10. CLINICAL EXPERIENCE

10.1. A PLACEBO-CONTROLLED STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PRELIMINARY PHARMACOKINETICS OF PL 14736 IN HEALTHY MALE VOLUNTEERS

10.1.1 Study design

The explorative, single-blind, placebo-controlled study was conducted to assess the safety, tolerability and preliminary pharmacokinetics of PL 14736 in thirty-two healthy male volunteers (A placebo controlled study to investigate the safety, tolerability and preliminary pharmacokinetics of PL 14736 in healthy male subjects. FOCUS Clinical Drug Development **GmbH;** FOCUS Report 20PV0673; 2001.). PL 14736 was administered in a form of rectal solution given through enema container at four different dose levels: 0.25 mg/kg, 0.5 mg/kg, 1 mg/kg and 2 mg/kg. Group one received 0.25 mg/kg, group two 0.5 mg/kg, group three 1 mg/kg and group four 2 mg/kg of PL 14736. Each group consisted of six subjects treated with PL 14736 and two subjects treated with isotonic NaCI solution (0.9%) that served as placebo, the treatments being selected in a randomised manner. Each volunteer received single PL 14736 or placebo dose followed by at least one-week wash out period and additional seven PL 14736 or placebo doses given once daily through 7 consecutive days.

10.1.2 Ethical considerations

The study was conducted in accordance with the local Regulatory Authority and Ethics Committee. The information on the study procedures was provided to the volunteers in their mother language and the informed consent was obtained from each volunteer at the start of the study.

The study was conducted in accordance with the German Drug Law (§40/41) and the European Directive 91/507/EEC and the following ICH GCP guidelines (CMP/ICH/135/95) which are based on the ethical principles laid down in then last revised version of the Declaration of the Helsinki (Somerset West, South Africa, 1996).

10.1.3 Safety

Safety was assessed by measuring blood pressure, pulse rate, ECG, biochemistry, haematology, urinalysis as well as by monitoring of adverse events. Blood pressure and pulse rate were measured pre-study, pre-dose and additionally on study days 2, 15, 16 and at the follow-up examination. ECG-recording was performed pre-study, pre-dose and additionally on study day 1 (1+ 4 h), on study days 2, 15, 16 and at the follow-up examination. Measurements of laboratory parameters were performed at the following time points: pre-study, pre-dose and on study days 2 (excluding urine), 15, 16 and at the follow-up examination. Adverse events questioning was performed pre-study, pre-dose and 1 h, 2 h, 4 h, 8 and 12 h post dose on each dosing day and additionally on study days 2, 15, 16 and at the follow-up examination. Safety parameters were summarised descriptively by frequency tables or summary statistics including n (number of observation), mean, standard deviation, minimum, median and maximum.

Single and repeated rectal doses of 0.25 to 2 mg/kg PL 14736 were very well tolerated. Headache and flatulence were the most frequently reported adverse events after PL 14736 as well as after placebo dosing. Laboratory monitoring, vital signs measurements, ECG and bowel evacuation recordings revealed no differences between any of the dosing steps of PL 14736 and placebo.

10.1.4 Pharmacokinetics

5 ml blood samples for pharmacokinetic measurements were taken pre-dose and 5 min, 10 min, 20 min, 40 min, 1 h, 1.5 h, 2 h, 4 h, 8 h and 12 h after the first dose. The same amount of blood was also taken at pre-dose, 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, and 12 h after the first and the last dosing in the repeated dose phase, and at pre-dose and after 12 h on the other dosing days in the repeated dose phase. Additional samples were taken on days 15, 16 and at the follow-up examination.

Where appropriate, the following pharmacokinetic parameters were to be calculated: $AUC_{(0-t)}$,

$AUC_{(0-inf)}$, C_{max}, t_{max}, and $t_{1/2}$ for plasma after the single dose

regimen and $AUC_{(0-\tau)}$, C_{maxSS}
T_{maxSS}, C_{min}, trough-value and $t_{1/2}$ for the repeated dose regimen.

