Pharmacodynamics and pharmacokinetics of BAY x 7195 aerosol, a new and selective receptor antagonist of cysteinyl-leukotrienes, in normal volunteers

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- 1 The safety, tolerability and pharmacokinetics of BAY x 7195 aerosol, a new selective receptor antagonist of cysteinyl-leukotrienes, were investigated in healthy male volunteers in two observational studies (1 and 2 mg; $n=5$ each) and two double blind, placebo-controlled two way crossover studies (4 and 8 mg; $n=6$ each) using the commercially available Inhaler Ingelheim M[®].
- 2 The pharmacodynamic effect was assessed by testing the ability of BAY x 7195 aerosol to inhibit leukotriene-D₄ (LTD₄) induced bronchoconstriction in healthy volunteers. Using a double-blind, placebo-controlled three way crossover design, volunteers received 2 and 4 mg of BAY x 7195 by means of a newly developed metered dose dry powder inhaler. Bronchoprovocation with nebulized LTD₄ was performed 20 min and 8 h ($n=6$ each) after drug administration. Specific airways conductance (SGaw) served to assess the airway's response.
- 3 BAY x 7195 aerosol was safe and well tolerated. Inhalation of the aerosol had no effect on baseline lung function. Only one volunteer reported cough following the inhalation of the 8 mg dose.
- 4 The pharmacokinetics of unchanged drug following the administration of BAY x 7195 aerosol were linear in the investigated range of doses and in general very similar to a previously investigated tablet formulation. Plasma-concentration *vs* time courses followed a two-compartment body model. Compared with oral administration of the tablet formulation absorption tended to be more rapid with the aerosol formulation.
- 5 Compared with placebo, 2 and 4 mg BAY x 7195 increased the concentration of LTD₄ needed to produce a 35% decrease in SGaw 20 min after drug administration by a mean (geometric) of 14.2 and 29.7 fold, respectively. For both doses only three volunteers showed a protective effect against LTD₄ induced bronchoconstriction 8 h after drug administration. Individual shifts in the concentration-response curve ranged between 0.4 and 7.2 fold.
- 6 In conclusion, the present results suggest that BAY x 7195 aerosol is a safe and potent but short acting receptor antagonist of cysteinyl leukotrienes in man.

Keywords powder aerosol bronchial challenge leukotriene-D₄

Introduction

Asthma is one of the most common chronic diseases in industrialized countries. Epidemiological studies show that despite optimized therapeutic regimens with antiin-

flammatory and bronchodilatory drugs its prevalence, morbidity and mortality are increasing [1, 2]. This stresses the need for new approaches to antiasthmatic therapy.

Cysteinyl-leukotrienes appear to be of major

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importance in the pathophysiology of asthma. Leukotrienes cause bronchoconstriction [3], lead to enhanced capillary permeability [4], and increase the formation of bronchial mucus [5]. They can be detected in plasma, urine, and bronchial lavage fluid of asthmatics [6, 7]. Acute exacerbations of the disease and antigen challenges are associated with their increased concentration in urine and in bronchial lavage fluid [8, 9]. Inhalation of leukotrienes leads to bronchoconstriction and can induce bronchial hyperreactivity both in the healthy subject and in the mild asthmatic [10–12].

BAY x 7195 [(S)-4-(4-carboxyphenylthio)-7-[4-(4-phenoxybutyloxy)-phenyl]-5-(Z)-heptenoic acid]] is a new and selective receptor antagonist of cysteinyl-leukotrienes [13] which in preclinical studies has been shown to be effective against leukotriene-D₄ (LTD₄) and antigen-induced bronchoconstriction *in vitro* and *in vivo* [14]. As tablet formulation, the compound has shown a moderate protective effect against LTD₄ induced bronchoconstriction in healthy volunteers in doses of 250 and 500 mg [15]. BAY x 7195 is also developed as a powder aerosol for the topical treatment of asthma. The aim of the present studies carried out in normal healthy male volunteers was to investigate the safety, tolerability and pharmacokinetics of the aerosol formulation of BAY x 7195, to assess its ability to protect against LTD₄ induced bronchoconstriction and to determine the duration of action.

