

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-395**

**CLINICAL PHARMACOLOGY AND  
BIOPHARMACEUTICS REVIEW(S)**

## CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

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NDA	21-395 (000)
Drug Substance	Tiotropium bromide Inhalation Powder
Drug Product	Spiriva® Inhalation Powder
Strengths	18 µg per capsule
Route of Administration	Inhalation
Sponsor	Boehringer Ingelheim Pharmaceuticals, Inc.
Type of submission	NME, 1S
Date of submission	12/12/01
OCPB Division	DPE-II
Clinical Division	Pulmonary and Allergy Drug Products (HFD-570)
Reviewer	Shinja R. Kim, Ph.D.
Team Leader	Emmanuel Fadiran, Ph.D.

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### 1. EXECUTIVE SUMMARY

Tiotropium bromide monohydrate is an anticholinergic drug with specificity for muscarinic receptors. Tiotropium bromide monohydrate is an anticholinergic drug with specificity for muscarinic receptors, proposed to be used for the long-term, once-daily, maintenance treatment of bronchospasm and dyspnea associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. The proposed dosage of tiotropium is inhalation of the contents of one capsule (18 µg of tiotropium cation), once daily, with the HandiHaler inhalation device.

Pharmacokinetic data for tiotropium were obtained from 15 clinical studies in a total of 600 subjects. In addition, many *in vitro* studies (metabolism pathways, stability of tiotropium, protein binding) were performed to support this NDA. Pharmacokinetic characteristics of tiotropium in humans are summarized follows:

The absolute bioavailability of tiotropium after dry powder inhalation is 19.5% and negligible after oral administration (2-3%). The drug is extensively distributed in the body and has a volume of distribution of 32 L/kg. The apparent terminal elimination half-life is between 5 and 6 days. An approximate steady state is achieved within 2-3 weeks by inhalation of 18 µg dry powder inhalation capsules and steady state plasma concentrations are about two times higher than concentrations after a single dose. Tiotropium plasma concentrations after an 18 µg inhalation range in steady state between a minimum of 2-4 pg/mL and a maximum of 15-20 pg/mL. Tiotropium is eliminated by renal excretion (73.6% of dose as parent compound in young healthy subjects) with a renal clearance exceeding the creatinine clearance, which suggests an active secretion by the kidney. Some of the drug (<30%) undergoes nonenzymatic ester cleavage as well as metabolism. As expected for any mainly renally eliminated drug, there is an increase of systemic exposure with renal dysfunction and thus also a slight increase in advanced age. Drug-drug interactions by tiotropium on other drugs are not expected due to the low dose of 18 µg and the lack of cytochrome P450 inhibition by tiotropium shown in *in vitro* studies. Since elimination of tiotropium by metabolism is minor, metabolic interactions of tiotropium are not expected.

During the drug development, the formulation was changed a few times (Phase I, II and III formulations). Also, device that delivers dry powder inhalation capsule was changed from

*Inhalator Ingelheim (FO2) to HandiHaler. Although a developmental formulation and device were used in dose-ranging studies, the to-be marketed formulation (Phase III formulation) and the device (HandiHaler) were used in Phase III studies and pivotal Phase I studies.*

Based on the submitted information, the Office of Clinical Pharmacology and Biopharmaceutics (OCPB) finds NDA 21-395 acceptable. The sponsor is requested to adopt the OCPB labeling as provided in this review.

#### **1.1 Recommendation**

The Office of Clinical Pharmacology and Biopharmaceutics has reviewed the Human Pharmacokinetics and Bioavailability section and found that NDA 21-395 is acceptable provided the sponsor accepts the labeling recommendations.

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Shinja R. Kim, Ph.D., DPE II

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Emmanuel Fadiran, Ph.D., Team Leader

## 2. Table of Contents

1 EXECUTIVE SUMMARY.....	1
1.1. Recommendation.....	2
2 TABLE OF CONTENTS.....	3
3 SUMMARY OF OCPB FINDINGS.....	4
4 QUESTION BASED REVIEW .....	6
4.1. General Attributes.....	6
4.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product? What is the proposed mechanism of drug action and therapeutic indications? What is the proposed dosage and route of administration?.....	6
4.1.2 What efficacy and safety information (e.g., biomarkers, surrogate endpoints, and clinical endpoints) contribute to the assessment of clinical pharmacology and biopharmaceutics study data (e.g., if disparate efficacy measurements or adverse event reports can be attributed to intrinsic or extrinsic factors that alter drug exposure/response relationships in patients)?.....	7
4.2. General Clinical Pharmacology.....	6
4.2.1 What is the basis for selecting the clinical-response endpoints (i.e., PD) and how are they measured in clinical pharmacology and clinical studies?.....	7
4.2.2 Are the active moieties in the plasma appropriately identified and measured to assess PK parameters and exposure response relationship?.....	8
4.2.3 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy and safety? Based on PK parameters, what is the degree of linearity or non-linearity in the dose-concentration relationship? Do PK parameters change with time following chronic dosing? How long is the time to the onset and offset of the pharmacological response or clinical endpoints? Are the dose and dosing regimen consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?.....	8
4.2.4 How does the PK of tiotropium in healthy volunteers compared to that in patients following dry powder inhalation? What are the basic PK parameters? Is this a high extraction ratio or a low extraction ratio drug?.....	10
4.2.5 Does mass balance suggest renal or hepatic the major route of elimination?.....	14
4.3. Intrinsic Factors.....	14
4.3.1 What are the relevant covariates that the pharmacokinetic variability of tiotropium?.....	14
4.4. Extrinsic Factors.....	16
4.4.1 What are the extrinsic factors (drugs, herbal products, diet, smoking, and alcohol) influence exposure and/or response of tiotropium? .....	16
4.4.2 Is there an in vitro basis to suspect in vivo drug-drug interactions?.....	16
4.4.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?.....	18
4.4.4 Are there other metabolic/transporter pathways that may be important?.....	18
4.5 General Biopharmaceutics.....	18
4.5.1 Based on BCS principle, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?.....	18
4.5.2 Has the proposed commercial formulation and device been adequately linked to the Phase III clinical trial formulation and device?.....	19
4.5.3 What is the effect of food on the bioavailability of tiotropium from the dosage form? What dosing recommendation should be made, if any regarding administration of the product in relation to meals or meal type?.....	19
4.6 Analytical.....	19
4.6.1 Were the analytical procedures used to determine drug concentrations in this NDA acceptable?.....	19
5. Labeling recommendations.....	19
6. Appendices.....	21
6.1. Proposed labeling.....	22
6.2 Individual Study Reviews (Available upon Request).....	30

### 3. Summary of Clinical Pharmacology and Biopharmaceutics Findings

The PK data for tiotropium were obtained in 15 clinical studies in a total of 600 subjects. In addition, many *in vitro* studies (7 reviewed) were performed to support this NDA.

**Absorption:** Tiotropium is a quaternary amine and it is not readily absorbed into the systemic circulation after oral administration of aqueous solutions as confirmed by the low bioavailability of 2-3% in young healthy subjects, while tiotropium showed an absolute bioavailability of 19.5% after dry powder inhalation in these subjects. This means that approximately 17% of the declared dose reach the lung, when the values for oral solutions and inhalation capsules are integrated.

Plasma profiles of tiotropium showed a rapid absorption and distribution with  $t_{max}$  at 5 min post inhalation (first sampling time), then, tiotropium plasma concentrations declined rapidly and 2-4 hours after inhalation they are often no longer quantifiable. Geometric mean tiotropium plasma concentrations 5 minutes after a first inhalation ( $C_{5min}$ ) of 18  $\mu\text{g}$  were often below the limit of quantification (—), while observed  $C_{5min}$  of 17-19  $\text{pg/mL}$  after multiple doses.

There was a trend to increased plasma concentrations with higher doses, which suggest the deviant behavior from the linear dose proportionality. However, the linearity of PK could not be confirmed due to insufficient data.

**Distribution:** 72% of tiotropium is bound to human plasma. The volume of distribution ( $V_{ss}$ ) after a 14.4  $\mu\text{g}$  intravenous infusion was 32 L/kg (205.105). This high  $V_{ss}$  indicates an extensive tissue binding of the drug.

**Metabolism:** Tiotropium is predominantly eliminated via renal secretion of unchanged drug (73.6% of dose as a parent drug was recovered in urine after intravenous infusion in healthy young male subjects). The fate of the remaining quarter of the dose in subjects is not exactly known.

Metabolism was investigated in *in vitro* studies and *in vivo* animal studies. *In vitro* studies indicated that (1) hydrolytic cleavage of the ester bond of tiotropium occurs non-enzymatically (converted to N-methylscopine and dithienylglycolic acid). (2) N-methylscopine and other metabolites (a variety of glutathione conjugates after oxidation of the thiophen ring system) were formed enzymatically via CYP 2D6 and probably CYP 3A4. (3) N-methylscopine and dithienylglycolic acid are pharmacologically inactive, and (4) high tiotropium concentrations of 1  $\mu\text{mol/L}$  did not inhibit cytochrome P450 1A1, 1A2, 2B1, 2C9, 2C19, 2D6, 2E1, or 3A4.

**Elimination:** In healthy young male subjects, urinary excretion of unchanged drug accounted for 73.6% and 14.4% of the dose after an intravenous infusion and dry powder inhalation, respectively. Total clearance was 880 mL/min with a renal clearance ( $CL_R$ ) of 669 mL/min after intravenous infusion.  $CL_R$  was 486 mL/min after inhalation. Renal clearance is greater than the creatinine clearance, which suggests that tiotropium undergoes active renal secretion. It is not known which cation transporter is responsible for the active renal secretion but *in vitro* investigations in CaCo2 cells showed that it is probably not p-glycoprotein. Tiotropium excreted in urine after chronic once daily inhalation by COPD patients was about 7% and the steady state was reached after 2-3 weeks in these patients. The terminal elimination half-life of tiotropium is between 5 and 6 days following inhalation. The high renal clearance as well as the urinary recovery of about 73.6% (intravenous) as unchanged substance indicates that the long elimination half-life may be due to a slow redistribution process.

**PK/PD relationship:** None of the clinical studies related the responses (efficacy or safety) to the pharmacokinetics of tiotropium. An  $E_{max}$  model established from dose-response ( $FEV_1$ ) relationship data obtained from Phase II studies was arrived to select the dose for Phase III

studies. Time-response plots were made using the data from studies with a single escalating inhalation doses. A second peak was seen at around 24 hrs in these studies. The reason of this 2<sup>nd</sup> peak is not known, however, it does not appear to be due to pharmacokinetic characteristics of tiotropium (i.e., no active metabolites, no enterohepatic recirculation). It could be, as the sponsor suggested, due to circadian rhythm.

#### **Pharmacokinetics in special populations**

Gender effect: Male and female COPD/asthma patients showed no relevant differences in drug plasma concentrations or urinary excretion of tiotropium.

Age Effect: Renal clearance was significantly decreased to 326 mL/min in COPD patients with a geometric mean age of 53 years to 163 mL/min (50% decrease) in patients with an mean age of 74 years following 18 µg by inhalation. The decrease in urinary excretion was associated with an increase of AUC<sub>0-4h</sub> values from 18.2 pg•h/mL (69% gCV) to 26.1 pg•h/mL (63% gCV) at the same time (~40% increase). However, a dose adjustment in advanced age is not considered necessary, because COPD patients of this age range are the target population, which was consequently studied regarding safety and efficacy.

Patients with renal impairment: Following an intravenous dose of 4.8 µg in healthy volunteers, tiotropium plasma concentrations increased with renal dysfunction with more pronounced changes in subjects with a CLcr < 50 mL/min; tiotropium AUC<sub>0-4h</sub> were 39, 81 and 94% higher in mild (CLcr >50-80 mL/min), moderate (CLcr >30-50 mL/min), and severe (CLcr <30 mL/min) renal impairment when compared to control subjects. The effect of a renal insufficiency on tiotropium concentrations after inhalation in COPD patients was also evaluated in Study 205.117. Trough plasma concentrations (C<sub>5min</sub>) at steady state (Day 92) increased by about 10% and 27% in mild and moderate impairment, respectively, compared to patients with normal renal function. C<sub>5min,ss (Day 92)</sub> increased by 84% and 188% in mild and moderate impairment, respectively, compared to patients with normal renal function. Increase of plasma concentrations was associated with a decrease of 20% and 50% in Ae<sub>0-24h,ss</sub> mild and moderate renal impairment, respectively, compared to patients with normal renal function. Therefore, tiotropium should be used with caution in patients with renal impairment, especially those with moderate and severe impairment.

Patients with hepatic impairment: The effect of hepatic impairment was not studied in human. Tiotropium was predominantly cleared by renal elimination as a parent drug (~70% in healthy young subjects), therefore, approximately 30% of dose (part of dose are degraded by ester cleavage) are expected to be eliminated as a metabolites. Thus, based on minor elimination by a metabolism route and low tiotropium plasma concentrations after 18 µg inhalation dose, a clinically significant effect due to hepatic dysfunction is not anticipated.

Effect of COPD and asthma: The effect of the pulmonary disease on the absorbed fraction of the inhaled dose is not exactly known, because this effect is hard to separate from the confounding effects of age and formulation on the urinary excretion.

Effect of different ethnicity: Urinary excretion data indicated no clinically significant difference between Caucasian (n = 95) and African-American (n = 9) COPD patients, however, the effect of different ethnicity was not conclusively determined since majority were Caucasians.

Drug-drug interactions: Less than 30% of tiotropium dose is expected to be metabolized by cytochrome CYP 2D6 and probably CYP 3A4. Therefore, potential interactions with the

inhibitors of these two enzymes (e.g., quinidine, gestodene, ketoconazole) are expected. No clinical studies have been performed to evaluate these interactions. However, based on the low extent of overall metabolism of the drug (<30%) and low tiotropium plasma concentrations (0.01 and 0.05 nmol/L) after a dose of 18 µg dry powder inhalation, a clinically significant metabolic interaction is not anticipated. Higher tiotropium plasma concentrations are expected in the 2D6 poor metabolizers. Indeed AUC<sub>0-4h</sub> was 33% higher in the poor 2D6 metabolizers (there were 4 subjects in the Study 205.222) in comparison to the extensive 2D6 metabolizers, however, this change does not warrant the lower dosing regimen. In addition, *in vitro* metabolic study showed that high tiotropium concentrations of 1 µmol/L did not inhibit cytochrome P 450 1A1, 1A2, 2B1, 2C9, 2C19, 2D6, 2E1, or 3A4 in human liver microsomes.

As many cationic drugs, tiotropium is actively secreted into urine, therefore, there is a possible interaction with drugs which are also actively secreted into urine. However, there were no clinically significant changes in pharmacokinetics of tiotropium when cimetidine (400-mg tid) or ranitidine (300 mg qd) was co-administered with tiotropium.

Food effect: Clinically significant food effects are not expected for this hydrophilic drug with its low oral bioavailability of 2-3%.

Formulation development: Formulation of a dry powder inhalation capsule was changed (Phase I, II and III formulation) along with inhalation devices during the drug development. Dose finding trial (Study 205.108) used a developmental formulation and device (i.e., Phase II formulation with FO2 device) was used, but the to-be marketed formulation with the intended device (i.e., Phase III formulation with HandiHaler) was used in Phase III and pivotal Phase I studies. In Study 205.108, the urinary excretion was lower compared to that in Phase III study, such as Study 205.117 (approximately A<sub>e0-24h</sub> of 4.5 vs. 7% of dose).

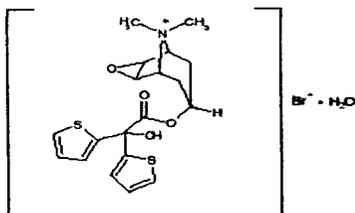
Analytical Methods: Tiotropium was quantified by the validated LC-MS/MS methods. The assay method was acceptable in terms of sensitivity and selectivity.

## 4. Question Based Review

### 4.1 General Attributes

#### 4.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product? What is the proposed mechanism of drug action and therapeutic indications? What is the proposed dosage and route of administration?