For most subjects included in this study at most time points plasma concentrations of PL 14736 were below the lower limit of quantification (LLQ 1.0 ng/ml). Sporadic positive concentrations were seen in few samples from several subjects but the measured plasma concentrations showed no clear pharmacokinetic profile for PL 14736. Since no PL 14736 concentrations in majority of analysed human plasma samples could be detected, these data were insufficient for the calculation of reliable pharmacokinetic parameters. These results indicate that very law amounts of PL 14736 are actually absorbed into the systemic circulation when the compound is administered intracolonicaly, using the above mentioned enema formulation.

10.1.5 Conclusion

Single and repeated intracolonic doses of PL 14736 to healthy male volunteers were very well tolerated. No difference to placebo dosing was observed for any of the safety parameters measured. Owing to the fact that most PL 14736 plasma concentration profiles were below the assay LLQ at all time points, it seems that very little PL 14736 is absorbed into the systemic circulation following rectal administration.

Reference:

1. A placebo controlled study to investigate the safety, tolerability and preliminary pharmacokinetics of PL 14736 in healthy male subjects. FOCUS Clinical Drug Development **GmbH**; FOCUS Report 20PV0673; 2001.

10.2. FOCUS - CLINICAL DRUG DEVELOPMENT GMBH STUDY NUMBER 21PV0786 FINAL VERSION 8TH FEBRUARY 2005

The primary aim of the study was to evaluate the effect of repeated rectal doses of 80 mg PL 14736 over 14 days in patients with distal ulcerative colitis. The effect was evaluated with regard to the overall clinical and histological symptoms by means of the DAI. This disease activity index was developed in cooperation with gastroenterologists, pathologists and specialists for laboratory medicine. It offered a quantitative evaluation of all categories, which characterize this disease entity.

Repeated rectal administration of 80 mg PL 14736 for 14 days resulted in a statistically significant improvement of the symptoms of the ulcerative colitis (DAI score), whereas placebo showed smaller and not significant improvement.

10.2.1. SAFETY EVALUATION

10.1.1. EXTENT OF EXPOSURE

In total 53 subjects were included in the study, 46 subjects completed the study. Twenty-one (21) subjects received 14 rectal doses of 80 mg PL 14736 and 24 subjects received 14 rectal placebo doses. One subject completed the study (ID 21), but received only 7 rectal doses of PL 14736 during the first week of treatment and then continued with 7 rectal placebo doses.

Seven subjects stopped the study prematurely. Subject 48 (PL 14736) was lost to follow-up after receiving the first dose, subject 41 (placebo) did not want to take the last dose.

Five subjects withdrew from the study due to adverse events:
PL 14736:
subject 17 received 3 doses and withdrew due to deterioration of the disease; subject 25 received 9 doses and withdrew due to fever and cold;
subject 30 received 7 doses and withdrew due to progression of the disease.

Placebo:

subject 11 received 11 doses and withdrew due to worsening of the symptoms subject 29 received 7 doses and withdrew from the study due to progression of the disease.

10.2.1.2 ADVERSE EVENTS (AE)

10.2.1.3 Brief summary of adverse events

During the study 10 (38.5%) and 15 (55.6%) subjects reported at least one adverse event in the PL 14736 and the placebo group, respectively. Four subjects of each treatment group reported drug related adverse events. The most frequently reported adverse events were gastro-intestinal system disorders: 9 events were reported by 7 subjects (26.9%) in the PL 14736 group and 8 events reported by 8 subjects in the placebo group (29.6%). The majority of adverse events was of mild to moderate intensity. The frequency of severe AEs was very low with 3 events in 2 patients of the PL 14736 group and 3 events in 1 patient of the placebo group. None of the severe adverse events was judged to be drug related. An overview is given in the next Table.

Table Overview of Adverse Events (Safety Population)

	PL 14736 (N=26)	Placebo (N=27)	Total (N=53)
Patients with any AEs	10 (38.5%)	15 (55.6%)	25 (47.2%)
Patients without any AEs	16 (61.5%)	12 (44.4%)	28 (52.8%)
Patients with SAEs	0 (0.0%)	2 (7.4%)	2 (3.8%)
Patients with drug related	4(15.4%)	4(14.8%)	8 (15.1%)
Patients with baseline AEs	1 (3.8%)	2 (7.4%)	3 (5.7%)
Patients with on-treatment	10 (38.5%)	12 (44.4%)	22 (41.5%)
Patients with follow-up AEs	1 (3.8%)	4 (14.8%)	5 (9.4%)

Two serious adverse events occurred during the study: subject 39 (placebo) had a circulatory collapse during an influenzal infection which required hospitalization. The patient recovered 6 days later. Subject 52 had a mesenterial embolism 17 days after the last placebo treatment. Although the patient had 2 operations he died 11 days later. For both serious adverse events the investigator saw no relation to the study drug.

