(pg/mL)	%
Human Plasma	0.5	462 ± 40.0	BLQ	ND
	5	4686 ± 203	59.9 ± 6.12	98.7 ± 0.15
	25	21378 ± 670	311 ± 28.0	98.5 ± 0.15
	100	96038 ± 3707	1126 ± 64.0	98.8 ± 0.07
	500	442925 ± 7033	5853 ± 191	98.7 ± 0.05
Human Albumin	0.5	471 ± 1.82	34.9 ± 1.14	92.6 ± 0.25
(40 mg/mL)	5	4520 ± 212	274 ± 12.5	93.9 ± 0.17
	25	22020 ± 1838	1384 ± 153	93.6 ± 0.81
	100	82430 ± 2712	4668 ± 163	94.3 ± 0.20
	500	389375 ± 9110	25775 ± 1084	93.5 ± 0.30
Human α1-Acid Glycoprotein	0.5	395 ± 15.6	19.2 ± 3.34	95.1 ± 0.81
(1 mg/mL)	5	3743 ± 85.1	213 ± 22.5	94.3 ± 0.52
	25	18525 ± 537	1153 ± 158	93.8 ± 0.99
	100	69106 ± 3598	6513 ± 2684	90.4 ± 4.39
	500	336281 ± 5232	31253 ± 3300	90.7 ± 1.08

*concentrations in human α1-acid glycoprotein solution and all dialysate were corrected for dilution during BSA sample collection. Bioanalytical reported concentrations without correction for BSA dilution.

BLQ, below the limit of quantitation of 125 pg/mL in dialysate | 10 pg/mL for BSA collected in dialysate samples|

ND, not determinable, concentration in dialysate below the limit of quantitation.

SUMMARY OF FINDINGS

1. The total compound recovery from the equilibrium dialysis system following 6 hr dialysis was not complete, indicating non-specific binding to the dialysis apparatus and the membrane. Therefore, the results of protein binding from this study should be interpreted with caution.
2. The in vitro protein binding of RPR 257970, calculated from the measured equilibrium concentrations of RPR 257970 in the dialysate and the study matrices, was high in plasma, over 97.5% in the plasma of all species.
3. At nominal RPR 257970 concentrations of 5 to 500 ng/mL, the mean percent protein binding in plasma followed the order of 97.5-98.0% in rat < 97.9-98.0% in dog < 98.5-98.8% in human < ~99.0% in mouse < 99.1-99.2% in rabbit. According to the sponsor, these results were very similar to those reported in the previous study using ultrafiltration.
4. At nominal RPR 257970 concentrations of 0.5 to 500 ng/mL, the mean percent binding in human albumin solution varied from 92.6 to 94.3%; the mean percent binding in human α1-acid glycoprotein solution varied from 90.4 to 95.1%. The results suggest that both albumin and α1-acid glycoprotein contributed to the protein binding of RPR 257970 in human plasma.
5. The total compound recoveries with human transcortin solution were very low, mostly below 10%. The protein binding of RPR 257970 to human transcortin could not be determined in the present study.

b(4)

"Plasma Protein Binding in Different Species, Blood Cell/plasma Partitioning in Human Blood and Drug Interaction in Human Plasma"

Clinical Study Report no.: DMPK/US 01-046 (79/2001)
Study Initiation Date: March 28, 2001
Study Director: _____
Study Leader: _____
Coordinator- Bioanalysis: _____

b(4)

Objectives

Primary:

- To determine the protein binding of RPR 257970 in various species including humans.
- To determine the human blood cell/plasma partitioning.

Secondary:

- to determine the changes in protein binding of RPR 257970 in the presence of warfarin and salicylic acid in renally impaired and hepatically impaired patients.

MATERIALS AND METHODS

Plasma from various species of animals was obtained from _____

_____ Human blood and plasma were supplied by _____

Human plasma samples (predose plasma samples) from healthy subjects and renally and hepatically impaired patients were obtained from AMP579 Clinical Studies 109 and 112.

Plasma protein binding of RPR 257970 were determined in different animal species and human plasma at 0.5, 5.0, 25, 100 and 500 ng/mL, which covers the concentration ranges anticipated in future human clinical studies. Free and bound drug was separated by the ultrafiltration using _____ Micropartition Device (DMPKIUS 98-029) and quantitated by scintillation counting.

b(4)

PREPARATION OF RPR 257970 SOLUTIONS

³H-RPR 257970 was custom-synthesized by _____ The specific activity of this lot (lot No. 324-158-005) was 1.0 Ci/mmol with a radioactivity purity of 99.9%.

b(4)

DETERMINATION OF INTERSPECIES PLASMA PROTEIN BINDING

One mL of the spiked plasma of each species and each concentration was loaded into a _____ Micropartition Device and centrifuged in a fixed angle rotor at 4,000 rpm for 25 min at room temperature to separate the unbound drug. Separation was conducted in quadruplicate for each species and each concentration except in triplicate for 500 ng/mL concentration. The total radioactivity was measured in the same way except using spiked plasma. All samples were then counted on a Tri-Carb Liquid Scintillation Analyzer.

The non-specific binding of RPR 257970 to the _____ Micropartition Device was determined in the same way using protein-free dog plasma (the filtrate of blank dog plasma).

For the predose plasma samples from AMP 579 Clinical Studies the total radioactivity and free radioactivity were determined using standard procedures

DETERMINATION OF DRUG INTERACTION IN HUMAN PLASMA

Spiked plasma samples with warfarin or salicylic acid were incubated into a _____ Micropartition Device. One mL of the spiked plasma of each species and each concentration was loaded into a _____ Micropartition Device and centrifuged. Free radioactivity was quantitated by taking aliquots of the filtrate and mixing with 15 mL of Monophase cocktail in a scintillation vial. The total radioactivity was measured by the same way except using spiked plasma. All samples were then counted on a _____ Liquid Scintillation Analyzer.

b(4)

DETERMINATION OF BLOOD CELL/PLASMA PARTITIONING

Spiked human blood sample was incubated and vortexed to get homogeneous whole blood. Four aliquots were taken into combustion cones and oxidized with a _____ Oxidizer. The rest of the incubated blood samples was immediately centrifuged. Three aliquots of the resultant plasma were mixed with 18 mL Monophase cocktail in a scintillation vial. All blood and plasma samples were then counted on a Liquid Scintillation Analyzer.

b(4)

Hematocrit was determined in triplicates using the same human blood sample with Micro-Hematocrit tubes and a Micro-Capillary Centrifuge.

DATA ANALYSIS

Data analysis was performed using Microsoft Excel® for the calculation and presentation of results.

The following equations were used:

Non-Specific Binding:

$$A = (CO - CO') / CO$$

Where,

A is non-specific binding

CO is the drug concentration in the deproteinated plasma

CO' is the drug concentration in the filtrate.

Plasma Protein Binding:

$$B = \{1 - F / [(1 - A) \times CO]\} \times 100\%$$

Where,

B is the percent protein binding (%)

CO is the drug concentration in the spiked plasma

F is the drug concentration in the filtrate of spiked plasma

A is the non-specific binding.

Blood Cell/Plasma Partitioning:

$$\text{Ratio of Blood/Plasma} = B/P$$

$$\text{Ratio of Blood Cell/Plasma} = [B - P(1 - H)] / (H \times P)$$

Where

B is the drug concentration in the spiked human blood

P is the drug concentration in the human plasma
H is Hematocrit

RESULTS AND DISCUSSION

Analytical Method

Pre-study validation

Stability and % of Recovery

INTERSPECIES PLASMA PROTEIN BINDING

The non-specific binding of RPR 257970 on _____ Micropartition Device was determined with protein-free human plasma and ranged from 27.5 % to 40.3% (Table 1).

The in vitro plasma protein binding results of RPR 257970 in different species, expressed as the mean percentage of drug bound to plasma proteins, are summarized in Table 1. In general, the plasma protein binding of RPR 257970 was high and was similar among species with slightly lower protein binding in the rat. The extent of protein binding was in the following order, rat (96.86-97.65%) <human (98.10-98.50%) <mouse (98.32-98.84%) <dog (98.58-98.78%) <monkey (98.77-98.98%). No apparent saturation of protein binding was observed in all species in the concentration range of 0.5 to 500 ng/mL of RPR 257970.

Table 1. Non-specific and in vitro plasma protein binding (%) of RPR 257970 in different species

Species	RPR 257970 Concentrations (ng/mL)				
	0.5	5	25	100	500
Non-Specific	40.34±3.81	34.18±1.56	32.00±0.62	33.67±1.40	27.52±2.08
Dog	98.78 ± 0.14	98.58 ± 0.01	98.66 ± 0.08	98.60 ± 0.08	98.68 ± 0.04
Monkey	98.92 ± 0.15	98.77 ± 0.07	98.85 ± 0.11	98.89 ± 0.05	98.98 ± 0.07
Rat	96.87 ± 0.45	96.86 ± 0.16	97.18 ± 0.12	96.90 ± 0.18	97.65 ± 0.22
Mouse	98.84 ± 0.29	98.51 ± 0.02	98.32 ± 0.45	98.54 ± 0.24	98.57 ± 0.43
Pooled Human	98.50 ± 0.14	98.19 ± 0.21	98.40 ± 0.09	98.10 ± 0.29	98.42 ± 0.08

BINDING TO THE PREDOSE PLASMA SAMPLES FROM HEALTH SUBJECTS AND RENALLY AND HEPATICALLY IMPAIRED PATIENTS

The in vitro plasma protein binding (mean ±SD) results of RPR 257970 in the predose plasma samples from healthy, renally and hepatically impaired volunteers (from AMP 579 Clinical Studies 109 and 112) are given in Table 2. At a concentration of 5.0 ng/mL, the protein binding of RPR 257970 in the predose plasma samples varied between 97.54 -99.00%. No significant differences in protein binding were observed between the plasma samples from healthy subjects and plasma samples from renally and hepatically impaired patients, suggesting the plasma protein binding of RPR 257970 may not be affected in patients with renal or hepatic disorders.

b(4)

Table 2. Mean plasma protein binding (%) of RPR 257970 in the predose plasma samples from AMP579 studies 109 and 112

	Binding (%)	N
Study 109	98.4 (0.4)	14
Study 112	98.3 (0.33)	13
Study 112	98.37 (0.32)	13

EFFECT OF SALICYLIC ACID AND WARFARIN ON THE BINDING OF RPR 257970

The effects of salicylic acid and warfarin on the protein binding of RPR 257970 in human plasma are presented in Table 3 and 4. At the concentration of 5.0 or 500 ng/mL, the protein binding of RPR 257970 in human plasma was not affected by presence of 2.0 or 10.0 µg/mL of warfarin and 100 or 300 µg/mL of salicylic acid.

Table 3. Effect of warfarin on the protein binding of RPR 257970 in pooled human plasma

RPR 257970(ng/mL)	Warfarin(µg/mL)	Binding of RPR 257970(%)
5.0	0	98.20 ± 0.04
5.0	2.0	98.28 ± 0.20
5.0	10	98.19 ± 0.17
500	0	98.53 ± 0.14
500	2.0	98.39 ± 0.36
500	10	98.50 ± 0.05

Table 4. Effect of warfarin on the protein binding of RPR 257970 in pooled human plasma

RPR 257970(ng/mL)	Salicylate(µg/mL)	Binding of RPR 257970(%)
5.0	0	98.20 ± 0.04
5.0	100	98.22 ± 0.20
5.0	300	98.26 ± 0.20
500	0	98.53 ± 0.14
500	100	98.56 ± 0.13
500	300	98.46 ± 0.13

HUMAN BLOOD CELL-PLASMA PARTITIONING

The determined hematocrit of the human blood was 0.28, which was much lower than normal blood. This value was used to calculate the partitioning results. The partitioning of RPR 257970 in human blood cell and plasma, expressed as the ratio of blood/plasma and ratio of

blood cell/plasma, are listed in Table 5. The blood to plasma binding ratio ranged from 77.87 to 80.77 % in the concentration range of 0.5 - 500 ng/mL. The blood cell to plasma binding ratio was in 24.03-41.50%. Since the hematocrit of this lot of blood was lower than normal blood, these results should only use as preliminary reference.

Table 5. In vitro blood cell/plasma partitioning (%) of RPR 257970 in human blood

	Concentration of RPR 257970 (ng/mL)				
	0.50	5.0	25	100	500
Cell/Plasma	41.50 ± 10.51	27.92 ± 5.25	24.03 ± 1.03	26.49 ± 2.21	27.47 ± 2.43
Blood/Plasma	80.77±2.84	80.10±1.47	77.87±0.28	79.35±0.62	79.08±0.67

SUMMARY OF FINDINGS

- The protein binding of RPR 257970 was high (~98.5%) in various species including humans.
- The human blood cell/plasma binding ratio was low (24.03-41.05%). However, the hematocrit of this lot of brood was much lower than normal blood, these results should be interpreted with caution.
- No changes were observed in protein binding of RPR 257970 in presence of warfarin and salicylic acid and in renally impaired and hepatically impaired patients.

GENERAL COMMENTS

- Data on analytical method validation was not provided.
- The sponsor did not provided raw data or study protocols for any of the analysis presented in this report.

CONCLUSIONS

- Because of the relatively high non-protein binding reported in this study and because of the hematocrit reported was much lower than normal blood, the protein binding values reported in this study should be interpreted with caution.

"Comparative investigation of the oropharyngeal deposition of ciclesonide, its active metabolite (RM1), and budesonide "

Clinical Study Report no.: (FHP024, 239/99)
Protocol No.: FHP 024
Development Phase of Study: Phase I

Objectives

- The purpose of this study was to investigate the oropharyngeal deposition of ciclesonide, RM1, and budesonide following inhalation of 800 µg (640 µg *ex-actuator*) ciclesonide and 800 µg budesonide.

Secondary:

- To gather further information on the safety and tolerability of ciclesonide.

Study Population

A total of 18 healthy male (9) and female (9) subjects participated in the study and 18 completed the study. The mean age of the 18 per-protocol subjects was 33 years; their median weight was 68.5 kg, their median height was 169 cm, and their median Broca Index was 98%.

STUDY DESIGN, TREATMENT AND ADMINISTRATION

Methods

This was an open label, two-treatment, two-sequence, five-period, randomized design in 18 healthy subjects. Subjects inhaled (MDI) a single dose of both ciclesonide and budesonide, followed by a rinsing and gargling procedure, and the sequence was randomly assigned. At the 5 visits, the time interval between inhalation and the commencement of mouthwash was staggered at the following time intervals: immediately post inhalation, 15, 30, 45, and 60 minutes postinhalation. The mouth rinsing solution was assessed for quantitative determination of ciclesonide, RM1, and budesonide. The AUC_{0-60min} for ciclesonide, RM1, and budesonide were calculated using noncompartmental methods. Point estimates and 95% confidence limits were calculated for the ratio of the AUC_{0-60min} (on a molar basis) population medians for RM1 and budesonide, RM1 plus ciclesonide and budesonide, as well as for RM1 and ciclesonide (to assess metabolite formation) using a multiplicative model and a parametric analysis. Tolerability and adverse events were monitored throughout the study and summarized with descriptive statistics.

Test Drugs

- Ciclesonide 800 µg (4 puffs of 200 µg each), batch number 131299
- Budesonide (Pulmicort) 800 µg (4 puffs of 200 µg each), batch number 132299

RESULTS

B9207-015 was detected in all mouth rinsing solutions. Maximum concentrations of B9207-015 were attained immediately after inhalation. The first available mean timepoint (minimum: 2 min., maximum: 5 min.) was 0.045 h (= 2.72 min.). The mean C_{max} value of B9207-015 at this time was about 1.8 mg/mL. The total mean amount of ciclesonide determined in the rinsing solution immediately following inhalation was 97.8 µg, corresponding to 12.2 % of the nominal inhaled dose of 800 µg. At the next available timepoint (0.25 h), the mean

concentration of B9207-015 was about 0.21 mg/L (398 nmol/l), thus illustrating a fast decrease in the oropharyngeal deposition. Mean (SEM) rinsing solution concentration vs. time profile of the parent compound B9207-01 5 following inhalative administrations of B9207-015 is shown in Figure 1. For a summary of mean (SEM, SD), median (mm, max) and geometric mean (68%-range) pharmacokinetic characteristics, see Table 1.

The results obtained are similar regardless of the use of the ITT population (n=18) or the per protocol population (n=17). There was one protocol violator who was omitted from the per protocol population because the total amount of budesonide in mouth rinsing solution immediately following inhalation was greater than the nominal dose administered. Data from the study reveal significant differences in the oropharyngeal deposition of the two study drugs. The point estimates and associated 95% confidence limits of molar $AUC_{0-60min}$ (test to reference) ratios for the per protocol population were 0.04 (0.02-0.05), 0.08 (0.06-0.11), and 0.47 (0.38-0.59) for the RM1 to budesonide, RM1 to ciclesonide, and ciclesonide plus RM1 to budesonide ratios, respectively.

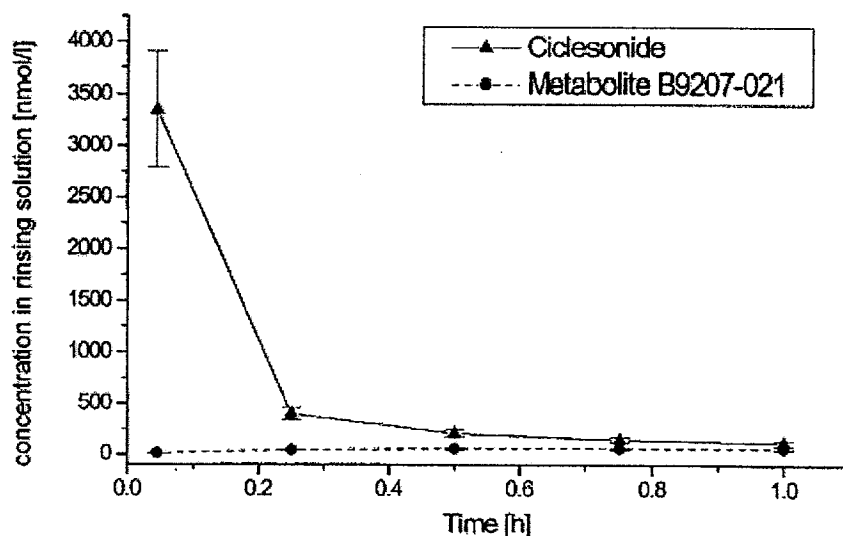


Figure 1. Mean (SEM) concentration of ciclesonide and its metabolite in mouth rinsing solutions of 18 healthy subjects following inhalation of 800 mcg ciclesonide and 800 mcg budesonide.

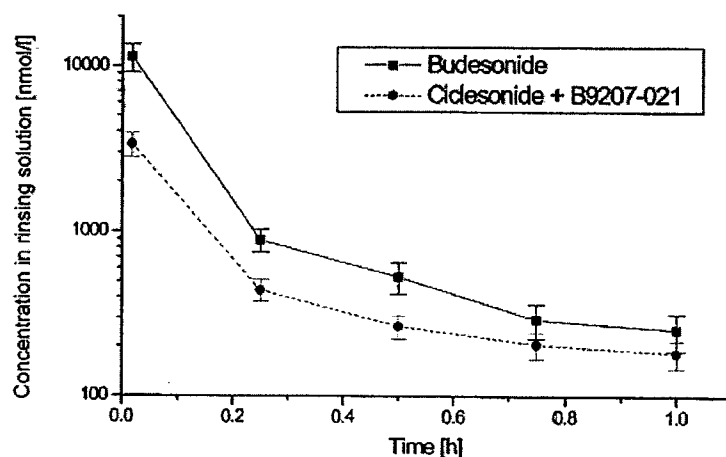


Figure 2. Mean (SEM) concentration of the sum of ciclesonide and its metabolite compared to budesonide in mouth rinsing solutions of 18 healthy subjects following inhalation of 800 mcg ciclesonide and 800 mcg budesonide.

Table 1. PK parameters of ciclesonide, its metabolite and budesonide in moth rinsing solutions following single administration of the treatments

Treatments (Dose)	Ciclesonide			RM1			Budesonide		
	Mean (SD)			Mean (SD)			Mean (SD)		
	AUC nM*h/L	Cmax nM/L	Tmax h	AUC nM*h/L	Cmax nM/L	Tmax h	AUC nM*h/L	Cmax nM/L	Tmax h
CiclesonideMDI 800µg (4x200µg)	611 (394.4)	3344.9 (2364.2)	0.06 (0.00)	47.6 (27.3)	76.1 (42.7)	0.74 (0.26)	1665.6 (1428.9)	11345.5 (9426.3)	0.06 (0.00)
BudesonideMDI 800µg (4x200µg)									
Test	RM1		RM1		RM1 + ciclesonide				
Reference	budesonide		ciclesonide		budesonide				
AUC (0-1h) Point estimate	0.04		0.06		0.47				
95% CI	0.02-0.05		0.06-0.11		0.38-0.99				

SUMMARY OF FINDINGS

- A point estimate of 0.04 and 95%-confidence limits of 0.02 and 0.05 for the ratio of the AUC for 89207-021 referenced to budesonide was obtained (see Table 1). This demonstrates significant difference in the oropharyngeal deposition of both pharmacologically active compounds.
- Only 4% activated ciclesonide as compared to budesonide was found in the rinsing solutions.
- About 50% less ciclesonide and its metabolite was detected in the mouth rising solutions within the first hour following inhalation as compared to budesonide.
- The activation of the prodrug ciclesonide within the oropharynx was concluded to be small as only about 8% of the active metabolite was detected in the rinsing solutions.

"Study assessing lung deposition of ^{99m}Tc-labeled ciclesonide in healthy volunteers"

Clinical Study Report no.: 74/2001
Protocol No.: FHP 014
Development Phase of Study: Phase I

Objectives

- The purpose of this study was to assess lung deposition following one or two inhalations of ^{99m}Tc (technetium)-labeled ciclesonide using the MDI formulation in healthy subjects, as well as to characterize and differentiate between two-dimensional and three dimensional imaging of lung deposition following inhalation of ^{99m}technetium-labeled ciclesonide

Secondary:

- To gather further information on the safety and tolerability of ciclesonide.

Study Population

Eight Caucasian healthy male/female volunteers were enrolled in this study. The table below summarizes the demographic disposition of the subjects.

Table 1. Summary of demographic and other baseline data

Age at screening (years)	mean: 37 SD: 6 median: 38 minimum: 29 maximum: 47
Gender	female: 1 (13%) male: 7 (88%)
Race	white: 8 (100%)
Tobacco use	past: 1 (13%) never: 7 (88%)
Alcohol use	current: 7 (88%) past: 1 (13%)
Drug allergies	no: 8 (100%)
Other allergies	yes: 2 (25%) no: 6 (75%)
Substance abuse	never: 8 (100%)
Height (cm)	mean: 181 SD: 9 median: 183 minimum: 164 maximum: 190
Weight at screening (kg)	mean: 80 SD: 10 median: 78 minimum: 68 maximum: 94
Weight at post-study (kg)	mean: 81 SD: 11 median: 78 minimum: 66 maximum: 95
Elbow breadth (cm)	mean: 6.8 SD: 0.5 median: 6.9 minimum: 5.9 maximum: 7.4
Frame size	small: 3 (38%) medium: 4 (50%) large: 1 (13%)

STUDY DESIGN, TREATMENT AND ADMINISTRATION

This was an open label, one-period, non-randomized study. Six subjects completed two-dimensional imaging and two completed both two- and three-dimensional imaging. Six subjects inhaled a single puff dose of radiolabeled ciclesonide containing 500 μ Ci of activity. The two subjects that completed both two- and three-dimensional imaging inhaled 10 puffs of radiolabeled ciclesonide containing 4 mCi of activity. The amount of radioactivity retained in the actuator and the exhalation filter was assessed. Deposition of labeled drug in the mouth, pharynx, lungs, mediastinum and abdomen was measured by planar gamma scintigraphy. SPECT imaging was then completed, followed by repeat planar images. The amount of radioactivity retained within the actuator and the amount collected on the filter was assessed by imaging the actuator and filter after the subject imaging had been completed. Tolerability and adverse events were monitored throughout the study and summarized with descriptive statistics.

The primary deposition variables were ex-valve and ex-actuator lung deposition. Secondary deposition variables were ex-valve actuator, mouth/pharynx, mediastinal, abdominal and exhaled deposition, ex-actuator mouth/pharynx, mediastinal, abdominal and exhaled deposition, and regional lung deposition (central, intermediate or middle, and peripheral regions).