Methods

Subjects

A total of 34 young healthy male volunteers were enrolled. The subjects were informed about the aims and the risks of the study and gave their written informed consent to participate. The study protocols were in agreement with the 1975 declaration of Helsinki and approved by the ethics committee of the North Rhine Medical Council, Düsseldorf, Germany. Before entering the study each volunteer underwent a complete medical examination including laboratory investigations, vital signs, ECG and a pulmonary function test. None of the volunteers had any history of asthma, allergic disease or other internal illnesses. No medication was allowed for 1 week before and during the study. The volunteers did not consume alcohol from day –1 until the end of the study and abstained from nicotine or caffeine 10 h prior to drug administration until after lunch of the study day (safety and pharmacokinetic studies) and 24 h after drug administration (pharmacodynamic studies), respectively.

Study design

Safety and pharmacokinetic studies The safety, tolerability and pharmacokinetics of BAY x 7195 aerosol were investigated in four separate dosing steps on a total of 22 volunteers. Volunteers inhaled single doses

of 1 mg ($n=5$; age: 34 ± 4 years, weight: 70 ± 9 kg, height: 181 ± 5 cm) and 2 mg ($n=5$; age: 34 ± 4 years, weight: 75 ± 8 kg, height: 181 ± 7 cm) in an uncontrolled not-blinded study design and 4 mg ($n=6$; age: 28 ± 4 years, weight: 87 ± 8 kg, height: 184 ± 7 cm) and 8 mg ($n=6$; age: 35 ± 9 years, weight: 83 ± 7 kg, height: 182 ± 7 cm) according to a placebo-controlled randomized double-blind two way crossover design. The powder was administered as a 5% adhesive mixture using lactose as carrier in capsules containing 0.5 (1 and 2 mg) and 1 mg (4 and 8 mg) of active substance, respectively, via the commercially available Inhaler Ingelheim M[®]. The wash-out phase between the two periods in the crossover studies was 7 days. Volunteers inhaled the different doses of BAY x 7195 at 08.00 after a 10 h overnight fast in sitting position. Thereafter specific airways conductance (SGaw) and the forced expiratory volume in 1 s (FEV₁) were recorded at 5 and 60 min as well as 6 and 24 h after drug administration (p.a.). A standardized breakfast was given 2 h p.a. (2 rolls, 20 g of butter, marmalade), lunch 5 h p.a. and dinner 10 h p.a. Blood samples for the determination of plasma concentrations of BAY x 7195 were taken at timed intervals up to 12 h. The safety and tolerability of the substance were monitored by non-leading questioning and the determination of laboratory parameters, vital signs, peak flow measurements and ECG up to 48 h.

Pharmacodynamic studies The ability of BAY x 7195 to inhibit a LTD₄ induced bronchoconstriction was tested in two separate studies on a total of 12 volunteers. In study 1 bronchoprovocation was started 20 min p.a. ($n=6$; age: 32 ± 6 years, weight: 79 ± 6 kg, height: 178 ± 5 cm), in study 2 8 h p.a. ($n=6$; age: 31 ± 6 years, weight: 76 ± 7 kg, height: 181 ± 3 cm). In both studies volunteers received 2 and 4 mg BAY x 7195 according to a randomized, double-blind, placebo-controlled, three way crossover design with a wash-out period of 7 days between two periods. The powder was administered as a 5% adhesive mixture using lactose as carrier and a newly developed dry powder metered dose inhaler delivering 0.5 mg of active substance per actuation. Volunteers inhaled the different doses of BAY x 7195 between 08.00 h and 10.00 h after a 10 h overnight fast in sitting position. Bronchoprovocation testing was carried out with synthetic leukotriene-D₄ (Cascade Ltd, Reading, England) and an Asthma Provocation System (Jaeger, Würzburg, Germany; nebulised solution 4–6 µl per inhalation) as described previously [15]. The LTD₄ concentrations tested on the study day were 0, 1, 5, 10, 50, 100, 500, 1000, 5000 µg ml⁻¹. All subjects were carefully monitored for adverse events throughout the study as described above. Plasma samples for the determination of BAY x 7195 were taken at time 0 and before and at the end of bronchoprovocation.