10.2.1.4. Analysis of adverse events

There were 3 subjects with baseline complaints: subject 2 had a mild high CKactivity, subject 6 had a severe intermittent hyperglucosaemia and subject 54 had mild intermittent joint pain. All events were ongoing during the treatment phase.

In the following table the on-treatment adverse events are summarized. During the treatment phase (until day 15) totally 17 adverse events were reported by 10 subjects (38.5%) of the PL 14736 group and 23 adverse events were reported by 12 subjects (44.4%) of the placebo group

Table Frequency of On-Treatment Adverse Events (Safety Population)

Body System	Preferred Term	PL 14736 (N = 26)	Placebo (N = 27)	Total (N = 53)
Musculoskeletal system disorders	Joint pain	**1 (1, 3.8%)**	0 (0, 0.0%)	1(1, 1.9%)
	Muscle cramp	1 (1, 3.8%)	0 (0, 0.0%)	1(1, 1.9%)
	Muscle pain	1 (1, 3.8%)	0 (0, 0.0%)	1(1, 1.9%)
	Total	3 (3, 11.5%)	0 (0, 0.0%)	3 (3, 5.7%)
Central & peripheral nervous system disorders	Headache	0 (0, 0.0%)	**2 (2, 7.4%)***	2 (2, 3.8%)
	Vertigo	0 (0, 0.0%)	1 (1, 3.7%)	1 (1, 1.9%)
	Total	0 (0, 0.0%)	3 (3, 11.1%)	3 (3, 5.7%)
Gastro-intestinal system disorders	Abdominal pain	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Anus disorder	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Blood in stool	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Colitis ulcerative aggravated	1 (1, 3.8%)	3(3, 11.1%)	4(4, 7.5%)
	Diarrhea bloody	0 (0, 0.0%)	1 (1, 3.7%)	1 (1, 1.9%)
	Flatulence	1 (1, 3.8%)	**2 (2, 7.4%)***	3 (3, 5.7%)
	Gastro-intestinal pain	0 (0, 0.0%)	1 (1, 3.7%)	1(1, 1.9%)
	Meteorism	1 (1, 3.8%)	0(0, 0.0%)	1(1, 1.9%)
	Nausea	2 (2, 7.7%)	1 (1, 3.7%)	3 (3, 5.7%)
	Rectal disorder	**1(1, 3.8%)**	0 (0, 0.0%)	1(1, 1.9%)
	Total	9 (7, 26.9%)	8 (8, 29.6%)	17 (15, 28.3%)
Respiratory system disorders	Rhinitis	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Throat sore	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Upper respiratory tract infection	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Total	3 (3, 11.5%)	0 (0, 0.0%)	3 (3, 5.7%)
Body as a whole - general disorders	Cheat pain	0 (0, 0.0%)	1 (1, 3.7%)	1 (1, 1.9%)
	Feeling cold	0 (0, 0.0%)	1 (1, 3.7%)	1 (1, 1.9%)
	Fever	1 (1, 3.8%)	0 (0, 0.0%)	1 (1, 1.9%)
	Malaise	0 (0, 0.0%)	1 (1, 3.7%)	1 (1, 1.9%)
	Tiredness	**1 (1, 3.8%)**	1 (1, 3.7%)	2 (2, 3.8%)
	Weakness generalized	0 (0, 0.0%)	2 (1, 3.7%)	2(1, 1.9%)
	Total	2 (2, 7.7%)	6 (3, 11.1%)	8 (5, 9.4%)

Only those body systems with adverse events reported by more than one subject

Bold: judged as possibly or probably related to study drug,

* one report judged as probably related

Gastro-intestinal adverse events were the most frequently reported adverse events and led to a stop of study treatment in 4 subjects:

- subject 11 had a moderate aggravation of the colitis symptoms, which started 11 days after start of placebo treatment. The subject was treated with rectal and oral cortisone and aminosalicylates and recovered 10 days later;

- subject 17 reported moderate blood on stool (more visible blood in stool) 3 days after start of treatment with PL 14736, the duration of the event was 6 days; the investigator judged this event as possibly related to study drug;
- subject 29 reported severe progression of bloody diarrhea together with severe weight loss and severe malaise, which started 4 days after start with placebo treatment and lasted for 26 days;
- subject 30 reported a severe worsening of the colitis symptoms together with severe burning pain at the anus, which started 6 and 7 days after start with PL 14736 treatment and lasted for 25 and 24 days, respectively.

A mild aggravation of colitis symptoms were reported by subjects 12 and 58 (both on placebo). In both subjects the worsening started on day 15 and lasted for 21 days in subject 12 and were not resolved before observation period in subject 58. Both subjects were treated with cortisone and aminosalicylates.

Flatulence or meteorism of mild to moderate intensity were reported by subjects 45 and 63 (PL 14736) and by subjects 1 and 47 (placebo). The event started on day 1 after treatment with PL 14736 and on days 3 and 10 after placebo dosing and resolved after 2 to 4 days without any concomitant treatment.

Two subjects (ID 2 and 63) reported moderate nausea on day 2 and 1, respectively, after dosing of PL 14736. The event resolved after 1 and 2 days without treatment. The relation to study drug was judged as unlikely and unrelated. Subject 24 reported moderate nausea together with moderate headache on day 3 of placebo dosing. Both events resolved 8 days later after concomitant treatment with novalgin drops. The relation to study drug was judged as probably.

Apart from the severe adverse events of subjects 29 and 30 only one further adverse event of severe intensity was reported: subject 32 (PL 14736) had severe abdominal pain on day 13, which was not resolved at the end of the observation period. No relation to the study drug was seen by the investigator.

There was one further drop-out due to adverse events: subject 25 had mild fever and cold on day 2, which resolved 13 days later. The events were judged as unrelated.

Two laboratory findings were reported as an adverse event: subject 6 (placebo) had a significant Hb-decrease on day 5. The Hb was 11.8 g/dl on day 1 and decrease to 9.3 g/dl on day 5 and further on to 7.9 g/dl on day 8. After blood constitution the values improved with 11.5 g/dl on day 9 (listed as the scheduled day 8 value) and 11.0 g/dl during a control three days later. On day 15 Hb slightly increased to 11.3 g/dl and was 12.8 at the follow-up.

Subject 31 (placebo) showed elevated liver enzymes on day 15. ASAT had increased from 32 U/I on day 1 to 57 U/I on day 15, ALAT had increased from 60 U/I to 102 U/I and total bilirubin had increased from 0.7 mg/dl to 1.4 mg/dl. At the follow-up the values had decreased again. Although the adverse event was judged by the investigator as possibly related to the study treatment, the laboratory values were commented as increase due to increased alcohol consumption during the holidays (Christmas days in between).

There were 4 adverse events reported during the follow-up the end of the period:
subject 25 (PL 14736) — moderate infection of the respiratory tract
subject 41 (placebo) — moderate bacterial bronchitis
subject 52 (placebo) — severe mesenterial embolism (see

Section 12.3).

subject 59 (placebo) — mild neurodystonia

10.2.1.5. DEATHS, OTHER SERIOUS ADVERSE EVENTS AND OTHER SIGNIFICANT ADVERSE EVENTS

10.2.1.6. Narratives of deaths, other serious adverse events and other significant adverse events

There were two serious adverse events, both in the placebo group.

Subject 39 (male, 62 years old, weight 76 kg, height 173 cm) had a moderate circulatory collapse (during an influenzal infection) on day 3 after start of dosing, which required hospitalization. The event resolved 6 days later. He received a mycolytic and a macrolide antibiotic for treatment of the bronchitis. The subject did not drop out, but took the study medication as planned. The subject had no further disease in the medical history, the colitis was diagnosed nearly 1 year before the start of the study and was treated with oral aminosalicylates (3 g per day). The subject reported no further adverse events. Laboratory values showed several values out of normal range, which were related to the colitis and to the influenza and were judged as not clinically significant by the investigator.