SPECT images were acquired in a 128 x 128 matrix using a dual-head gamma camera with a high resolution collimator. There was an automatic correction for non-uniformity included in the manufacturer's software. The camera had two heads mounted opposite to each other on a gantry. This allowed the camera to acquire SPECT images by rotating around the subject.

b(4)

RESULTS

FEV1 testing results are shown in Table 2. Two-dimensional imaging demonstrated high lung deposition and low mouth/pharynx deposition of 1 puff (6 subjects) or 10 puffs (2 subjects) of radiolabeled ciclesonide. The mean $\pm$ SD *ex-valve* lung deposition was $38 \pm 11\%$, with mouth/pharynx deposition of $26 \pm 5\%$ and actuator deposition of $29 \pm 8\%$ (Table 3). *Ex-actuator* lung deposition was $52 \pm 11\%$, with mouth/pharynx deposition of $38 \pm 14\%$ (Table 4). According to the sponsor, the flow through the actuator at the time of actuation (MDIF) for Subject 003 was 228 mL/sec, while the flows for other subjects were all greater than 2000 mL/sec. The investigators felt that this was a possible explanation for the low lung (13% *ex-valve*, 25% *ex-actuator*), high mouth/pharynx (37% *ex-valve*, 71% *ex-actuator*), and high actuator (48% *ex-valve*) deposition results for this subject. With the data from Subject 003 excluded, mean lung deposition was 41% *ex-valve* and 55% *ex-actuator*. Table 5 shows regional lung deposition summary statistics. Radiolabeled ciclesonide was deposited in all regions of the lung.

Three-dimensional deposition data showed higher peripheral ($47 \pm 13\%$) and lower central ($17 \pm 8\%$) deposition than two-dimensional methods (Table 6). Table 7 summarizes the lung deposition finding from this study.

Table 2. Summary of FEV₁ testing

	Measured FEV ₁ (liters)	Predicted FEV ₁ (liters)	Percent Predicted (%)
mean	4.71	4.31	109.5
SD	0.67	0.61	8.3
median	4.78	4.44	108.8
min	3.44	3.01	98.9
max	5.48	4.90	124.0

Table 3. Ex-valve planar deposition data (n=8)

	Actuator (%)	Mouth/pharynx (%)	Lungs (%)	Mediastinum (%)	Abdomen (%)	Exhaled (%)
mean	29	26	38	1	0	6
SD	8	5	11	1	0	3
median	27	25	39	1	0	7
min	20	21	13	0	0	1
max	48	37	47	2	1	10

Table 4. Ex-actuator planar deposition data (n=8)

	Mouth/pharynx (%)	Lungs (%)	Mediastinum (%)	Abdomen (%)	Exhaled (%)
mean	38	52	2	0	9
SD	14	11	1	0	4
median	34	55	2	0	10
min	28	25	1	0	2
max	71	60	3	1	14

Table 5. Planar regional lung deposition (n=8)

Lung Region	Mean	SD	Median	Minimum	Maximum
Peripheral	34	8.5	36	20	45
Intermediate (middle)	36	3.4	36	32	41
Central	30	6.9	29	23	39

Table 6. SPECT regional deposition (percent of total lung count in each region)

Subject ID Number	Lung	Region	3D SPECT Regions	Summed Coronal Slices	Anterior/Posterior Planar Averages (3D Regions)	Anterior/Posterior Planar Averages (2D Regions)
001	Right	Central	10	21	23	24
		Middle (Intermediate)	32	32	32	33
		Peripheral	58	47	45	43
	Left	Central	9	21	20	21
		Middle (Intermediate)	31	31	31	32
		Peripheral	59	48	49	47
002	Right	Central	25	41	34	41
		Middle (Intermediate)	39	33	35	36
		Peripheral	35	26	31	23
	Left	Central	22	39	40	38
		Middle (Intermediate)	42	33	32	36
		Peripheral	36	28	28	26

Table 7. Summary of lung deposition findings

2-D Data											
Batch # (NME)	Study Objective	Study Design	Subjects (M/F)	HV/P (Age: range)	Treatments (Dose)		Mouth/ Pharynx	Lungs %	Mediastinum %	Abdomen %	Exhaled %
PT-00-01 PT-00-03	Lung deposition, 2D, 3 D imaging	Single Dose, Inh	7M/1F	HV (29-47)	40 mcg ex-actuator (1 puff)	Mean (SD)	38 (14)	52 (11)	2 (1)	0 (0)	9 (4)
2-D Lung Data											
Batch # (NME)	Study Objective	Study Design	Subjects (M/F)	HV/P (Age: range)	Treatments (Dose)	Lung Region	Mean %	SD	Median %	Minimum %	Maximum %
PT-00-01 PT-00-03	Lung deposition, 2D, imaging	Single Dose, Inh	7M/1F	HV (29-47)	40 mcg ex-actuator (1 puff)	Peripheral	34	8.5	36	20	45
						Intermediate	36	3.4	36	32	41
						Central	30	6.9	29	23	39
3-D Lung Data											
Batch # (NME)	Study Objective	Study Design	Subjects (M/F)	HV/P (Age: range)	Treatments (Dose)	Lung Region	Mean %	SD	Median %	Minimum %	Maximum %
PT-00-01 PT-00-03	Lung deposition,3D imaging	Single Dose, Inh	2 (Both Lungs)	HV (29-47)	40 mcg ex-actuator (10 puffs)	Peripheral	47	13	18	9	25
						Intermediate	36	5	36	31	42
						Central	17	8	47	35	59

SUMMARY OF FINDINGS

- Planar imaging demonstrated high lung deposition and low mouth/pharynx deposition of 1 puff (6 subjects) or 10 puffs (2 subjects) of radiolabeled ciclesonide. Ex-valve lung deposition was $38 \pm 11\%$ (mean $\pm$ SD) with mouth/pharynx deposition of $26 \pm 5\%$ and actuator deposition of $29 \pm 8\%$. Ex-actuator lung deposition was $52 \pm 11\%$ with mouthpharynx deposition of $38 \pm 14\%$.
- SPECT provided 3D images of radiolabeled CIC deposition. A comparison of pre-SPECT and post-SPECT planar images showed a decreased amount of technetium in the lungs.
- Planar and SPECT imaging showed that radiolabeled ciclesonide was deposited in the peripheral, middle (intermediate), and central regions of the lung. 3D regional deposition data showed higher peripheral ($47 \pm 13\%$, mean $\pm$ SD) and lower central ($17 \pm 8\%$) deposition than 2D methods ($37 \pm 10\%$ peripheral deposition, $30 \pm 9\%$ central deposition). Radioactive counts in the 2D central regions included overlapping peripheral

region counts while the 3D central regions did not.

"Systemic availability of the metabolite RM1 after administration of ciclesonide as powder capsules p. o., inhalation via MDI as compared to intravenous administration, and pharmacodynamics (cortisol in serum and urine) in healthy male volunteers"

Clinical Study Report no.: 172/97
Protocol No.: BY9010/FHP015
Development Phase of Study: Phase I
Study Initiation Date: April 27, 2001
Study Completion Date: May 22, 2001
Chief Investigator: Dr Wilhelm Wurst, Byk Gulden Pharmaceuticals,
Constance (Germany)

Objectives

- to investigate the pharmacokinetics and pharmacodynamics of the metabolite RM1 after oral administration of ciclesonide (10 mg) as powder capsules p.o., oral inhalation of 1600 µg (1280 µg *ex-actuator*) ciclesonide *via* the MDI, powder inhalation of 1600 µg ciclesonide *via* the ~~MDI~~ and intravenous administration

b(1)

Study Population

Fifteen healthy male subjects were enrolled in this study and 12 subjects completed the study.

STUDY DESIGN, TREATMENT AND ADMINISTRATION

This study was an open-labeled, randomized, 4-period crossover study. Subjects received the following treatments:

- Treatment A consisted of 25 placebo powder capsules on Day 1 and 10 mg of ciclesonide (25 capsules of 400 µg each) on Day 2.
- Treatment B consisted of 8 puffs of placebo MDI on Day 1 and 1600 µg (1280 µg *ex-actuator*) of ciclesonide inhaled by MDI (8 puffs of 200 µg each) on Day 2.
- Treatment C consisted of 4 placebo powder capsules inhaled by means of Cyclohaler on Day 1 and 1600 µg of ciclesonide (4 capsules of 400 µg each inhaled by means of Cyclohaler on Day 2.
- Treatment D consisted of placebo solution (0.9% NaCl) infused over 10 minutes on Day 1 and 0.8 mg of ciclesonide as a solution in 20% HAS infused over 10 minutes on Day 2.

PD Evaluations

24 h profiles of cortisol in serum were evaluated by taking samples repeatedly on study Days 1 and 2 (placebo) and Days 2 and 3 (ciclesonide) of each period. Urinary excretion of cortisol was also measured by collecting cortisol urine fractions in 4 h intervals on study Days 1 and 2 (placebo) and Days 2 and 3 (ciclesonide) of each period.

PK Measurements

For determination of the serum concentrations of ciclesonide and its active metabolite RM1, repeated blood samplings were performed on study Days 2 and 3 of each period up to 24 h

after dosing: predose, 0.17 h (Treatment D: end of infusion), 0.33 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 7 h, 12 h and 24 h after administration (Treatment D: start of infusion)

Analytical Method

Samples were analyzed by HPLC with _____, spectrometric detection. The limit of quantification was 0.25 ng/mL for both compounds.

b(4)

Safety Measurements

Repeated measurements of blood pressure, heart rate and 12-lead resting ECG, clinical laboratory investigation, pharyngoscopy, physical examination, and continuous recording of adverse events were performed.

Analysis of Data

PK/PD Analysis

Plasma concentration-time data for each subject in each regimen were analyzed by non-compartmental analysis

Primary pharmacokinetic variable was the AUC_{0-∞} of metabolite RM1 for each treatment. The respective values were referenced to the AUC_{0-∞} of RM1 after intravenous administration of 0.8 mg ciclesonide. Primary pharmacodynamic parameters were the cortisol serum mesor during the 24 hours post dosing and the 24-hour urinary cortisol excretion compared to the respective baseline profile.

Statistical Analysis

Geometric mean and 95% confidence limits were given for the respective ratios ciclesonide/placebo following calculation of a standard analysis of variance for the crossover design using a multiplicative model.

RESULTS

Analytical Method

In-Study Validation

Determination of B9207-015 and B9207-021 in Serum

Calibration data, quality control data and chromatograms indicated that the method performed acceptably during the sample analyses. Intra-batch precision values, based upon coefficients of variation of quality control samples (60, 800, 1600 pg/mL), were less than or equal to _____% for B9207-015 and _____% for B9207-021. Intra-batch accuracy of quality control samples ranged from _____% for B9207-015 and _____% for B9207-021.

b(4)

Pharmacokinetic Results

Following oral administration, the concentration of ciclesonide was detected only in 4 samples of two volunteers, with concentration being close to the limit of quantification. Mean serum concentrations of B927-015 and B-9207-021 in healthy subjects following i.v. and inhalation of B9207-015 are shown in Figures 1 and 2, respectively.

Geometric means of pharmacokinetic parameters of ciclesonide and RM1 are given in Table 2. Point estimates and 90%-confidence intervals of pharmacokinetic parameters following a single dose inhalation of ciclesonide *via* MDI as referenced to intravenous infusion (dose-adjusted) are illustrated in Table 3.

The clearance of both ciclesonide and RM1 exceeded the hepatic blood flow of 90 L/h. The volume of distribution of ciclesonide and RM1 exceeded the value for total body water in humans of 42 L. Thus, ciclesonide and RM1 have a high apparent clearance and large apparent volume of distribution in humans.

Inhaled bioavailability of RM1 following inhalation of ciclesonide *via* MDI was >50 %, as compared to RM1 concentrations following administration of intravenous ciclesonide. The pharmacokinetic profile for the MDI device showed a fast delivery of the parent compound and a maximum value of the biologically active metabolite, RM1, observed at 1.04 h (mean T_{max}). Systemic oral and inhaled bioavailability, based on AUC_{0-12h} values of ciclesonide, was <1% (n=10) and 11% (n=6), respectively.

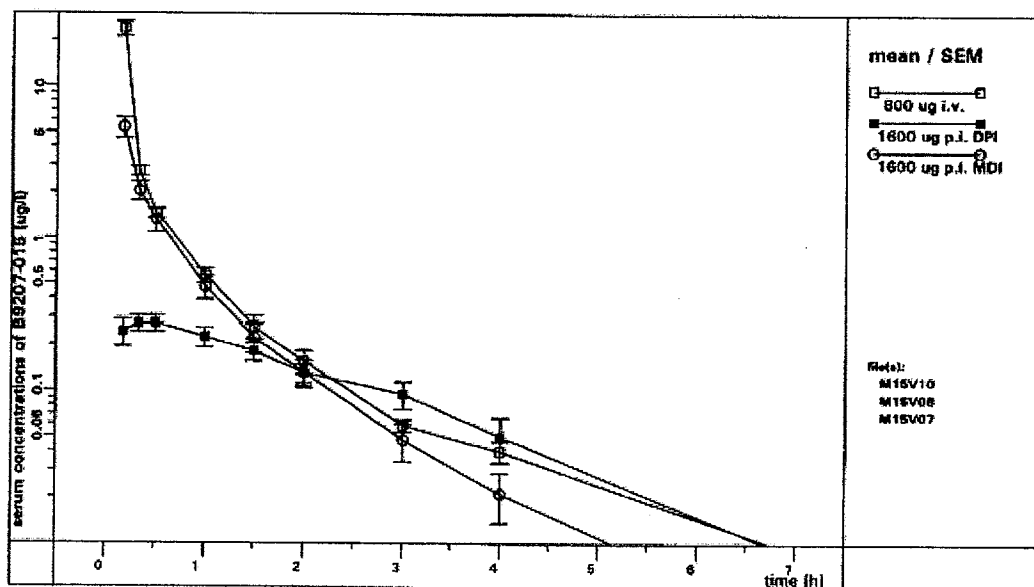


Figure 1. Mean serum concentrations of B927-015 in 12 healthy subjects following i.v. and inhalation of B927-015.

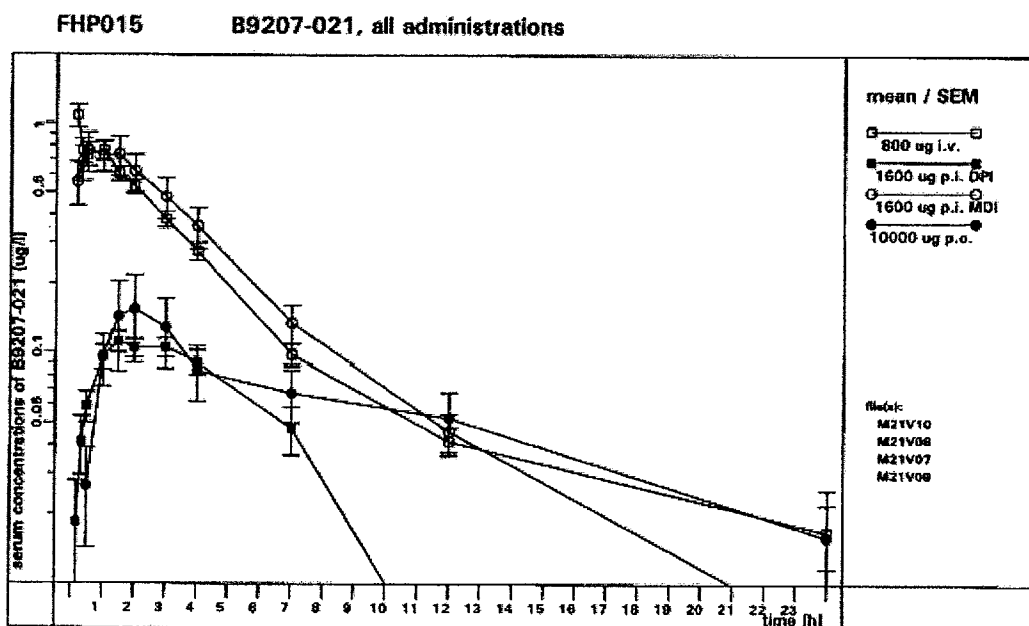


Figure 2. Mean serum concentrations of B927-021 in 12 healthy subjects following i.v., oral, and inhalation of B9207-015.

Table 2: Geometric means of pharmacokinetic parameters of ciclesonide and RM1 (n=12)

Parameter	Intravenous (800 µg) ciclesonide	Intravenous (800 µg) RM1	Oral (10000 µg) ciclesonide	Oral (10000 µg) RM1	MDI (1280 µg <i>ex-actuator</i>) ciclesonide	MDI (1280 µg <i>ex-actuator</i>) RM1
AUC _{0-12h} (ng·h/mL)	5.41	3.17	not ascertainable	0.95 ^a	1.92	3.17
C _{max} (ng/mL)	21.911	1.070	not ascertainable	0.138	4.890	0.698
T _{max} ¹ (h)	0.17 ± 0.0	0.43 ± 0.46	not ascertainable	3.92 ± 4.11	0.18 ± 0.05	1.04 ± 0.50
T _{1/2} ¹ (h)	0.94 ± 0.53	2.8 ± 0.51	not ascertainable	not ascertainable	0.71 ± 0.45	2.8 ± 0.30
CL ¹ (L/h)	152.34 ± 37.29	227.73 ± 65.01	NA	NA	NA	NA
V _{darea} ¹ (L)	206.79 ± 149.89	896.78 ± 236.79	NA	NA	NA	NA

a: AUC_{0-12h} (n=10)

1: Mean ± SD

NA: Not Applicable

Table 3: Point estimates and 90%-confidence intervals of pharmacokinetic characteristics following a single dose inhalation of ciclesonide *via* MDI as referenced to intravenous infusion

Parameter	Compound	Point Estimate	90 % CI
AUC _{0-∞}	Ciclesonide	0.18	(0.13, 0.24)
AUC _{0-∞}	RM1	0.50	(0.35, 0.73)
C _{max}	RM1	0.33	(0.22, 0.49)
T _{1/2}	RM1	1.01	(0.94, 1.09)

PD Results

Reduction of serum cortisol by the different ciclesonide treatments is provided as percentage decreases of the 12 h and 24 h serum cortisol mesor compared to placebo. Following administration of 1000 µg oral, 1280 µg (*ex-actuator*) MDI and 800 µg intravenous ciclesonide, the reduction in 12 h cortisol mesor was 5%, 7% and 14%, respectively. Following administration of 1000 µg oral, 1280 µg (*ex-actuator*) MDI and 800 µg intravenous ciclesonide, the reduction in 24 h cortisol mesor was 5%, 4% and 0%, respectively. The changes in the serum cortisol mesors were statistically not significant at the 5% alpha level of significance. Several values for cortisol in urine samples were below the lower limit of quantification, and hence the summary statistics on urine cortisol values were not calculated.

CONCLUSIONS

- The greatest decrease of the 12-hour cortisol mesor was 14%, and this was caused by the intravenous administration of 0.8 mg ciclesonide. MDI caused the same degree of suppression.
- The absolute BA of ciclesonide is lower than 1%
- The systemic bioavailability of ciclesonide and RM1 following inhalation using the MDI formulation was 11% and 30%, respectively.

APPEARS THIS WAY ON ORIGINAL

**"SAFETY, TOLERABILITY AND PHARMACODYNAMICS OF THE NEW TOPICAL
STEROID CICLESONIDE IN HEALTHY MALE VOLUNTEERS FOLLOWING
ASCENDING SINGLE DOSE AND REPEATED DOSE INHALATIONS OVER 7 DAYS"**

Clinical Study Report no.: 117E/97
Protocol No.: BY9010/FHP009
Development Phase of Study: Phase I
Study Initiation Date: Nov 27, 1996
Study Completion Date: Oct 1, 1997
Chief Investigator: Dr. Th. Bethke
Wilhelm Wurst, Byk Gulden Pharmaceuticals, Constance
(Germany)

Objectives

- to investigate the safety (ECG, BP, HR, clinical laboratory, physical examination, pharyngoscopy) and tolerability of ciclesonide after single and repeated dose inhalations
- to determine the pharmacokinetics and pharmacodynamics (cortisol concentration in serum and urinary cortisol) after single and multiple administration

Study Design

Single Dose Part (n=12): an open, single dose, 4-period change-over study with strictly increasing ciclesonide doses and randomly interspersed placebo.

Repeated Dose Part (n=12): A double-blind repeated dose, 3-period change-over study with strictly increasing ciclesonide doses and randomly interspersed placebo.

The allocation of the subjects to the respective treatment sequences was randomized.

There was a wash-out period of 2 weeks between treatments of two subsequent periods

Volunteers population: Caucasians (males) ages 18-40 years

- | | |
|----------------------------------|----|
| • Number planned: | 24 |
| • Number included: | 25 |
| • Number of drop-outs: | 1 |
| • Subjects valid for evaluation: | 24 |

The participants, males (n=12) were between 19 and 40 years old (median: 31 years). Their body weight (median: 79 kg, range 66-93 kg) corresponded to their height (median: 183 cm, range 173-191 cm). All individual values of the Body Mass Index (median: 24 kg/m², range: 20-29 kg/m²) were within the required range of 19-30 kg/m²

Study Medication:

Metered dose inhalers (MDI) containing 120 puffs of placebo or ciclesonide; 50 µg, 100 µg, 200 µg ciclesonide or placebo-aerosol were applied per puff.

Dosage:

Single dose: Single administration on Day 1 of each period (n=12) of the following doses:

- 0 µg of ciclesonide

- 400 µg of ciclesonide
- 1200 µg of ciclesonide
- 3600 µg of ciclesonide
-

Repeated dose: Repeated dose administration on days 1-6 of each period

- 0 µg of ciclesonide BID
- 250 µg of ciclesonide BID
- 1000 µg of ciclesonide BID

On Day 7:

- 0 µg of ciclesonide single dose
- 400 µg of ciclesonide single dose
- 1200 µg of ciclesonide single dose

PK Measurements

Determinations of serum concentration of ciclesonide and its metabolite (B9207-021) were done in the single dose part of the study after morning administration at the following time points:

- 400 mg and 1200 mg ciclesonide: predose (-10 to -5min), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h a.a.
- 3600 mg ciclesonide: predose (-10 to -5 mm.), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, 28, 32 and 36 h

In the repeated dose part of the study serum concentrations of ciclesonide and its metabolite/placebo were determined on day 7 at the following time points:

- predose (-10 to -5 mm.), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, 28, 32 and 36 h after morning administration.

Analytical Method

The developed method utilized _____ for sample preparation and high pressure liquid chromatography (HPLC) with _____ spectrometric detection. The method previously validated ad a lower limit of quantification (LLQQ) of 0.025 ng/mL for ciclesonide and its metabolite

b(4)

ECG Measurements

On each pre- and final check a 12-lead ECG was recorded after 15 mm rest in supine position and additionally on day 1 of the single dose part and on day 7 of the repeated dose part at the following time points: predose, 1 h, 6 h, 12 h and 23.5 h after morning inhalation. Additionally on pre- and final check, a 3-lead ECG was recorded over 1 min to check for arrhythmia.

In case of the administration of a single dose of 0, 400 and 1200 mcg ciclesonide the measurements at 23.5 h after administration corresponded to the measurements at final check. In case of repeated dose administration the measurements at 23.5 h after administration on day 8 corresponded to the measurements at final check.

PD Measurements

The morning levels of serum cortisol were determined in the repeated dose part on days 1 - 6 before inhalation to assess the drug effect on the HPA-axis.

On day 1 in the single dose part and on day 7 in the repeated dose part cortisol was determined at the following time points: predose, 2, 4, 6, 8, 10, 12, 14, 24 hrs, after administration.

Cortisol in Urine

Urine was collected at the following intervals:

Single Dose Part: On study day 1:

-1h to 4 h a.a. (interval 1)

4 h to 9 h a.a. (interval 2)

9 h to 14 h a.a. (interval 3)

14 h to 23 h a.a. (interval 4)

Repeated Dose: On study day 1 and day 6:

-1 h to 4 h a.a. (interval 1)

4 h to 9 h a.a. (interval 2)

9 h to 14 h a.a. (interval 3)

14 h to 23 h a.a. (interval 4)

Data Analysis

PK Analysis

PK parameters were calculated using non-compartmental methods. Dose proportionality was analyzed by dose-adjusted ratio analysis. In the single dose part the 1200 µg dose served as reference, in the repeated dose part the 500 µg dose served as reference. Dose proportionality between two doses was concluded if the 90% confidence interval for the respective ratio was entirely within the equivalence range of 0.80 to 1.25.