SPIRIVA™ consists of a hard gelatin capsule containing a dry powder for use with the HandiHaler<sup>®</sup> inhalation device. Each hard gelatin capsule contains 18 µg tiotropium (equivalent to 22.5 µg tiotropium bromide monohydrate) blended with lactose monohydrate as the carrier. The drug substance, tiotropium bromide (monohydrate) has a molecular mass of 490.4 and a molecular formula of C<sub>19</sub>H<sub>22</sub>NO<sub>4</sub>S<sub>2</sub>Br•H<sub>2</sub>O. It is sparingly soluble in water and soluble in methanol, and has the following structural formula;



Tiotropium bromide is developed as a long-acting anticholinergic bronchodilator, around 24 hrs of duration of action (*in vitro* study showed that tiotropium binds to all five muscarinic receptor subtypes and the dissociation from the  $m_3$  receptor seems to be slower than from  $m_1$  and  $m_2$  receptors). Ipratropium bromide is currently on the market as a short acting (6 hours of duration of action) bronchodilator. Tiotropium bromide is intended for the long-term maintenance treatment of bronchospasm and dyspnea associated with COPD including chronic bronchitis and emphysema with one capsule inhalation powder once-daily dosing regimen.

**4.1.2. What efficacy and safety information (e.g., biomarkers, surrogate endpoints, and clinical endpoints) contribute to the assessment of clinical pharmacology and biopharmaceutics study data (e.g., if disparate efficacy measurements or adverse event reports can be attributed to intrinsic or extrinsic factors that alter drug exposure/response relationships in patients)?**

Data from a study in asthma patients provided urinary excretion rates over a broader range of FEV<sub>1</sub> baseline values and suggested some minor influence of the disease progression on the urinary excretion (decreasing). This effect was also evaluated in COPD patients of different disease progression. There was no consistent effect of the decrease in lung function on tiotropium plasma concentrations or on the urinary excretion of tiotropium. Overall, the effect of the chronic pulmonary disease on the absorbed fraction of the inhaled dose is not exactly known, because this effect is hard to separate from the confounding effects of age on the urinary excretion.

**4.2. General Clinical Pharmacology**

**4.2.1 What is the basis for selecting the clinical-response endpoints (i.e., clinical or surrogate endpoints or biomarkers) and how are they measured in clinical pharmacology and clinical studies?**

The clinical pharmacology of tiotropium was evaluated in 22 completed clinical trials (reviewed 15 studies which contained the PK). The tolerability and bronchoactive properties of tiotropium in relation to dose were evaluated in healthy volunteers, COPD patients and asthma patients.

**Efficacy variables:** Tiotropium is an anticholinergic bronchodilator. The effects of tiotropium is evidenced by improvements in FEV<sub>1</sub> (Forced Expiratory Volume in one second), FVC (Forced Vital Capacity), PEER (Peak Expiratory Flow Rate) or decrease in airway resistance ( $R_{aw}$ ). In the clinical trials, the bronchodilator efficacy of inhaled tiotropium was primarily determined by trough FEV<sub>1</sub> response; defined as change from base line in trough FEV<sub>1</sub>; trough FEV<sub>1</sub> was calculated as the mean of the two FEV<sub>1</sub> readings prior to the first administration of study medication and at the end of the dosing interval. Secondary spirometric endpoints and the long term effectiveness includes FVC, PEER,  $R_{aw}$ , PEFs, COPD/asthma symptoms, physician's

global evaluation, albuterol use as needed, oral and inhaled steroid use, theophylline use, the number of awakenings, transitional Dyspnea Index (measure dyspnea) and quality of life measures collected over the treatment period. To assess the quality of life, the Impact score from the St. George's Hospital Respiratory Questionnaire (SGRQ) was used.

**Safety Measures:** Adverse events, fluctuation in the patient's COPD/asthma symptoms, Clinical and laboratory tests, ECG and vital signs were monitored.

**4.2.2 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess PK parameters and exposure response relationships?**

Only the parent compound, tiotropium, was measured in the plasma and urine. Tiotropium concentrations in plasma could only be measured up to 2 hrs post dose in most of the studies in healthy volunteers even with the adequate assay method (LC/MS/MS). This could be due to the high volume of distribution and the small dose. Therefore, PK parameters were estimated using the urine data. It should be noted that even urine data were not optimally collected in most of studies (e.g., urine was collected usually up to 4, 8, 24 hrs, not long enough compared to  $t_{1/2}$  of 5-6 days). There is only one study (Study 205.105) that measured urine up to 25 days after tiotropium dose.

**4.2.3. What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy and safety?**

The sponsor conducted a dose-ranging study (Study 205.108) that was used to select the dose for Phase III program (see section 4.2.3.4, page 9).

**4.2.3.1. Based on PK parameters, what is the degree of linearity or non-linearity in the dose- concentration relationship?**

Dose proportionality was evaluated using the data after iv infusion, oral administration and dry powder inhalation of tiotropium.

*Intravenous doses:* As shown in Table 1, there was a trend to increased urinary excretion after doses of 2.4, 4.8, 9.6 and 14.4 µg tiotropium. A similar trend was observed for plasma concentrations (Study 205.107).

**Table 1. Geometric mean (% gCV): data from Study 205.107 after single iv infusion**

Dose	2.4 µg	4.8 µg	9.6 µg	14.4 µg
$A_{e0-24h}$ (% of dose)	39.3 (7)	42.0 (17)	46.0 (6)	54.3 (4)
$C_{15min}$ (pg/mL) <sup>a</sup>	322.8 (9)	375 (16)	306 (24)	390 (22)

<sup>a</sup>Dose normalized to 14.4 µg

*Oral doses:* Tiotropium plasma concentrations were regularly below the limit of quantification and are therefore not discussed. Urinary excretion seemed to increase slightly with higher doses; 0.72%, 0.84%, 0.69% and 0.99% of dose were renally excreted over 24 hours for 8, 16, 32 and 64 µg respectively (Study 205.106).

*Inhalation doses:* The geometric mean urinary excretion data ( $A_{e0-4h}$ ) after a single and multiple doses are summarized in Table 2.

**Table 2.** Geometric mean % of tiotropium dose excreted unchanged in urine in the interval 0-4 h after dry powder inhalation by young healthy subjects

Study	8.8 µg	17.6 µg	35.2 µg	70.4 µg	108 µg	141 µg	282 µg
<b>Single dose</b>							
205.102	--	--	1.88	1.67	--	2.36	3.19
205.103	--	--	--	1.33	--	1.78	--
205.104	1.34	1.61	1.31	--	--	--	--
205.105	--	--	--	--	1.84	--	--
<b>Multiple dose</b>							
205.104	3.13	3.63	4.28	--	--	--	--
205.103	--	--	--	4.03	--	4.22	--

Note: multiple dose measured on Day 14 (Study 205.104) and 7 (Study 205.103)

As show in Table 2, there is a trend to higher urinary excretion after the higher doses. A similar trend was observed for urinary excretion, such as, 10.5%, 12.1% and 14.7% of the dose were excreted unchanged within 24 hours after doses of 8.8, 17.6 and 35.2 µg tiotropium measured on Day 14, respectively (Study 205.104). The reason(s) for this trend is not known. The sponsor speculates that an easier elimination could occur from the binding sites (including muscarinic receptors) for the higher doses due to saturation of the binding sites. Dose proportionality within the therapeutic range can not be determined due to insufficient data.

**4.2.3.2. Do PK parameters change with time following chronic dosing?**

No, the second once daily tiotropium inhalation dose (at a low dose range) generates consistently slightly higher AUC values than expected from the first dose (also seen it after intravenous infusion in Study 205.107). The reason for this finding is not know, however the sponsor suggested that this might be due to incomplete saturation of binding sites (including muscarinic receptors) after the first dose and a very slow dissociation constant of the tiotropium binding site complex. Once all binding sites are at least near to saturation, more tiotropium can escape from the tissue and the drug appears faster in the systemic circulation, which leads to higher systemic plasma concentrations. Urinary excretion indicated an accumulation by the factor 2-3 from first to the fourteenth inhalation.

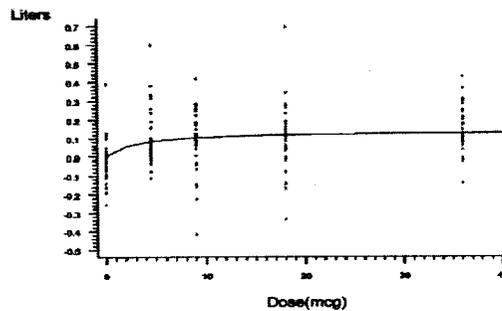
**4.2.3.3. How long is the time to the onset and offset of the pharmacological response or clinical endpoint?**

See Figure 2 on page 10 for study that measure pharmacodynamic endpoint (FEV<sub>1</sub>).

**4.2.3.4. Are the dose and dosing regimen consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?**

Dose-Response relationship (Efficacy): A dose ranging study with a four-week multiple dose design (Study 205.108) showed that tiotropium administered once a day by oral inhalation via the FO<sub>2</sub> inhalation device over a range of doses (4.4, 8.8, 17.6 and 35.2 µg) was considered safe and effective. E<sub>max</sub> model was fitted to the dose-response data (Figure 1). As shown in Figure 1, there is no apparent relationship (may be trends) between dose and response (FEV<sub>1</sub>). Nevertheless, this study and two other dose-ranging studies (Study 205.119 and Study 205.120) provided support for a selection of 18 µg dose of tiotropium for the phase III program.

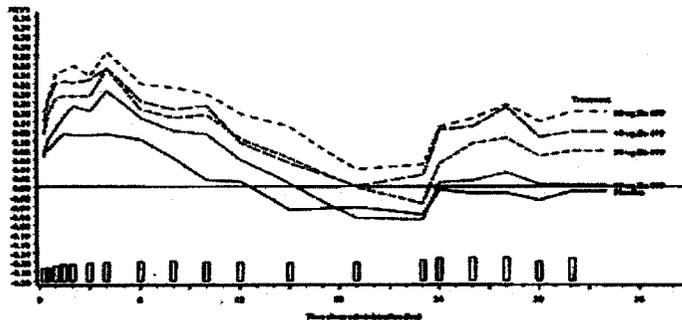
Figure 1.  $E_{max}$  Model of Dose-Response Data (205.108)



The relationship between the pharmacokinetics of tiotropium and the efficacy or safety variables was not explored in this submission. Safety parameters (see page 8 under 4.2.1 question) were measured at various times throughout the trial.

2<sup>nd</sup> Peak in Time-Response data: Approximately 24 hrs after a single dose inhalation of tiotropium, response (measured by mean FEV<sub>1</sub>) was increased (2<sup>nd</sup> peak) in dose ranging studies (Figure 2). The 2<sup>nd</sup> peak was shown also in patients who received a placebo (smaller peak compared to the patients who received tiotropium). This 2<sup>nd</sup> peak could not be attributed to PK of tiotropium (no active metabolites, no enterohepatic recirculation) or to the intrinsic/extrinsic factors. It could be, as the sponsor suggested, due to the circadian rhythm.

Figure 2. Increase in Mean FEV<sub>1</sub> (L)



**4.2.4. How does the PK of tiotropium in healthy volunteers compared to that in patients following dry powder inhalation? What are the basic PK parameters? Is this a high extraction ratio or a low extraction ratio drug?**

Pharmacokinetics after intravenous doses: Tiotropium was infused in doses ranging from 2.4 to 14.4  $\mu$ g over 15 minutes in healthy male subjects (Studies 205.105, 107 and 134). Tiotropium plasma concentrations at the end of the infusion ( $C_{max}$ ) reached dose-normalized (to 18  $\mu$ g) values of 320-440 pg/mL for 14.4  $\mu$ g depending on the subject group tested. Study 205.105 provided the most complete pharmacokinetics of tiotropium among studies conducted (in this study, tiotropium was administered also by inhalation as well as orally): the mean (% gCV) values of volume of distribution ( $V_{ss}$ ), total clearance, renal clearance, half-life and the residence time of tiotropium after 14.4  $\mu$ g iv dose are of 32 L/kg (27.8% gCV), 880 mL/min (22.1% gCV), 669 mL/min (16.5% gCV), 5.7 days (26% gCV) and 50.6 hrs (31.5% gCV), respectively. Urinary excretion as an unchanged tiotropium was 73.6% in young healthy subjects after intravenous

infusions and 14.4% after dry powder inhalation. Urinary excretion in the first 4 (or 24) hours was already 43.8% (53.6%) of the dose (thus 59% and 72% of the total urinary excretion are achieved within 4 hours and 24 hours). The long  $t_{1/2}$  was not explained by the binding of tiotropium to erythrocytes, but expected due to binding of tiotropium to tissues.

**Table 3.** Geometric mean (% gCV) tiotropium PK parameters after intravenous infusion of 14.4 µg, dry powder (DP) inhalation of 108 µg or oral solution of 64 µg tiotropium to different groups of twelve healthy male subjects,

		n	group comparison (n=12)					
			intraven. 14.4 µg		DP inhalation 108 µg		oral solution 64 µg	
			gMean	%gCV	gMean	%gCV	gMean	%gCV
$C_{max}$	[pg/mL]	11/11/12	378	25.4	65.4	58.0	2.35&	87.7
$t_{max}$		~11/5	#	---	5 min	##	§ 2 h	§ 1-8 h
$NC_{max}$	[pg/mL] for 18 µg	11/11/12	473	25.4	10.9	58.0	0.66	87.7
$AUC_{0-2h}$	[pg.h/mL]	9/11/-	143	18.7	39.9	38.7	--	--
$AUC_{0-4h}$	[pg.h/mL]	9/11/-	161	19.4	61.7	34.1	--	--
$AUC_{0-8h}$	[pg.h/mL]	5/11/-	186	25.0	92.6	28.4	--	--
$NAUC_{0-2h}$	[pg.h/mL] for 18 µg	9/11/-	179	18.7	6.65	38.7	--	--
$NAUC_{0-4h}$	[pg.h/mL] for 18 µg	9/11/-	201	19.4	10.3	34.1	--	--
$NAUC_{0-8h}$	[pg.h/mL] for 18 µg	5/11/-	233	25.0	15.4	28.4	--	--
$Ae_{0-4h}$	[% of dose]	11/10/9	43.8	10.2	1.84	40.7	0.39	39.8
$Ae_{0-8h}$	[% of dose]	11/11/9	48.0	10.6	2.83	33.6	0.67	30.1
$Ae_{0-24h}$	[% of dose]	11/8/9	53.6	11.4	4.99	22.4	1.04	31.2
$Ae_{0-96h}$	[% of dose]	11/6/9	61.0	11.7	8.93	9.4	1.60	30.0
$Ae_{0-\infty}$	[% of dose]	11/9/9	73.6	13.7	14.4	7.8	--	--
F	[%]	(11)/9/9	--	--	19.5	--	2.6	--
$CL_r$ (0-4h)	[mL/min]	8/11/-	669	16.5	486	14.0	--	--
$t_{1/2}$	[h]	11/11/-	137	26.0	116	16.1	--	--
$t_{1/2}$	[days]	11/11/-	5.71	26.0	4.84	16.1	--	--
Parameters calculated from plasma concentrations that were obtained from urinary excretion data via renal clearance (only values > BLQ):								
$AUC_{0-\infty}$	[pg.h/mL]	8/9/-	273	22.1	518	16.6	--	--
$NAUC_{0-\infty}$	[pg.h/mL] for 18 µg	8/9/-	341	22.1	86.3	16.6	--	--
CL, CL/f	[mL/min]	8/9/-	880	22.1	3474	16.6	--	--
$V_{ss}$	[L]	8/-/-	2665	27.8	--	--	--	--
$V_z$	[L/kg]	8/-/-	138	27.6	--	--	--	--
$MRT_{dis/tot}$	[h]	8/9/-	50.6	31.5	110	18.2	--	--

# at the end of infusion (15 min).

## always in the first plasma sampling 5 min post dose, § median and range

& values below — replaced by 1/2 this LOQ. Source data in U99-1315

**Pharmacokinetics after inhalation:** Tiotropium was rapidly absorbed after inhalation regardless the inhalation device used in any studies. The earliest blood sample was always scheduled 5 minutes after inhalation and this plasma sample contained with a few exceptions always the highest tiotropium concentration ( $C_{max}$ ) of the complete profile, suggesting a very rapid absorption. Then, tiotropium concentrations fell rapidly afterwards in a polyexponential (the sponsor used 4-5 compartment model) manner and were usually not quantifiable after 2 to 8 hours post inhalation, while urinary excretion was quantifiable for days after a single inhalation. Urinary excretion rates declined polyexponentially until they reached an apparent terminal

elimination phase at about 96 hours post dose. Urinary excretion after a single dose ( $Ae_{0-\infty}$ ) was 14.4% (7.8% gCV) for a dose of 108  $\mu$ g in Study 205.105. This was the only study which collected urine long enough for a reliable calculation. The terminal elimination half-life was 4.84 days in the Study 205.105 and geometric mean values ranged from 5 to 7 days in other studies. The pharmacokinetic parameter values from Study 205.105 are shown in Table 3.