BAY x 7195 assay

Plasma was prepared by centrifugation and stored at –20° C until the time of analysis. BAY x 7195 plasma

concentrations were determined with a fully validated specific h.p.l.c. assay with post-column photoderivatisation and subsequent fluorescence detection [16]. The limit of quantification was $0.6 \mu\text{g l}^{-1}$. Quality control (QC) samples analysed concurrently with study samples were determined with an inter-day precision of 5.9–8.9% ($n(\text{total})=60$, QC concentrations were 2, 5 and $12 \mu\text{g l}^{-1}$).

Data evaluation

The pharmacokinetics of BAYx 7195 were evaluated using non-compartmental techniques (Kincalc 2.32 software for PC, Institute of Clinical Pharmacology, Bayer AG). Maximum plasma-concentrations (C_{max}) and the times needed to reach these concentrations (t_{max}) were assessed by inspection of the individual plasma-concentration *vs* time plots. Area under the plasma-concentration *vs* time curve (AUC) was calculated using the log-linear trapezoidal rule up to the last data point (t_n) plus the extrapolated portion ($\text{AUC}(t_n, \infty)$). The latter was calculated by the terminal phase rate constant (λ_z) which was the slope of the log-linear regression of the last n data points (where $n > 3$) and the estimated concentration at t_n ($C'(t_n)$) using the formula $C'(t_n)/\lambda_z$. Apparent oral clearance (CL/F) was estimated as dose/AUC , where F is the fraction of dose reaching the systemic circulation. Mean residence time (MRT) was calculated as ratio area under the first moment *vs* time curve (AUMC/AUC).

The ability of BAYx 7195 to inhibit LTD₄ induced bronchoconstriction was assessed by comparing the concentration of LTD₄ needed to cause a 35% reduction in SGaw (PC35SGaw) for the two doses of BAYx 7195 with placebo. This concentration was derived by linear interpolation of the logarithmic dose-response curve

between the two concentrations above and below a 35% decrease in SGaw.

All parameters with the exception of t_{max} are presented as mean and s.d. t_{max} is given as median and range. Multiple group comparisons between placebo and treatment groups as well as baseline and follow-up were made by the Friedman test. In case of significance, the Wilcoxon signed rank test was used to detect differences between single groups. These tests were applied under the assumption that the results were not biased by period effects and carry over effects, respectively. The relationship between BAYx 7195 plasma concentrations and the PC35SGaw was investigated by means of linear regression analysis. $P < 0.05$ was taken as the cut-off point for significance.

Results

Safety and pharmacokinetic studies

Inhalation of BAYx 7195 did not cause bronchoconstriction (Figure 1). The mean differences in SGaw between active treatment (8 mg) and placebo were 0.14 (95% confidence -0.14 ; 0.42), 0.16 (95% CI 0 ; 0.32) and 0.01 (95% CI -0.32 ; 0.35) at 5 min, 1 and 6 h respectively. Only with the inhalation of the 8 mg dose, one volunteer reported cough starting shortly after the inhalation of the test substance and reoccurring during the study day. No relevant changes in any of the other safety parameters were observed.

Figure 2 shows BAYx 7195 plasma-concentration *vs* time courses in six subjects receiving 4 mg drug by inhalation. The drug was rapidly absorbed and maximum concentrations of 12.4 to $31.5 \mu\text{g l}^{-1}$ were reached 0.75 – 2.00 h after administration. Following t_{max} , concen-

Figure 1 Lung function assessed by specific airways conductance after inhalation of a single dose of 8 mg BAYx 7195 aerosol and placebo. Results between baseline and follow-up are not significantly different as analysed by multiple group comparison. The same holds true if the difference between active treatment and placebo is calculated.