PD Analysis

The cortisol plasma levels of drug and placebo phases were expressed as percentage of the respective 8.00 hour-values:

$$S(t) = \left(1 - \frac{C_{t,Dx}}{C_{(8,Dx)}} : \frac{C_{t,P}}{C_{(8,P)}}\right) \times 100\%$$

$S(t)$ = Cortisol suppression (%) compared to placebo

$C_{t,P}$ = Cortisol level at time t after inhalation of placebo (day 2 - 6)

$C_{t,Dx}$ = Cortisol level at time t after inhalation of a dose x of ciclesonide (day 2 - 6)

$\frac{C_{(8,Dx)}}{C_{(8,P)}}$ = respective cortisol levels before inhalation at 8.00 of day 1

Cortisol in Urine

Results of the cortisol in urine were presented as individual values together with median, the 68% range, mean, standard deviation, minimum and maximum.

The free cortisol in urine was presented as raw values and standardized for creatinine in urine. The 24-hour urinary excretion of cortisol was calculated using the fraction from 7.00 h (days 1 and 6) to 7.00 h (days 2 and 7). The 24-hour cortisol excretion in urine was also compared by means of standardized ratio analysis.

Statistical Analysis

Geometric mean and 95% confidence limits were given for the respective ratios ciclesonide/placebo following calculation of a standard analysis of variance for the crossover design using a multiplicative model.

Ratio analysis (ANOVA) including geometric mean and 90%-confidence limits using a logarithm transformation for cortisol.

In the case that equivalence within the above setting could not be demonstrated, the 95%-confidence interval was additionally calculated. If this interval did not include the value of 1 a significant difference at the 5%-level, two-sided was indicated.

RESULTS

Analytical Method

In-Study Validation

Determination of B9207-015 and B9207-021 in Serum

Ciclesonide and metabolite B9207-021 were assayed in serum using _____ for sample preparation and HPLC with _____ spectrometric detection. Calibration data, quality control data and chromatograms indicated that the method performed acceptably during the sample analyses. Intra-batch precision values, based upon coefficient of variation of quality control samples (60, 800, 1600 pg/mL) were less than or equal to _____ % for B9207-015 and _____ % for B9207-021. Intra-batch accuracy of quality control samples ranged from _____ % for B9207-015 and from _____ % to _____ % for B9207-021.

b(4)

Pharmacokinetic Results

Figure 1 and 2 show the mean concentration time-profiles for ciclesonide and its metabolite, respectively following single administration of the treatments. Likewise, Figures 3 and 4 showed after multiple administering of the treatments. Table 1 shows the median PK parameters for ciclesonide and its metabolite. The individual AUC values following single and multiple administration of the treatments are shown in Figures 5 and 6, respectively. Table 2 shows the geometric means and 90% confidence intervals for 24 hr serum cortisol following single and multiple administration of the treatments. Figure 7 illustrates the mean serum cortisol levels as a function of the single dose treatments.

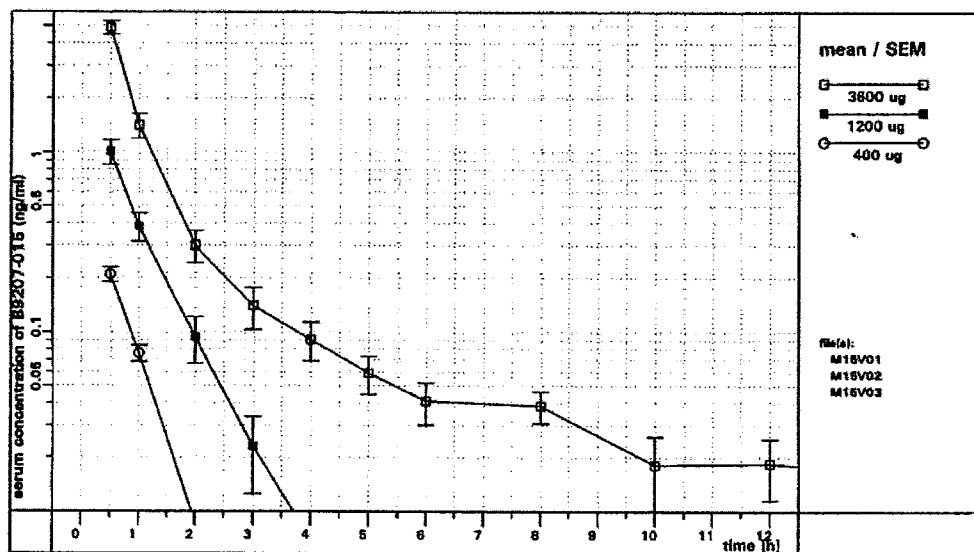


Figure 1. Mean serum concentrations of B9207-015 in healthy volunteers following single inhalation of 400, 1200, or 3600 μ g of B2907-015 via MDI

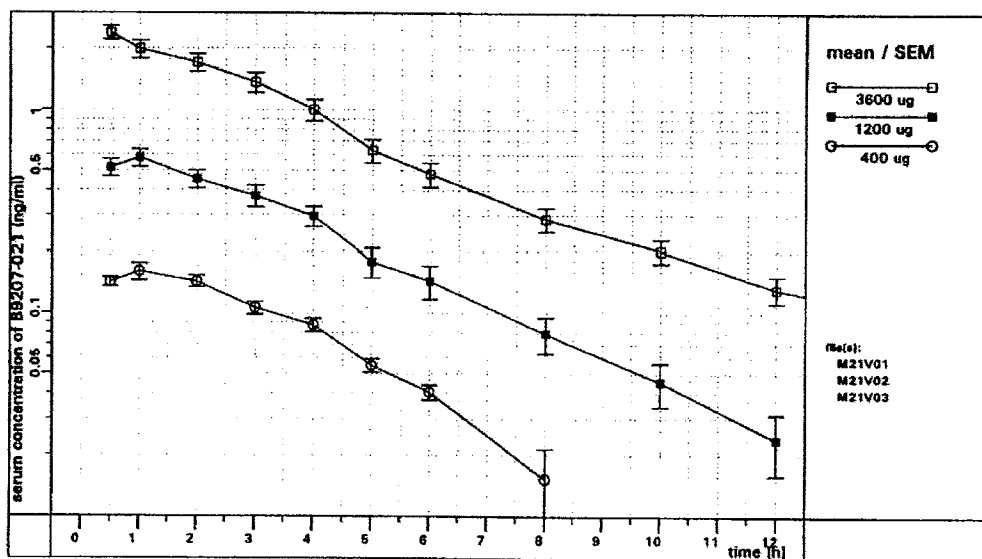


Figure 2. Mean serum concentrations of B9207-021 in healthy volunteers following single inhalation of 400, 1200, or 3600 μ g of B2907-015 via MDI

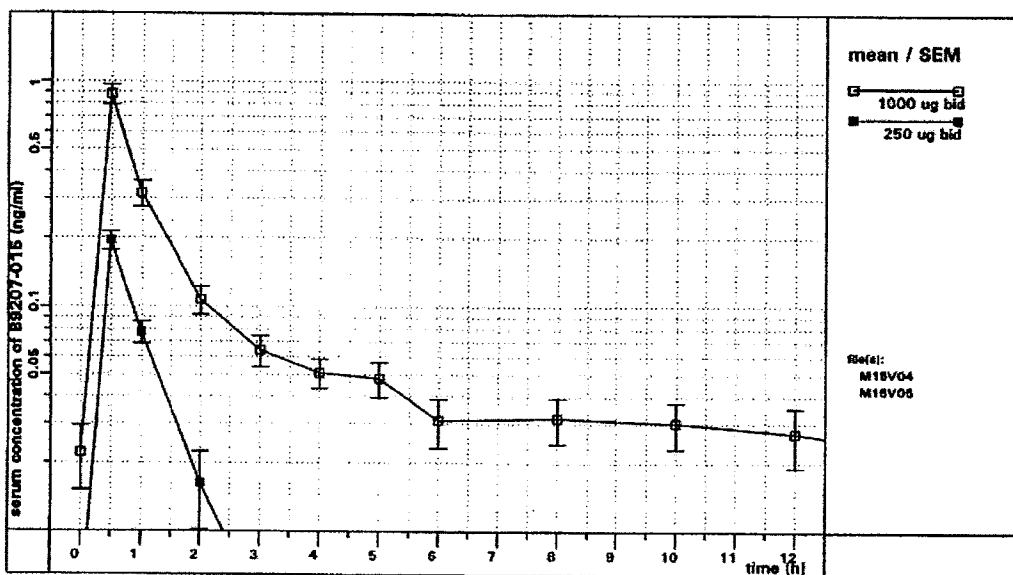


Figure 3. Mean serum concentrations of B9207-015 in healthy volunteers following multiple BID inhalation of 250 and, 1000 μ g of B2907-015 via MDI (DAY 7).

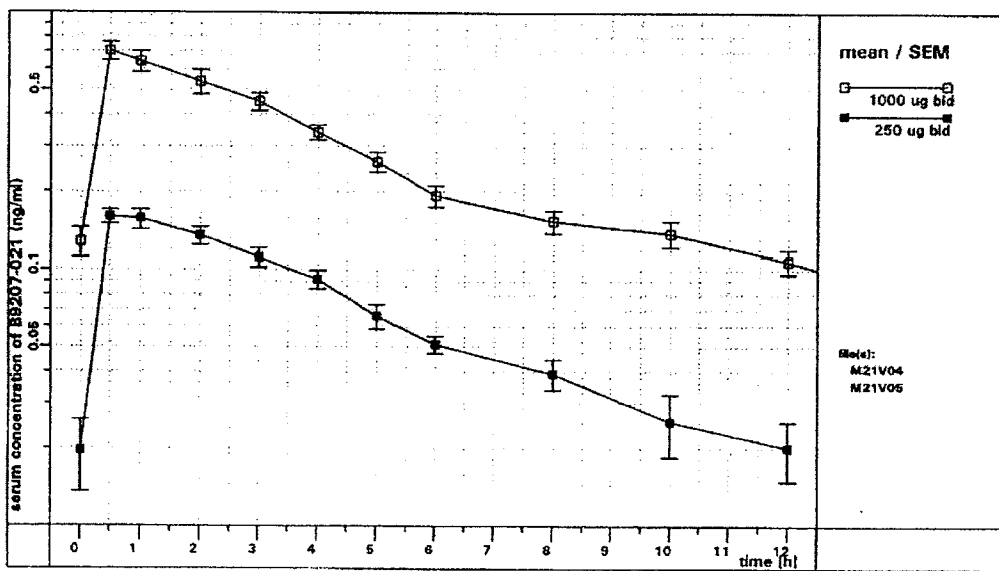


Figure 4. Mean serum concentrations of B9207-021 in healthy volunteers following multiple BID inhalation of 250 and, 1000 μ g of B2907-015 via MDI (DAY 7).

Table 1. Median values for the major PK parameters of ciclesonide and its metabolite following single and multiple administration of the treatments.

Part S		B9207-015		B9207-021			
Dose µg	Study Day	AUC _(0,last) µg/l* ^a	C _{max} µg/l	AUC _(0,inf) µg/l* ^a	point estimate	C _{max} µg/l	point estimate
400	Day 1	n.a.	0.250	0.67 ^a	0.94	0.153	0.82
1200	Day 1	0.91	0.854	2.49	reference	0.605	reference
3600	Day 1	4.24	4.890	10.98	1.36	2.668	1.41
Part R		AUC _(0,12)		AUC _(0,12)			
250	Day 7	0.24 ^b	0.207	0.83	reference	0.170	reference
1000	Day 7	1.12	0.891	2.93	0.98	0.679	1.06

b: N=5

a = AUC_(0,12)

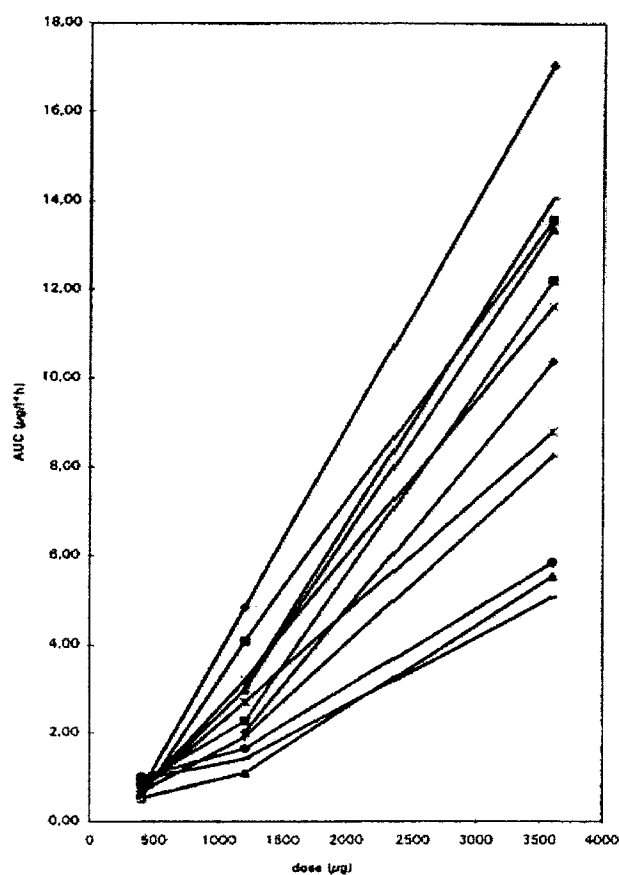


Figure 5. Individual AUC of B9207-021 in healthy volunteers following single inhalation of 400, 1200 and, 3600 µg of B9207-015 via MDI.

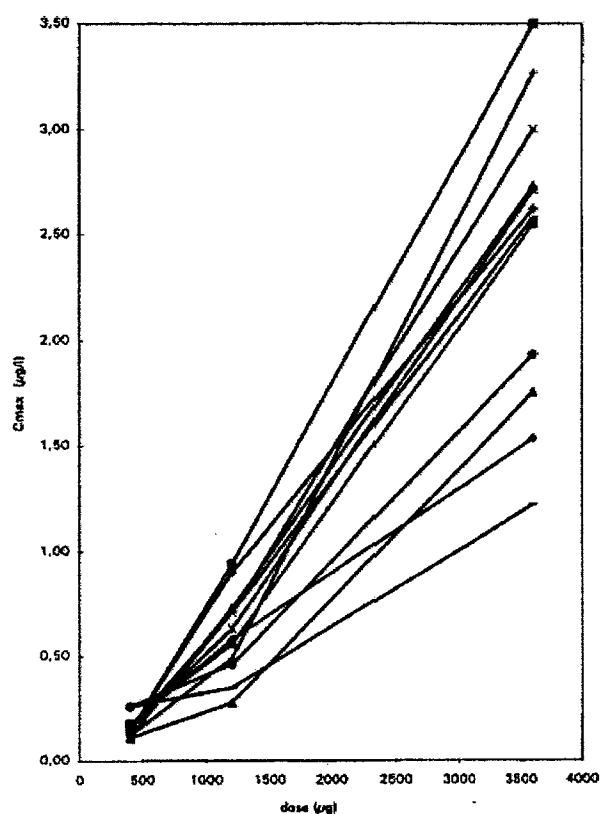


Figure 6. Individual Cmax of B9207-021 in healthy volunteers following single inhalation of 400, 1200 and, 3600 µg of B9207-015 via MDI.

PD Results

- A 24 h cortisol profile was determined on day 1 after single dose application of placebo, 400, 1200, and 3600 pg ciclesonide. there were no missing values and no implausible results so that all data could be included in the evaluation.
- Comparing the means (n=12) of serum cortisol after ciclesonide application with placebo the following can be stated:
 - Precheck levels of cortisol in serum measured on day -1 were comparable for all treatments.
 - The same can be stated for the predose Levels on day 1 of each period.
 - During the period of time from 2 h up to 8 h after inhalation, all mean cortisol concentrations at a particular measuring time point show a dose-dependent decrease with rising ciclesonide dose level.
 - For all treatments the minimum cortisol levels found 14 h after application reflecting the normal circadian variation.
 - 24 h after inhalation mean serum cortisol was on a similar level as predose in each treatment group.

The mean 24 h-profiles of cortisol in serum are shown in Figure 7. A dose-dependent

decrease is obvious for the period of time from 2 up to 8h after morning inhalation. Calculation of the geometric mean cortisol mesor reflecting the AUC0-24h also indicates a dose-dependent decrease of serum cortisol (Table 2).

Table 2. Geometric means and 90% confidence intervals for 24 hr serum cortisol following single and multiple administration of the treatments.

Study Design	Subjects (M/F)	HV/P ¹ (Age: Mean, range)	Product ID/Batch # (NME)	Treatments (Dose)	Reference (Dose)	Serum cortisol (µg/dL)	
						geometric mean cortisol mesor	point estimate to reference (90% confidence interval)
4 period change-over single dose	12 (12/0)	HV 30 years (19-40 years)	CT960811/1	400µg sid	placebo	9.8	0.94 (0.85-1.05)
			CT960812/1	1200µg sid	placebo	8.8	0.83 (0.76-0.92)
			CT960812/1	3600µg sid	placebo	7.3	0.62 (0.56-0.69)
			CT960809/1	Placebo	xxxx	10.6	xxxx
repeated dose	12/0	HV 31 (19-39)	CT960810/1	250µg sid day 7/ bid day 1-6	placebo	10.2	1.11 1.03-1.20
			CT960812/1	1000µg sid day 7/ bid day 1-6	placebo	7.9	0.92 0.85-0.99
			CT960809/1	Placebo	xxxx	8.9	xxxx

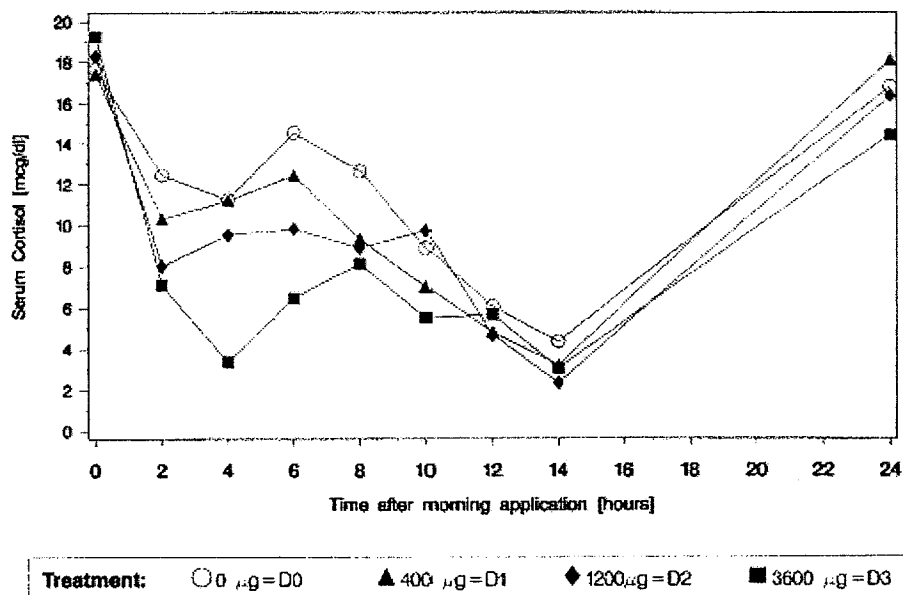


Figure 7. Means 24 hr serum cortisol following single administration of the treatments.

QTc Findings

Figure 8 shows the QTc versus RR relationship for the Bazett and Fredericia corrections. Figure 9 shows the individual change from baseline as a function of ECG measurements and as a function of dose. Table 3 shows the mean (SD) QTc change from baseline as a function of time. Table 4 summarizes the mean of maximum QTb change from baseline as a function of dose.

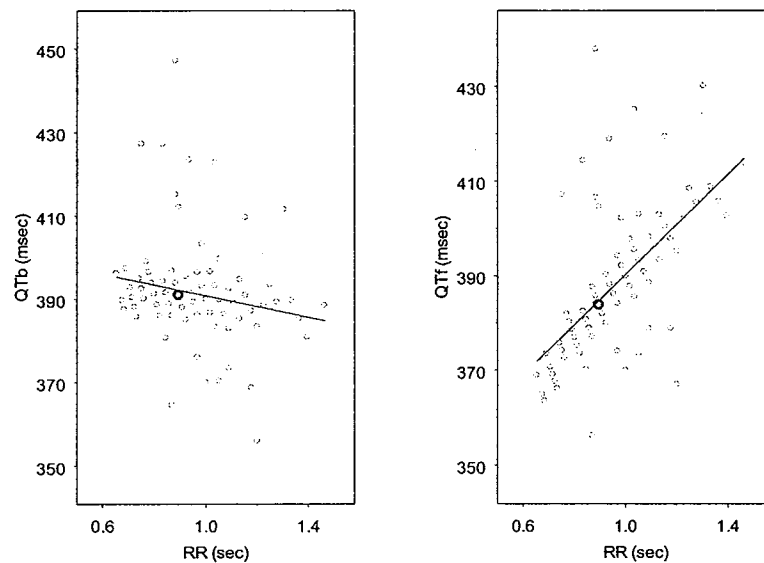


Figure 6. QT versus RR relationship based on no correction, Bazett's and Fredericia correction formula.

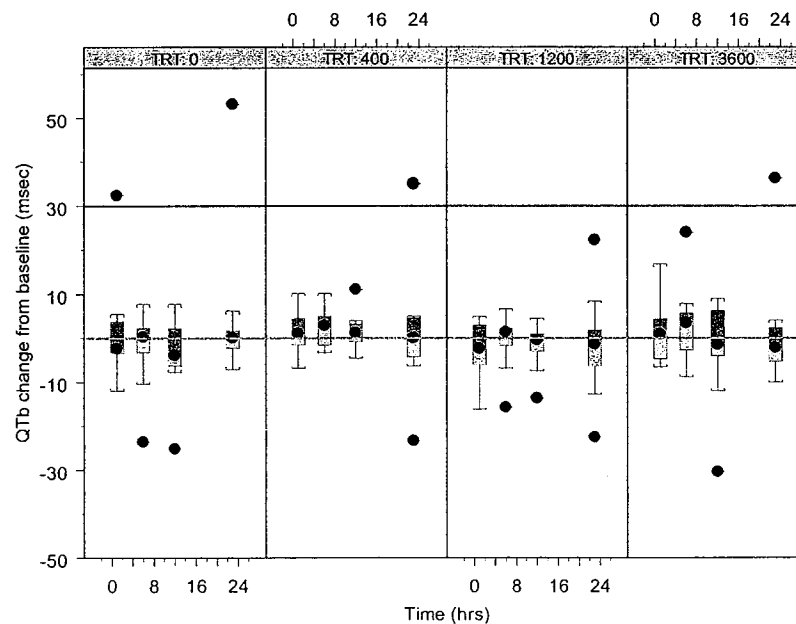


Figure 9. Individual change from baseline (Bazett's correction)-time of ECG measurement relationship as a function of treatment.

Table 3. Mean (SD) QTc change from baseline as a function of time

Time	Delta QTc (msec) Based on Bazett's correction				Delta QTc (msec) Based on Fredericia's correction			
	0	400	1200	3600	0	400	1200	3600
0	0 (2.3)	0 (1.8)	0 (7.3)	0 (4.48)	0.48 (9.2)	1.5 (7.4)	1.7 (11.5)	-0.8 (9.7)
1	1.28 (10.2)	1.4 (4.7)	-2.7 (6)	0.84 (6.6)	-2.7 (12)	-1.9 (7)	-4.7 (9.6)	-3.6 (11.8)
6	-1.9 (8.2)	2.5 (4.3)	-0.4 (6)	2.8 (8.5)	-7.5 (10.6)	-3.1 (5.9)	-4.9 (9.8)	-3.02 (14)
12	-3.6 (8.6)	1.3 (4)	-1.6 (5)	-2.1 (10.9)	-7.0 (12.4)	-3.7 (7)	-8 (9.9)	-7.7 (13.9)
23.5	3.8 (15.9)	2.9 (15.9)	-1.6 (10.9)	0.7 (11.9)	3.9 (15.2)	1.8 (14.5)	1.8 (12.8)	4.3 (12)
maximum	3.8	2.9	0	2.8	3.9	1.8	1.8	4.3

Table 4. Mean of maximum QTb change from baseline following treatments (n=12)

Dose (µg)	Based on Bazett's correction
	Mean of maximum Delta QTb
0	10.05 (16.1)
400	9.2 (11.9)
1200	7.4 (7.4)
3600	8.7 (10.7)

SUMMARY OF FINDINGS

PD Findings

- In the single dose part of the study, a significant adrenal suppression was found for 1200 µg and 3600 µg ciclesonide.
- In the early phase of the cortisol profile a dose-response relation exists for the tested doses of 400 µg, 1200 µg and 3600 µg ciclesonide.
- In the repeated dose part of the study cortisol serum levels were equivalent to those under placebo.