PK parameters from individual studies following a single and multiple doses of tiotropium by inhalation in healthy subjects and patients with COPD or asthma are summarized in Table 4.

**Table 4.** Tabular overview of geometric mean (% gCV) PK parameters of individual single or multiple dose tiotropium studies after inhalation doses in healthy subjects (shaded area) and patients with COPD or asthma ( $C_{5min}$  and  $AUC_{0-2h}$  are dose normalized to 18  $\mu$ g).

Study	Dose ( $\mu$ g)	$C_{5min}$ (pg/mL)	$AUC_{0-2h}$ (pg•h/mL)	$Ae_{0-4h}$ % of dose	$Ae_{0-24h}$ % of dose	CLr (mL/min)	$t_{1/2}$ (days)
Single Dose							
205.103	70.4	8.61 (63)	6.4 (64)	1.33 (54)	-	-	-
	141	13.7 (75)	9.4 (58)	1.78 (62)	-	-	-
205.104	8.8	-	-	1.34 (35)	3.30 (27)	-	-
	17.6	15.1 (94)	-	1.61 (65)	4.64 (48)	-	-
	35.2	6.9 (32)	-	1.31 (50)	3.70 (6)	-	-
205.105	108	10.9 (58)	6.65 (39)	1.84 (41)	4.99 (22)	486 (14)	4.8 (16)
205.108	8.8	-	-	-	-	-	-
	17.6	6.53 (44)	-	-	-	-	-
	35.2	10.07 (80)	-	-	-	-	-
205.120	8.8	-	-	0.85 (89)	-	-	-
	17.6	-	-	1.50 (55)	-	-	-
	35.2	7.52 (21)	-	1.70 (27)	-	-	-
	70.4	8.57 (56)	-	1.69 (25)	-	-	-
205.133	18 <sup>a</sup>	4.87 (69)	5.99 (28)	0.61 (143)	-	-	-
	18 <sup>b</sup>	7.06 (83)	8.12 (46)	0.66 (66)	-	-	-
205.201	18	-	-	0.53 (66) <sup>c</sup>	-	-	-
	36	-	-	0.92 (105) <sup>c</sup>	-	-	-
Multiple Dose							
205.103	70.4	16.4 (49)					
	141	25.4 (57)					
205.104	8.8	16.2 (50)	-	3.13 (50)	10.5 (26)		5.8 (23)
	17.6	25.1 (58)	-	3.63 (39)	12.1 (20)		7.7 (31)
	35.2	16.5 (16)	16.4 (7)	4.28 (25)	14.7 (14)	407 (8)	6.0 (21)
205.108	8.8	13.5 (39) <sup>d</sup>			4.8 (28) <sup>d</sup>		
	17.6	7.68 (36) <sup>d</sup>			3.97 (48) <sup>d</sup>		
	35.2	18.31(35) <sup>d</sup>			4.75 (38) <sup>d</sup>		
205.117	18	15.3 (63) <sup>e</sup>	-	2.02 (52) <sup>c</sup>	6.63 (66)	-	-
		19.2 (73) <sup>f</sup>	-	1.46 (82) <sup>c</sup>	7.01 (38)	-	-
		16.1 (72) <sup>g</sup>	-	1.32 (54) <sup>c</sup>	6.95 (45)	-	-
		19.0 (45) <sup>h</sup>	-	0.98 (77) <sup>c</sup>	7.43 (59)	-	-
205.133	18 <sup>a</sup>	9.63 (142)	10.8 (84)	1.97 (74)	-	326 (60)	5.5 (29)
	18 <sup>b</sup>	15.3 (60)	15.7 (67)	1.42 (89)	-	163 (93)	6.5 (29)
205.201	18	-	-	1.19 (122) <sup>c</sup>	-	-	-
	36	-	-	2.15 (61) <sup>c</sup>	-	-	-

<sup>a</sup>Younger patients (45-58 yrs age)      <sup>b</sup>Older patients (69-80 yrs age)

<sup>d</sup>day 22      <sup>e</sup>40-49 yrs

<sup>f</sup>50-59 yrs

<sup>g</sup>60-69 yrs

<sup>c</sup> $Ae_{0-2h}$

<sup>h</sup>>69 yrs

*Single dose:* Geometric mean tiotropium plasma concentrations 5 minutes after ( $C_{5min}$ ) a single 17.6 µg dose in healthy young male subjects were 14.8 pg/mL (94.3% gCV) in Study 205.104 and 10.9 pg/mL (58% gCV) in Study 205.105. In COPD patients, calculated  $C_{5min}$  of tiotropium was 4.9-7.1 pg/mL (69-83% gCV) after a single dose (Study 205.133).

Urinary excretion of tiotropium were 1.61% of dose (64.8% gCV) in the interval 0-4 h and 4.64% (47.6% gCV) in the fraction 0-24 h in Study 205.104, while those values in Study 205.105 were 1.84% (40.7% gCV) and 4.99% (22.4% gCV), respectively. In COPD patients, urinary excretion of tiotropium showed lower values with 0.61-0.66% in the 4 h interval (Study 205.133).

A comparison of  $Ae_{0-4h}$  (1.84%) and  $Ae_{0-24h}$  (4.99%) values with the total urinary excretion (14.4%) in Study 205.105 showed a different behavior in contrast to the excretion after an intravenous dose. About 12.8% (= 1.84/0.144) and 34.7% (= 4.99/0.144) of the total excretion was complete 4 and 24 hours after inhalation. This is much less than for infusion with 59.5% and 72.8% for the same time intervals. There was therefore a clear difference in tiotropium disposition between intravenous infusion and inhalation. The reason is not known, however, as the sponsor suggested, this could be due to a more pronounced tissue binding sites in the lung after inhalation. The fraction of an intravenous infusion, which gets access to lung tissue is smaller and the load for the kidney becomes much higher, which results in a faster excretion. The longer mean residence time of 110 h (18.2% gCV) after inhalation vs 50.6 hours (31.5% gCV) after infusion fits in this view.

*Multiple doses:* Pharmacokinetic profiles after 7 (Study 205.103 and 133) or 14 days (Study 205.104 and 133) were investigated in Phase I studies. Urinary excretion in asthma patients was also studied after 21 days in Study 205.201. The following results are summarized based on these studies:

- The accumulation factor did not exceed the 2-3 despite a terminal elimination half-life of 5-7 days and a once daily dosing regimen. This suggests that long terminal elimination half-life is not dominant (Study 205.103). It was shown that there was no further accumulation with continued tiotropium inhalation (e.g. over weeks and months). Peak as well as trough plasma concentrations and urinary excretion remained approximately constant over months once a steady state was achieved (Study 205.117).
- Based on  $t_{1/2}$  of 5-7 days, steady state conditions are expected to be reached 3-5 weeks of continued treatment and such a long treatment time was only reached in the 4-week Study 205.108 and the 1-year safety and efficacy studies (Study 205.114/117). However, the scatter in the data does often not allow differentiating between data after 7 and 14 days treatment. Thus approximate steady state conditions is achieved after 7-14 days treatment.
- Geometric mean tiotropium concentrations in healthy subjects 5 minutes after a 17.6 µg tiotropium dose given for 14-days inhalation were 24.6 pg/mL (58% gCV) after a 17.6 µg tiotropium dose given for 14-days (Study 205.104). Corresponding values in COPD patients were 9.63 pg/mL (142% gCV) and 15.3 pg/mL (60.0% gCV) in younger (mean 53 years of age) and older patients (mean 74 years of age), respectively (Study 205.133). In COPD patients (Study 205.117),  $C_{5min}$  and  $C_{-5min}$  (trough) at true steady state conditions (e.g., measurements on Day 50 or 92) were about 18.6 and 5.8 pg/mL, respectively. 24-h urine samples at steady state conditions (Day 50) showed 7.01% of dose (62.6% gCV) for female and 7.12% of dose (38.2% gCV) for male patients were excreted in urine (Study 205.117). Therefore, this data suggests that steady state conditions are not much different from the status achieved within 2 weeks of treatment.
- Urinary excretion of tiotropium in healthy subjects reached 3.63% of dose (39.1% gCV) in the interval 0-4 h after two weeks and 12.1% of dose in the interval 0-24 h (Study 205.104). Corresponding values in COPD patients for the interval 0-4 h were 1.42% (88.7% gCV) to 1.97% (74.4% gCV) in older and younger patients of Study 205.133, respectively.

*Absolute bioavailability:* Absolute bioavailability was 19.5% after an inhalation (Study 205.105). The respective value for an oral dose is between 2% and 3%. This means that about 17% of the inhaled dose reached the lung, while up to 83% were swallowed (assume full bioavailability from the lung).

Overall summary: Tiotropium is a quaternary amine and it is not readily absorbed into the systemic circulation. This was confirmed by the low bioavailability of 2-3% for oral solutions in young healthy subjects, while tiotropium showed an absolute bioavailability of 19.5% in these subjects. Urinary excretion of unchanged drug was 73.6% and 14.4% ( $Ae_{0-\infty}$ ) of the dose after an intravenous and inhalation dose respectively in healthy subjects and 7% ( $Ae_{0-24h}$ ) after inhalation of tiotropium in COPD patients. The drug has a high volume of distribution of 32 L/kg, a total clearance of 880 mL/min and a terminal elimination half-life of 5-6 days. The renal clearance of tiotropium (669 mL/min after an iv dose) exceeds the creatinine clearance, which suggests the presence of active secretion into kidney tubules. After multiple administration, (approximate) steady state was reached after 2-3 weeks with an accumulation factor of about two to three. Pharmacokinetics linearity of tiotropium could not be confirmed due to insufficient data.

Plasma concentrations (e.g.,  $C_{5min}$ , AUC) and urinary excretion of tiotropium in urine were lower in patients with COPD or asthma compared those in healthy subjects (Table 3). Absorption of the drug could be affected by the Disease State (COPD/asthma), however, it is not exactly known, because this effect is hard to separate from the confounding effects of age on the urinary excretion.

#### **4.2.5. Does mass balance suggest renal or hepatic the major route of elimination?**

A mass balance study of tiotropium in humans was not conducted. The sponsor stated that this was not possible due to the combination of analytical problems and the PK characteristics of tiotropium (i.e., large  $V_{ss}$ , long  $t_{1/2}$ , metabolism play a minor role in the excretion of tiotropium, inhalation route of administration, etc.). However, following iv infusion 73.6% of the dose is excreted in urine as unchanged drug. The remaining 25% of the dose undergo nonenzymatic hydrolysis and CYP 450 metabolism (see section 4.4.2, page 17).

### **4.3 Intrinsic Factors**

#### **4.3.1 What are the relevant covariates that influence the pharmacokinetic variability of tiotropium?**

Pharmacokinetics in elderly subjects with COPD: Study 205.133 evaluated age factor on PK of tiotropium in patients with COPD, and the results are summarized in Table 5. Renal clearance of tiotropium was significantly lower in the elderly patients (163 mL/min) compared with younger patients (326 mL/min).  $C_{5min}$  and  $AUC_{0-4h}$  were 59% and 43% higher, respectively, in the elderly than the younger COPD patients (Day 14). However, age factor on the PK of tiotropium can not be confirmed due to the confounding factor of old age (compromised renal function). Table 6 lists multiple dose pharmacokinetic parameters in subjects of various age groups.

**Table 5.** Geometric mean PK parameters in elderly (69-80 yrs) and young (45-58 yrs) patients

		elderly patients		young patients		ratio
		gMean	95% CI	gMean	95% CI	
<b>Day 1</b>						
$C_{5min}$	pg/mL	7.06	3.68-13.6	(4.87)	2.71-8.74	(1.43)
$AUC_{0-24}$	pg h/mL	(13.7)	(10.4-18.0)	(11.2)	(9.34-13.4)	(1.22)
$Ae_{0-24}$	% of dose	0.661	0.384-1.14	0.606	0.224-1.64	1.09
$CL_{ren}$	mL/min	(141)	83.2-239	(162)	62.9-417	(0.870)
<b>Day 7</b>						
$C_{5min}$	pg/mL	13.2	6.76-25.8	11.6	4.86-27.7	1.14
$AUC_{0-24}$	pg h/mL	21.8	14.3-33.3	17.9	10.7-29.9	1.22
$Ae_{0-24}$	% of dose	1.42	0.936-2.15	1.61	0.704-3.68	0.882
$CL_{ren}$	mL/min	194	113-333	268	136-527	0.724
<b>Day 14</b>						
$C_{5min}$	pg/mL	15.3	9.27-25.3	9.63	3.58-25.9	1.59
$AUC_{0-24}$	pg h/mL	26.1	15.5-43.9	18.2	10.1-32.8	1.43
$Ae_{0-24}$	% of dose	1.42	0.713-2.83	1.97	1.03-3.68	0.721
$CL_{ren}$	mL/min	163	79.9-333	326	193-550	0.500
<b>Overall</b>						
$t_{1/2}$	days	6.5	4.93-8.37	5.5	4.16-7.27	1.18

Note: (1) Elderly = mean age of 74 years (range 69-80 years); Young = mean age of 53 years (range 45-58 years). (2) Drug plasma concentrations below  $LOQ$  were replaced by  $\frac{1}{2}$  the  $LOQ$  to calculate PK parameters. Parameter values with a high incidence of replaced values were set into brackets.

**Table 6.** Multiple dose tiotropium pharmacokinetic parameters (geometric means) for dry powder inhalation and different age groups

study	age [yrs] *	dose	n	$C_{5min}$		$C_{2h}$		$Ae_{0-24}$		$Ae_{0-24h}$	
				[pg/mL]	%CV	[pg/mL]	%CV	[% of dose]	%CV	[% of dose]	%CV
205.105	28-42	108	-/-/+/9	--	--	--	--	--	--	14.4 &	8
205.104	24-45	17.6 §	2/-/5/5	25	58	--	--	3.63	39	12.1	20
205.104	24-45	35.2 §	3/4/5/5	17	16	4.4	22	4.28	25	14.7	14
205.117#	40-49	18	7/9/-/6	15	63	7.9	30	--	--	6.63	66
205.133	45-58	18	12/12/12/-	10 §	142	4.0 §	68	1.97	74	--	--
205.117#	50-59	18	20/24/-/23	19	73	8.2	52	--	--	7.01	38
205.117#	60-69	18	38/39/-/42	16	72	7.8	42	--	--	6.95	45
205.133	69-80	18	13/13/13/-	15 §	60	5.7 §	69	1.42	89	--	--
205.117#	70-85	18	19/24/-/28	19	45	12	48	--	--	7.43	59

§: BQL replaced by  $\frac{1}{2}$  BQL

&: single dose, to strengthen the data base for young subjects,  $Ae_{0-24h}$  corresponds to  $Ae_{0-24h}$  in steady state

§: normalized to a 18  $\mu$ g dose

#: data for Day 50

\* range

**Pharmacokinetics in subjects with renal impairment:** PK of tiotropium was compared in four different groups of subjects with normal to severe renal impairment following an intravenous dose of 4.8  $\mu$ g (Study 205.134). The results are summarized in Table 7.