Figure 2 Individual time vs BAY x 7195 plasma-concentration profiles following administration of 4 mg BAY x 7195 as powder aerosol to six healthy male volunteers (● 1; □ 2; ▲ 3; ▽ 4; ◆ 5; ● 6).

trations declined in a distinctly biphasic fashion. The apparent half-life ($t_{1/2\alpha}$) of the first rapid distribution phase was approximately 1 h, whereas the mean terminal elimination half-life ($t_{1/2z}$) was estimated at 5.1 h following the 4 mg dose (Table 1). $t_{1/2z}$ could not be determined following administration of a 1 mg dose where only the first more rapid distribution phase was assessable and concentrations in the terminal elimination phase were below the limit of quantification of the analytical method ($0.6 \mu\text{g l}^{-1}$). Pharmacokinetics were linear in the dose range 1–8 mg, $C_{\max}$ and AUC increasing proportionately with dose (Figure 3). The apparent oral clearance CL/F (geometric mean) was relatively constant (52.2 – 60.4 l h^{-1}) across the different doses investigated in the dose escalation study.

Pharmacodynamic studies

One volunteer dropped out after two periods of study one in which he received 2 and 4 mg BAY x 7195 due to reasons unrelated to the study procedure. Two volunteers reported headache and one volunteer a 'cardiac lancinating pain'. The latter is a known and frequently occurring event to the volunteer and most likely related to certain movements. No other adverse events due to the study medication occurred. Bronchial challenge testing was also well tolerated. Volunteers reported only mild complaints related to the inhalation of leukotrienes as for instance cough, chest tightness and increased mucus production. All symptoms resolved spontaneously after the end of the bronchoprovocation and

Table 1 Non-compartmental pharmacokinetic parameters of BAY x 7195 aerosol following single-dose administration to healthy volunteers. Data are presented as geometric mean value and s.d. or median, minimum and maximum value

Dose of BAY x 7195 (mg)	1	2	4	8
Number of subjects	5	5	6	6
Parameter				
$C_{\max}$ ($\mu\text{g l}^{-1}$)	6.0 (1.40)	7.1 (1.71)	24.6 (1.42)	51.9 (1.35)
AUC ($\mu\text{g l}^{-1} \text{ h}$)	16.5 (2.37)	34.0 (1.40)	76.6 (1.65)	150.7 (1.25)
$t_{\max}^*$ (h)	1.5 (1.0–1.5)	1.5 (0.8–3.0)	1.3 (0.8–2.0)	1.0 (0.8–2.0)
$t_{1/2z}$ (h)	n.c.	6.9 (1.92)	5.1 (1.26)	3.5 (1.76)
MRT (h)	5.1 (3.65)	7.6 (1.82)	5.1 (1.27)	4.0 (1.40)
CL/F (l h^{-1})	60.4 (2.37)	58.9 (1.40)	52.2 (1.65)	53.1 (1.25)

* Median (range), n.c.: not calculated.

Figure 3 Plot of dose vs geometric mean $\pm$ s.d. $C_{\max}$ (■) and AUC (●) of BAY x 7195 and linear regression line (y-intercept fixed to zero).

no medication was required to reverse bronchoconstriction. Lung function was normalized within 60 min in all volunteers. The placebo and verum PC35SGaw in individual volunteers receiving 2 and 4 mg BAY x 7195 are depicted in Figures 4 (20 min p.a.) and 5 (8 h p.a.). The respective concentrations in the volunteer who dropped out were 79.5 (2 mg) and 517.0 (4 mg) $\mu\text{g ml}^{-1}$. Inhalation of 2 and 4 mg of BAY x 7195 significantly increased the concentration of LTD₄ needed to produce a 35% decrease in SGaw in all volunteers 20 min p.a. ($P < 0.015$ for the multiple group comparison; $P < 0.043$ for both doses compared to placebo). Individual shifts

Figure 4 LTD₄-concentrations needed to produce a 35% decrease in specific airways conductance 20 min after inhalation of 2 and 4 mg BAY x 7195 compared with placebo. Results for the 2 and the 4 mg dose are significantly different from placebo ($P < 0.043$ for both doses).