QT findings

- The maximum mean QT change from baseline using on Bazzet's correction was observed at 23.5 hrs post administration for the placebo group (3.8 msec).
- There were 2 subjects from the placebo group, one subject in the 400 mcg dose and one subject in the 3600 mg dose whose QTb change from baseline was greater than 30 msec. The highest QTb individual change from baseline was 53 msec following placebo treatment.
- The highest QTb absolute value was 447.12 following placebo treatment
- The mean of maximum QTb change from baseline was highest for the placebo group (10.05 msec ± 16)

QT Conclusion

- Ciclesonide did not significantly prolong QT or QTb interval. This data should be interpreted with caution since it is based on single dosing, male subjects, and lack of robustness of the data provided.

PK Findings

- In the single dose part AUC and Cmax of both compounds increase more than dose-proportionately. In the repeated dose part these characteristics are well in proportion with the dose.

GENERAL COMMENTS

- It seems that the Bazzet's formula corrected the QT values appropriately. The QTc at baseline was the result of the average of 2 values per dose per subject. Although two subject taking the treatment had a delta change in QTc higher than 30 msec, the relevance of this is difficult to assess since two subjects under placebo treatment had the highest delta changes (approx 50 msec).

APPEARS THIS WAY ON ORIGINAL

"A single-dose study to investigate the epimeric stability of ciclesonide following inhalation of 1600 µg ciclesonide"

Clinical Study Report no.: 201/2001
Protocol No.: BY9010/FHP 028
Development Phase of Study: Phase I
Study Initiation Date: April 27, 2001
Study Completion Date: May 22, 2001
Chief Investigator: Dr Wilhelm Wurst, Byk Gulden Pharmaceuticals,
Constance (Germany)

Objectives

Primary:

- To investigate the epimeric stability of R-ciclesonide (B9207-015) and its pharmacodynamic active metabolite M1 (B9207-021) in healthy volunteers after inhalation of 1600 µg ciclesonide.

Secondary:

- To gather further information on the safety and tolerability of ciclesonide.

Study Population

Six Caucasian healthy volunteers were enrolled in this study. The table below summarizes the demographic disposition of the subjects.

Demographic characteristics for the study population (n=6)	
Age, years	28 (24, 33)
median (range)	
Height, cm	176 (169, 180)
median (range)	
Weight, kg	69 (56, 75)
median (range)	
Broca-Index	0.93 (0.81, 0.95)
median (range)	
Gender, n (%)	
Male	5 (83)
Female	1 (17)
Alcohol consumption, n (%)	
Yes	3 (50)
no	3 (50)
Smoking, n (%)	
Yes	4 (67)
No	2 (33)

STUDY DESIGN, TREATMENT AND ADMINISTRATION

This study was conducted as an open, single-dose, monocenter Phase I study. It consisted of a screening examination (within 4 weeks before administration day), an administration day, and a post-study examination (3-14 days after administration day).

FORMULATION

The following study medication was used in this study:

Test product: Ciclesonide, 200 µg/actuation

Dose: one single dose of 1600 µg (8 puffs in the morning)
Mode of administration: MDI for oral inhalation
Batch No.: 4BG008
Duration of treatment: 1 day (single inhalation)

PHARMACOKINETIC MEASUREMENTS

Blood Sampling

Blood samples were collected 0.25 h prior to ciclesonide inhalation as well as 0.17 h (10 min), 0.50 h (30 min), 1 h, 2 h, 4 h and 8 h thereafter.

Analytical Method

The analytical evaluation of R-ciclesonide (B9207-015), S-ciclesonide (B9207-026), and their respective metabolites M1 in serum was performed using a validated LC-MS/MS assay.

SAFETY MEASUREMENTS

The safety and tolerability assessment was based on the following:

- Adverse events reporting during the entire study,
- Clinical laboratory (clinical chemistry, hematology, urinalysis),
- 12-lead resting ECG, blood pressure and pulse rate, body temperature, physical examination.

DATA ANALYSIS

Pharmacokinetic Data Analysis

Plasma concentration-time data for each subject in each regimen were analyzed by non-compartmental analysis

Statistical Analysis

Biometric analysis was performed for C_{max} ratios of the epimers of ciclesonide (primary variable) using a multiplicative model and parametric analysis. The program BIOQPC (Version 1.2.2) was used for statistical evaluations. Point estimate and 95%-confidence limits were reported for C_{max}-ratios (S/R) of ciclesonide and of the metabolites M1. Other variables were analyzed in a descriptive manner.

RESULTS

Pharmacokinetic Results

Serum concentration-time profiles of the parent compound B9207-015 (parent compound R-ciclesonide), B9207-016 (S-ciclesonide) and R-M1 (R-ciclesonide's metabolite) following inhalative administrations of 1600 µg ciclesonide are shown in Figure 1. B9207-015 was detected in all serum samples up to 2 h postdose (Figure 1). Maximum concentrations of B9207-015 were attained immediately after inhalation at the first available mean timepoint (0.17 h = 10 min). The mean C_{max} value of B9207-015 at this time was about 11.1 µg/L (see Table 1). At the next available timepoint (0.5 h), the mean concentration of B9207-015 was about 1.7 µg/L, thus illustrating a fast distribution and metabolism. Serum concentrations of S-ciclesonide were attained only at 0.17 h following inhalation. Mean C_{max} was 0.16 µg/L.

Concentrations of the main pharmacologically active metabolite B9207-021 following administration of 1600 µg B9207-015 were obtained for all samples. Individual maximum serum concentrations of B9207-021 were attained between 0.5 h (N=3) and 1 h (N=3). The maximal individual concentration of B9207-021 was 1.20 µg/l. A summary of median (min, max) serum concentrations is given in Table 1. No serum concentrations above the LLOQ of 46.17 pg/ml were obtained for the B9207-022, the S-epimer of metabolite M1.

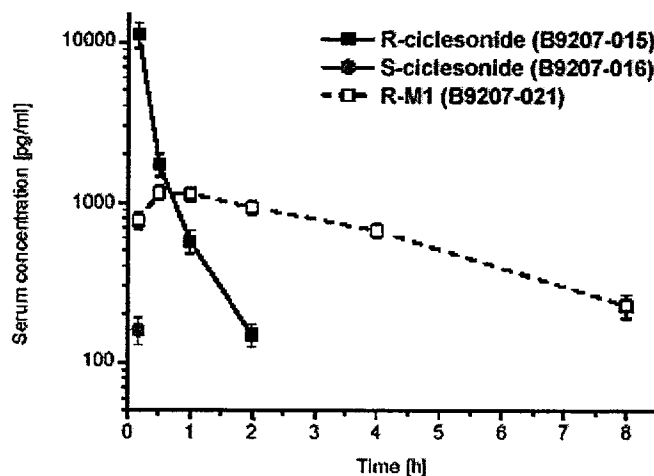


Figure 1. Mean (SEM) serum concentrations of R- and S-ciclesonide and their metabolite M1 in 6 healthy subjects following inhalation of 1600 µg ciclesonide.

Table 1. Median (min, max) serum concentrations of the parent compound ciclesonide (B9207-015 and B9207-016), the metabolite B9207-021 and B9207-022 in 6 healthy subjects following a single administration of 1600 µg B9207-015.

Sampling Time [h]	R-Epimer B9207-015 [pg/ml]	S-Epimer B9207-016 [pg/ml]	R-Epimer B9207-021 [pg/ml]	S-Epimer B9207-022 [pg/ml]
0.17	10984 (5579, 19216)	158.2 (67.23, 287.6)	817.6 (371.0, 962.5)	< 46.17 (< 46.17, <46.17)
0.5	1788 (845.4, 2584)	< 38.78 (< 38.78, < 38.78)	1231 (713.9, 1478)	< 46.17 (< 46.17, <46.17)
1	600.0 (252.6, 903.9)	< 38.78 (< 38.78, < 38.78)	1276 (635.2, 1314)	< 46.17 (< 46.17, <46.17)
2	141.5 (74.89, 246.7)	< 38.78 (< 38.78, < 38.78)	983.5 (560.9, 1253)	< 46.17 (< 46.17, <46.17)
4	< 50.00 (< 50.00, < 50.00)	< 38.78 (< 38.78, < 38.78)	695.5 (471.9, 851.7)	< 46.17 (< 46.17, <46.17)
8	< 50.00 (< 50.00, < 50.00)	< 38.78 (< 38.78, < 38.78)	209.2 (123.9, 361.0)	< 46.17 (< 46.17, <46.17)

Table 2. Individual maximum serum concentrations C_{max} of the parent compound ciclesonide (B9207-015 and B9207-016), the respective main metabolites (B9207-021 and B9207-022) in 6 healthy subjects following a single administration of 1600 µg B9207-015.

Subject No.	Sampling time [h]	R-Epimer B9207-015 [pg/ml]	S-Epimer B9207-016 [pg/ml]	[%] ^a
1	0.17	10256	143.4	1.4
2	0.17	5579	67.23	1.2
3	0.17	19216	287.6	1.5
4	0.17	11712	173.0	1.5
5	0.17	6968	100.3	1.4
6	0.17	12627	181.2	1.4
Mean		11060	158.8	1.4
		Metabolite B9207-021	Metabolite B9207-022	
1	1.0	1314	< 46.17	n.a. ^b
2	1.0	977.0	< 46.17	n.a. ^b
3	1.0	1285	< 46.17	n.a. ^b
4	0.5	1401	< 46.17	n.a. ^b
5	0.5	713.9	< 46.17	n.a. ^b
6	0.5	1478	< 46.17	n.a. ^b
Mean		1195	< 46.17	n.a.

^aPercentage refers to the sum of both epimers.

^bFor individual data, C_{max} was below the LLOQ for B9207-022, so that no percentage was calculated (n.a. = not applicable)

A point estimate of 0.014 and 95%-confidence limits of 0.013 and 0.015 for the ratio of the C_{max} population medians for S-ciclesonide (B9207-016) referenced to R-ciclesonide (B9207-015) was obtained. Only 1.4 % S-ciclesonide as compared to R-ciclesonide was found in the serum samples. The ratio of C_{max} of R-ciclesonide to S-ciclesonide are shown in Table 3. Based on this approach a PE of 71.2 and 95%-confidence limits of 65.5 and 77.3 for the ratio of the C_{max} population medians for R-ciclesonide (B9207-015) referenced to S-ciclesonide (B9207-016) was obtained (Note the reciprocal value of 71.2 is 0.014 corresponding to 1.4 %).

No values above the LLOQ were obtained for B9207-022, the S-epimer of metabolite M1. Therefore, no ratios could be calculated for values below the LLOQ. However, when the mean values of B9207-021 (1195 pg/ml) and B9207-022 (< 46.17 pg/ml) are used to calculate percentages, clearly less than 3.7 % of S-M1 (B9207-022) compared to the sum of both epimers was present in the study samples (Table 2).

Table 3. Point estimates (95%-CI) of C_{max} ratios following single inhalative doses of 1600 µg ciclesonide via MDI

Reference S-epimer	Test R-epimer	Point estimate (95% CI)
S-ciclesonide (B9207-016)	R-ciclesonide (B9207-015)	71.2 (65.5-77.3)
S-M1 (B9207-22)	R-M1 (B9207-021)	not applicable

GENERAL COMMENTS

- According to the sponsor, ciclesonide Batch R02990 was used to manufacture the ciclesonide MDIs (Batch number 4BG008) which were used in the present study. The ciclesonide batch specification is ~~1.0~~ % B9207-016 and ~~1.0~~ % B9207-021. The content of S-ciclesonide (B9207-016) and R-M1 (B9207-021) was ~~0.5~~ % and ~~0.5~~ %, respectively. Therefore, about ~~0.5~~ % S-ciclesonide could be expected in serum samples following inhalation of the above mentioned medication. The obtained value of ~~0.5~~ % S-epimer is fairly in good agreement with the expectation showing that in vivo conversion does not occurs to a measurable extent.
- The sponsor believes that S-ciclesonide concentrations were overestimated in the presence of an excess amount of R-ciclesonide, in such a way that the ~~0.5~~ % value reported could be corrected to less than ~~0.5~~ %.

b(4)

CONCLUSIONS

- ~~0.5~~ % S-ciclesonide as compared to R-ciclesonide was found in the serum samples.
- Since the content of S-ciclesonide (B9207-016) and R-M1 (B9207-021) in the drug product used for this study was ~~0.5~~ % and ~~0.5~~ % respectively, the obtained value of ~~0.5~~ % for S-ciclesonide may not be clinically relevant and may be due to variability of the data or a lack of sensitive enough analytical method for the detection of this compound.

b(4)

APPEARS THIS WAY ON ORIGINAL

"Study on the bioequivalence of two different ciclesonide dosing regimen using different inhaler strengths (4 puffs of 200 µg vs. 8 puffs of 100 µg)"

Clinical Study Report no.: 128/2000
Protocol No.: BY9010/FHP026
Development Phase of Study: Phase I
Study Initiation Date: Sep 29, 1999
Study Completion Date: Nov 11, 1999
Chief Investigator: Dr. Th. Bethke
Wilhelm Wurst, Byk Gulden Pharmaceuticals, Constance
(Germany)

Objectives

- to investigate the bioequivalence of two different ciclesonide dosing regimen using different inhaler strengths.
- Secondary objectives were safety and tolerability.

Study Design

The study was conducted according to a randomized, open, two-period, and crossover design. Twenty four (24) healthy subjects entered the study and were randomly allocated to the two treatment sequences with 12 subjects per sequence.

The following treatments were administered according to the randomized, open, two-period, crossover design.

Treatment A (Test): Ciclesonide in metered dose inhalers (MDI), each puff containing 100 µg dose: 800 µg ciclesonide (8 puffs of 200 µg each) Batch No.: 033499

Treatment B (Reference): Ciclesonide in metered dose inhalers (MDI), each puff containing 200 µg dose: 800 µg ciclesonide (4 puffs of 50 µg each) Batch No.: 034499

The duration of the wash out period between the two study periods was at least 7 days, but not more than 2 weeks.

Study Subjects

A total of 24 male/female subjects were enrolled and 24 subjects completed the study. The median age of the study participants was 28 years; their median weight was 72 kg, their median height was 178 cm, and their median Broca Index was 96%.

PK Measurements

Blood samples were taken at predose and at 10 min, 20 min, 0.5h, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12hrs after study drug administration.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 in serum was performed using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for B9207-015 and B9207-021, respectively.

Data Analysis

PK Analysis

Relevant PK parameters were calculated using non-compartmental methods.

Statistical Analysis

Point estimate and 90%-confidence limits were given for the ratio of the population medians for Test and Reference using a multiplicative model and a parametric analysis, no substantive deviations from this model called for a nonparametric analysis. b(4)

Equivalence between Test and Reference was concluded when the 90% confidence interval was entirely within the equivalence range of ~~0.8 to 1.25~~.

RESULTS

Analytical Method

In-Study Validation

The between-batch accuracy for Ciclesonide metabolite was in the range from 96.9 % to 100.6 % (number of replicates = 19-20) and the between-batch accuracy for Ciclesonide was between 100.1 % and 102.3 % (number of replicates = 18-20). The between-batch precision for Ciclesonide metabolite and Ciclesonide were from 3.8 % to 7.4 % and from 3.3 % to 7.1 %, respectively.

Pharmacokinetic Results

Figure 1 and 2 show the mean serum concentrations of B9207-015 and metabolite, respectively in healthy volunteers following single inhalative doses of 800 mcg B907-015 using 16 puffs of 50 mcg or 4 puff of 200 mcg. Tables 1 summarizes the PK characteristics of B9207-015 and its metabolite as well as 90% CI of the relevant PK parameters for the ratio lower to higher strength.

B9207-015 (parent compound ciclesonide) was detected in most serum samples up to 4 hours post dose.

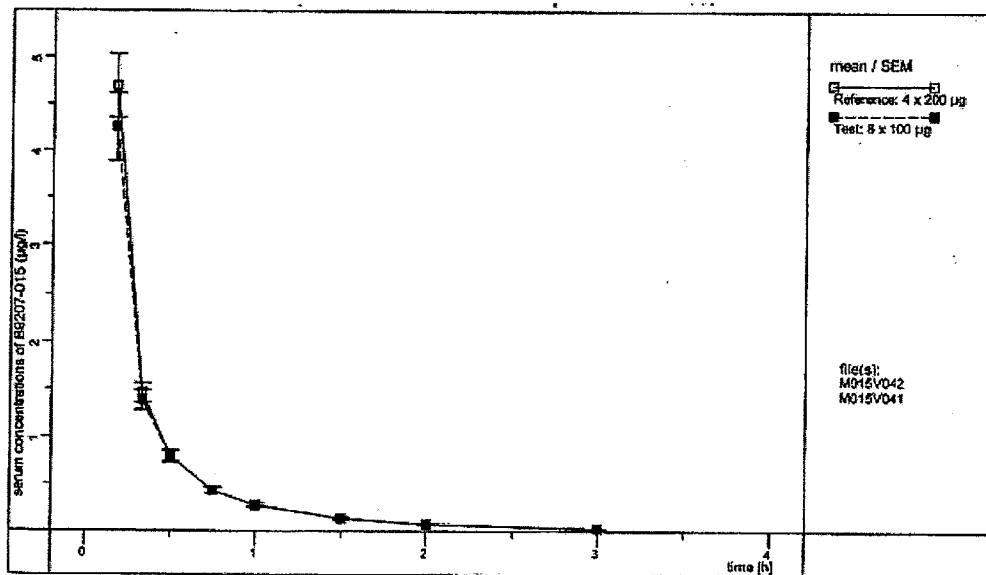


Figure 1. Mean serum concentrations of B9207-015 in healthy volunteers following single inhalative doses of 800 mcg B907-015 using 8 puffs of 1000 mcg or 4 puff of 200 mcg.

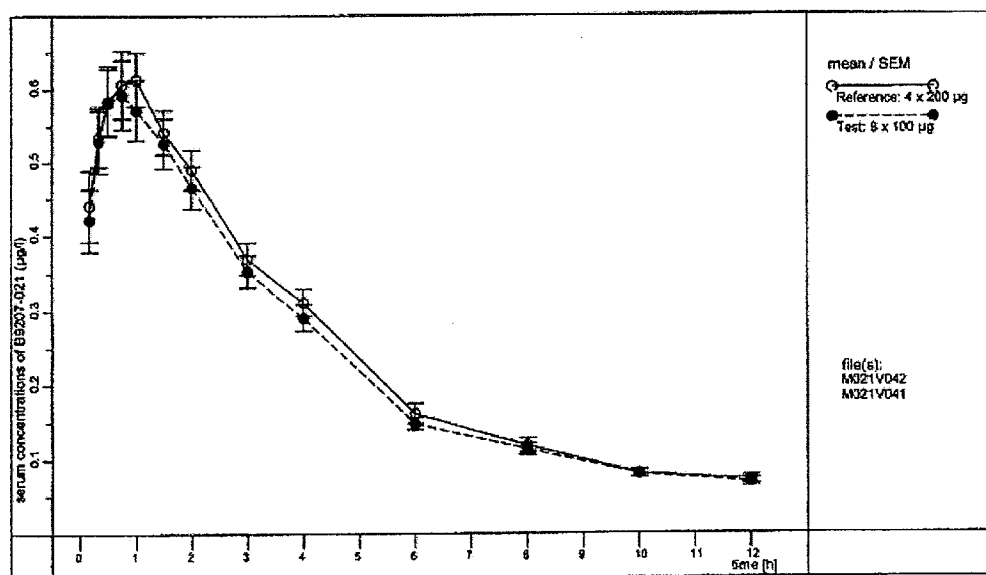


Figure 2. Mean serum concentrations of B9207-021 in healthy volunteers following single inhalative doses of 800 mcg B907-015 using 8 puffs of 100 mcg or 4 puff of 200 mcg.

Table 1. PK characteristics of B9207-015 and its metabolite and point estimates and 90% CI of the PK parameters following single administration of 800 mcg B9207-015.

Product ID/ Batch # (NME)	Study Objective	Study Design	Subjects (M/F)	HWP (Age: Mean, range)	Treatments (Dose)	Ciclesonide		Active Principle RM1			
						Geometric mean Pharmacokinetic Parameters (68% range)		Geometric mean Pharmacokinetic Parameters (68% range)			Mean (SEM)
						Cmax $\mu\text{g/L}$	AUC $\mu\text{g}\cdot\text{h/L}$	Cmax $\mu\text{g/L}$	AUC $\mu\text{g}\cdot\text{h/L}$	T1/2 h	Tmax h
033499	BE inhaler strength	Open, 2-period, single dose	24 (20M/4F)	HV 29 (21-44)	CIC 800 μg (8x100) SD p=nh	3.764 (2.110, 6.716)	1.32 (0.77, 2.29)	0.995 (0.402, 0.661)	2.86 (2.16, 4.11)	3.7 (3.0, 4.4)	0.73 (0.07)
034499					CIC 800 μg (8x200) SD p=nh	4.446 (3.175, 6.224)	1.49 (1.10, 2.01)	0.624 (0.445, 0.874)	3.14 (2.39, 4.13)	3.6 (3.1, 4.2)	0.8 (0.07)
				Parameter	AUC (0-4) ($\mu\text{g}\cdot\text{h/L}$)	Cmax ($\mu\text{g/L}$)		Cmax / AUC (h ⁻¹)		T1/2 (h)	
				Reference	RM1 (200 μg puf)	RM1 (200 μg puf)		RM1 (200 μg puf)		RM1 (200 μg puf)	
				Point estimate	0.95	0.95		1.01		1.01	
				90% CI	0.84-1.08	0.84-1.08		0.96-1.06		0.96-1.07	

SUMMARY OF FINDINGS

- 90% CI applied to the geometric means for AUC and Cmax of the metabolite B9207-021 were within $\pm 20\%$ limits. Therefore, the 100 mcg strengths and the 200 mcg strength give proportional similar systemic exposure of the metabolite.

b(4)

GENERAL COMMENTS

- The term BE should not be applied to two different strengths of an inhaled product; instead the terms relative systemic BA or dose proportionality should be used in the comparison of two products under an IND.
- The 90% CI limits for two product to be considered having dose proportionality in terms of systemic exposure should be $\pm 20\%$ instead of $\pm 10\%$.

b(4)

"Study on the bioequivalence of two different ciclesonide dosing regimen using different inhaler strengths (4 puffs of 200 µg vs. 16 puffs of 50 µg)"

Clinical Study Report no.: 307/98
Protocol No.: BY9010/FHP022
Development Phase of Study: Phase I
Study Initiation Date: Jan 27, 2000
Study Completion Date: March 22, 2000
Chief Investigator: Dr. Th. Bethke
Wilhelm Wurst, Byk Gulden Pharmaceuticals, Constance
(Germany)

Objectives

- to investigate the bioequivalence of two different ciclesonide dosing regimen using different inhaler strengths.
- Secondary objectives were safety and tolerability.

Study Design

The study was conducted according to a randomized, open, two-period, crossover design. Twenty four (24) healthy subjects entered the study and were randomly allocated to the two treatment sequences with 12 subjects per sequence.

The following treatments were administered according to the randomized, open, two-period, crossover design.

Treatment A (Test): Ciclesonide in metered dose inhalers (MDI), each puff containing 200 µg dose: 800 µg ciclesonide (4 puffs of 200 µg each) Batch No.: 147298

Treatment B (Reference): Ciclesonide in metered dose inhalers (MDI), each puff containing 50 µg dose: 800 µg ciclesonide (16 puffs of 50 µg each) Batch No.: 148298

The duration of the wash out period between the two study periods was at least 7 days, but not more than 2 weeks.

Study Subjects

A total of 24 male/female subjects were enrolled and 24 subjects completed the study. The median age of the study participants was 29 years; their median weight was 72 kg, their median height was 176 cm, and their median Broca Index was 89%.

PK Measurements

Blood samples were taken at predose and at 10 min, 20 min, 0.5h, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12hrs after study drug administration.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 in serum was performed using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for B9207-015 and B9207-021, respectively.

Data Analysis

PK Analysis

Relevant PK parameters were calculated using non-compartmental methods.