The effect of a renal insufficiency on tiotropium plasma concentrations after inhalation was also evaluated in Study 205.117 (COPD patients). Trough tiotropium plasma concentrations ( $C_{5min}$ ) at steady state (Day 92) increased by about 10% and 27% in mild and moderate impairment, respectively, compared to patients with normal renal function.  $C_{5min,ss}$  (Day 92) increased by 90% and 188% in mild and moderate impairment, respectively, compared to patients with normal renal function. Increase in tiotropium plasma concentrations was associated with a decrease of  $Ae_{0-24h,ss}$  0.3% and 33% in mild and moderate renal impairment, respectively, compared to patients with normal renal function. Therefore tiotropium should be used with caution in patients with renal impairment, especially those with moderate and severe impairment.

**Table 7.** Geometric mean (% gCV) tiotropium pharmacokinetic parameters after intravenous infusion of 4.8 µg tiotropium to subjects with varying degrees of renal impairment.

	CL <sub>CR</sub> [mL/min]		C <sub>max</sub> [pg/mL]	AUC <sub>0-4 h</sub> [pg.h/mL]	Ae <sub>0-4h</sub> [% of dose]	Ae <sub>0-∞</sub> [% of dose]	t <sub>1/2</sub> [days]	CL <sub>ren</sub> [mL/min]
>80 mL/min n=6	108	gMean (%gCV)	147 (21.3)	55.5 (16.2)	30.2 (11.4)	60.1 (17.7)	4.03 (19.1)	435 (12.7)
50-80 mL/min n=5	70.4	gMean (%gCV)	200 (30.1)	77.1 (20.1)	23.7 (20.1)	59.3 (14.4)	5.02 (45.1)	246 (34.8)
30-50 mL/min n=7	44.1	gMean (%gCV)	223 (26.5)	101 (29.8)	15.1# (31.4)	39.9 (34.5)	3.96 (32.3)	124 (29.9)
<30 mL/min n=6	23.5	gMean (%gCV)	223 (17.5)	108 (27.3)	11.0* (14.6)	37.4 (10.2)	5.95 (29.3)	85.7 (35.5)

#: n=5, \*: n=3

**Pharmacokinetics in subjects with hepatic impairment:** No study was performed in patients with hepatic impairment. Tiotropium was predominantly cleared by renal elimination as a parent drug (~70% of the dose is excreted in urine in healthy young subjects), therefore, approximately 30% of dose are expected to be eliminated as metabolites. Tiotropium was degraded by nonenzymatic ester cleavage (U98-2865). Also, tiotropium was metabolized by CYP 2D6 and probably by CYP 3A4. Therefore, there is possible interaction with drug(s) that are substrate of CYP 2D6 and 3A4, such as quinidine and ketoconazole, and concern for CYP 2D6 poor metabolizers. Four subjects from the study 205.222 were identified (by genotype). AUC<sub>0-4h</sub> was 33% higher in the poor 2D6 metabolizers in comparison to the extensive 2D6 metabolizers.

However, overall, based on the low extent of overall metabolism of the drug (<30%) and low tiotropium plasma concentrations (0.01 and 0.05 nmol/L) after 18 µg dry powder inhalation dose, a clinically significant change due to metabolic interaction or hepatic dysfunction is not anticipated. Similarly, PK change shown in poor 2D6 metabolizers does not warrant the lower dosing regimen.

**Pharmacokinetics in subjects of different human races:** Urinary excretion data in Study 205.201 indicated no clinically significant difference between Caucasian and African-American COPD patients. However, the majority of patients were Caucasians (95 Caucasians vs 9 African-Americans), therefore, the ethnicity factor is not conclusively confirmed.

**PK in Pediatric patients:** Pharmacokinetic data in subjects under an age of 18 years are not available. Tiotropium inhalation powder was intended for the maintenance treatment of bronchospasm and dyspnea associated with COPD including chronic bronchitis and emphysema. Since the disease being treated is typically found in older patients, the lack of data in subjects with an age of less than 18 years is not considered to be an issue.

#### 4.4 Extrinsic Factors

##### 4.4.1. What are the extrinsic factors (drugs, herbal products, diet, smoking, and alcohol) influence exposure and/or response of tiotropium?

The influence of above mentioned extrinsic factors on the PK and/or PD were not evaluated.

##### 4.4.2. Is there an *in vitro* basis to suspect *in vivo* drug-drug interactions?

Some of the administered dose (<30%) of tiotropium is metabolized by cytochrome CYP 2D6 and probably CYP 3A4. Therefore, there may be potential interactions with inhibitors of

these two enzymes (e.g., quinidine, gestodene, ketoconazole). No clinical studies have been performed to evaluate the interactions. However, based on the low extent of overall metabolism of the drug (<30%) and low tiotropium plasma concentrations (between — nmol/L) after a dose of 18 µg by dry powder inhalation, clinically significant metabolic interaction is not anticipated.

Since mass balance study in human was not conducted, metabolism was investigated using *in vitro* studies (and *in vivo* animal studies). *In vitro* metabolism of tiotropium was investigated in human liver microsomes and in liver microsomes from mice, rats, and dogs to compare the metabolic pattern between species (U99-1348). The results from this study are summarized as follows:

- Ba 679 BR is metabolized by CYP, and the site of metabolic attack was the dithienylglycolic acid moiety.
- The metabolic pathways leading to the metabolites observed *in vitro* are oxidation in the thiophene ring systems, glutathione conjugation and oxidative cleavage of thiophene ring systems.
- Enzymatic cleavage of the ester linkage either by esterases or by CYP does not occur.
- Use of enzyme specific chemical inhibitors (e.g., ketoconazole, quinidine), recombinant CYPs, and correlation analysis showed the involvement of CYP 3A4 (minor) and CYP 2D6 (dominant) the metabolism of Ba 679 BR.
- These principal pathways were also observed with rat and human hepatocytes.
- Metabolism is marginal in rat lung microsomes compared to rat liver microsomes.

Stability of tiotropium bromide in plasma was evaluated *in vitro*. Tiotropium bromide is stable in acidic aqueous solutions (pH 2). The hydrolytic cleavage becomes more rapid with increasing pH and had a hydrolysis half-life of 17 h at 37 °C in pH 7.4 plasma as well as in 0.1 M phosphate buffer pH 7.25 (U91-0236). The hydrolysis of the ester bond was temperature-sensitive, as the rate of hydrolysis was threefold lower at 25°C (U91-0236).

The possible involvement of esterases as well as the possible species specificity of tiotropium cleavage in plasma was investigated in EDTA-plasma of mice, rats, dogs, rabbits and humans (U98-2865). Neither physostigmine, paraoxon, PMSF nor PCMB (esterase inhibitors) influenced the hydrolysis of tiotropium bromide.

\_\_\_\_\_, had also no effect on tiotropium bromide hydrolysis. The results indicated that plasma esterase enzymes did not contribute to the hydrolysis of tiotropium bromide. This study showed that hydrolytic cleavage of the ester bond of tiotropium (formed to N-methylscopine and dithienylglycolic acid) was occurred in plasma, therefore, it occurred via nonenzymatic reaction. Inhibition of CYP450 by tiotropium was investigated (U97-2651). The results showed that tiotropium (used concentrations of 1 µmol/L) did not inhibit cytochrome P 450 1A1, 1A2, 2B1, 2C9, 2C19, 2D6, 2E1, or 3A4 in human liver microsomes.

In summary, metabolism plays a minor role in the elimination of the drug from the body (73.6% renal excretion of unchanged drug after an iv dose). It showed that hydrolytic cleavage of the ester bond of tiotropium occurred in plasma (formed to N-methylscopine and dithienylglycolic acid). A (minor) amounts are metabolized by the cytochrome CYP 450 2D6 and probably CYP 450 3A4 involving the formation of a variety of glutathione conjugates after oxidation of the thiophen ring system and N-methylscopine.

### 4.4.3. Is the drug an inhibitor and/or an inducer of CYP enzymes?

*In vitro* study showed that high tiotropium concentrations of 1 µmol/L did not inhibit cytochrome P 450 1A1, 1A2, 2B1, 2C9, 2C19, 2D6, 2E1, or 3A4 (U97-2651) in human liver microsomes.

### 4.4.4. Are there other metabolic/transporter pathways that may be important?

Interactions via p-glycoprotein: Tiotropium may be secreted into the gastrointestinal tract (like in animals) as well as active secretion into the kidney, then, there is possible interactions with p-glycoprotein (i.e., p-glycoprotein may inhibit tiotropium secretion into gut and the renal proximal tubules). Thus it was investigated in CaCo2 cells *in vitro*, whether cyclosporine (well known competitive inhibitor of p-glycoprotein) did change tiotropium uptake in CaCo2 cells or not. No effect was found, thus, it appears that tiotropium is not dependent on p-glycoprotein to be transported to the kidney (U00-1350).

Interactions via renal elimination: Since tiotropium is expected to be actively secreted by the renal tubule (because tiotropium renal clearance is relevantly high compared to the creatinine clearance), interaction study was carried out to elucidate the effect of concomitant administration of tiotropium (iv infusion) with cimetidine (400 mg tid)/ranitidine (300 mg qd), which are also actively secreted (Study 205.222). As shown in Table 8, no clinically significant interaction occurred between thiotropium and cimetidine or ranitidine.

**Table 8.** Summary of geometric mean (% gCV) PK parameters

Tiotropium 14.4 µg		with cimetidine			tiotropium alone			Point estimate	90% CI
		gMean	%gCV	n	gMean	%gCV	n		
AUC <sub>0-4h</sub>	pg.h/mL	304	26.0	6	253	23.3	6	1.20	1.03-1.4
C <sub>max</sub>	pg/mL	664	26.8	6	635	10.6	6	1.05	0.8-1.37
Ae <sub>0-96h</sub>	% dose	47.2	15.5	5	48.2	14.8	5	-	-
CLr	mL/min	277	26.4	5	355	19.6	5	-	-
Tiotropium 14.4 µg		with ranitidine			tiotropium alone			Point estimate	90% CI
		gMean	%gCV	n	gMean	%gCV	n		
AUC <sub>0-4h</sub>	pg.h/mL	254	19.6	12	256	19.4	12	0.99	0.9-1.08
C <sub>max</sub>	pg/mL	596	24.1	12	683	16.3	12	0.87	0.73-1.04
Ae <sub>0-96h</sub>	% dose	50.4	11.1	11	50.6	9.7	11	-	-
CLr	mL/min	343	28.0	11	342	25.7	11	-	-

## 4.5 General Biopharmaceutics

### 4.5.1. Based on BCS principle, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

The aqueous solubility of the compound is about — mg/ml at room temperature, independent of pH. The drug substance is more soluble in polar organic solvents, such as methanol — but practically insoluble in — Tiotropium is a quaternary ammonium compound and was poorly absorbed from the gastrointestinal tract (oral bioavailability is about 2-3%), but was well absorbed from the lung. As per the sponsor suggested, the difference might be due to the fact that absorption via the gut requires penetration of several cell layers with tight junctions, while absorption via the lung is facilitated by the very few membranes that have to be penetrated to reach the systemic circulation. Moreover, a variety of efficient transporters in the gut pump drugs out of the cells in the gut lumen thereby eliminating the drug from the body and

reducing net absorption. Since tiotropium is inhalation route of administration, dissolution is not relevant.

**4.5.2. Has the proposed commercial formulation and device been adequately linked to the Phase III clinical trial formulation and device?**

Tiotropium was administered in several formulations throughout the clinical development including intravenous infusions, oral solutions, and solutions for dispersion by a piezoelectric device as well as by dry powder inhalation from inhalation capsules.

Inhalation solution was administered via piezoelectric dispersion (Study 205.101), BINEB (Study 205.112) or RESPIMAT device (205.127). Dry powder inhalation was administered via Inhalator Ingelheim (FO2 device) or HandiHaler device.

During the development, the contents of dry powder inhalation capsule were changed (Phase I, II and III formulation). Dry powder inhalation capsule with Phase III formulation is the to-be marketed formulation and this will be delivered by the HandiHaler device. The to-be marketed formulation with the HandiHaler device was used in pivotal clinical trials, such as 205.105, 114/117, 133, 139 (on going study) and 201.

**4.5.3. What is the effect of food on the bioavailability of tiotropium from the dosage form? What dosing recommendation should be made, if any regarding administration of the product in relation to meals or meal type?**

Food effect was not evaluated, however, it is expected not significant since this hydrophilic drug has low oral bioavailability of 2-3% and the drug will be administered by inhalation route.

**4.6 Analytical**

**4.6.1. Were the analytical procedures used to determine drug concentrations in this NDA acceptable?**

Tiotropium was quantified by the validated LC-MS/MS assay method. This validated LC-MS/MS assay for tiotropium after \_\_\_\_\_ was able to measure concentrations down to \_\_\_\_\_ pg/mL in human plasma and \_\_\_\_\_ pg/mL in human urine. The performance of the assay during study sample analysis was acceptable as evidenced by QC sample precision and accuracy within \_\_\_\_\_

**5. Labeling Recommendations:** Underlined words are addition and the crossed out words/sentences are for deletion, as follows:

Distribution:

Tiotropium shows a volume of distribution of 32 L/kg indicating that the drug binds extensively to tissues. The drug is bound by 72% to plasma proteins \_\_\_\_\_

\_\_\_\_\_ At steady state, tiotropium plasma levels in COPD patients at peak were 17-19 pg/mL when measured 5 minutes after dry powder inhalation of an 18 mcg dose and decreased rapidly in a multi-compartmental manner. Steady state trough plasma concentrations were 3-4 pg/mL. Local concentrations in the lung are not known, but the mode of administration suggests substantially higher concentrations in the lung. Studies in rats have shown that tiotropium does not \_\_\_\_\_

### Biotransformation

The extent of biotransformation appears to be small. This is evident from a urinary excretion of 74% of unchanged substance after an intravenous dose to young healthy volunteers. Tiotropium, an ester, is nonenzymatically cleaved to the alcohol N-methylscopine and dithienylglycolic acid, neither of which binds to muscarinic receptors. *In vitro* experiments with human liver microsomes and human hepatocytes suggest that

is metabolized by cytochrome P450 dependent oxidation and subsequent glutathione conjugation to a variety of Phase II-metabolites. This enzymatic pathway can be inhibited by the CYP450 2D6 (and 3A4) inhibitors, quinidine, ketoconazole and gestodene. Thus CYP450 2D6 and 3A4 are involved in the metabolic pathway that is responsible for the elimination of a small part of the dose. Tiotropium

does not inhibit CYP450 1A1, 1A2, 2B6, 2C9, 2C19, 2D6, 2E1 or

### Elimination:

The terminal elimination half-life of tiotropium is between 5 and 6 days following inhalation. Total clearance was 880 mL/min after an intravenous dose in young healthy volunteers with an interindividual variability of 22%. Intravenously administered tiotropium is mainly excreted unchanged in urine (74%). After dry powder inhalation urinary excretion is 14% of the dose, the remainder being mainly non-absorbed drug in the gut that is eliminated via the feces. The renal clearance of tiotropium exceeds the creatinine clearance, indicating secretion into the urine. After chronic once daily inhalation by COPD patients, pharmacokinetic steady state was reached after 2-3 weeks with no accumulation thereafter.

### Drug Interactions

Interaction study with tiotropium (14.4 µg intravenous infusion over 15 minutes) and cimetidine 400 mg tid or ranitidine 300 mg qd was conducted. Concomitant administration of cimetidine with tiotropium resulted in a 20% increase in the AUC<sub>0-4h</sub>, a 28% decrease in the renal clearance of tiotropium and but no significant change in the C<sub>max</sub> and amount excreted in urine over 96 hours. Coadministration of tiotropium with ranitidine did not affect the pharmacokinetics of tiotropium. Therefore, no clinically significant interaction occurred between tiotropium and cimetidine or ranitidine.

### PRECAUTIONS

#### Drug Interactions

SPIRIVA has been used concomitantly with other drugs without adverse drug reactions. These include sympathomimetic bronchodilators, methylxanthines, and oral and inhaled steroids,

**APPENDICES**

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8 Draft Labeling Page(s) Withheld

**Protocol 205.101 (Study Report #U93-0252)**

**Study Type:** Tolerability & PK of single increasing doses with inhalation solution via piezoelectric.

**Title:** Tolerability and preliminary pharmacokinetics of Ba 679 BR after inhalation of single increasing doses (1-200 µg) by healthy volunteers.