Figure 5 LTD₄-concentrations needed to produce a 35% decrease in specific airways conductance 8 h after inhalation of 2 and 4 mg BAY x 7195 compared with placebo. Results between the two doses and placebo are not significantly different.

in the concentration response curve varied between 4.5 and 42.6 fold (geometric mean 14.2) for the 2 mg dose and 13.2 and 149.2 fold (geometric mean 29.7) for the 4 mg dose. The geometric mean PC35SGaw were 98.0, 1390 and 2905 $\mu\text{g ml}^{-1}$ for placebo and 2 and 4 mg BAY x 7195, respectively. Only three volunteers showed a protective effect against LTD₄ induced bronchoconstriction 8 h after administration. Individual shifts ranged from 1.0 to 6.2 and 0.4 to 7.2 fold for the 2 and the 4 mg dose, respectively. Geometric mean PC35SGaw were 21 $\mu\text{g ml}^{-1}$ for placebo and 54 and 41 $\mu\text{g ml}^{-1}$ for doses of 2 and 4 mg. As expected for a drug administered locally as aerosol, individual shifts in the concentration response curve were not correlated to plasma concentrations of BAY x 7195 measured before the start or at the end of the bronchoprovocation testing.

Discussion

Bronchial challenges with specific mediators are generally used to determine the pharmacological effect of a new antiasthmatic drug early in the course of clinical development. They provide a useful tool to verify pharmacological effects known from preclinical studies in healthy volunteers or asthmatics. In addition, they have a role in selecting doses and dose regimens for

clinical trials. Specific mediator challenges have been used in the development of a number of antiasthmatic drugs such as receptor antagonists of thromboxane [17], platelet activating factor [18] or cysteinyl-leukotrienes [19]. For bronchoprovocation with synthetic leukotriene- D_4 reliable protocols for use in normal volunteers are available [10, 20]. LTD $_4$ challenges have been shown to be safe and reproducible both in normal volunteers and in asthmatics with a rapid onset and a prolonged duration of bronchoconstriction [21–23].

Bronchoprovocation testing with synthetic LTD $_4$ has recently been used to determine the pharmacological activity of oral BAY x 7195 in healthy volunteers [15]. The results indicated an only moderate activity of the compound. This was surprising as compared with other new receptor antagonists of cysteinyl-leukotrienes for which a potent pharmacological effect in man could be demonstrated [19, 24] BAY x 7195 showed a comparable pharmacological activity in preclinical studies [14]. The reason for the discrepancy between preclinical and clinical pharmacological activity was unclear. Although up to now no data on absolute bioavailability in man are available, the observed plasma concentrations appeared to be high enough to exert a powerful pharmacodynamic effect based on preclinical data. Moreover, there was no relationship between the plasma concentrations of BAY x 7195 and shifts in the LTD $_4$ concentration-response curves. An alternative explanation for the only moderate pharmacodynamic effect was the lack of availability of substance at the site of action in the lungs, which in part may be due to the high plasma protein binding of the compound of >99% [25].

The present results argue in favour of this explanation. Compared with the oral formulation, BAY x 7195 when directly administered to the lungs showed a markedly improved protection against LTD $_4$ induced bronchoconstriction in this small group of unselected volunteers. For the 4 mg dose, a 35% decrease in specific airways conductance could only be induced with the highest concentration of 5000 $\mu\text{g ml}^{-1}$ in two volunteers and could not be achieved in one volunteer who showed an interpolated approximate 150 fold shift in the concentration response curve. For the two doses tested a clear dose-response relationship was observed. Geometric mean shifts were about 15 fold for the 2 mg dose and 30 fold for the 4 mg dose indicating that the administration of higher doses may cause an even more pronounced protection against LTD $_4$ induced bronchoconstriction.

Despite the potent receptor blocking activity of the aerosol its pharmacodynamic half-life appears to be short. Only three out of six volunteers showed a shift in the concentration-response curve 8 h after inhalation of the compound and in these volunteers the magnitude of shift was not dose dependent. Nevertheless, the receptor blocking potency in these three volunteers exceeded the inhibitory effect seen in studies with the tablet formulation for which a significant shift in the concentration-response curve 8 h p.a. could be demonstrated [15]. To get a more precise estimate of the duration of action more information about the

pharmacodynamic effect between 20 min and 8 h should be obtained.