Statistical Analysis

Point estimate and 90%-confidence limits were given for the ratio of the population medians for Test and Reference using a multiplicative model and a parametric analysis, no substantive deviations from this model called for a nonparametric analysis.

Equivalence between Test and Reference was concluded when the 90% confidence interval was entirely within the equivalence range of

b(4)

RESULTS

Analytical Method

In-Study Validation

The between-batch accuracy for Ciclesonide metabolite was in the range from 96.9 % to 100.6 % (number of replicates = 19-20) and the between-batch accuracy for Ciclesonide was between 100.1 % and 102.3 % (number of replicates = 18-20). The between-batch precision for Ciclesonide metabolite and Ciclesonide were from 3.8 % to 7.4 % and from 3.3 % to 7.1 %, respectively.

b(4)

Pharmacokinetic Results

Figure 1 and 2 show the mean serum concentrations of B9207-015 and metabolite, respectively in healthy volunteers following single inhalative doses of 800 mcg B907-015 using 16 puffs of 50 mcg or 4 puff of 200 mcg.. Tables 1 and 2 summarize the PK characteristics of B9207-015 and its metabolite, respectively, in serum of healthy subjects following single administration of 800 mcg B9207-015.

B9207-015 (parent compound ciclesonide) was detected in most serum samples up to 3 hours post dose.

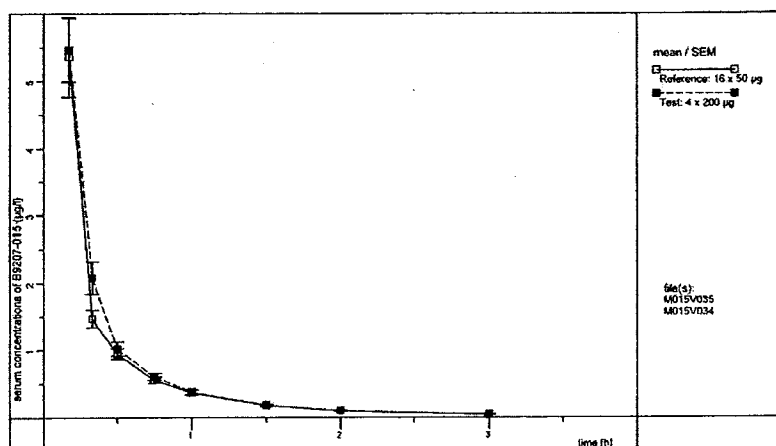


Figure 1. Mean serum concentrations of B9207-015 in healthy volunteers following single inhalative doses of 800 mcg B9207-015 using 16 puffs of 50 mcg or 4 puffs of 200 mcg.

Table 1. PK characteristics of CIC in serum healthy subjects following single administration of 800 mcg B9207-015.

dosing regimen	N	AUC _{0-∞} [µg·h/l]						geom. mean	lower range	upper range
		mean	SD	SEM	median	min	max			
16 x 50 µg	21	1.79	0.64	0.14	1.83	0.31	2.92	1.64	1.01	2.67
4 x 200 µg	22	1.98	0.73	0.16	1.93	0.58	3.75	1.82	1.21	2.76
dosing regimen	N	C _{max} [µg/l]						geom. mean	lower range	upper range
		mean	SD	SEM	median	min	max			
16 x 50 µg	21	4.839	2.757	0.59	5.116	0.340	11.73	3.835	1.692	8.693
4 x 200 µg	22	5.279	2.265	0.48	5.194	1.460	10.27	4.782	2.960	7.724
dosing regimen	N	C _{0.17h} [µg/l]						geom. mean	lower range	upper range
		mean	SD	SEM	median	min	max			
16 x 50 µg	19	5.358	2.577	0.59	5.419	1.208	11.73	4.638	2.518	8.542
4 x 200 µg	21	5.461	2.150	0.47	5.429	1.999	10.27	5.060	3.360	7.620
dosing regimen	N	t _{1/2} [h]						geom. mean	lower range	upper range
		mean	SD	SEM	median	min	max			
16 x 50 µg	21	0.82	0.67	0.15	0.70	0.26	3.54	0.70	0.42	1.16
4 x 200 µg	22	0.73	0.34	0.07	0.71	0.38	2.11	0.69	0.48	0.97

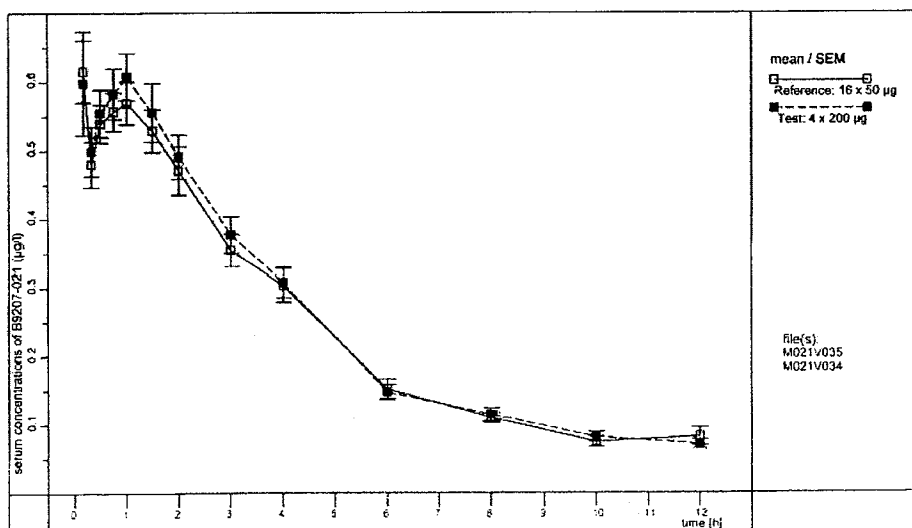


Figure 2. Mean serum concentrations of RM1 in healthy volunteers following single inhalative doses of 800 mcg B907-015 using 16 puffs of 50 mcg or 4 puff of 200 mcg.

Table 2. PK characteristics of RM1 in serum healthy subjects following single administration of 800 mcg CIC

dosing regimen	AUC _(0,inf) [µg·h/l]								
	mean	SD	SEM	median	min	max	geom. mean	lower range	upper range
16 x 50 µg	3.06	0.91	0.19	3.35	0.60	4.35	2.88	1.88	4.38
4 x 200 µg	3.25	1.03	0.21	3.37	1.11	5.30	3.07	2.12	4.44
	C _{max} [µg/l]								
	mean	SD	SEM	median	min	max	geom. mean	lower range	upper range
16 x 50 µg	0.711	0.195	0.040	0.701	0.223	1.050	0.679	0.486	0.949
4 x 200 µg	0.735	0.348	0.071	0.672	0.274	1.735	0.672	0.439	1.028
	C _{max} /AUC [1/h]								
	mean	SD	SEM	median	min	max	geom. mean	lower range	upper range
16 x 50 µg	0.244	0.061	0.012	0.215	0.171	0.388	0.237	0.188	0.299
4 x 200 µg	0.226	0.057	0.012	0.212	0.118	0.327	0.219	0.169	0.283
	t _{1/2} [h]								
	mean	SD	SEM	median	min	max	geom. mean	lower range	upper range
16 x 50 µg	3.47	0.57	0.12	3.42	1.90	4.82	3.42	2.86	4.08
4 x 200 µg	3.66	0.60	0.12	3.45	2.92	5.19	3.61	3.09	4.23
	t _{max} [h]								
	mean	SD	SEM	median	min	max	geom. mean	lower range	upper range
16 x 50 µg	0.66	0.56	0.11	0.50	0.17	2.00			
4 x 200 µg	0.85	0.54	0.11	0.88	0.17	2.00			

Table 3. Point estimates and 90% CI of the PK parameters following single administration of 800 mcg B9207-015.

Characteristics	Point estimate	90% confidence interval
AUC _(0,inf)	1.07	(0.99, 1.16)
C _{max}	0.99	(0.88, 1.11)
C _{max} / AUC _(0,inf)	0.92	(0.83, 1.02)
t _{1/2}	1.06	(1.00, 1.12)
t _{max} [h]	0.125	(0.000, 0.415)

t_{max} = additive statistical model, non-parametric analysis

SUMMARY OF FINDINGS

- 90% CI applied to the geometric means for AUC and Cmax of the metabolite B9207-021 were within $\pm 10\%$ limits. Therefore, the 50 mcg strengths and the 200 mcg strength give proportional similar systemic exposure of the metabolite.

GENERAL COMMENTS

- The term BE should not be applied to two different strengths of an inhaled product; instead the terms relative systemic BA or dose proportionality should be used in the comparison of two products under an IND.
- The 90% CI limits for two product to be considered having dose proportionality in terms of systemic exposure should be ' $\pm 10\%$ ' instead of ' $\pm 20\%$ '.

Comparison of the pharmacokinetics (active metabolite M1 and parent compound) of inhaled ciclesonide when administered via a MDI with and without AeroChamber Plus™ spacer"

Protocol No.: BY9010/ CP-030 (41/2003)
Development Phase of Study: Phase I
Study Period: 22 July 2002 – 27 August 2002
Study Completion Date: Nov 11, 1999
Chief Investigator: _____

b(4)

Objectives

- to compare the pharmacokinetics (active metabolite and parent compound) of inhaled ciclesonide when administered via the MDI with and without the AeroChamber Plus spacer in subjects suffering from mild to moderate bronchial asthma.

Study Design

The study was conducted as an open, randomized single-dose, two-period crossover study. It consisted of a screening examination (within 4 weeks before administration day), two treatment periods of 1 day each, separated by a wash-out phase of 7 to 14 days and a poststudy examination within 5 days after termination of the study but at least 24 hours after the last administration. The following treatments were administered according to the randomized, open, two-period, crossover design.

	Treatment period 1 (Administration day 1)	Treatment period 2* (Administration day 2)
Sequence 1:	Treatment A (400 µg ciclesonide using a MDI with the AeroChamber Plus™)	Treatment B (400 µg ciclesonide using a MDI without the AeroChamber Plus™)
Sequence 2:	Treatment B (400 µg ciclesonide using a MDI without the Chamber Plus™)	Treatment A (400 µg ciclesonide using a MDI with the AeroChamber Plus™)

*After a washout period of at least 7 days but not more than 14 days

Batch No.: 4BGA001

Study Subjects

A total of 30 male/female subjects were enrolled and 30 subjects completed the study. A summary of the demographic characteristics is depicted in the following table:

Demographic characteristics for the study population (n=30)	
Age, years	
Mean	28
Median (range)	26 (19, 52)
Height, cm	
Mean	175
Median (range)	173 (161, 199)
Weight, kg	
Mean	71.9
Median (range)	72.5 (53, 103)
Broca-index	
Mean	0.97
Median (range)	0.95 (0.79, 1.25)
Gender, n (%)	
Male	14 (47)
Female	16 (53)
Alcohol consumption, n (%)	
Yes	27 (90)
No	3 (10)
Smoking, n (%)	
Yes	0 (0)
No	30 (100)

PK Measurements

Blood samples were drawn by means of an indwelling catheter in treatment periods 1 and 2, prior to ciclesonide inhalation as well as 0.25 h (15 min), 0.50 h (30 min), 0.75 h (45 min), 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, and 14 h thereafter.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 was performed using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for B9207-015 and B9207-021, respectively.

Data Analysis

PK Analysis

Relevant PK parameters were calculated using non-compartmental methods.

Statistical Analysis

For the primary variable AUC(0,inf) of the active metabolite B9207-021 point estimate and 90%-confidence limits was given for the ratio of the population medians for Test (ciclesonide via MDI with spacer) / Reference (ciclesonide via MDI without spacer) using a multiplicative model and a parametric analysis. The secondary variables C_{max}, t_{max} and t_{1/2} of the active ciclesonide metabolite B9207-021 were analyzed in analogy to the primary variables. For t_{max} point estimates and 90%-confidence intervals were given for the test - reference differences. The secondary variables AUC(0,inf), C_{max}, t_{max} and t_{1/2} of the parent compound ciclesonide (B9207-015) were presented in a descriptive manner including summary statistics such as median, mean, standard deviation (SD), standard error of the mean (SEM), geometric mean and geometric 68%-range, where appropriate. The safety variables were analyzed in a descriptive manner using summary statistics (e.g. median, 68%-range, mean, SD) where appropriate.

RESULTS

Analytical Method

In-Study Validation

Calibration data, quality control data and chromatograms indicated that the method performed acceptably during the sample analyses. The calibration curves for ciclesonide and its active metabolite (B9207-021) were linear with a coefficient of correlation equal or better than $r = 0.999$ for ciclesonide and equal or better than $r = 0.999$ for B9207-021. The within-study (total study) accuracy as from the analysis of QC samples spiked in human serum for ciclesonide was in the range from -1.5% to 1.5% , the corresponding within-study accuracy for B9207-021 was between -1.5% and 1.5% . The total precision for ciclesonide and B9207-021 ranged from 1.5% and from 1.5% respectively.

b(4)

Pharmacokinetic Results

Mean (SEM) serum concentration vs time profiles of the parent compound ciclesonide (B9207-015) and its metabolite following single inhaled administration of ciclesonide in both study periods are shown in Figures 1 and 2. A summary of mean (SD) pharmacokinetic characteristics is given in Table 2. Table 3 shows 90% confidence intervals for the geometric mean ratios of the relevant PK parameters of the metabolite Distribution of ciclesonide following inhalation was fast ($t_{1/2}$ of distribution about 0.5 h). Three hours after administration, the serum concentrations of ciclesonide were mostly below the lower limit of detection.

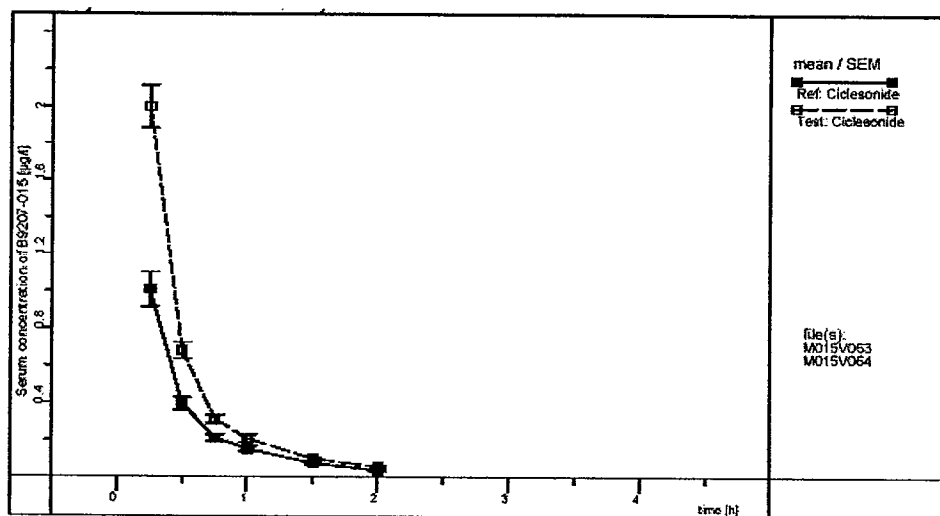


Figure 1. Mean serum concentrations of ciclesonide in 30 asthmatic patients following a single inhaled dose of ciclesonide 400 µg using MDI with (Test) and without (Reference) use of a spacer

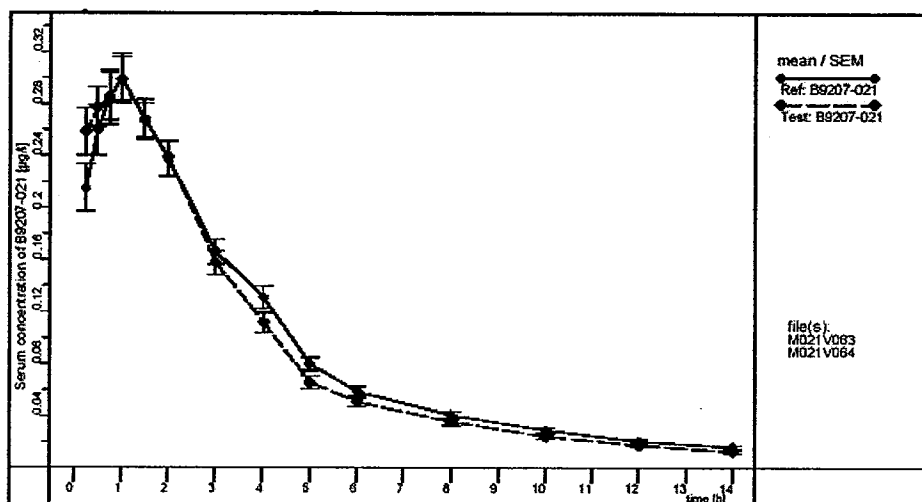


Figure 2. Mean serum concentrations of the active metabolite of ciclesonide (B9207-021) in 30 asthmatic patients following a single inhaled dose of ciclesonide 400 µg using MDI (Test) and without (Reference) use of a spacer

Table 1: Pharmacokinetic characteristics of ciclesonide (B9207-015) and its metabolite in serum of patients suffering from mild to moderate bronchial asthma following a single inhaled dose of ciclesonide 400 µg administered using MDI with and without the AeroChamber Plus™ spacer

Product ID Batch # (NME)	Study Objective	Study Design	Subjects (M/F)	HVIP (Age: Mean, range)	Treatments (Dose)	Ciclesonide		Active Principle RM1				
						Geometric mean Pharmacokinetic Parameters (68% range)		Geometric mean Pharmacokinetic Parameters (68% range)				Mean (SEM)
						Cmax µg/L	AUC µg·h/L	Cmax µg/L	AUC µg·h/L	T1/2 h	Tmax h	
4BGA001	BE with or without spacer	Open, 2-period, single dose	30 (14M/16F)	Bronchial asthma 28 (19-52)	CIC 400 µg (2x200)SD p.i.inh with spacer	1.915 (1.434, 2.566)	0.89 (0.65, 1.21)	0.313 (0.229, 0.426)	1.26 (0.94, 1.70)	4.79 (3.42, 6.72)		0.84 (0.08)
4BGA001					CIC 400 µg (2x200)SD p.i.inh without spacer	0.680 (0.527, 1.001)	0.49 (0.31, 0.78)	0.298 (0.207, 0.426)	1.32 (0.91, 1.90)	4.59 (3.40, 6.21)		0.91 (0.07)

Table 3: Summary of equivalence assessment of the active metabolite of ciclesonide (B9207-021): Geometric means (68% range) and point estimates (90% confidence interval) for the ratios of the pharmacokinetic characteristics of Test (MDI with a spacer) vs reference (MDI without spacer) [tmax: median]

Pharmacokinetic characteristic	Treatment A (Test) With Spacer	Treatment B (Reference) Without Spacer	Equivalence Ratio (Test/Reference)	
	Geometric mean	Geometric mean	Point Estimate	(90% CI)
AUC _(0,inf) [µg·h/l]	1.26	1.32	0.96	(0.85, 1.07)
(68% range)	(0.93-1.70)	(0.91-1.90)		
C _{max} [µg/l]	0.313	0.298	1.05	(0.94, 1.18)
(68% range)	(0.230-0.427)	(0.206-0.429)		
t _{1/2} [h]	4.79	4.59	1.04	(0.92, 1.18)
(68% range)	(3.44-6.67)	(3.38-6.24)		
t _{max} [h]*	1.00 (median)	1.00 (median)	-0.125	(-0.250, 0.125)

* Additive statistical model, non-parametric analysis. Point estimate and 90% CI are given for the Test - Reference differences.

SUMMARY OF FINDINGS

- Mean serum concentration of ciclesonide was higher when using the AeroChamber Plus™ spacer resulting in about twofold higher C_{max} and AUC values. T_{max} was at 0.25 h, the first time point of pharmacokinetic analysis after dose administration. The elimination half life was not influenced by using the spacer.
- The use of the spacer did not affect the pharmacokinetics of the active metabolite B9207-021. AUC increase by 6% and C_{max} decreased by 4% when no spacer was used. 90% CI were within 80-125% limits.

CONCLUSION

- The systemic exposure of the active metabolite is not affected by the use of AeroChamber Plus spacer.

APPEARS THIS WAY ON ORIGINAL

"Investigation of the pharmacokinetics of ciclesonide and its active metabolite B9207-021 after single and repeated once-daily inhalation of 400 µg ciclesonide using the MDI device"

Clinical Study Report no.: 211/2000
Protocol No.: BY9010/FHP027
Development Phase of Study: Phase I
Study Initiation Date: Jan 27, 2000
Study Completion Date: March 22, 2000
Chief Investigator: Dr. Th. Bethke
Wilhelm Wurst, Byk Gulden Pharmaceuticals, Constance
(Germany)

Objectives

- to investigate the pharmacokinetics of the active compound B9207-021 after first and repeated inhalation of 400 µg ciclesonide once daily using the MDI device in healthy volunteers. Further, descriptive pharmacokinetics as well as safety and tolerability of ciclesonide were assessed.

Study Design

This was an open, non-controlled, one-period, single-center Phase I study in 18 healthy subjects. The study consisted of a screening examination (within four weeks prior to study entry), a treatment period of 7 days, and a post-study examination (two weeks after end of treatment). During the treatment period all subjects received 400 µg ciclesonide in the morning on each study day.

Study Subjects

A total of 18 subjects were enrolled and 18 subjects completed the study. The Table below summarizes the demographic characteristics of the subjects included in this study.

Characteristic	Study Population (n = 18)
Age, years	
median (range)	28 (22 - 43)
Gender, N (%)	
male	15 (83)
female	3 (17)
Weight, kg	
median (range)	77.5 (57 - 96)
Height, cm	
median (range)	178 (166 - 190)
Broca	
median (range)	0.98 (0.84 - 1.19)
Tobacco use, n (%)	
yes	6 (33)
no	12 (67)
Alcohol consumption, n (%)	
no	4 (22)
occasionally	13 (72)
daily	1 (6)

Study Medication:

Ciclesonide (Batch no.: 46G003 Dosage form: MDI) at a dose of 400 µg was

administered once daily in the morning at approximately 8a.m. Subjects were asked to take two inhalations (200 µg /puff) each time.

PK Measurements

Blood samples were taken at study Days 1 and 7, immediately (within 5 min) prior to ciclesonide inhalation and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 5, 10, 12, and 24 hours after study drug administration.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 in serum was performed by _____; using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for B9207-015 and B9207-021, respectively.

b(4)

Data Analysis

PK Analysis

Relevant PK parameters were calculated using non-compartmental methods.

Statistical Analysis

Geometric mean and 90% confidence limits were provided for the ratio of the population means for AUC following repeated dosing (test) and first application using a multiplicative model and a parametric analysis.

RESULTS

Analytical Method

In-Study Validation

Determination of B9207-015 and B9207.021 in Serum

The between-batch accuracy for Ciclesonide metabolite was in the range from 96.9 % to 100.6 % (number of replicates = 19-20) and the between-batch accuracy for Ciclesonide was between 100.1 % and 102.3 % (number of replicates = 18-20). The between-batch precision for Ciclesonide metabolite and Ciclesonide were from 3.8 % to 7.4 % and from 3.3 % to 7.1 %, respectively.

Pharmacokinetic Results

Figure 1 shows the mean concentration time-profiles for ciclesonide and its metabolite, respectively following single and multiple administration of the treatments. Tables 1 and 2 summarize the PK characteristics of B9207-015 and its metabolite, respectively, in serum of healthy subjects following single and multiple (QD) (Day 7) administration of 400 mcg B9207-015. Figure 2 compares the individual AUCs of B9207-021 on Day I and Day 7 following inhalation of 400 mcg ciclesonide once daily. Table 3 shows the point estimates and 90% CI of the PK parameters following single versus multiple administration of 400 mcg once daily of ciclesonide.