**Clinical Investigators:** — M.D., Ingelheim, Germany.

**Objectives:** To characterize the tolerability and preliminary pharmacokinetics of tiotropium after single inhalations of either placebo, 0.8, 4, 8, 20, 40, 80 or 160 µg tiotropium.

**Formulations:** Tiotropium was administered as tiotropium bromide monohydrate (Ba 679 BR) in 15 µl inhalation solution by a piezoelectric device over a deep slow inspiration. Treatment groups are as follows:

- 0.8 µg tiotropium (= 1 µg Ba 679 BR, solution with 5 µg Ba 679 BR, batch 102203 diluted 1+4 with 0.9% saline).
- 4.0 µg tiotropium (= 5 µg Ba 679 BR, batch 102203).
- 8.0 µg tiotropium (= 10 µg Ba 679 BR, batch 102204).
- 20 µg tiotropium (= 25 µg Ba 679 BR, solution with 50 µg Ba 679 BR, batch 102205 diluted 1+1 with 0.9% saline).
- 40 µg tiotropium (= 50 µg Ba 679 BR, batch 102205).
- 80 µg tiotropium (= 100 µg Ba 679 BR, solution with 200 µg Ba 679 BR, batch 102206 diluted 1+1 with 0.9% saline).
- 160 µg tiotropium (= 200 µg Ba 679 BR, batch 102206).
- identically appearing placebo solution (batch 102202)

15 µl solution was placed by the investigator on to the nebulizer plate of a piezoelectric device at the beginning of a slow deep inhalation of the subject. Nostrils were closed by a nasal clip and breath was held for 10 seconds at the end of inspiration.

**Study design:** Single-blind randomized, placebo controlled rising dose group comparison in 24 healthy male Caucasian subjects (mean age of 31 years with range of 24 to 43 years). Each subject received 2-3 doses, either, 0.8, 20, and 160 µg (subjects 1-8) or 4 and 40 µg (subjects 9-16) or 8 and 80 µg (subjects 17-24). Each subject inhaled one dose per period with an at least 14 days washout period before the next dose.

**Sampling times:** Blood samples were collected at t = 0, 5, 20, 60 and 120 min after the inhalation of 40, 80 and 160 µg tiotropium. Urine at t = 0-4h and 4-8h after the inhalation of 40, 80 and 160 µg tiotropium only.

**Assays:** Plasma samples and urine samples were assayed by a — assay with a limit of quantification of — tiotropium. Assay precision in plasma samples was within — and assay accuracy was within —. Respective values for urine samples were — for precision and — of accuracy.

**Results:** Tiotropium plasma concentrations were below the limit of quantification in all plasma samples. Tiotropium concentrations in urine were regularly above the limit of quantification, and the data is summarized in Table 1.

**Table 1. Geometric mean (% gCV) urinary excretion (% of dose) of tiotropium in subjects receiving single doses of inhalation solution with 40, 80 and 160 µg tiotropium.**

		40 µg n=3 of 6	80 µg n=4 of 6	160 µg n=6 of 6
Ae <sub>0-4h</sub>	% of dose	0.788 (64.5)	0.783 (62.0)	0.498 (150)*
Ae <sub>0-8h</sub>	% of dose	-- #	1.29 (46.7)	0.981 (65.9)

Source data TABLE 8 in U93-0252,  
 \*: one outlier with 0.0759% of dose, median = 0.585% of dose,  
 #: n=1, 0.818% of dose

Ae<sub>0-4h</sub> values following intravenous infusion and inhalation of tiotropium in study 205.105 were about 44% and 1.84% of the dose, respectively. Ae<sub>0-4h</sub> of 1.84% of the dose corresponded to an absolute bioavailability of 19.5% (based on study 205.105). Thus absolute bioavailability of the inhalation solution is approximately 8.6% (0.8% / 1.84% ≈ 44%. 44% of 19.5% are 8.6%). Low assay sensitivity could attributes to the low bioavailability (among other possible reasons).

**Conclusions:** (1) Tiotropium plasma concentrations were below the limit of quantification in all plasma samples. (2) Urinary excretion of tiotropium inhalation solution reached about 0.5-0.8% of the dose in sampling interval 0-4 h and 1-1.3% in the interval 0-8 h.

**Comment:** Assay methods need to be more sensitive to be satisfactory. Washout period was too short (t<sub>1/2</sub> = 5-7 days), therefore, data is not quite reliable (carry-over effect). Effect of dilution of tiotropium is not discussed (e.g, stability of tiotropium in 0.9% saline, concentration difference between doses).

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Protocol 205.102 (Study Report #U99-0774)

**Study Type:** Tolerability & PK of Single increasing doses using inhalation capsules via FO2

**Title:** Tolerability and preliminary pharmacokinetics of Ba 679 BR after inhalation of single increasing doses (40-320 µg) by healthy volunteers.

**Clinical Investigators:** \_\_\_\_\_, M.D., Ingelheim, Germany.

**Objectives:** To characterize the tolerability and preliminary pharmacokinetics of tiotropium after single dry powder inhalations of either placebo, 35.2, 70.4, 141 or 282 µg tiotropium.

**Formulations:** Tiotropium was administered as tiotropium bromide monohydrate (Ba 679 BR) lactose inhalation capsules corresponding to

- 2 x 17.6 µg tiotropium (= nominally 20 µg Ba 679 BR, batch 203002, 22% < \_\_\_\_\_ or
- 2 x 35.2 µg tiotropium (= nominally 40 µg Ba 679 BR, batch 203003, 29% < \_\_\_\_\_ or
- 2 x 70.4 µg tiotropium (= nominally 80 µg Ba 679 BR, batch 203004, 29% < \_\_\_\_\_ or
- 2 x 141 µg tiotropium (= nominally 160 µg Ba 679 BR, batch 203005, 32% < \_\_\_\_\_ or
- identically appearing placebo capsules (batch 204001)

Two inhalation capsules were administered one hour after a light breakfast by aid of the FO2 device (= Inhalator Ingelheim), while nostrils of the subjects were closed with clamps. Breath was held for 10 sec after inhalation. The second inhalation capsule was inhaled approximately 25 sec after the first capsule.

**Study design:** Single-blind randomized, placebo controlled, two 2-way cross-over periods (7 days washout period) using 16 healthy male Caucasian subjects (mean age of 30 years, with range 23 to 44 years). Each period was performed in eight subjects, six subjects receiving drug and two placebo. Each subject received two doses, either 35.2 µg and 141 µg or 70.4 µg and 282 µg tiotropium.

**Sampling times:** Blood samples were collected at t = 0, 5, 20, 60 and 120 min after each inhalation. Urine was collected at t = 0-4h and 4-8h after the inhalation.

**Assays:** Plasma samples and urine samples were assayed by a \_\_\_\_\_ assay with a limit of quantification of \_\_\_\_\_ tiotropium. Assay precision in plasma samples was within \_\_\_\_\_ and assay accuracy was within : \_\_\_\_\_ Respective values for urine samples were \_\_\_\_\_ for precision and \_\_\_\_\_ of accuracy.

**Results:** Two plasma samples collected 5 minutes after the highest dose of 282 µg tiotropium showed tiotropium concentrations just above the limit of quantification. All other samples were below the limit of quantification. Tiotropium concentrations in urine were regularly above the limit of quantification. Table 1 shows that higher amount (expressed as % of dose) of unchanged tiotropium was excreted in urine as dose increased (i.e., deviation from dose-proportionality). The sponsor suggested that this could be due to oral absorption. A comparison with data from Study 205.101 with inhalation solution suggested a higher urinary excretion and thus most likely a higher bioavailability of the dry powder inhalation with lactose inhalation capsules.

**Table 1.** Geometric mean (% gCV) urinary excretion of tiotropium in subjects receiving single doses of lactose inhalation capsules with 35.2, 70.4, 141 or 282 µg tiotropium.

		35.2 µg n=3	70.4 µg n=5	141 µg n=5	282 µg n=6
Aeq <sub>0-4h</sub>	% of dose	1.88 (53.1)	1.67 (55.2)	2.36 (47.5)	3.19 (50.2)
Aeq <sub>0-8h</sub>	% of dose	2.35 (74.0)	2.41 (66.6)	3.34 (41.6)	4.83 (48.6)

**Conclusion:** Tiotropium plasma concentrations were below 1 µg/mL. The amount excreted unchanged in urine within 4 hours was 1.7-3.2% of dose and within 8 hours 2.4-4.8% of dose with a trend to increase with dose in more than dose-proportional manner.

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## Protocol 205.103 (Study Report #U930939)

**Study Type:** Tolerability & Multiple dose PK using FO2

**Title:** Tolerability and PK of Ba 679 BR after inhalation of multiple doses of 80 µg QD or 160 µg QD Ba 679 BR or placebo over 7 days by healthy volunteers.

**Clinical Investigators:** \_\_\_\_\_, M.D., Ingelheim, Germany.

**Objectives:** To characterize the tolerability and preliminary pharmacokinetics of tiotropium after seven once daily dry powder inhalations of either placebo, 70.4 or 141 µg tiotropium cation.

**Formulations:** Tiotropium was administered as lactose inhalation capsules corresponding to

- 2 x 35.2 µg tiotropium (= nominally 40 µg Ba 679 BR, batch 203003, 29% < \_\_\_\_\_, or
- 2 x 70.4 µg tiotropium (= nominally 80 µg Ba 679 BR, batch 203004, 29% < \_\_\_\_\_ or
- identically appearing placebo capsules (batch 204001)

Two inhalation capsules were administered one hour after a light breakfast by FO2 device (= Inhalator Ingelheim), while nostrils of the subjects were closed with clamps. Breath was held for 10 sec after inhalation. The second inhalation capsule was inhaled approximately 25 sec after the first capsule.

**Study design:** Double-blind randomized three-way cross-over in twelve healthy male Caucasian subjects (mean age of 29.5 years, range 21-43 years). Each subject inhaled once daily two capsules for seven days with a washout of three days between the three treatment periods.

**Sampling times:** Blood samples were collected at t = 0, 5, 20, 60 and 120 min after 1<sup>st</sup> (Day 1) and last (Day 7) inhalations. Urine at t = 0-4, 4-8 and 8-12 h after the inhalations on Day 1, 3 and 7.

**Assays:** Plasma samples were assayed by \_\_\_\_\_ with a limit of quantification of \_\_\_\_\_ pg/mL, while urine samples were assayed by a \_\_\_\_\_ assay with a limit of quantification of \_\_\_\_\_ g/mL tiotropium. Assay precision in plasma samples was within \_\_\_\_\_ and assay accuracy was within ± \_\_\_\_\_. Respective values for urine samples were \_\_\_\_\_ for precision and: \_\_\_\_\_ of accuracy.

**Results:** The sponsor stated that the long terminal elimination half-life of tiotropium in the range of several days was not yet known at the time of the study performance. Therefore, the primary evaluation included only subject, who did not receive tiotropium in the treatment period before. This applied to seven subjects receiving 70.4 µg doses and to eight subjects receiving 141 µg doses. Three subjects provided profiles for both 70.4 µg and 141 µg doses with a 10 days washout period between these two active treatments (subjects 2, 5 and 12). A secondary evaluation included the data from the subjects with only three days washout: the sponsor stated that it is reasonable because the carry over effect was minor after 7 days of tiotropium treatment and thus a comparison of Day 7 data was considered to be relatively unbiased. For geometric mean PK parameters refer to the Table 1 and geometric mean values are plotted in Figure 1

**Table 1. Geometric mean tiotropium PK parameters for subjects with a minimum washout period of ten days and once daily doses of 70.4 µg or 141 µg tiotropium by dry powder inhalation for seven days**

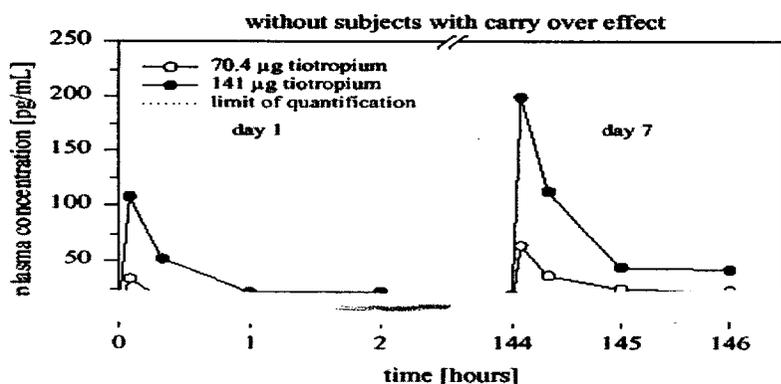
		n	70.4 µg		141 µg		gMean ratio
			gMean	%gCV	gMean	%gCV	
$C_{max,Day1}$	[pg/mL]	6/8	33.7	62.9	108	74.9	---
$C_{max,Day7}$	[pg/mL]	5/6	64.2	48.7	199	57.4	---
Accumulation Day 1-7			1.91		1.85		
$C_{max,Day7}$ #	[pg/mL]	8/10	83.1	63.5	184	45.2	---
$NC_{max,Day1}$	[pg/mL]	6/8	8.61	62.9	13.7	74.9	1.59
$NC_{max,Day7}$	[pg/mL]	5/6	16.4	48.7	25.4	57.4	1.55
$NC_{max,Day7}$ #	[pg/mL]	8/10	21.2	63.5	23.4	45.2	1.10
$C_{preDay7}$	[pg/mL]	3/2	10.4	12.7	19.4	15.4	---
$C_{preDay7}$ #	[pg/mL]	6/5	9.82	25.3	18.4	15.1	---
$NC_{preDay7}$	[pg/mL]	3/2	2.66	12.7	2.48	15.4	0.93
$NC_{preDay7}$ #	[pg/mL]	6/5	2.51	25.3	2.35	15.1	0.94

		n	70.4 µg		141 µg		gMean ratio
			gMean	%gCV	gMean	%gCV	
$AUC_{0-2h,Day1}$	[pg.h/mL]	5/6	25.2	64.0	73.9	57.6	---
$AUC_{0-2h,Day7}$	[pg.h/mL]	3/2	43.6	18.1	86.8	27.6	---
Accumulation Day 1-7			1.73		1.17		
$NAUC_{0-2h,Day1}$	[pg.h/mL]	5/6	6.43	64.0	9.44	57.6	1.47
$NAUC_{0-7h,Day7}$	[pg.h/mL]	3/2	11.1	18.1	11.1	27.9	0.99
$AUC_{0-2h,Day7}$ #	[pg.h/mL]	7/5	55.1	39.7	114	40.8	---
$NAUC_{0-7h,Day7}$ #	[pg.h/mL]	7/5	14.1	39.7	14.6	40.8	1.04
$Ae_{0-4h,Day1}$	[% of dose]	7/8	1.33	53.9	1.78	62.2	1.34
$Ae_{0-4h,Day3}$	[% of dose]	7/7	2.26	91.1	4.40	41.0	1.95
$Ae_{0-4h,Day7}$	[% of dose]	7/8	4.03	33.5	4.22	33.6	1.05
Accumulation Day 1-7			3.03		2.37		
$Ae_{0-12h,Day1}$	[% of dose]	7/7	2.67	36.3	3.56	53.2	1.33
$Ae_{0-12h,Day3}$	[% of dose]	7/7	6.41	31.9	7.85	29.7	1.22
$Ae_{0-12h,Day7}$	[% of dose]	7/8	7.72	39.3	7.77	28.3	1.01
$Ae_{0-4h,Day7}$ #	[% of dose]	11/12	4.35	32.5	4.31	36.5	0.99
$Ae_{0-12h,Day7}$ #	[% of dose]	11/12	8.37	33.7	7.86	29.9	0.94

Source data in U93-0939, # over all subjects

Note: prefixed PK parameters with N means PK parameters are normalized to 18 µg

**Figure 1.** Geometric mean tiotropium plasma concentration time-profiles for subjects with a minimum washout period of 10 days and once daily doses of 70.4 µg or 141 µg tiotropium for 7 days.