Subject 52 (male, 76 years old, weight 83 kg, height 180 cm) had a mesenterial embolism 16 days after the end of study treatment. The subject had two bowel resections but died finally due multi organ failure. The subject had an essential hypertension for 3 years and a cataract for 22 years; the colitis was diagnosed 34 years ago. The hypertension was treated with bisoprolol (5 mg) and 12.5 mg HOT. The colitis was treated with 3 g aminosalicylates and 9 mg cortisone. The

subject felt well during the study and reported no adverse events. The systolic blood pressure varied between 130 and 140 mmHg and the diastolic between 75 and 90 mmHg. The patient left the study at follow-up with no clinically relevant laboratory findings.

10.2.1.7. Analysis and discussion of deaths, other serious adverse events and other significant adverse events

Both serious adverse events were not related to the study drug. In both cases the reason was a concomitant disease.

10.2.2 LABORATORY EVALUATION

10.2.2.1 Listings of individual laboratory measurements by subject and each abnormal laboratory value

The tabular listings by subject of all laboratory values are presented in Section 16.2.9, including a listing of all abnormal or commented values.

10.2.2.2 Evaluation of each laboratory parameter

Descriptive statistics for each safety laboratory parameter as well as the shift tables showed no clinically relevant time or dose related changes.
There were two laboratory findings, which were reported as adverse event.
Mean values for albumin as well as for Hb, ESR and ferritin, all indicating the extent of inflammation, showed no clinically relevant differences between the two treatment groups. For orosomucoid the mean slightly decreased after PL 14736 treatment and slightly increased after treatment with placebo, whereas the mean values for CRP showed a trend into the opposite direction: for PL 14736 they showed a clear increase, but

decreased for the placebo group. However, the worsening of the mean CRP value in the PL 14736 treatment group has mainly to be attributed to the extremely high CRP values of one patient (ID-No. 25) on day 15 (17,04 mg/dl), which is also reflected in the high standard deviation for the active treatment group. This patient presented at this time a respiratory tract infection, which is usually associated with increased values of inflammation markers like CRP. Excluding the values for patient 1D-No. 25 mean (0,83 mg/dl) and standard deviation (± 0.98 mg/dl) are similar to that of the placebo group.

Table Summary Statistics for Selective Laboratory Values (Safety Population)

		Day 1			Day 15		
		n	mean	(SD)	N	mean	(SD)
CRP	PL 14736	25	0.88	(1.00)	23	1.54	(3.51)
(mg/dl)	placebo	25	1.13	(1.64)	23	0.91	(1.00)
Albumin	PL 14736	24	4.11	(0.42)	21	4.28	(0.36)
(g/dl)	placebo	26	4.14	(0.51)	26	4.24	(0.39)
HB	PL 14736	26	8.53	(0.80)	24	8.32	(0.94)
(mnno1/1)	placebo	27	8.71	(0.84)	27	8.54	(0.88)
ESR 1h	PL 14736	24	13.1	(14.3)	22	13.6	(15.5)
(mm/h)	placebo	25	21.1	(19.9)	26	25.0	(22.5)
Ferritin	PL 14736	20	45.7	(29.6)	18	46.4	(41.5)
(pg/1)	placebo	23	92.5	(69.6)	23	84.2	(68.2)
Orosomucold	PL 14736	25	97.4	(29.0)	23	94.7	(29.2)
(mg/d1)	placebo	27	107.4	(31.9)	27	115.0	(38.1)
		Day 1			Day 15		
		n	mean	(SD)	N	mean	(SD)

10.2.3. VITAL SIGNS, PHYSICAL FINDINGS AND OTHER OBSERVATIONS RELATED TO SAFETY

Mean values for blood pressure and pulse rate showed no clinically relevant time or dose related changes.

No relevant changes were detected between the different study days.

10.2.4 SAFETY CONCLUSIONS

Repeated rectal doses of 80 mg PL 14736 over 14 days were safe and well tolerated.