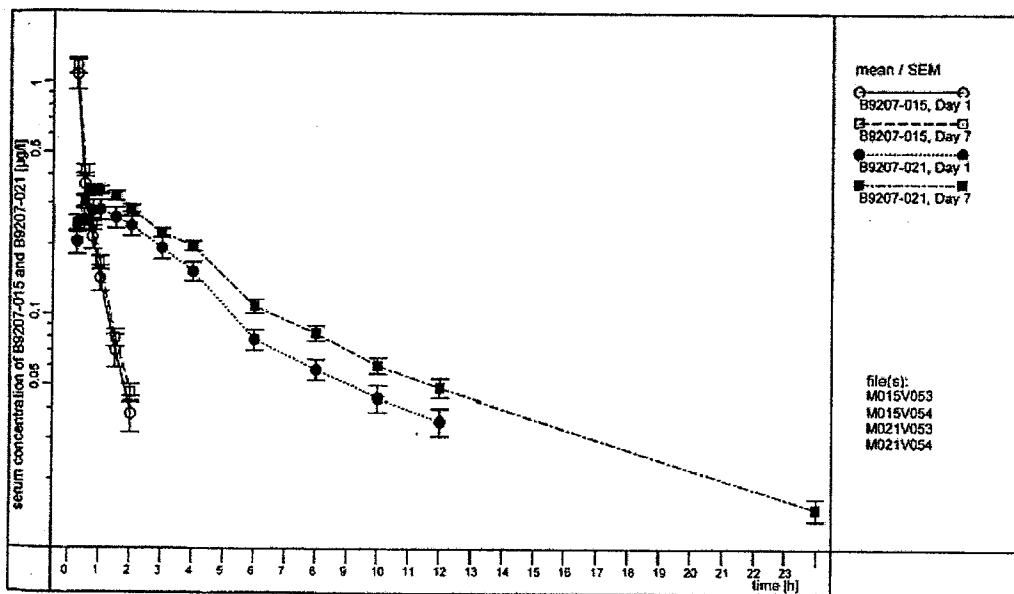


Figure 1. Mean serum concentrations of B9207-015 and B9207-021 in healthy volunteers following single and multiple inhalation of 400 µg of B2907-015 via MDI

Table 1. PK characteristics of B9207-015 in serum of 10 healthy subjects following single and multiple (QD) (Day 7) administration of 400 mcg B9207-015.

	Mean	SD	SEM	Median	Min	Max	Geom. mean	68% range	
AUC^a (µg·h/l)									
Day 1	0.54	0.30	0.07	0.49	0.09	1.28	0.46	0.23	0.89
Day 7	0.61	0.16	0.04	0.58	0.35	0.99	0.59	0.45	0.76
C_{max} (µg/l)									
Day 1	1.076	0.659	0.155	0.951	0.173	2.724	0.873	0.423	1.803
Day 7	1.164	0.369	0.087	1.166	0.510	1.813	1.106	0.790	1.548
t_{1/2} (h)									
Day 1	0.52	0.09	0.02	0.50	0.32	0.68	0.51	0.42	0.61
Day 7	0.54	0.11	0.03	0.51	0.41	0.83	0.53	0.44	0.65

^a Day 1 = AUC_(0, ∞), Day 7 = AUC_(0, 24h).

Table 2. PK characteristics of B9207-021 in serum of 10 healthy subjects following single and multiple (QD) (Day 7) administration of 400 mcg B9207-015

	Mean	SD	SEM	Median	Min	Max	Geom. mean	68% range	
AUC^a (µg·h/l)									
Day 1	1.72	0.73	0.17	1.93	0.30	2.56	1.49	0.78	2.83
Day 7	2.18	0.42	0.10	2.18	1.62	3.04	2.14	1.77	2.59
C_{max} (µg/l)									
Day 1	0.299	0.130	0.031	0.329	0.069	0.554	0.263	0.148	0.468
Day 7	0.369	0.063	0.015	0.354	0.259	0.499	0.364	0.308	0.430
C_{max}/AUC (1/h)									
Day 1	0.18	0.03	0.01	0.18	0.12	0.24	0.18	0.15	0.21
Day 7	0.17	0.03	0.01	0.17	0.13	0.22	0.17	0.14	0.20
t_{1/2} (h)									
Day 1	5.23	1.28	0.30	5.40	2.37	7.11	5.05	3.82	6.59
Day 7	6.72	1.04	0.25	6.59	5.13	8.33	6.65	5.68	7.77
t_{max} (h)									
Day 1	1.08	0.62	0.15	1.00	0.25	3.00			
Day 7	0.94	0.44	0.10	0.75	0.25	2.00			

^a Day 1 = AUC_(0,inf); Day 7 = AUC_(0,24h)

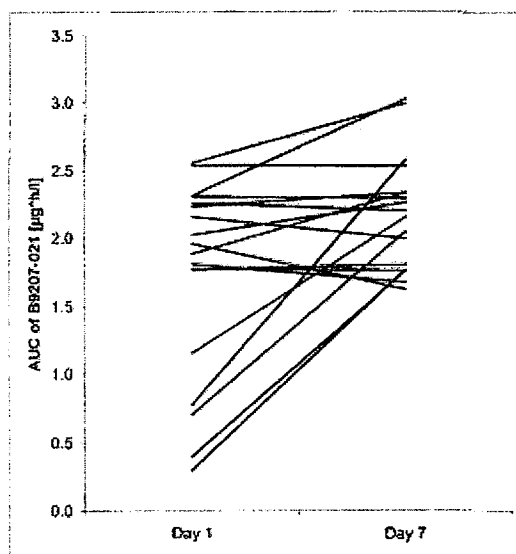


Figure 2. Comparison of individual AUCs of B9207-021 on Day 1 and Day 7 following inhalation of 400 mcg ciclesonide once daily.

Table 3. Point estimates and 90% CI of the PK parameters following single versus multiple administration of 400 mcg once daily of ciclesonide

Pharmacokinetic characteristics	Point estimates (90% CI)	
	Full analysis set (n = 18)	Limited analysis set (n = 14)
AUC	1.44 (1.13, 1.84)	1.08 (0.98, 1.19)
C _{max}	1.38 (1.09, 1.76)	1.05 (0.94, 1.18)
t _{1/2}	1.32 (1.18, 1.48)	1.20 (1.09, 1.32)
t _{max} (h) (additive statistical model, non-parametric analysis)	0.00 (-0.25, 0.13)	0.00 (-0.38, 0.13)

SUMMARY OF FINDINGS

- The AUC and C_{max} geometric means of ciclesonide increased by 28% and 30%, respectively following multiple administration of ciclesonide compared to that after single administration.
- The half-life of ciclesonide remained unchanged following multiple administration compared to that after single administration.
- The AUC and C_{max} geometric means of the metabolite B9207-021 increased by 44% and 38%, respectively following multiple administration of ciclesonide compared to that after single administration.
- The half-life of the metabolite increased by 1.6 following multiple administration compared to that after single administration.

APPEARS THIS WAY ON ORIGINAL

"Investigation of the pharmacokinetics of the active metabolite in asthmatic patients and healthy subjects after single dose inhalation of 1600 mcg of ciclesonide"

Clinical Study Report no.: 56E/99
Protocol No.: BY9010/FHP023
Development Phase of Study: Phase I
Study Initiation Date: Jan 27, 2000
Study Completion Date: March 22, 2000
Chief Investigator: _____

b(4)

Objectives

- to investigate the pharmacokinetics of the active metabolite 89207-021 in asthmatic patients and in healthy subjects after single dose inhalation of 1600 mcg ciclesonide via the MDI device.
- Secondary objectives were to determine the PK characteristics of ciclesonide (B9207-015) and
- to assess the safety and tolerability.

Study Design

The study was conducted according to a parallel group design. To each of the 12 asthmatic patients one healthy subject of the same sex and comparable in age, height and weight was allocated (matched pairs). Both the 12 asthmatics patients and the 12 healthy subjects received a single dose of 1600 mcg ciclesonide to be inhaled by means of the MDI device. The following treatments were administered:

- Each asthmatic patient or a normal subject received a single dose of 1600 mcg ciclesonide as a single dose (8 puffs of 200 mcg each) from a metered dose inhaler (MDI): Batch No.: 090398

Study Subjects

Twelve (12) asthmatic patients and 12 healthy subjects of either sex were included in the study and 12 completed the study. To each asthmatic patient who completed the study one healthy subject was allocated according to the following matching criteria:

Same sex; Age within ± 5 years; Height within $\pm 10\%$; Weight within $\pm 10\%$

The median age of the asthmatic patients was 36 years (range 20 - 59 years); their median weight was 80 kg (range 55 - 89 kg), their median height was 173 cm (range 152-181 cm), and their median Broca index was 104% (range 97% - 122%). For the healthy subjects the median age was 35 years (range 21 - 60 years); their median weight was 80.5 kg (range 55 - 93 kg), their median height was 175 cm (range 152- 184 cm), and their median Broca Index was 109% (range 93%-117%)

PK Measurements

Blood samples were taken at predose and at 10 min, 20 min, 0.5h, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12hrs after study drug administration.

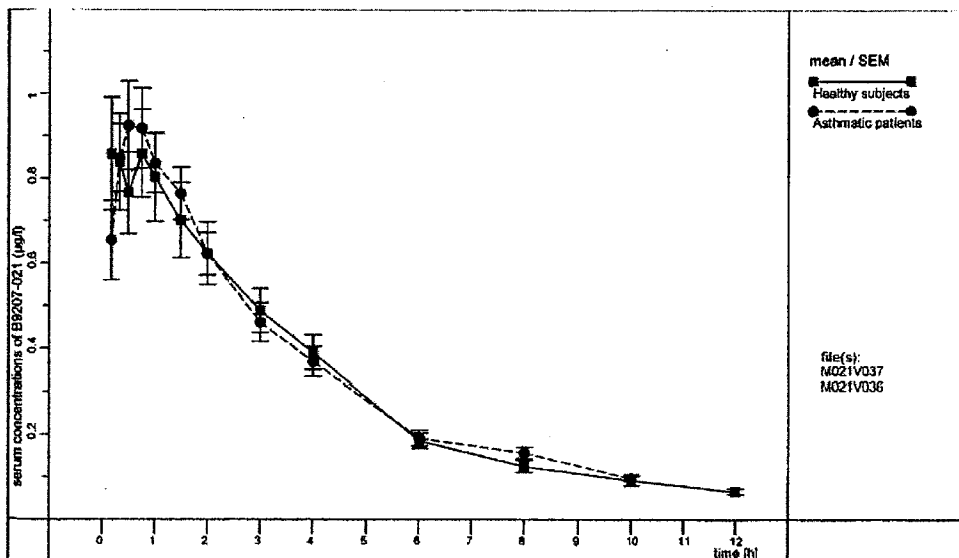


Figure 1. Mean serum concentrations of B9207-021 in healthy volunteers and asthmatics following single inhalative doses of 1600 mcg B907-015.

Table 1. PK characteristics of B9207-015 and its major metabolite in healthy subjects and asthmatic patients following single administration of 1600 mcg B9207-015.

Product ID/ Batch # (NME)	Study Objective	Study Design	Subjects (M/F)	HV/P (Age: Mean, range)	Treatments (Dose)	Ciclesonide		Active Principle RM1			Mean (SEM)
						Geometric mean Pharmacokinetic Parameters (58% range)		Geometric mean Pharmacokinetic Parameters (68% range)			
						Cmax µg/L	AUC µg*h/L	Cmax µg/L	AUC µg*h/L	T1/2 h	
090386	PK, Healthy	Parallel group,	12 (8M/4F)	HV 36 (21-60)	CIC 1600 µg (8x200) SD p.inh	3.696 (1.627, 8.397)	1.35 (0.70, 2.59)	0.875 (0.457, 1.677)	3.91 (2.78, 5.51)	3.24 (2.55, 4.12)	0.67 (0.09)
	PK, Asthmatics	matched pairs	12 (8M/4F)	P (Asthmatics) 37 (20-59)	CIC 1600 µg (8x200) SD p.inh	2.926 (1.631, 5.250)	1.00 (0.60, 1.67)	0.976 (0.716, 1.330)	3.93 (3.21, 4.80)	3.09 (2.53, 3.78)	0.71 (0.12)

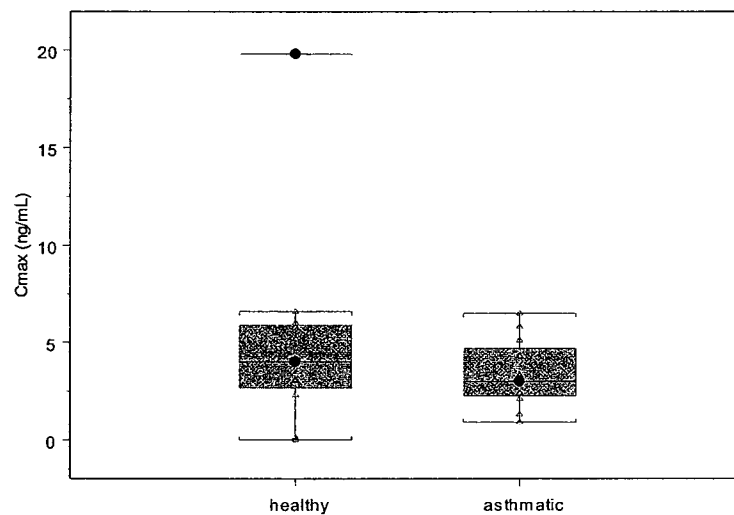


Figure 2. Individual C_{max} values for ciclesonide in healthy volunteers and is patients with asthma following single inhalative doses of 1600 mcg B907-015.

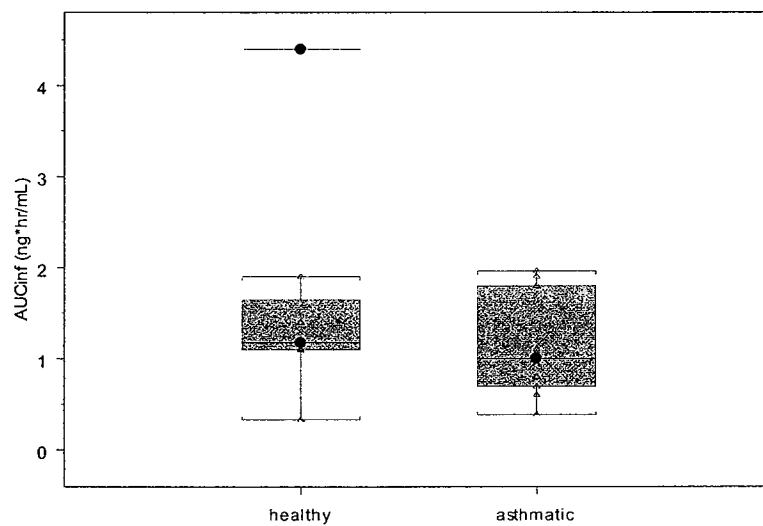


Figure 3. Individual AUC values for ciclesonide in healthy volunteers and is patients with asthma following single inhalative doses of 1600 mcg B907-015.

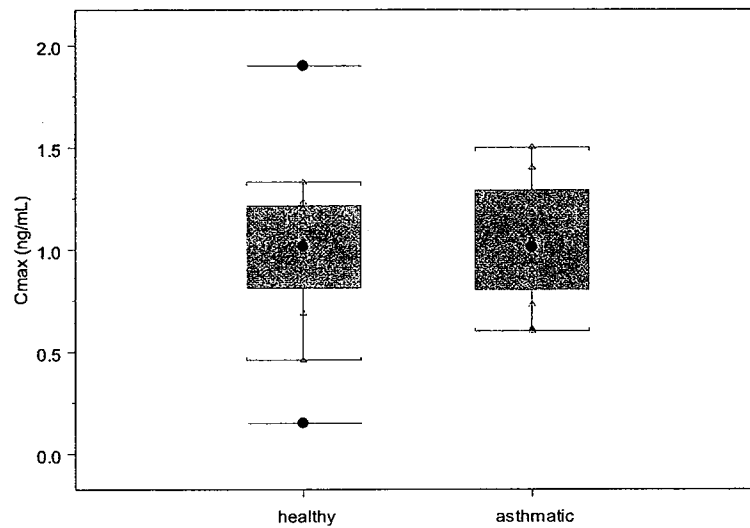


Figure 4. Individual Cmax values for the metabolite RM1 in healthy volunteers and is patients with asthma following single inhalative doses of 1600 mcg B907-015.

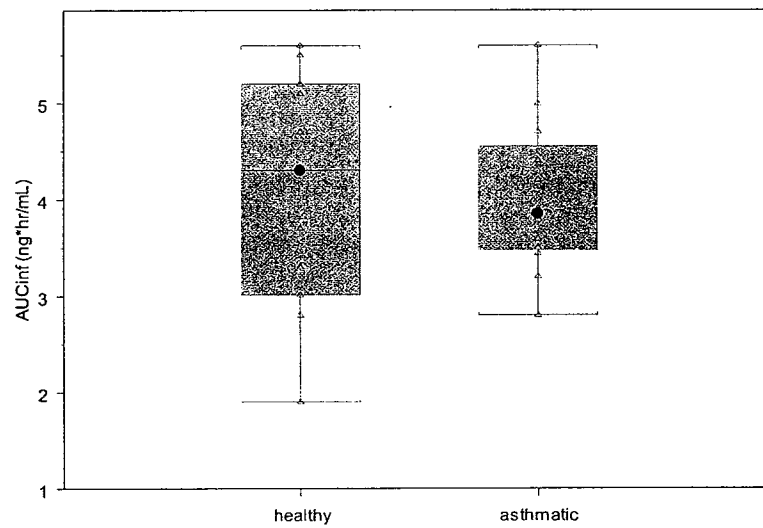


Figure 4. Individual AUCinf values for the metabolite RM1 in healthy volunteers and is patients with asthma following single inhalative doses of 1600 mcg B907-015 using 8 puffs of 200 mcg.

Table 3. Point estimates and 90% CI of the PK parameters of B9207-021 following single administration of 1600 mcg B9207-015 to healthy subjects and asthmatic patients.

Characteristics	Point estimate	90%-Confidence Interval
$AUC_{(0,168)}$	1.003	(0.815, 1.234)
$C_{max} / AUC_{(0,168)}$	0.949	(0.779, 1.157)
$t_{1/2}$	0.954	(0.815, 1.117)
t_{max} (additive statistical model)		
t_{max} [h]	0.048	(-0.218, 0.313)

SUMMARY OF FINDINGS

- The C_{max} and AUC of the active metabolite B9207-021 increased in patients with asthma by 12% and 1%, respectively.
- The C_{max} and AUC of ciclesonide decreased in patients with asthma by 21% and 25%, respectively, as compared to that from healthy controls.
- The $t_{1/2}$ and t_{max} of the active metabolite B9207-021 remained unchanged in asthmatic patients as compared with the healthy controls.

GENERAL COMMENTS

- This study was conducted as a single dose. However, because little accumulation of the metabolite and drug product occurs (<50%) single dose PK may predict multiple dose PK.

CONCLUSION

- The systemic exposure of ciclesonide and its metabolite in asthmatic patients receiving a single dose of ciclesonide 1600 mcg is similar to that observed in healthy subjects.

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"Investigation of the pharmacokinetics of the active metabolite after single dose inhalation of 1600 mcg of ciclesonide in healthy elderly subjects"

Clinical Study Report no.: 253E/99
Protocol No.: BY9010/FHP025
Development Phase of Study: Phase I
Study Initiation Date: Jan 26, 1999
Study Completion Date: Nov 5, 1999
Chief Investigator: _____

b(4)

Objectives

- to investigate the pharmacokinetics of the active metabolite 89207-021 in elderly subjects after single dose inhalation of 1600 mcg ciclesonide via the MDI device.
- Secondary objectives were to determine the PK characteristics of ciclesonide (B9207-015) and
- to assess the safety and tolerability.

Study Design

The study was conducted according to a an open one period, single dose design, in twelve male elderly subjects. The following treatments were administered:

- Each subject received a single dose of 1600 mcg ciclesonide as a single dose (8 puffs of 200 mcg each) from a metered dose inhaler (MDI): Batch No.: 080498

Study Subjects

In total 14 male subjects ≥ 65 years were screened and 12 completed the study. A summary of the demographic variables for this group is included in the table below:

Variable	Unit	N	Mean	SD
Age	years	12	72	3.9
Height	cm	12	172	7.0
Weight pre-study	kg	12	79.2	6.9
post-study	kg	12	79.1	6.8
Broca pre-study	%	12	1.11	0.10
post-study	%	12	1.11	0.09

PK Measurements

Blood samples were taken at predose and at 10 min, 20 min, 0.5h, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24hrs after study drug administration.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 in serum was performed using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for

B9207-015 and B9207-021, respectively.

Data Analysis

PK Analysis

Relevant PK parameters were calculated using non-compartmental methods.

Statistical Analysis

Point estimate and 90%-confidence limits were given for the ratio of the population medians (young to middle-aged subjects from study FH015/ elderly subjects) of the two study groups.

RESULTS

Analytical Method

In-Study Validation

The between-batch accuracy for Ciclesonide metabolite was in the range from 96.9 % to 100.6 % and the between-batch accuracy for Ciclesonide was between 96.5 % and 108.1 %. The between-batch precision for Ciclesonide metabolite and Ciclesonide were from 4.9 % to 8.9 % and from 8.8 % to 23.7 %, respectively.

Pharmacokinetic Results

B9207-015 (parent compound ciclesonide) was detected in most serum samples up to 4 hours post dose. Mean (SEM) serum concentration vs. time profiles of ciclesonide and its metabolite in young and elderly healthy subjects following a single inhalative administration of B9207-015 at a dose level of 1600 µg are shown in Figures 1. Figure 2 shows the concentration-time profile of the metabolite in elderly and young volunteers. Table 1 summarizes the PK characteristics of B9207-015 and its metabolite in healthy elderly versus young subjects following single administration of 1600 mcg B9207-015. Figures 3 and 4 show the individual C_{max} and AUC_{inf} of ciclesonide following administration of the treatments. Likewise, Figures 5 and 6 show it for the metabolite. Table 2 summarizes the point estimates and 90% CI of the ratios of PK parameters of B9207-021 following single administration of 1600 mcg B9207-015.

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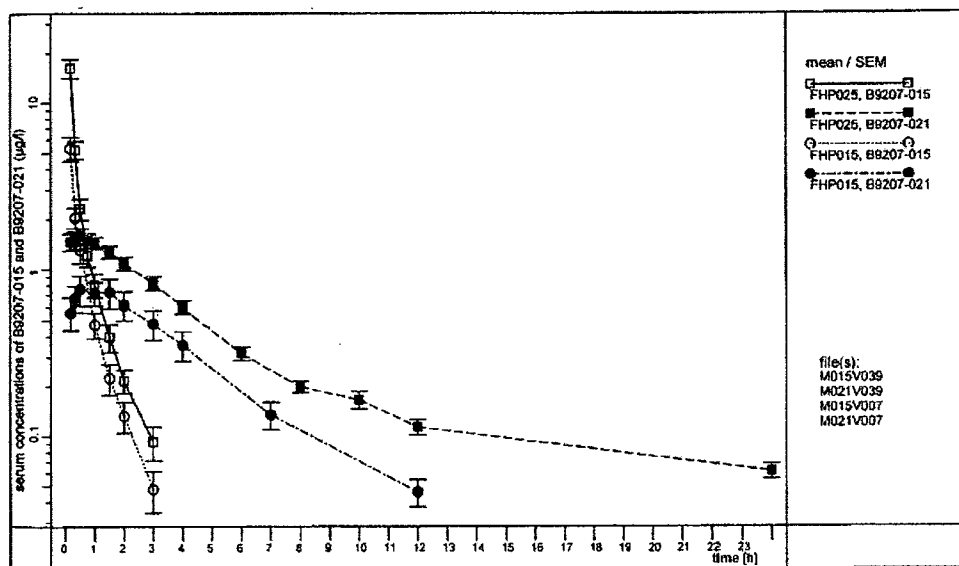


Figure 1. Serum concentrations of ciclosonide and its metabolite in healthy subjects (FHP025, elderly) and healthy subjects (FHP015, young and middle age) following single inhalative dose of 1600 mcg of ciclosonide.

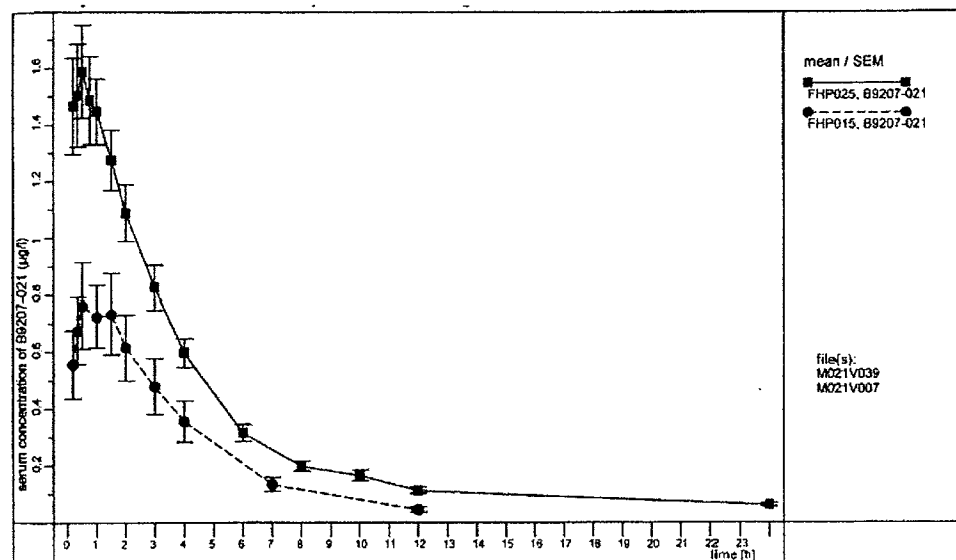


Figure 2. Serum concentrations of metabolite in healthy subjects (FHP025, elderly) and healthy subjects (FHP015, young and middle age) following single inhalative dose of 1600 mcg of ciclosonide.