**Accumulation factor:** Geometric mean  $C_{max}$  values increased from the 1<sup>st</sup> to the 7<sup>th</sup> inhalation by a factor of about two.  $AUC_{0-2h}$  values accumulated similarly in 70.4 µg.

**Peak/trough ratio:** Predose concentrations ( $C_{pre}$ ) were quantifiable on Day 7 and indicating that peak concentrations were about 6 times (64.2 vs. 10.4 pg/mL) for a dose of 70.4 µg to 10 times (199 vs. 19.4 pg/mL) for a dose of 141 µg higher than trough concentrations.

**$T_{max}$ :** Maximum drug concentrations were always observed in the first plasma samples collected 5 minutes post dose.

**Dose-proportionality:**  $C_{max}$  and AUC values showed consistently higher values for 141 µg vs 70.4 µg for Day 1, suggested a deviation from dose-proportionality in this group (comparison on Day 1). However, differences were nearly absent based on data from Day 7 (e.g.,  $C_{preDay7}$ ,  $C_{max, day 7}$ ,  $AUC_{day 7}$  and  $Ae_{day 7}$ ; nearly dose-proportionality for  $C_{max, day 7}$  and AUC on Day 7). Thus, the sponsor stated, a relevant carry over effect is expected to be minimal after seven days treatment.

**Steady state:** The long terminal elimination half-life inferred from the carry-over effects makes it unlikely that it is achieved by Day 7, but the overall relatively small increase in  $Ae_{0-12h}$  from Day 3 to Day 7 suggests no gross change after seven days.

#### Summary:

- Tiotropium is rapidly absorbed by the lungs with peak plasma concentrations occurring at 5 min after inhalation.
- Drug plasma concentrations accumulated by a factor of two within 7 days.
- A positive deviation from dose-proportionality was observed on Day 1 for  $C_{max}$ , AUC and  $Ae$  values, but the trend was decreased on Day 7.
- Urinary excretion in the first four hours increased from 1.3-1.8% (Day 1) to 4.0-4.2% of dose on Day 7.
- 18 µg tiotropium doses on Day 7 are expected from this study to fluctuate between trough concentrations of about 2.5 pg/mL ( $C_{preDay7}$ ) and peak concentrations of about 20 pg/mL ( $C_{max, day 7}$ ).

## Protocol 205.104 (Study Report #U93-0940)

**Study Type:** Tolerability & Multiple doses using inhalation caps via FO2

**Title of study:** Tolerability and preliminary PK of Ba 679 BR after multiple inhalation administration of 1x10 µg Ba 679 BR/24 hours or 20 µg Ba 679 BR/24 hours or 40 µg Ba 679 BR/24 hours over 14 days to healthy volunteers.

**Clinical Investigator:** \_\_\_\_\_ Dr. med.

**Objectives:** To characterize the tolerability and preliminary PK of tiotropium after 14 once daily dry powder inhalations of either, 8.8, 17.6, or 35.2 µg tiotropium as dry powder inhalation.

**Study design & Formulations:** Double-blind randomized parallel group comparison in 15 healthy male Caucasian subjects. Tiotropium bromide monohydrate was administered once daily for 14 days as lactose inhalation capsules corresponding to;

- 1 x 8.8 µg tiotropium (= 10 µg Ba 679 BR, batch 203001, 23% < \_\_\_\_\_ ) or
- 1 x 17.6 µg tiotropium (= 20 µg Ba 679 BR, batch 203002, 22% < \_\_\_\_\_ ) or
- 1 x 35.2 µg tiotropium (= 40 µg Ba 679 BR, batch 203003, 29% < \_\_\_\_\_ )

The inhalation capsules were administered one hour after a light breakfast using the FO2 device (= Inhalator Ingelheim), while nostrils of the subjects were closed with clamps. Breath was held for 10 sec after inhalation.

**Subjects:** Fifteen healthy male Caucasian subjects participated in this study. Groups of five subjects received one of the three treatments. Mean age, weight and height were 34.5 years (range 24 to 45 years), 75.2 kg (range 65 to 89 kg) and 177 cm (range 169 to 184 cm), respectively.

### **Sampling times:**

**Plasma:** Collected at t = 5, 20 minutes and 1, 2, 4 and 8 hours after the first inhalation, before the 7<sup>th</sup> and 13<sup>th</sup> as well as before and 5, 20 minutes and 1, 2, 4 and 8 hours after the last inhalation on Day 14.

**Urine:** Collected for 24 hours after inhalation on Days 1, 7, 13, and 14 in fractions 0-4h, 4-8h, 8-12h and 12-24h. The terminal urinary elimination of tiotropium was monitored in urine collected in fractions 0-4h and 4-8h on Days 17, 20, 23, 30, 37 and 44.

### **Analytical Methodology:**

**Assay Method:** Plasma samples were assayed by a validated \_\_\_\_\_ assay with a limit of quantification of \_\_\_\_\_, while urine samples were assayed by a r \_\_\_\_\_ assay with a limit of quantification of \_\_\_\_\_ for tiotropium. Lower urine concentrations were measured by LC-MS/MS with a limit of quantification of \_\_\_\_\_ pg/mL tiotropium.

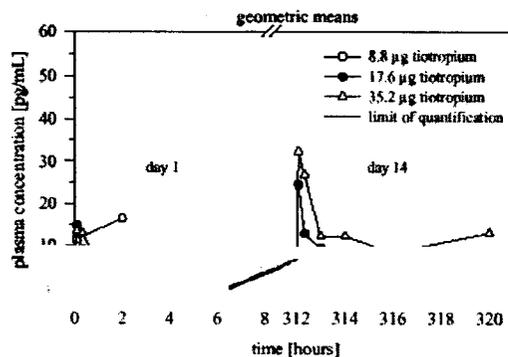
**Accuracy and Precision:** Assay precision in plasma samples was within \_\_\_\_\_, and assay accuracy was within ± \_\_\_\_\_. Respective values for urine samples were \_\_\_\_\_ and 15% (LC-MS) for precision and ± \_\_\_\_\_ and ± \_\_\_\_\_ (LC-MS) for accuracy.

**Data analysis:** Plasma concentrations obtained in this study were used to derive the maximum drug plasma concentration ( $C_{max} = C_{5min}$ ), the area under the tiotropium plasma concentration-time curve from the time of inhalation to 2 hours post dose ( $AUC_{0-2h}$ ) as well as these parameters normalized to a tiotropium dose of 18 µg tiotropium ( $NC_{5min}$  or  $NAUC_{0-2h}$ ). Tiotropium concentrations in urine were used to calculate the amounts excreted unchanged in urine (e.g.,  $Ae_{0-4h}$ ). Renal clearance was calculated from the amount excreted unchanged in urine divided by the

respective AUC for this interval. The terminal elimination half-life was calculated from the linear trapezoidal rule urinary excretion rates.

**Results:** For geometric mean pharmacokinetic parameters refer to the Table 1, geometric mean values are plotted in Figure 1.

**Figure 1.** Geometric mean tiotropium plasma concentration time-profiles after once daily dry powder inhalation of 8.8, 17.6 or 35.2 µg tiotropium for 14 days.



**Plasma:** Tiotropium plasma concentrations were rarely above the limit of quantification after doses of 8.8 µg tiotropium. Only two drug concentrations were above 10 µg/ml on Day 1, while geometric mean  $C_{5min}$  ( $= C_{max}$ ) was 7.91 pg/mL on Day 14. The subsequent plasma samples showed often no further concentration above 10 µg/mL. The accumulation factor for  $C_{5min}$  following 17.6 and 35.2 µg dose were 1.7 and 2.4, respectively. Predose concentrations on Days 7 and 14 were below the limit of quantification for the two lower doses. A quantifiable predose concentration (geometric mean = 7.43 pg/mL) was obtained on Day 14 in all five subjects from the dose of 35.2 µg tiotropium. The peak to trough concentration ratio on Day 14 was about 4-5 (i.e., 32.3 vs. 7.43 pg/mL). Based on geometric mean tiotropium plasma concentrations on day 14 (peak and trough level at dose level 35.2 µg) normalized for an intended dose of 18 µg tiotropium cation one can expect a steady state predose concentration of ~3 pg/mL and a steady state peak concentration ( $C_{5min}$ ) of ~20 pg/mL.

**Urine:**  $Ae_{0-4h}$  was between 1.3% and 1.6% of dose on Day 1 and increased to 3.1% - 4.3% of dose on Day 14. However,  $Ae_{0-4h}$  was similar between Day 7 and 14 following 3 doses, suggests that for practical purposes steady state was reached on day 7 while the estimated  $t_{1/2}$  of 6-8 days expecting steady state conditions formally after about 3-5 weeks. Accumulation for  $Ae_{0-4h}$  from Day 1 to Day 14 showed a factor between 2.3 and 3.3. Accumulation for  $Ae_{0-24h}$  from Day 1 to Day 14 reached a factor of 2.6 to 4.0.  $Ae$  values (amount excreted in urine) increased more than proportional with dose after multiple doses in this group comparison (e.g.,  $Ae_{0-24h}$  increased from 10.5% (8.8 µg) to 14.7% (35.2 µg) of dose on Day 14).

**Terminal elimination half-life and Renal clearance:** Geometric mean elimination half-lives (obtained linear regression using log-transformed renal excretion rates at the midpoint of the time interval) were between 6 and 8 days (139-184 hours), which were calculated from the urinary excretion rates obtained after the last inhalation on day 14. The renal clearance could be calculated for the 35.2 µg dose (using  $Ae_{0-8h}$  and  $AUC_{0-8h}$ ) and had a geometric mean value of 407 mL/min, which is greater than the creatinine clearance and indicates an active excretion process.

**Table 1.** Geometric mean (% gCV) tiotropium PK parameters after QD dry powder Inhalation of 8.8, 17.6 or 35.2 µg tiotropium for 14 days.

		day		tiotropium dose (n=5 for 8.8 µg, 17.6 µg and 35.2 µg)					
				8.8 µg		17.6 µg		35.2 µg	
				gMean	%gCV	gMean	%gCV	gMean	%gCV
C <sub>5min</sub>	[pg/mL]	1	1/4/2	*	---	14.8	94.3	13.4	31.7
		14	4/2/2	7.91	50.5	24.6	58.0	32.3	16.2
Accumul. C <sub>7re</sub>	Day 1-14 [pg/mL]	14	0/0/5	---	---	---	---	7.43‡	15.1
NC <sub>5min</sub>	[pg/mL]	1	1/4/5	---	---	15.1	94.3	6.87	31.7
		14	4/2/5	16.2	50.4	25.1	58.0	16.5	16.2
AUC <sub>0-2h</sub>	[pg.h/mL]	14	0/0/2	---	---	---	---	32.1	7.2
NAUC <sub>0-2h</sub>	[pg.h/mL]	14	0/0/2	---	---	---	---	16.4	7.2
AUC <sub>0-24h</sub>	[pg.h/mL]	14	0/0/3	---	---	---	---	87.7	19.7
Ae <sub>0-∞</sub>	[% of dose]	1	5/5/5	1.34	35.3	1.61	64.8	1.31	50.3
		7	5/5/5	2.56	24.6	3.66	76.4	4.38	40.3
		14	5/5/5	3.13	49.7	3.63	39.1	4.28	25.0
Accumul. Ae <sub>0-∞</sub>	Day 1-7			1.91	---	2.27	---	3.34	---
Accumul. Ae <sub>0-∞</sub>	Day 1-14			2.34	---	2.25	---	3.27	---
Ae <sub>0-24h</sub>	[% of dose]	1	5/4/5	1.87	30.1	2.60	54.8	2.05	30.5
		14	5/5/5	5.13	40.4	5.66	33.0	6.83	18.4
Ae <sub>0-24h</sub>	[% of dose]	1	5/3/4	3.30	26.9	4.64	47.6	3.70	6.0
		14	5/5/5	10.5	25.9	12.1	20.4	14.7	13.6
Accumul. CL <sub>r, 0-∞</sub>	Day 1-14 [mL/min]	14	0/0/3	---	---	---	---	407	8.3
t <sub>1/2</sub>	[days]		5/4/5	5.8	23.0	7.7	31.4	6.0	21.4

\* only one value of 11.4 pg/mL all other below --- g/mL,

source data in U93-0940

‡ NC<sub>7re</sub> for 18 µg = 3.80 pg/mL

### Conclusions:

- The apparent terminal urinary elimination half-life of tiotropium was 6 to 8 days.
- Approximate steady state conditions were nevertheless achieved within 7 days of tiotropium inhalation.
- Tiotropium is actively secreted by the kidney, as seen by a CL<sub>r</sub> value of 407 mL/min.
- The amount excreted unchanged in urine increased moderately more than proportional with dose.
- Tiotropium plasma concentrations for an 18 µg dose by dry powder inhalation are expected to fluctuate between 3 and 20 pg/mL in steady state.

## Protocol 205.105 (Study Report #U99-1315)

**Study Type:** Absolute bioavailability/Single dose PK (Phase I).

**Title:** Pharmacokinetics and bioavailability of tiotropium after intravenous (14.4 µg), oral (64 µg) and inhalational (108 µg) administration in healthy male volunteers (open randomized three parallel groups).

**Clinical Investigators:** — M.D., BI Pharma KG, Germany

**Objectives:** To investigate the PK and absolute bioavailability (BA) of tiotropium after inhalational and oral administration.

**Drug Products:** All doses were given one hour after a light breakfast.

**Test Product:** (1) 108 µg tiotropium in three 36 µg dry powder inhalation capsules. Inhalation capsules by means of the HandiHaler device with clamped nostrils, breath held for 10s after inhalation and each capsule inhaled twice. Batch no = 9602005

(2) 64 µg tiotropium as oral solution. Oral solution was prepared from ampoules for intravenous injection. Batch no = 9707203 (ampoules)

**Reference product:** Intravenous infusion of 14.4 µg over 15 minutes. Batch no = 9707203

**Study Design and Method:** This study had a single dose, open-label, randomized with 3 paralleled groups. Different groups of twelve healthy male subjects received single tiotropium doses as tiotropium bromide monohydrate. A dose of 14.4 µg tiotropium was infused intravenously over 15 minutes, 64 µg were administered as oral solution and three 36 µg inhalation capsules (108 µg tiotropium) were inhaled via the HandiHaler device. Plasma samples were collected up to 8 hours post dose and urine samples for up to 25 days. Tiotropium was quantified in plasma and urine. PK parameters were calculated by non-compartmental procedures.

**Trial Subjects:** Thirty six healthy male Caucasian subjects completed the trial. The mean age was 31 years (range 24-42 years), the mean height and weight were 181 cm (172- 195 cm) and 82.5 kg (67 - 105 kg), respectively.

**Criteria for Evaluation:** PK parameters (AUC,  $C_{max}$ ,  $T_{max}$ ,  $V_z$ , CL,  $t_{1/2}$ , MRT and F).

### **Sampling times:**

**Blood:** IV infusion,  $t = 0, 7, 15, 20, 25, 35, 45$  min, 1, 2, 4 and 8 hrs post infusion; oral,  $t = 0, 15, 30$  min, 1, 2, 4 and 8 hrs post dose; Inhalation,  $t = 0, 5, 20$  min, 1, 2, 4 and 8 hrs post start of inhalation.

**Urine:** pre-dose, 0-4, 4-8, 8-24 hr on days 1-4; 0-4, 4-8 hr on day 8, 11, 15, 18, 22 and 25

### **Analytical Methodology**

**Assay Method:** LCMS/MS

**Assay Sensitivity:** The LOQ for tiotropium was — pg/mL in plasma and — pg/mL in urine.

**Accuracy and Precision:** An assay precision and accuracy for QC were within — (plasma) and — (urine) and  $\pm$  — (plasma) and — (urine), respectively.