In total 17 adverse events were reported by 10 subjects (38.5%) of the PL 14736 group and 23 adverse events were reported by 12 subjects (44.4%) of the placebo group during the treatment phase (until day 15). Gastro-intestinal adverse events were the most frequently reported adverse events and led to premature termination of the study for 4 subjects (2 of each treatment group).

Most adverse events were of mild to moderate intensity. Only 4 adverse events reported by 2 subjects after treatment with PL 14736 and 4 events reported by 3 subjects after placebo dosing were judged as possibly or probably related to study drug. For the few severe adverse events experienced during the study (3 in 2 patients and 3 in 1 patient, respectively) no relationship with the treatment was assumed.

There were two serious adverse events, both in the placebo group: subject 39 had a moderate circulatory collapse (during an influenzal infection) on day 3 after start of dosing, which required hospitalization. The event resolved 6 days later. Subject 52 had a mesenterial embolism 16 days after the end of study treatment. The subject had two bowel resections but died

finally due multi organ failure. For both serious adverse events no relation to the study drug or study procedure was expected.

Laboratory and vital sign data showed no time or dose related changes.

10.2.5 DISCUSSION

The primary aim of the study was to evaluate the effect of repeated rectal doses of 80 mg PL 14736 over 14 days in patients with distal ulcerative colitis. The effect was evaluated with regard to the overall clinical and histological symptoms by means of the DAI. This disease activity index was developed in cooperation with gastroenterologists, pathologists and specialists for laboratory medicine. It offered a quantitative evaluation of all categories, which characterize this disease entity.

Comparison of the pre-dose DAI score with the score obtained after the end of treatment resulted in a statistically significant improvement of the symptoms in the patients of PL 14736 group, but not in the patients of the placebo group.

The comparison between the two treatment groups failed to be statistically significant. This may be due to the low number of patients on the one side, but possibly also due to the short treatment duration.

Clinical symptoms, mainly stool frequency and stool consistency, but also 'blood in stool' and abdominal pain, began to improve already with the first days of dosing and showed an ongoing positive trend during the second week of treatment. This positive trend in the subjective, patient-derived parameters was confirmed after 14 days of treatment by the histopathological scoring, which showed an improvement after treatment with PL 14736, but remained constant after placebo dosing.

These results are of special importance in the light of the PL

14736 plasma concentrations, which were always below the LLQ, thus possibly indicating that the substance acts locally and not via systemic absorption.
Repeated rectal doses of 80 mg PL 14736 over 14 days showed an excellent tolerability.

The total incidence of adverse events was higher in the placebo group. Most adverse events were related to gastro-intestinal disorders; for these events the incidence was similar for both treatment groups. There were no adverse events which can be attributed to the PL 14736 treatment. Adverse events which led to a patient's drop out can be characterised for the majority as a deterioration of the disease (in two patients of each treatment group), i.e. as treatment failures.

The only two serious adverse events reported in this study, occurred in the placebo group, both were not related to the study treatment or the study procedures.
Laboratory and vital sign assessments showed no clinically relevant drug or time related changes.
Overall, this first study with locally applied PL°14736 indicated, that the substance is safe and well tolerated and has the potential of an effective **treatment alternative for both, induction and maintenance therapy of patients with ulcerative colitis.**

10.2.6. CONCLUSION

Repeated rectal administration of 80 mg PL 14736 for 14 days resulted in a statistically significant improvement of the symptoms of the ulcerative colitis (DAI score), whereas placebo showed smaller and not significant improvement. The difference between the two treatment groups did not reach statistical significance, maybe due to the small sample size.
Repeated rectal doses of 80 mg PL 14736 for 14 days were well tolerated. In total 10 subjects (38.5%) of the PL 14736 group

and 12 subjects (44.4%) of the placebo group reported adverse events during the treatment phase. Gastrointestinal adverse events were most frequently reported and led to premature termination of the study for 4 subjects (2 of each treatment group).

Referencee

1. ***FOCUS - CLINICAL DRUG DEVELOPMENT GMBH STUDY NUMBER 21PV0786 FINAL VERSION 8TH FEBRUARY 2005***