Table 1. PK characteristics of B9207-015 and its major metabolite in healthy subjects and asthmatic patients following single administration of 1600 mcg B9207-015.

Treatments (Dose)	Ciclesonide		Active Principle R881			
	Geometric mean Pharmacokinetic Parameters (88% range)		Geometric mean Pharmacokinetic Parameters (88% range)			Mean (SEM)
	C _{max} µg/L	AUC µg*hr/L	C _{max} µg/L	AUC µg*hr/L	T _{1/2} h	T _{max} h
CIC 1600 µg (8x200) SD p inh	4.860 (2.615, 9.141)	1.92 (1.06, 3.48)	0.693 (0.374, 1.304)	3.17 (1.81, 5.53)	2.8 (2.5, 3.1)	1.04 (0.14)
CIC 1600 µg (8x200) SD p inh	14.742 (9.220, 23.571)	4.76 (2.93, 7.76)	1.667 (1.125, 2.368)	6.53 (4.75, 8.98)	3.02 (2.83, 3.22)	0.63 (0.14)

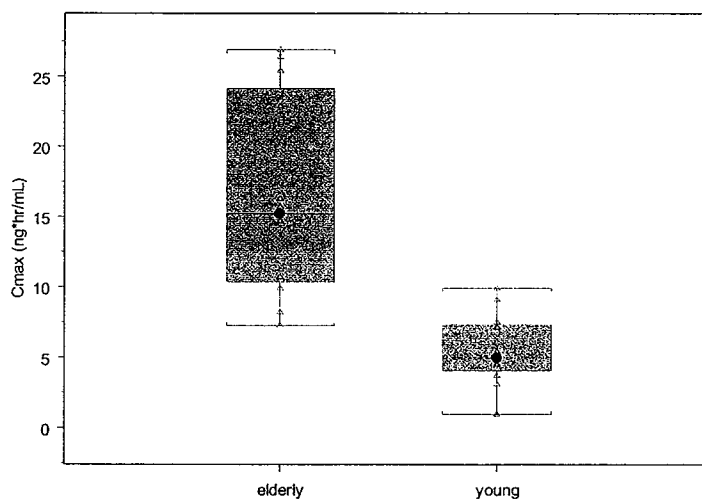


Figure 3. Individual C_{max} values for ciclesonide in healthy elderly volunteers and in young volunteers (data from study 253E/99) following single inhalative doses of 1600 mcg B907-015.

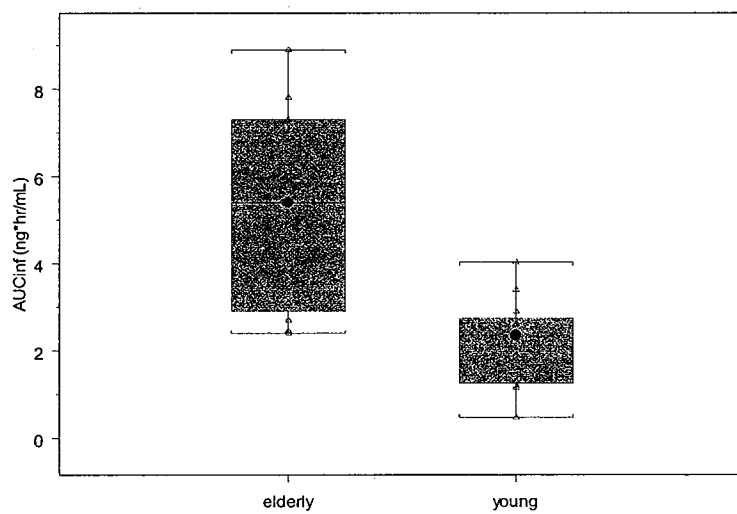


Figure 4. Individual AUC values for ciclesonide in healthy elderly volunteers and in young volunteers (data from study 253E/99) following single inhalative doses of 1600 mcg B907-015.

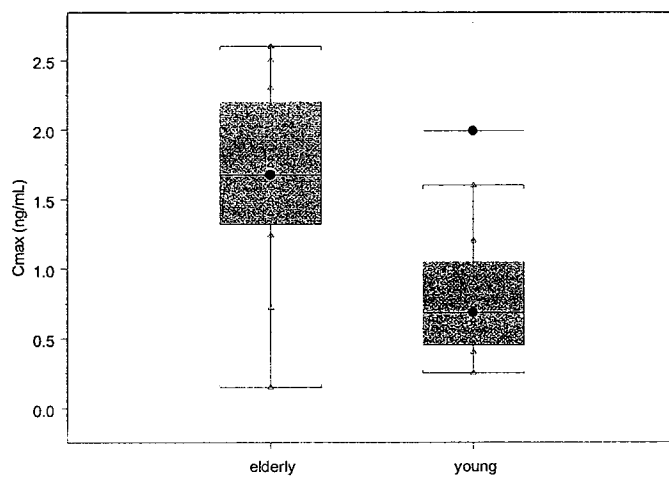


Figure 5. Individual Cmax values for RM1 in healthy elderly volunteers and in young volunteers (data from study 253E/99) following single inhalative doses of 1600 mcg B907-015.

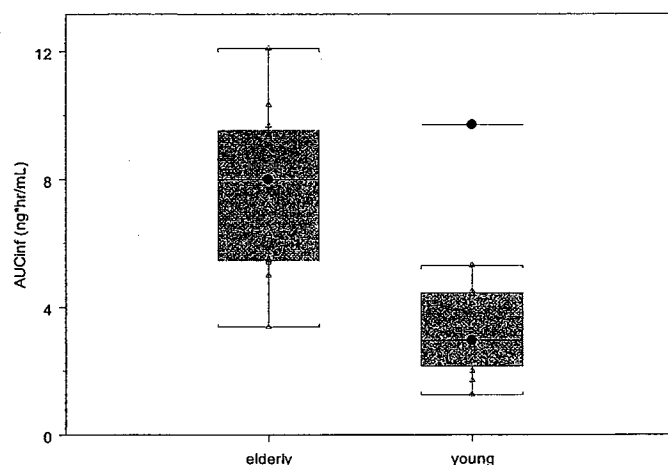


Figure 6. Individual AUCinf values for RM1 in healthy elderly volunteers and in young volunteers (data from study 253E/99) following single inhalative doses of 1600 mcg B907-015.

Table 3. Point estimates and 90% CI of the PK parameters of B9207-021 following single administration of 1600 mcg B9207-015 to healthy young t middle age subjects (reference) and to elderly subjects (test).

Pharmacokinetic Characteristics	Point estimate	90 % confidence interval
AUC _{0-24h} *	2.06	1.49, 2.85
C _{max}	2.39	1.67, 3.42
t _{1/2} *	1.08	1.02, 1.16
t _{1/2} (h) (additive statistical model)	-0.41	-0.76, -0.07

SUMMARY OF FINDINGS

- The mean C_{max} and AUC of ciclesonide increased in elderly subjects by 3-fold and 2.5 fold, respectively, as compared with that observed in young healthy controls.
- The mean C_{max} and AUC of RM1 increased in healthy elderly subjects by 2.4-fold and 2-fold respectively, as compared with the young healthy controls.

GENERAL COMMENTS

- This study was conducted as a single dose. However, because little accumulation of RM1 and parent (<50%) single dose PK may predict multiple dose PK.

CONCLUSION

- The systemic exposure of ciclesonide and its metabolite in healthy elderly subjects receiving a single dose of ciclesonide 1600 mcg increased more than 2-fold compared to that observed in healthy young subjects. However, these findings should be interpreted with caution since the observations are based on cross-study comparisons.
- Dose adjustment in the elderly based on these PK findings may not be necessary since the dose-response curve for ciclesonide was flat in the range of 100-800 µg/day and the safety profile of the drug for doses up to 3600 µg/day.

"Comparison of the serum pharmacokinetic characteristics of RM1 in Caucasians and Japanese subjects following a single inhaled dose of ciclesonide using the MDI device"

Clinical Study Report no.: 200/2000
 Development Phase of Study: Phase I
 Study Initiation Date: April 28, 1998
 Study Completion Date: Jul 23, 1998
 Chief Investigator: Thomas B. MD. Byk Gulden Pharmaceuticals

Objectives. To compare the serum pharmacokinetic characteristics of RM1 in Caucasians and Japanese subjects following a single inhaled dose of ciclesonide using the MDI device

Methods. A meta-analysis of data from healthy young Caucasian subjects was conducted using data from Study Numbers FHP009, FHP015, FHP018, FHP019, FHP023, FHP026 (128/2000, FHP027, as well as from healthy young Japanese subjects in Study Numbers BTR-15/001 and BTR-15/002. In order to obtain an independent database, individual subjects were included only once per study. Student t-test for inter-individual C_{max} and AUC₀₋ ratios (log-transformed) following a single dose of 800 µg ciclesonide or adjusted to a dose of 10 µg/kg.

Results. Statistical evaluation of the inter-individual C_{max} and AUC_{0-∞} values of RM1 in Caucasians and Japanese following single inhalation of 800 µg ciclesonide yielded point estimates (90% confidence interval) of 0.87 (0.77, 0.99) and 0.90 (0.77, 1.05), respectively. Equivalence of the pharmacokinetics in Japanese and Caucasians could be demonstrated for C_{max} and AUC_{0-∞} values, using the extended equivalence limits of 0.67 to 1.50. Moreover, the upper 90% confidence limits were also within the conventional equivalence range of oral administered drugs of 0.80 to 1.25.

Conclusions. The pharmacokinetics of the active metabolite RM1 in Japanese following single inhalations of ciclesonide *via* MDI were similar to Caucasians.

Table 1. Comparison of the PK in Japanese and in Caucasians healthy subjects

Subjects	HWP (Age: Mean, range)	Treatments (Dose)	Ciclesonide		RM1		
			Geometric mean Pharmacokinetic Parameters (68% range)		Geometric mean Pharmacokinetic Parameters (68% range)		
			C _{max} µg/L*	AUC µg*h/L*	C _{max} µg/L	AUC µg*h/L	Point of estimate
N= 66 Caucasian (Reference)	HV* {32, 24-43}	OC 1600 µg (8x200) SD push	-	-	0.699 {0.374, 1.304}	3.17 {1.81, 5.53}	C _{max} 0.90
N= 21 Japanese (Test)	HV {22, 20-37}	OC 1600 µg (8x200) SD push	-	-	1.667 {1.175, 2.365}	6.53 {4.75, 8.98}	AUC 0.60

"Repeated dose study of ciclesonide in healthy male"

Clinical Study Report no.:	BTR/15/002 (50-2000)
Development Phase of Study:	Phase I
Study Initiation Date:	April 28, 1998
Study Completion Date:	Jul 23, 1998
Chief Investigator:	Thomas B. MD. Byk Gulden Pharmaceuticals

Objectives

- to investigate the safety and pharmacokinetics after repeated inhalation of ciclesonide for 7 days in healthy, adult, Japanese male volunteers;
- to evaluate the safety parameters specific to inhaled steroids such as the effect on HPA-axis function and local safety by the pharynx test; and
- to evaluate drug accumulation in regard to pharmacokinetics.

Methods

The study was conducted in a single blind, repeated dose, placebo controlled, design with two groups of 12 healthy male volunteers. Two doses of ciclesonide (400 µg and 800 µg b.i.d.; 320 and 640 µg *ex-actuator*) were sequentially administered after confirmation of safety. In each dose group nine subjects received active medication and three subjects received placebo for 7 days. Safety was evaluated by the appearance of adverse events, clinically significant changes in the laboratory assessments, and serum and urine cortisol changes. Pharmacokinetic evaluations included the determination of C_{max}, T_{max}, AUC, and T_{1/2} values for ciclesonide and RM1.

Results

The absorption of the ciclesonide was very rapid with a T_{max} value of 10 minutes after inhalation. The mean T_{1/2} values for ciclesonide for the 400 µg bid group were 0.272 h on day 1, 0.3265 h on day 4 and 0.329 h on day 7, and the for 800 µg bid group were 0.304 h on Day 1, 0.853 h on day 4 and 0.627 h on Day 7, indicating rapid elimination. The serum ciclesonide and RM1 concentrations showed that the AUC_{0-∞} and C_{max} increased depending on dose, and that there was no significant change found on each treatment day in each dose group (Table 1). The AUC_{0-t} and C_{max} values for RM1 were approximately identical on Days 4 and 7, indicating that a steady-state was already achieved at Day 4. Further, no appreciable accumulation was observed upon repeated dosing.

No abnormalities or variations were observed in serum cortisol at any dosage. In the group receiving 400 µg bid, the mean values on Day 7 were significantly lower than on Day 1, but this decrease was considered to be within physiological variations and not likely to be clinically important. No significant differences between Day 1 and Day 7 serum cortisol were observed in the 800 µg bid group. No abnormalities in urinary cortisol were observed. Comparison of the 24 hour urinary cortisol excretion between administration Day 1 and administration Day 6 showed no significant decrease due to study drug administration at any dose.

Table 1: Pharmacokinetic characteristics of RM1 after inhaled administration of 400 µg and 800 µg (320 and 640 µg *ex-actuator*) b. i. d. in 9 healthy Japanese volunteers

Parameter	400 µg (320 µg <i>ex-actuator</i>)			800 µg (640 µg <i>ex-actuator</i>)		
	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
AUC _{0-t} (ng.h/mL)	1.230 ± 0.742	2.021 ± 1.153	2.026 ± 0.979	3.178 ± 0.604	4.561 ± 0.850	4.529 ± 0.677
C _{max} (ng/mL)	0.281 ± 0.147	0.395 ± 0.209	0.390 ± 0.188	0.704 ± 0.143	0.854 ± 0.133	0.830 ± 0.085
T _{max} (h)	0.833 ± 0.595	0.833 ± 0.595	0.833 ± 0.595	0.852 ± 0.757	0.926 ± 0.541	1.333 ± 0.500
T _s (h)	3.131 ± 0.483	3.410 ± 0.345	3.567 ± 0.226	2.967 ± 0.266	3.694 ± 0.615	3.645 ± 0.462

*t: time point of last quantifiable serum concentration

Conclusions

- The C_{max} and AUC_{0-t} values for RM1 increased in proportion to dose.
- There was no significant change found on each treatment day in each group.
- No clinically significant decrease in serum cortisol, urinary cortisol excretion, or suppression of HPA-axis function was observed for doses up to 800 µg bid in Japanese subjects.

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"Single dose study of ciclesonide in healthy male volunteers - Phase I study"

Clinical Study Report no.: 49/2000 (BRT-15/001)
Development Phase of Study: Phase I
Study Completion Date: 26/02/2000
Chief Investigator: _____

b(4)

Objectives

- Investigation of the safety and the pharmacokinetics after single administration of ciclesonide (Teijin substance code BTR-15) in healthy adult male volunteers.

Methodology

This was an open, placebo-controlled single dose study with rising dose level. 48 healthy Japanese volunteers (males, age 20-37 years) received ciclesonide and placebo. On the first day all subjects received placebo. On the second day all subjects received ciclesonide: 12 subjects received 200µg, 12 subjects received 400µg, 12 subjects received 800µg and 12 subjects received 1600µg ciclesonide via the MDI as a single dose. Criteria for evaluation were the pharmacokinetic characteristics (AUC(0,Inf), AUC(0,t), t_{1/2}, C_{max}, t_{max}) of the metabolite B9207-021 and ciclesonide and the safety of ciclesonide assessed by adverse events, laboratory parameters (including urine and serum cortisol and osteocalcine) ECG, vital signs and physical examination.

Study Subjects

Total: 48 for placebo and ciclesonide; 12 for each dose level.

Test product: ciclesonide (Teijin substance code BTR-15, corresponds to Byk Gulden substance code B9207-015)

Dose: 200 µg – 1600 µg, single dose

Mode of administration: Inhalation via a metered dose inhaler

Batch No.: BTRX920A

Duration of treatment: single dose

Criteria for evaluation:

Pharmacokinetic characteristics (AUC(0, inf), AUC(0,t), t_{1/2}, C_{max}, t_{max}) of the metabolite B9207-021 and ciclesonide and the safety of ciclesonide

Statistical methods:

Mainly descriptive

SUMMARY - CONCLUSIONS

In the table below the pharmacokinetic characteristics of the active principle of ciclesonide B9207-021 are provided. 46 subjects were evaluable for the pharmacokinetic characterization.

Dose	200µg (n=11)		400µg (n=11)		800µg (n=12)		1600µg (n=12)	
	mean	SD	mean	SD	mean	SD	mean	SD
AUC _(0,inf) [µg×h/l]	0.669	0.213	1.485	0.650	2.542	0.523	4.429	1.956
t _{1/2} [h]	2.63	1.07	2.84	0.80	3.04	0.43	2.91	0.82
C _{max} [µg/l]	0.177	0.063	0.384	0.106	0.638	0.310	1.149	0.398
t _{max} [h]	0.53	0.58	0.38	0.25	0.54	0.62	0.63	0.68

The t_{max} of ciclesonide (B9207-015) was after 10 minutes (first time point of pharmacokinetic analysis). C_{max} and AUC values of B9207-015 increased approximately in proportion with the dose. Pharmacokinetic characteristics of B9207-021 increased in proportion with the dose.

Safety results

Serum cortisol and urine cortisol were not significantly decreased at any dose. Even the mean 24h-serum cortisol profile of the 1600µg dose seemed to be without an apparent difference to the placebo profile of the volunteers. There was one adverse event (muscle pain) which was considered to be related to the administration of ciclesonide. The other clinical safety parameters did not reveal any relevant findings.

APPEARS THIS WAY ON ORIGINAL

"Influence of liver cirrhosis on the pharmacokinetics of the active metabolite B9207-021 of ciclesonide in comparison to a control group of healthy subjects"

Clinical Study Report no.: 210/2000
Protocol No.: BY9010FHP018
Development Phase of Study: Phase I
Study Initiation Date: Aug 02, 1999
Study Completion Date: Nov 11, 1999
Chief Investigator: Dr. Th. Bethke
Wilhelm Wurst, Byk Gulden Pharmaceuticals, Constance
(Germany)

Objectives

- To investigate the pharmacokinetics of the pharmacologically active metabolite 69207-021 of ciclesonide in patients with impaired hepatic function due to liver cirrhosis in comparison to healthy subjects after single dose inhalation of 1600 µg ciclesonide.
- To determine the pharmacokinetic characteristics of ciclesonide (B9207-015). Further secondary objectives were safety and tolerability.

Study Design

This was an open label 3-parallel-group study comparison in 24 subjects with different degrees of liver impairment (8 healthy, 8 with severe hepatic impairment and 8 with moderate hepatic impairment). The 8 healthy subjects reflected the gender distribution of the patients; age and weight were comparable to that of the patients, i.e. within the respective ranges observed for the patients.

The following treatments were administered:

Test product: ciclesonide
Dose: 1600 µg (8 puffs)
Mode of administration: metered dose inhaler; 8 puffs containing 200 µg each
Batch no.: 034498

Study Subjects

A total of 24 male/female subjects were enrolled and 24 subjects completed the study.

PK Measurements

Blood samples were taken at predose and at 10 min, 20 min, 0.5h, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hrs after study drug administration in all treatment groups for metabolite and if possible ciclesonide determination.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 in serum was performed using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for B9207-015 and B9207-021, respectively.

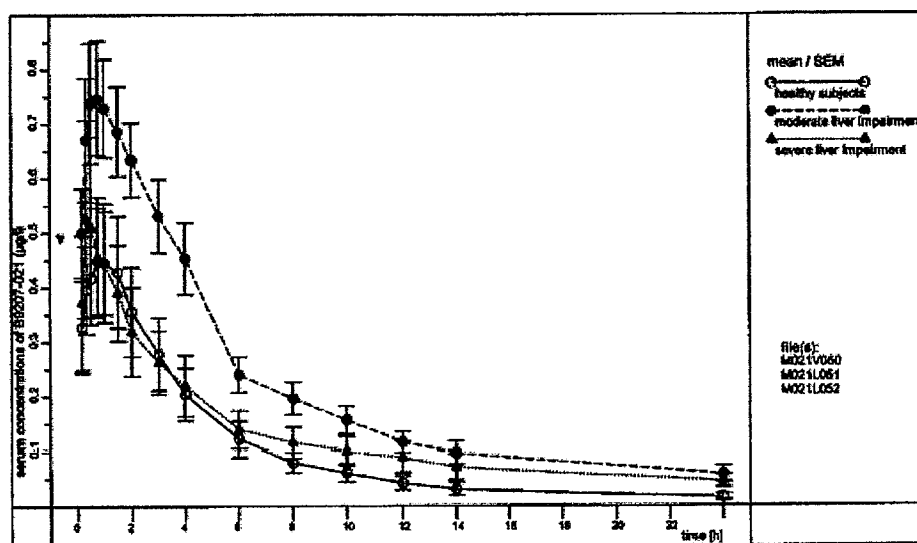


Figure 1. Mean serum concentrations of B9207-021 in healthy volunteers and is patients with moderate and severe hepatic impairment following single inhalative doses of 1600 mcg B907-015 using 8 puffs of 200 mcg.

Table 1. Mean (SEM) PK characteristics of B9207-015 in healthy volunteers and is patients with moderate and severe hepatic impairment following single inhalative doses of 1600 mcg B907-015.

Subjects (M/F)	HVIP (Age: Mean, range)	Treatments (Dose)	Ciclesonide		Active Principle RM1			
			Geometric mean Pharmacokinetic Parameters (68% range)		Geometric mean Pharmacokinetic Parameters (68% range)			Mean (SEM)
			Cmax µg/L	AUC µg·h/L	Cmax µg/L	AUC µg·h/L	T1/2 h	Tmax h
6 (7M/1F)	HV (49, 46-52)	CIC 1600 µg {Bx200} SD p.r.h	2.612 {0.823, 9.606}	0.93 {0.27, 3.16}	0.399 {0.157, 1.012}	1.95 {0.83, 4.74}	6.1 {5.2, 7.1}	0.75 {0.15}
6 (6M/0F)	P, moderate (48, 43-53)	CIC 1600 µg {Bx200} SD p.r.h	4.567 {2.026, 11.633}	2.34 {1.23, 4.45}	0.784 {0.525, 1.171}	5.31 {3.33, 8.57}	8.35 {6.43, 10.64}	0.92 {0.20}
6 (5M/1F)	P, severe (52, 40-60)	CIC 1600 µg {Bx200} SD p.r.h	1.599 {0.474, 5.355}	0.54 {0.16, 1.84}	0.552 {0.304, 1.005}	3.45 {1.95, 6.14}	10.70 {8.65, 12.95}	0.52 {0.10}

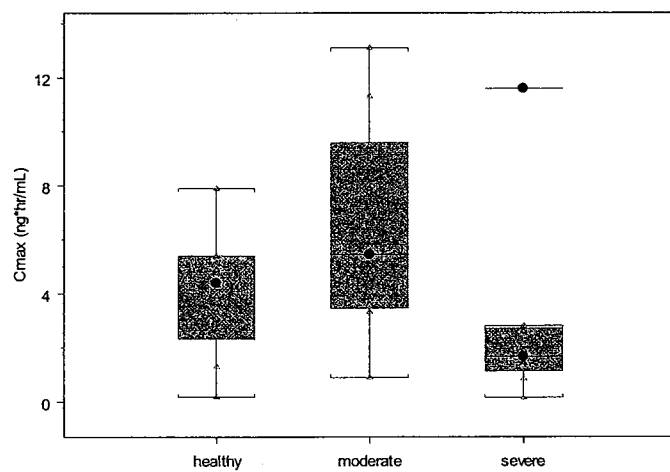


Figure 2. Individual Cmax values for ciclesonide in healthy volunteers and is patients with moderate and severe hepatic impairment following single inhalative doses of 1600 mcg B907-015.