**Data analysis:** Renal clearance was calculated from the amount excreted unchanged in urine divided by the respective AUC for this interval. Total AUC values ( $AUC_{0-\infty}$ ) were obtained by converting urinary excretion rates to plasma concentrations by means of the renal clearance and adding these values to the AUC values measured in plasma directly. Apparent terminal elimination rate constant,  $\lambda_z$  and the terminal elimination half-life was calculated from the log-linear urinary excretion rates. Renal clearance and the urinary excretion values were used to

calculate the later drug plasma concentrations in order to obtain total clearance, the volume of distribution and the mean residence time by noncompartmental procedures. Therefore,  $AUC_{0-\infty}$ ,  $V_{ss}$  (and  $V_z$ ) and MRT values are prone to some more variability (because they rely on analytical precision, accuracy, and accuracy of urine collection and the accurate determination of the individual renal clearance value). In order to aid these evaluations further the sponsor fitted a 4-compartment model to the tiotropium plasma concentrations and urinary excretion data together.

**Results:** Summary of the results refers to Table 1 and Figure 1.

**Table 1.** Geometric mean (% gCV) tiotropium PK parameters after intravenous infusion of 14.4 µg, dry powder (DP) inhalation of 108 µg or oral solution of 64 µg tiotropium to different groups of twelve healthy male subjects,

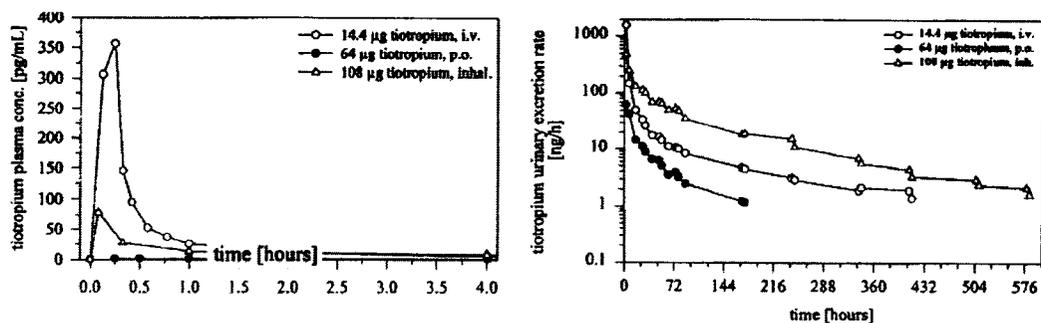
		n	group comparison (n=12)					
			intraven. 14.4 µg		DP inhalation 108 µg		oral solution 64 µg	
			gMean	%gCV	gMean	%gCV	gMean	%gCV
$C_{max}$	[pg/mL]	11/11/12	378	25.4	65.4	58.0	2.35&	87.7
$t_{max}$		-/11/5	#	---	5 min	##	§ 2 h	§ 1-8 h
$NC_{max}$	[pg/mL] for 18 µg	11/11/12	473	25.4	10.9	58.0	0.66	87.7
$AUC_{0-2h}$	[pg.h/mL]	9/11/-	143	18.7	39.9	38.7	--	--
$AUC_{0-4h}$	[pg.h/mL]	9/11/-	161	19.4	61.7	34.1	--	--
$AUC_{0-8h}$	[pg.h/mL]	5/11/-	186	25.0	92.6	28.4	--	--
$NAUC_{0-2h}$	[pg.h/mL] for 18 µg	9/11/-	179	18.7	6.65	38.7	--	--
$NAUC_{0-4h}$	[pg.h/mL] for 18 µg	9/11/-	201	19.4	10.3	34.1	--	--
$NAUC_{0-8h}$	[pg.h/mL] for 18 µg	5/11/-	233	25.0	15.4	28.4	--	--
$Ae_{0-4h}$	[% of dose]	11/10/9	43.8	10.2	1.84	40.7	0.39	39.8
$Ae_{0-8h}$	[% of dose]	11/11/9	48.0	10.6	2.83	33.6	0.67	30.1
$Ae_{0-24h}$	[% of dose]	11/8/9	53.6	11.4	4.99	22.4	1.04	31.2
$Ae_{0-96h}$	[% of dose]	11/6/9	61.0	11.7	8.93	9.4	1.60	30.0
$Ae_{0-\infty}$	[% of dose]	11/9/9	73.6	13.7	14.4	7.8	--	--
F	[%]	(11)/9/9	--	--	19.5	--	2.6	--
$CL_T(0-4h)$	[mL/min]	8/11/-	669	16.5	486	14.0	--	--
$t_{1/2}$	[h]	11/11/-	137	26.0	116	16.1	--	--
$t_{1/2}$	[days]	11/11/-	5.71	26.0	4.84	16.1	--	--
Parameters calculated from plasma concentrations that were obtained from urinary excretion data via renal clearance (only values > BLQ):								
$AUC_{0-\infty}$	[pg.h/mL]	8/9/-	273	22.1	518	16.6	--	--
$NAUC_{0-\infty}$	[pg.h/mL] for 18 µg	8/9/-	341	22.1	86.3	16.6	--	--
CL, CL/f	[mL/min]	8/9/-	880	22.1	3474	16.6	--	--
$V_{ss}$	[L]	8/-/-	2665	27.8	--	--	--	--
$V_z$	[L/kg]	8/-/-	138	27.6	--	--	--	--
MRT <sub>dis/tot</sub>	[h]	8/9/-	50.6	31.5	110	18.2	--	--

# at the end of infusion (15 min),

## always in the first plasma sampling 5 min post dose, § median and range

& values below replaced by 1/2 this LOQ. Source data in U99-1315

**Figure 1.** Geometric mean profiles for plasma concentrations (left panel) and Urinary excretion rates (right panel)



The  $C_{max}$  of the tiotropium plasma concentrations following intravenous infusion was reached at the end of the infusion (15 minutes) and its geometric mean was 378 pg/mL. Following inhalation, the  $C_{max}$  (65.4 pg/mL) was observed already at the first time of blood sampling 5 minutes after the inhalation. Following oral administration, the tiotropium plasma concentrations were mostly below the assay limit.  $C_{max}$  was in the range of 2 pg/mL and was observed about 2 hours after administration.

Estimates for the absolute BA were calculated from the total urinary excretion and were 19.5 % for inhalation ( $14.4 * 100/73.6 = 19.5\%$ ) and 2.6% ( $1.6 (Ae_{0-96h}) * 100/61 = 2.6\%$ ) for oral administration. A minimum of ~17% of the inhaled dose is expected to reach the lung.

Total and renal clearance were 880 and 669 mL/min, respectively. This means that  $(880-669) / 880 = \sim 24\%$  of the clearance were non-renal clearance.

The volume of distribution ( $V_{ss}$ ) after intravenous infusion was high with 2665 L (n=8), which corresponds to 32 L/kg. This demonstrates the high tissue binding of tiotropium. This is further supported by the even much higher volume of distribution during the terminal phase (11510 L = 138 L/kg). The high clearance together with the high volumes of distribution results in a very long elimination half-life and suggest that terminal elimination at later times is primarily driven by diffusion from tissue to the systemic circulation.

**Conclusion:** Tiotropium showed an absolute bioavailability of 19.5 % after dry powder inhalation and of 2 - 3 % after oral solution. Urinary excretion of unchanged drug accounted for 73.6% of the dose after intravenous infusion and 14.4% of the dose after inhalation. Renal clearance was 669 mL/min after intravenous infusion and 495 mL/min after dry powder inhalation. Total clearance,  $V_{ss}$ ,  $V_z$  and  $t_{1/2}$  were 880 mL/min, 32 L/kg, 138 L/kg and 5 to 6 days, respectively.

**Comment:**  $CL_r$  is overestimated, consequently, plasma concentration (obtained by calculation using  $CL_r$ ) related PK parameters such as  $AUC_{\infty}$ ,  $CL$ ,  $V_{ss}$ ,  $V_z$  and MRT are over estimated.

## Protocol 205.106 (Study Report #U97-2337)

**Study Type:** Tolerability & Single increasing doses of oral solution

**Title:** A single increasing dose tolerance study in healthy volunteers after oral administration of Ba 679 BR (dosage: 10-80 µg).

**Clinical Investigator:** \_\_\_\_\_ . Dr. med.

**Objectives:** Safety, tolerability and preliminary PK of tiotropium bromide monohydrate (Ba 679 BR) after oral administration.

**Study Design:** Single rising doses, double blind within the respective dose groups, placebo controlled. 4 days washout period in between doses.

**Subjects:** 15 healthy male subjects; 4 on placebo, 5 with 10 and 40 µg tiotropium bromide monohydrate (8 and 32 µg tiotropium cation) and 6 with 20 and 80 µg (16 and 64 µg)

**Dose:** Nominally 10, 20, 40 or 80 µg tiotropium bromide monohydrate once daily in the morning, corresponding to 8, 16, 32 or 64 µg tiotropium cation.

**Mode of admin.:** oral solution (60 µg tiotropium bromide monohydrate/mL 0.9 % NaCl = 48 µg/mL tiotropium cation; diluted with water; administered volume 100 mL), one hour after light breakfast; each subject received two doses: either 8 and 32 µg or 16 and 64 µg tiotropium cation or two doses of placebo.

**Test product:** 10, 20, 40 or 80 µg Ba 679 BR (tiotropium bromide monohydrate) solution, corresponding to 8, 16, 32 or 64 µg tiotropium cation. Batch no. = 60 µg tiotropium bromide monohydrate/1 mL 0.9% NaCl (batch no. 9501203) corresponding to 48 µg/mL tiotropium cation.

**Reference therapy:** Placebo solution in 100 mL water; oral solution prepared from placebo ampules for intravenous injection (1.0 mL 0.9% NaCl). Batch no. 9501204

### **Sampling times:**

**Plasma:** t = predose, 15, 30 minutes and 1, 2, 4, 8 and 24 hours after the dose (additional sample at 12 hr after 64 µg).

**Urine:** predose, 0-4h, 4-8h, and 8-24h after drug administration. The terminal urinary elimination of tiotropium was monitored in urine collected in fractions 0-4h and 4-8h on Days 17, 20, 23, 30, 37 and 44.

### **Analytical Methodology:**

**Assay Method:** Plasma and urine samples were assayed by a validated HPLC \_\_\_\_\_ with a limit of quantification of \_\_\_\_\_ tiotropium cation in plasma and \_\_\_\_\_ urine.

**Accuracy and Precision:** Assay precision in plasma samples was within \_\_\_\_\_ and assay accuracy was within ± \_\_\_\_\_. Respective values for urine samples were \_\_\_\_\_ for precision and \_\_\_\_\_ for accuracy.

### **Criteria for evaluation:**

**PK:**  $C_{max}$ ,  $t_{max}$ ,  $AUC_{0-24h}$  and amount excreted unchanged via urine (Ae).

**Safety:** Blood pressure, pulse and respiratory rates, ECG, salivary secretion, vital functions, laboratory parameters, adverse events.

**Statistical methods:** Descriptive analysis

**Results:** The tiotropium plasma concentrations following oral administration of doses up to 32 µg tiotropium cation were below the limit of quantification. Following administration of the highest dose (64 µg tiotropium cation) some quantifiable tiotropium plasma concentrations were found in the range of the limit of quantification (therefore, no PK parameters were estimated). The tiotropium concentrations in the urine samples could be quantified following administration of all doses (Table 1). The geometric mean of the cumulative urinary excretion of tiotropium within 24 h after administration of tiotropium was about 0.7-1.0%. A historical comparison with intravenous data suggested a low absolute bioavailability in the range of 2% after oral administration.

**Table 1.** Urinary excretion of tiotropium (in urine) after oral administration.

		8.0 µg N = 3		16 µg N = 6		32 µg N = 4		64 µg N = 5	
		gmean	% gCV	gmean	% gCV	gmean	% gCV	gmean	% gCV
Ae <sub>0-4h</sub>	ng	17.6	25.1	51.6	31.0	70.3	47.7	296	26.7
Ae <sub>0-24h</sub>	ng	57.6	43.7	135	29.6	220	34.6	635	29.8
Ae <sub>0-4h</sub>	% of dose	0.219	25.0	0.323	31.0	0.220	47.9	0.462	26.7
Ae <sub>0-24h</sub>	% of dose	0.720	43.9	0.841	29.6	0.689	34.6	0.991	29.8

Source data: APPENDIX 15.9.3.3, TABLES PK6: 3 and PK6: 4

**Conclusions:** (1) Oral administration of up to 64 µg tiotropium did not generate quantifiable drug plasma concentrations. (2) The geometric mean of the cumulative urinary excretion of tiotropium within 24 h after administration of tiotropium was about 0.7-1.0%.

**APPEARS THIS WAY  
ON ORIGINAL**

**Protocol 205.107 (Study Report #U98-2282)**

**Study type:** Tolerability & PK after IV dose.

**Title of study:** A controlled randomized and double blind increasing dose tolerance study after intravenous administration of Ba 679 BR (dosage: 3-18 µg) in male healthy volunteers.

**Investigator:** ← Dr. med.

**Objectives:** To investigate the tolerability, safety and PK of tiotropium (Ba 679 BR) after increasing intravenous doses.

**Methodology:** Double-blind, placebo-controlled increasing dose of iv infusion over 15 min. A total of 16 healthy male subjects (mean age 36.5 years; each subject was tested twice), divided in two equal groups of 8 subjects participated in this study (at least 7 days wash out period between the administration of successive dose levels). The scheduled treatments are shown in the table below.

Dose level	D1	D2*	D3*	D4
Dose (µg tiotropium bromide monohydrate)	3	6	12	18
Dose (µg tiotropium cation)	2.4	4.8	9.6	14.4
No. of subjects	8	8	8	8
Volunteers receiving placebo	2	2	2	2
Volunteers receiving drug	6	6	6	6
Volunteer no.	1, 102, 3 - 8	9 - 16	1, 102, 3 - 8	9 - 16

\* D2 and D3 were administered twice, once on two consecutive days.

**Test Product:** Batch no. = 60 µg tiotropium bromide monohydrate /mL saline (batch no. 9604207) corresponding to 48 µg tiotropium cation/mL 0.9% saline.

**Reference therapy:** Placebo solution (batch no 9604206)

**Sampling times:** Blood samples were taken before start of infusion and 7, 15, 20, 25, 35, 45, 60 and 120 min after infusion for 2.4 µg. The same sampling was used with an additional sample 240 min post infusion for 4.8 µg and for all other treatments a further sample 480 min post infusion taken. Urine was collected fractions up to 24 h or 48 h post infusion depends on the dose.

**Criteria for evaluation:**

**Pharmacokinetics:**  $C_{max}$ ,  $t_{max}$ , AUCs and amounts of drug excreted unchanged in urine.

**Safety:** Blood pressure, pulse and respiratory rates, ECG, salivary secretion, local tolerability, laboratory parameters, adverse events.

**Analytical Methodology:**

**Assay Method:** Plasma and urine samples were assayed by LC-MS/MS with a limit of quantification of — and — µg/mL tiotropium for plasma and urine, respectively.

**Accuracy and Precision:** Assay precision in plasma samples was within — and assay accuracy was within —. Respective values for urine samples were — for precision and — for accuracy.

**Statistical methods:** Descriptive analysis.

**Results:** During intravenous infusion of tiotropium of doses of 2.4, 4.8, 9.6 and 14.4 µg tiotropium cation over 15 minutes the plasma levels increased rapidly. Maximum drug plasma concentrations were achieved at the end of infusion with geometric mean values of 54-390 pg/mL (dose 2.4 - 14.4 µg) after the first infusion. Geometric mean maximum drug concentrations after the second infusion on the next day were 168 pg/mL for 4.8 µg and 241 pg/mL for 9.6 µg. After the end of infusion the tiotropium plasma concentrations dropped rapidly within the first 5 minutes to about 30- 50% of the maximum value. The geometric mean urinary excretion of unchanged tiotropium in the interval 0-24 h was 39.3% of dose for the 2.4 µg dose and 54.3% of dose for 14.4 µg. The urinary excretion of unchanged tiotropium increased from first infusion to second infusion from 41.9% to 52.4% of dose for the 4.8 µg dose and from 46.0% to 57.0% of dose for the 9.6 µg dose. The second infusion of the doses of 4.8 µg and 9.6 µg after 24 hours resulted in slightly higher AUC<sub>0-2h</sub> values (probably due to some accumulation from the first infusion). The mean renal clearance calculated from the urinary excretion in the interval from 0-4 h and AUC<sub>0-4h</sub> was about 600 - 700 mL/min. The results of the noncompartmental evaluation standardized for all tiotropium studies is provided Table 1.