A short pharmacodynamic half-life of the aerosol formulation was also indicated for zafirlukast (ICI 204,219). While the tablet formulation is known to block both the early and the late allergic response in patients with asthma, the aerosol has been reported to only block the early allergic response [26]. These findings are readily explainable by a rapid clearance of aerosols from the lungs while high local initial concentrations are responsible for the potent early pharmacodynamic effect. High local concentrations, however, may also cause unspecific effects in the airways. While an unwanted bronchoconstriction can be excluded by the present results, the specificity of BAY x 7195 so far has only been tested in preclinical studies. Data for SK&F 104353, however, indicate that the specificity of cysteinyl-leukotrienes when given by inhalation is preserved in man [27].

The relevance of challenge studies with LTD $_4$ for the prediction of efficacy in bronchial asthma remains to be established. The outcome of bronchoprovocation testing in phase I is usually taken as a go/no go decision for further drug development. A negative outcome of a challenge questions the pharmacological activity of the drug and usually precludes further clinical development. On the other hand, a positive outcome does not necessarily mean that the drug is active in clinical asthma and may not even predict the outcome in indirect or allergen challenge studies. Zafirlukast and MK 886 are both effective receptor antagonists of cysteinyl-leukotrienes but show variable response to the allergen induced increase in bronchial hyperresponsiveness [28, 29]. To date, only little data on long term studies in clinical asthma are available. In asthmatics, zafirlukast blocked the LTD $_4$ induced bronchoconstriction in doses from 10 to 100 mg [30]. In a 6 weeks study in patients with bronchial asthma daily doses of 10 and 20 mg failed to improve the FEV $_1$ while 40 mg showed a moderate 11 % improvement [31]. First data on a 3 months treatment period in asthmatic patients indicate that even the 40 and the 80 mg doses show only little efficacy in improving lung function parameters [32]. Therefore, the results of further studies in clinical asthma must be awaited before the value of LTD $_4$ challenge studies for the outcome in clinical asthma can be assessed.

In previous studies with oral administration of a tablet formulation BAY x 7195 had shown biphasic disposition which could be well described by a two-compartment body model [25]. Terminal elimination half-life in these studies was 5.0–9.6 h, which is comparable with the values estimated for the aerosol formulation. BAY x 7195 exhibits linear pharmacokinetics both as aerosol (dose range: 1–8 mg) and as oral tablet formulation (dose range: 50–1000 mg). The only notable difference in plasma-concentration *vs* time course was a trend to somewhat more rapid absorption of the drug in the aerosol formulation corresponding to smaller t_{max} values (median t_{max} : 1.0–1.5 h (aerosol) compared with 1.9–2.5 h (tablet)). Early pulmonary absorption of the drug seems to be a ready explanation.

However, the faster absorption following inhalation also may at least partly be due to a difference in particle size of BAY x 7195 i.e. a more rapid gastrointestinal dissolution of the smaller particles used in the powder aerosol formulation. Apparent oral clearance of BAY x 7195 aerosol tended to be somewhat smaller compared with the tablet formulation (geo. mean CL/F: 52.2–60.4 l h⁻¹ (1–8 mg aerosol) compared to 64.5–95.3 l h⁻¹ (50–1000 mg tablet)) corresponding with an increase in systemic bioavailability of the aerosol formulation. The extent to which pulmonary absorption contributes to the overall systemic bioavailability of BAY x 7195 remains to be established. Further studies investigating the pulmonary absorption of BAY x 7195 aerosol more specifically e.g. by preventing gastrointestinal absorption with concomitant charcoal administration need to be conducted.

In conclusion, BAY x 7195 given as powder aerosol has been shown to be a potent receptor antagonist of cysteinyl leukotrienes in man with a short pharmacodynamic half-life. As the compound was safe and well tolerated and a close dose-effect relationship was observed higher doses of BAY x 7195 might be administered and show a more pronounced and longer lasting LTD₄ antagonism.

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