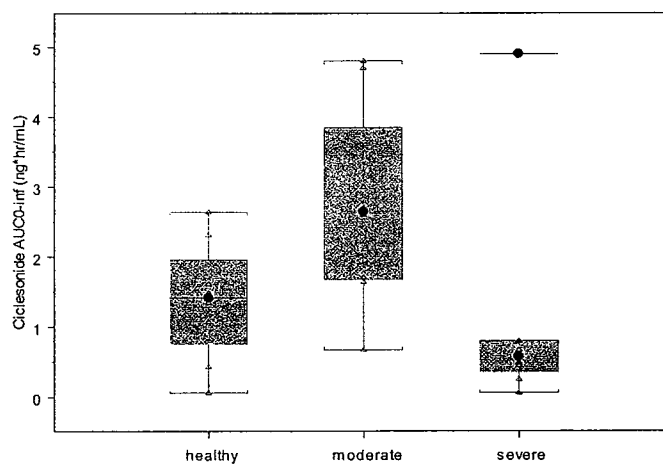


Figure 3. Individual AUCinf values for ciclesonide in healthy volunteers and is patients with moderate and severe hepatic impairment following single inhalative doses of 1600 mcg B907-015.

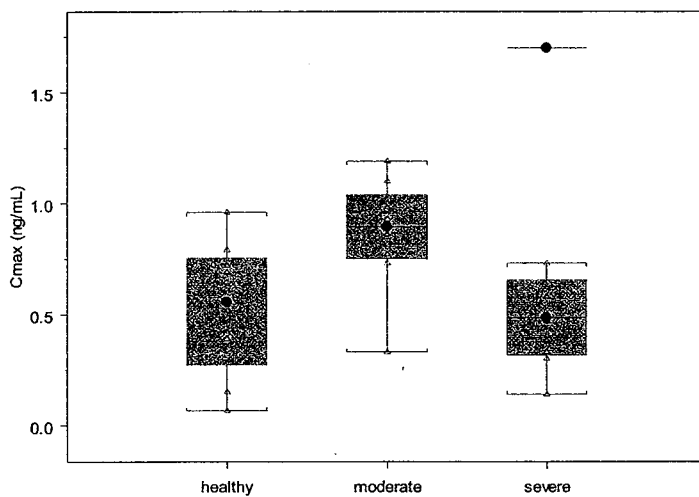


Figure 4. Individual Cmax values for the metabolite RM1 in healthy volunteers and is patients with moderate and severe hepatic impairment following single inhalative doses of 1600 mcg B907-015.

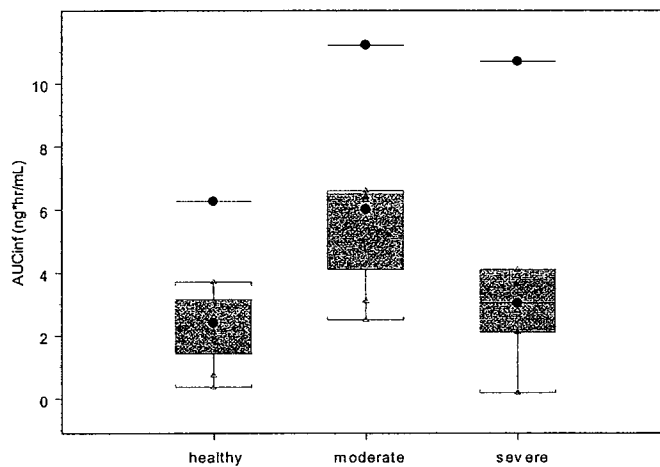


Figure 4. Individual AUCinf values for the metabolite RM1 in healthy volunteers and is patients with moderate and severe hepatic impairment following single inhalative doses of 1600 mcg B907-015.

Table 2. Point estimates and 90% CI of the ratios of PK parameters of B9207-021 following single administration of 1600 mcg B9207-015 to patients with various degrees of hepatic impairment.

Pharmacokinetic Characteristics	Liver impairment / healthy subjects	
	Moderate hepatic impairment (N=8)	Severe hepatic impairment (N=7*)
AUC _(0-∞)	2.73 (1.45, 5.12)	1.77 (0.88, 3.57)
C _{max} / AUC _(0-∞)	0.73 (0.59, 0.92)	0.79 (0.63, 1.00)
t _{1/2}	1.37 (1.14, 1.66)	1.76 (1.50, 2.06)
t _{max} (additive statistical model)		
t _{max} [h]	0.17 (-0.28, 0.61)	-0.30 (-0.62, 0.02)

* N=7, excluding subject No. 8

SUMMARY OF FINDINGS

- The AUCs of the active metabolite B9207-021 increased in patients with moderate and severe liver impairment by factors of 2.73 and 1.77, respectively, as compared with the healthy controls.
- The C_{max} of the active metabolite B9207-021 increased in patients with moderate and severe liver impairment by factors of 1.96 and 1.38, respectively, as compared with the healthy controls.
- The t_{1/2} of the active metabolite B9207-021 increased in patients with moderate and severe liver impairment by 2.3h and 4.6h, respectively, as compared with the healthy controls.
- The C_{max} of ciclesonide increased 1.76-fold in patients with moderate liver impairment and decreased by 43% in severe liver patients as compared with the healthy controls.
- The AUC_{inf} of ciclesonide increased 2.52-fold in patients with moderate liver impairment and decreased by 42% in severe liver patients as compared with the healthy controls.

GENERAL COMMENTS

- This study was conducted as a single dose. However, because little accumulation of the metabolite and drug product occurs (<50%) single dose PK may predict multiple dose PK.
- This data should be interpreted with caution since there is inconsistency in the results observed in patients with moderate and severe HI.

CONCLUSION

Dose adjustment may not be needed patients with moderate and severe HI.

"Investigation of a possible pharmacokinetic interaction between ciclesonide and erythromycin in healthy subjects"

Clinical Study Report no.: 304/98
Protocol No.: BY9010/FHP019
Development Phase of Study: Phase I
Study Initiation Date: April 28, 1998
Study Completion Date: Jul 23, 1998
Chief Investigator: Thomas B. MD.
Byk Gulden Pharmaceuticals

Objectives

Primary: to investigate the influence of concomitant ciclesonide inhalation on the pharmacokinetics of orally administered erythromycin.

Secondary: to determine the influence of concomitant dosing of erythromycin on the pharmacokinetics of the active metabolite B9207-021; safety and tolerability of concomitant administration of ciclesonide and erythromycin

Study Design

The study was conducted according to a randomized, open, 3-period change-over design. In one study period, the subjects received ciclesonide alone and in another study period, the subjects received erythromycin alone. In a third study period, both drugs were given together. Repeated measurements of pharmacokinetic and safety parameters were performed in each study period. Subjects were randomized to the following treatments:

Treatment A: 800 µg ciclesonide inhaled via MDI (batch no. 015298)+ 500 mg erythromycin p.o. (Test)

Treatment B: 500 mg erythromycin p.o. (film tablets batch no. 017298) (Reference 1)

Treatment C: 800 µg ciclesonide inhaled via MDI (Reference 2)

The duration of the wash out period between two subsequent study periods was at least 7 days, but not more than two weeks.

Study Subjects

In total 18 male/female subjects 18-45 years were screened and 17 completed the study. The median age of the study participants was 31 years; their median weight was 66 kg, their median height was 171 cm, and their median Broca Index was 91% .

PK Measurements

Blood samples were taken at predose and at 0.25h, 0.5h, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12hrs after study drug administration.

Analytical Method

Determination of ciclesonide and its metabolite B9207-021 in serum was performed using a validated LC-MS/MS assay. The lower limit of quantitation were 25 and 10 pg/mL for B9207-015 and B9207-021, respectively.

Data Analysis

PK Analysis

Relevant PK parameters were calculated using non-compartmental methods.

Statistical Analysis

Point estimate and 90%-confidence limits were given for the ratio of the population medians for erythromycin after administration of Test and Reference 1 using a multiplicative model and a parametric analysis.

Equivalence of the pharmacokinetic characteristics of erythromycin after administration of Test and Reference 1 was concluded when the 90%-confidence interval for the Test/Reference ratio was entirely within the predefined equivalence range of 0.67 to 1.50. According to the sponsor, this extended range was based on the safety and tolerability of erythromycin.

RESULTS

Analytical Method

In-Study Validation

The method had a lower limit of quantification (LLOQ) of 25 ng/mL for ciclesonide and its metabolite Using 1 ml human serum. Intra-batch precision values, based upon coefficients of variation of quality control samples (60, 800, 1600 pg/mL) were less than or equal to % for B9207-015 and % for B9207-021. Intra-batch accuracy of quality control samples ranged from % for B9207-015 from % for 99207-021.

n(4)

Pharmacokinetic Results

B9207-015 (parent compound ciclesonide) was detected in most serum samples up to 4 hours post dose. Mean (SEM) serum concentration vs. time profiles of erythromycin in healthy subjects following a single oral administration of erythromycin at a dose level of 500 mg with and without ciclesonide 800 µg are shown in Figure 1. Figure 2 shows the concentration-time profile of ciclesonide following single inhalative dose of 800 µg with and without erythromycin. Likewise Figure 3 shows the same for the metabolite. Table 1 summarizes the PK characteristics of B9207-015 and its metabolite in the presence and absence of erythromycin and the PK of erythromycin with and without ciclesonide following single administration of the treatments. Figures 4 and 5 show the individual C_{max} and AUC_{inf} of ciclesonide following administration of the treatments. Likewise, Figures 6 and 7 show it for the metabolite. Table 2 summarizes the point estimates and 90% CI of the ratios of PK parameters of B9207-021 following single administration of 800 mcg B9207-015 with and without erythromycin.

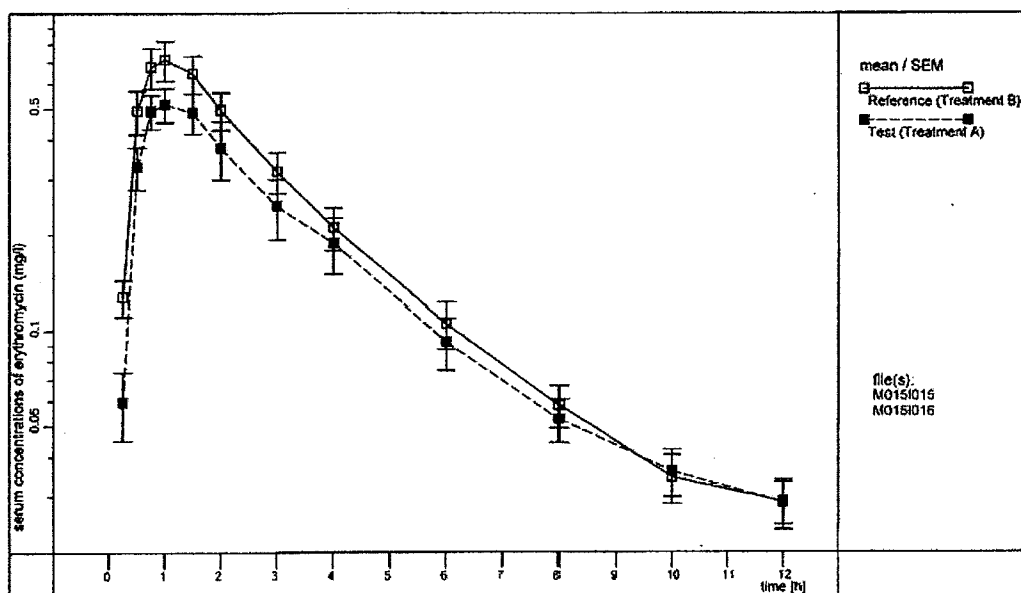


Figure 1. Serum concentrations of erythromycin in 18 healthy subjects following single oral dose of 500 mg erythromycin with and without ciclesonide as a single inhalative dose of 800 mcg.

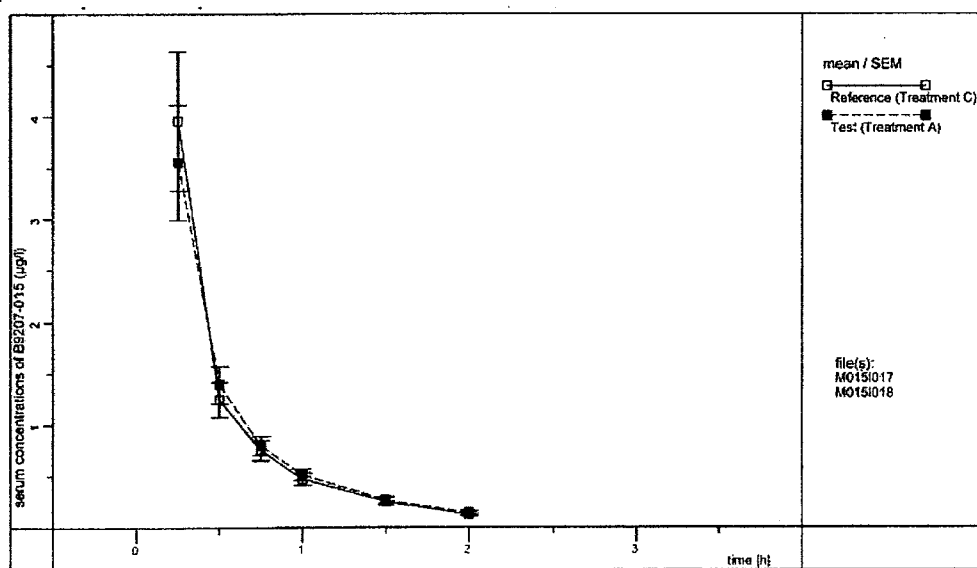


Figure 2. Serum concentrations of ciclesonide in 17 healthy subjects following single oral dose of 800 mcg ciclesonide with and without a single oral dose of erythromycin 500 mg.

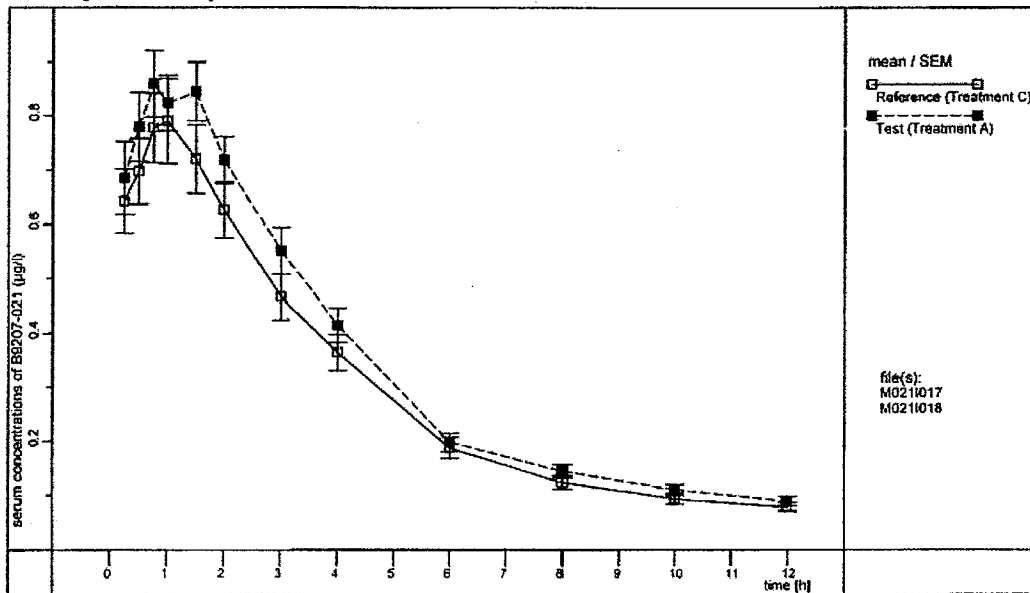


Figure 3. Serum concentrations of RM1 in 17 healthy subjects following single oral dose of 800 mcg ciclesonide with and without a single oral dose of erythromycin 500 mg.

Table 1. PK characteristics of B9207-015 and its major metabolite in healthy subjects following single administration of the treatments

	Ciclesonide		RM1		*Erythromycin	
	Without ERY	With ERY	Without ERY	With ERY	Without CIC	With CIC
Cmax (µg/L)	3.74 (2.57)	3.1 (1.97)	0.892 (0.288)	0.936 (0.249)	0.801 (0.42)	0.61 (0.337)
AUCinf (µg*hr/L)	1.84 (1.0)	1.75 (0.92)	3.91 (1.25)	4.39 (1.17)	2.35 (1.37)	1.86 (1.2)
Tmax (hrs)	0.28 (0.08)	0.31 (0.11)	0.82 (0.46)	1.16 (0.7)	1.03 (0.37)	1.04 (0.4)
T1/2 (hrs)	0.5 (0.1)	0.52 (0.08)	3.54 (0.74)	3.61 (0.44)	1.63	2.67

*units are mg/L

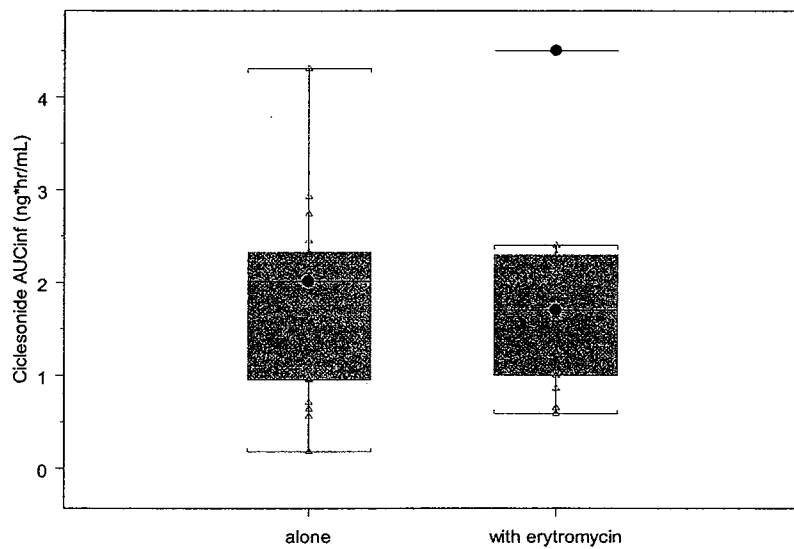


Figure 4. Individual AUCinf values for ciclesonide in healthy volunteers following single administration of ciclesonide with and without erythromycin 500 mg

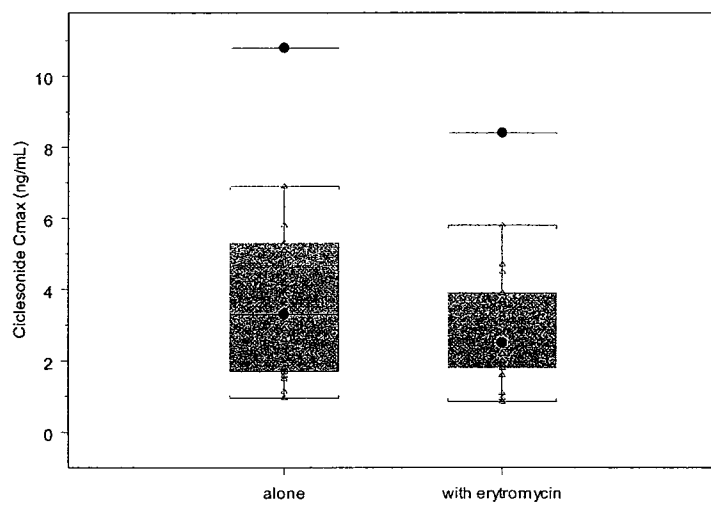


Figure 5. Individual Cmax values for ciclesonide in healthy volunteers following single administration of ciclesonide with and without erythromycin 500 mg

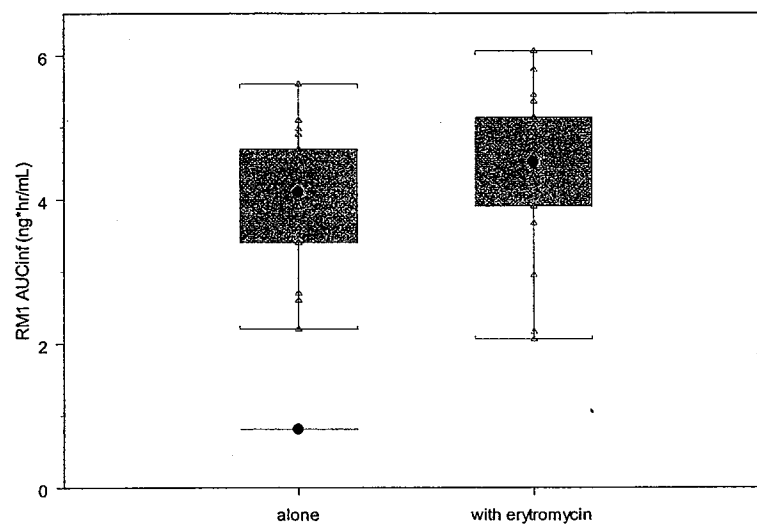


Figure 6. Individual AUCinf values for RM1 in healthy volunteers following single administration of ciclesonide with and without erythromycin 500 mg

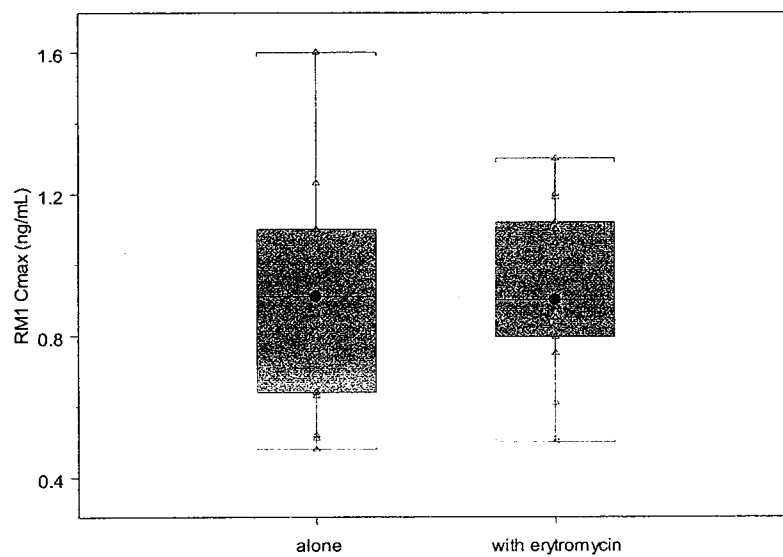


Figure 7. Individual Cmax values for RM1 in healthy volunteers following single administration of ciclesonide with and without erythromycin 500 mg.

Table 2. Geometric means, point estimates and 90% CI of the PK parameters of B9207-021 following single administration of 800 mcg B9207-015 to healthy subjects with and without erythromycin 500 mg.

Pharmacokinetic characteristic	Geometric mean (68%-range)		Point estimate (90%-confidence interval) of ratio Test/Reference
	without erythromycin (Reference 2)	with erythromycin (Test)	
AUC _(0,inf) [µg*h/l]	3.66 (2.36, 5.68)	4.24 (3.18, 5.65)	1.16 (1.03-1.30)
C _{max} [µg/l]	0.858 (0.648, 1.134)	0.909 (0.700, 1.181)	1.06 (0.98-1.15)
t _½ [h]	3.5 (2.7, 4.4)	3.6 (3.15, 4.10)	1.04 (0.96-1.13)

SUMMARY OF FINDINGS

- The arithmetic mean C_{max} and AUC of ciclesonide decreased in the presence of erythromycin 500 mg by 17% and 5%, respectively following single administration of the treatments.
- The arithmetic mean C_{max} and AUC of the active metabolite B9207-021 increased in the presence of erythromycin 500 mg by 5 % and 12%, respectively following single administration of the treatments.
- The arithmetic mean C_{max} and AUC of erythromycin decreased in the presence of ciclesonide by 24 % and 21%, respectively following single administration of the treatments.

GENERAL COMMENTS

- This study was conducted as a single dose. However, because little accumulation of the metabolite and drug product occurs (<50%) single dose PK may predict multiple dose PK.
- In the assessment of this DDI study with erythromycin the sponsor did not use the maximum planned dose (1240 mcg) of ciclesonide to be administered to patients with asthma

CONCLUSION

- The systemic exposure of ciclesonide and its metabolite in healthy subjects receiving a single dose of ciclesonide 800 mcg was not statistically significantly altered by the its coadministration with erythromycin 500 mg oral single dose.
- The systemic exposure of erythromycin in healthy subjects receiving a single dose of 500 mg was not statistically significantly altered by its coadministration with ciclesonide 800 mcg single dose.