**Table 1.** Noncompartmental evaluation, expressed in tiotropium cation.

		tiotropium (n = 6 if not indicated otherwise)									
		infusion		2.4 µg		4.8 µg		9.6 µg		14.4 µg	
		day	gmean	%CV	gmean	%CV	gmean	%CV	gmean	%CV	
*C <sub>15min</sub>	[pg/mL]	1	53.8	9.4	125 (n = 5)	16.3	204	24.0	390	22.4	
		2	--	--	168 (n = 5)	15.8	241	10.0	--	--	
AUC <sub>0-2h</sub>	[pg-h/mL]	1	19.0	8.8	40.0 (n = 2)	1.1	78.2	14.1	158	18.3	
		2	--	--	48.8 (n = 4)	8.4	93.4	18.0	--	--	
AUC <sub>0-4h</sub>	[pg-h/mL]	1	--	--	--	--	88.8	14.9	177	17.3	
		2	--	--	--	--	102	12.9	--	--	
Ae <sub>0-4h</sub>	% of dose	1	29.5	9.1	34.0 (n = 4)	13.1	37.1 (n = 5)	6.3	43.4 (n = 3)	8.8	
Ae <sub>0-24h</sub>	% of dose	2	--	--	39.5	16.5	43.8	9.0	--	--	
		1	39.3	7.0	41.9 (n = 4)	16.8	46.0 (n = 5)	5.7	54.3 (n = 3)	3.9	
Cl <sub>ren0-4h</sub>	[mL/min]	2	--	--	52.4	18.0	57.0	11.6	--	--	
		1	--	--	--	--	657 (n = 5)	12.1	610 (n = 3)	14.6	
		2	--	--	--	--	690	19.4	--	--	

\*at the end of infusion (15 minute)

Source data APPENDIX 15.9.3.3, TABLE PK6: 6.1 - 6.4

### Conclusions:

- Tiotropium showed a slight to moderate deviation from dose proportional behavior after iv infusion. Notes that the slight change in disposition from the first to a second once daily dose was also observed after inhalation administration.
- C<sub>max</sub>, AUC<sub>0-2h</sub>, AUC<sub>0-4h</sub> and the urinary excretion (Ae<sub>0-4h</sub> and Ae<sub>0-24h</sub>) were higher after the second compared to the first infusion.
- High urinary excretion of tiotropium (600-700 ml/min) shows that metabolism of tiotropium is not the determining factor in the PK of tiotropium. Tiotropium is actively renally excreted.
- Terminal half-life was not evaluated in this study (t<sub>1/2</sub> is about 5-6 days, therefore urine should be collected about 4 weeks).

## Protocol 205.108 (Study Report #U96-3068)

**Study type:** Dose finding & Multiple dose PK in COPD patients (Phase II).

**Title of study:** Randomized multiple dose, double blind parallel group study to determine the optimal dose of Ba 679 BR inhaled as a dry powder in patients with COPD.

**Investigator:** \_\_\_\_\_

**Objectives:** To evaluate the optimal dose of tiotropium bromide monohydrate as dry powder inhalation once daily for four weeks in patients with COPD.

**Study Design:** Multi-center, randomized double blind placebo controlled parallel group comparison study evaluating the bronchodilative response of tiotropium by spirometry in COPD patients >40 years of age. Following an initial screening and a 2 week baseline period, the patients were randomized into 4 week, double blind portion of the study in which they inhaled the contents of one capsule of test drug each day at noon. Following the 4-week treatment period patients were evaluated for an additional 3 weeks (post-treatment period) for safety and efficacy evaluation (e.g., increase symptoms after the discontinuation of the drug). Blood was collected at 2 of the 9 centers 5-15 min before and 5-min after the daily inhalation at various Visits. The results of the PK findings for the 37 patients (plasma) and 35 patients (urine) were presented in this report. The total study population consisted of 169 patients (the overall mean age was 65.8 years, 56.8% of the trial population was male and 95% were white).

**Fermentations:** Tiotropium bromide monohydrate was administered once daily for four weeks as lactose inhalation capsules corresponding to

- 4.4 µg tiotropium (= 5.5 µg Ba 679 BR, batch 408001, 13% < \_\_\_\_\_ or
- 8.8 µg tiotropium (= 11 µg Ba 679 BR, batch 408005, 17% < \_\_\_\_\_ or
- 17.6 µg tiotropium (= 22 µg Ba 679 BR, batch 408007, 16% < \_\_\_\_\_ or
- 35.2 µg tiotropium (= 44 µg Ba 679 BR, batch 408011, 20% < \_\_\_\_\_ or
- placebo (batch 300117)

Phase II formulation of inhalation capsules was used via FO2 device in this trial.

### **Analytical Methodology:**

**Assay Method:** Plasma and urine samples were assayed by a validated HPLC \_\_\_\_\_ with a LOQ of \_\_\_\_\_ and \_\_\_\_\_ µg/mL tiotropium cation in plasma and urine, respectively.

**Accuracy and Precision:** Assay precision in plasma and urine samples was within \_\_\_\_\_ and \_\_\_\_\_ for precision and \_\_\_\_\_ and \_\_\_\_\_ for accuracy, respectively.

**Sampling times:** Plasma samples were obtained 5 minutes before and after inhalation on Days 1, 8, 15, 22 and 29 as well as on Day 50. Urine was collected in 24 hours intervals after the doses on Days 8, 15 and 29 (last dose) as well as in the interval 6-24 h on Days 22, 36, 43, and 50.

### **Criteria for evaluation:**

**PK:** Predose concentrations 10 to 15 min before dose (reported as  $C_{-5min}$ ) and 5 min after ( $C_{5min}$ ) the dose,  $t_{1/2}$ , and amount of drug excreted unchanged in urine ( $A_e$ ). Terminal elimination rate constant was calculated by linear regression using log-transformed renal excretion rates (ng/h) at the midpoint of the time interval.

**PD:** FEV<sub>1</sub>, Pulmonary function test (FVC, PEF, etc.).

### **Results:**

**Plasma:** As illustrated in Table 1, tiotropium plasma concentrations measured at trough ( $C_{5min}$ ) were mostly below the level of quantification with the exception of the highest dose of 35.2 µg. Tiotropium plasma concentrations 5 minutes after inhalation increased with dose (however, not dose proportional fashion) and were approximately constant from Day 8 to Day 29 with a trend to increase, which reflex of long half-life ( $t_{1/2}$  of ~5-7 days).

In Study 205.104, geometric mean of  $C_{5min}$  on Day 14 were 7.9, 24.6 and 32.3 µg/mL following 8.8, 17.6 and 35.2 µg, respectively. Therefore, it appears that  $C_{5min}$  after 17.6 µg dose is different between these two studies, while those after the two other doses are similar.

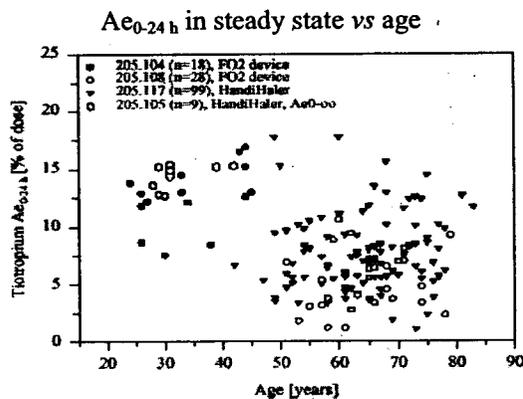
**Table 1.** Geometric mean (% gCV) tiotropium plasma concentrations and tiotropium excretion in urine in COPD patients.

		4.4 µg		8.8 µg		17.6 µg		35.2 µg	
		gMean	%gCV	gMean	%gCV	gMean	%gCV	gMean	%gCV
C-5min, Day 8	[pg/mL]	<3		<3		<3		5.42	27.8
C-5min, Day 15	[pg/mL]	<3		<3		<3		8.50	62.8
C-5min, Day 22	[pg/mL]	<3		<3		<3		4.81	49.0
C-5min, Day 29	[pg/mL]	<3		<3		<3		7.53	40.7
C-5min, Day 50	[pg/mL]	<3		<3		<3		<3	
C5min, Day 1	[pg/mL]	<3		--	--	6.38	43.5	19.7	80.1
C5min, Day 8	[pg/mL]	<3		7.98	38.1	14.8	56.6	24.6	51.7
C5min, Day 15	[pg/mL]	<3		7.82	64.8	9.20	81.8	21.3	114
C5min, Day 22	[pg/mL]	<3		6.60	38.9	7.51	36.0	35.8	35.3
C5min, Day 29	[pg/mL]	<3		8.73	36.0	13.2	58.1	--	--
Ae0-24 h, Day 8	[% of dose]	3.50	44.0	4.54	40.3	3.81	40.1	4.06	71.6
Ae0-24 h, Day 15	[% of dose]	3.88	88.2	4.80	27.5	3.97	47.6	4.75	38.4
Ae0-24 h, Day 29	[% of dose]	3.67	38.9	4.39	70.6	3.41	73.1	6.39	54.4
Ae6-24 h, Day 22	[% of dose]	2.68	37.5	3.39	50.5	2.56	121	4.18	48.1
Ae6-24 h, Day 36	[% of dose]	0.775	204	0.526	66.1	0.434	52.1	0.503	68.6
Ae6-24 h, Day 41	[% of dose]	--	--	0.359	60.8	0.186	69.1	0.245	51.1
Ae6-24 h, Day 50	[% of dose]	--	--	0.210	69.8	0.150	23.8	0.108	31.3
t1/2	[days]	--	--	7.30	17.9	--	--	5.43	8.6

Urine: Approximately 3-6% of the drug was excreted unchanged in the urine within 24 h intervals. Approximate steady state conditions were reached by treatment Day 8 for the 3 lower doses. The geometric mean excreted unchanged measured on Day 29 was 35% higher than that of Day 15 (reasons unknown). The amount declined in a log-linear fashion following the last dose (29<sup>th</sup>) with a t<sub>1/2</sub> of 5-7 days. Note that geometric mean t<sub>1/2</sub> was not calculated when the data points in the elimination phase were <3 pg/mL.

Individual and geometric mean values of urinary excretion values are plotted along with from other studies in Figure 1 below.

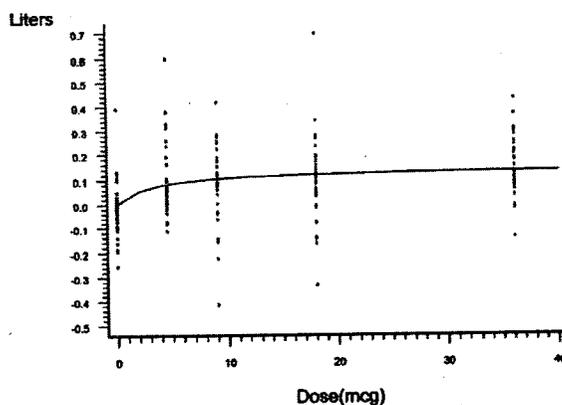
**Figure 1.** Dose-normalized urinary excretion in Study 205.108 in comparison to Studies 205.104, 205.105 and 205.117



The urinary excretion over the 24 hour period in steady state from this study was lower (3.4-6.4% of dose) than expected from Study 205.104 (10-15%). The sponsor explained that this might be due to formulation effects and/or age effects. Lactose inhalation capsules in this study had a lower 'respirable fraction' ( —  $\mu\text{m}$ : 13, 16, 17 and 20%) than those in Study 205.104 (23, 22 and 29%). In addition, patients in study had a mean age of 67 years, those of Study 205.104 a mean age of 35 years. The high scatter in all values with inconsistent increases and decreases could be due to compliance issues.

**Pharmacodynamics:** A maximum efficacy ( $E_{\text{max}}$ ) model was fitted to the dose-response data (Figure 2). The sponsor claimed that 8.8  $\mu\text{g}$  dose provided 75%, the 17.6  $\mu\text{g}$  dose provided 86% and the 35.2  $\mu\text{g}$  dose provided 92% of the maximum effect, based on the  $E_{\text{max}}$  model estimates.

Figure 2.  $E_{\text{max}}$  Model of Dose-Response



**Conclusions:**

- Tiotropium maximum plasma concentrations increased with dose in COPD patients and were similar to those in healthy volunteers, except the 17.6 $\mu\text{g}$  dose.
- Urinary excretion was about 4% for a 24 h dose interval and was lower than in healthy male subjects.
- Urinary excretion half-lives ranged from 5 to 7 days. Approximate steady state conditions were reached within 8 to 15 days of therapy and there appears no further drug accumulation for doses up to 17.6  $\mu\text{g}$  tiotropium cation.
- There was no clear dose-response relationship (Figure 2).

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## Protocol 205.112 (Study Report #U97-2425)

**Study type:** Tolerability & single/multiple rising dose PK, inhalation solution via \_\_\_\_\_  
**Title of study:** A multiple increasing dose tolerance study after inhalant administration of 10, 20 and 40 µg/24 h of Ba 679 BR \_\_\_\_\_ for 14 days in healthy volunteers.

**Investigator:** \_\_\_\_\_, Dr. med.

**Objectives:** To determine the tolerability and safety as well as the preliminary PK of tiotropium given by the BINEB device to healthy male volunteers.

**Study Design:** Randomized placebo controlled group comparison in four different groups of healthy male Caucasian subjects. 36 healthy male Caucasian subjects (range of age 21-43 years) participated in this study. Groups of nine subjects received one of the three treatments.

**Formulations:** Tiotropium was administered as tiotropium bromide monohydrate in three different doses to different groups of subjects one hour after a light breakfast.

- 8 µg tiotropium (= 10 µg Ba 679 BR, batch 9508206) or
- 16 µg tiotropium (= 20 µg Ba 679 BR, batch 9508207) or
- 32 µg tiotropium (= 40 µg Ba 679 BR, batch 9508209) or
- placebo inhalation capsules (batch 9508204)

Tiotropium was administered as a nebulized solution by the BINEB device. The BINEB device is pressure actuated like metered dose inhalers and not driven by inspiration force.

### **Analytical Methodology:**

**Assay Method:** Plasma and urine samples were assayed by HPLC- \_\_\_\_\_ with a limit of quantification of \_\_\_\_\_ tiotropium cation in plasma and \_\_\_\_\_ µg/mL in urine.

**Accuracy and Precision:** Assay precision in plasma samples was within \_\_\_\_\_ and accuracy was within ± \_\_\_\_\_. Respective values for urine samples were \_\_\_\_\_ for precision and ± \_\_\_\_\_ for accuracy.

**Sampling times:** Plasma was collected before and 5 and 20 minutes after inhalation on Days 1, 7 and 14 and 24 h on Day 1. Urine was collected in fractions 0-4, 4-8 and 8-24 hours post dose on Days 1, 7 and 14.

### **Criteria for evaluation:**

**Pharmacokinetics:**  $C_{max}$ ,  $C_{pre}$ , AUCs and amount of drug excreted unchanged in urine ( $A_e$ ).

**Safety:** Blood pressure, pulse and respiratory rates, ECG, salivary secretion, local tolerability, laboratory parameters, adverse events.

**Statistical methods:** Descriptive analysis.

**Results:** Maximum drug plasma concentrations and urinary excretion increased with increasing dose. The increase from Day 7 to Day 14 was much less than the increase from Day 1 to Day 7. This is in line with observations in other studies and suggests that nearly steady state conditions are achieved within 7-14 days at least with this device, while formal steady state ( $5-t_{1/2}$ ) were achieved within 25-30 days.

Tiotropium solution administered with the BINEB device resulted a higher bioavailability compared to that after dry powder inhalation: e.g.  $NC_{5min}$  values of 16-25 µg/mL on Day 14 (Study 205.104) vs 25-38 µg/mL in this study.  $A_{e0-24h}$  values ranged from 20-24.5% of dose in this study vs 10.5-14.7% of dose in Study 205.104. The total urinary excretion after intravenous administration was 73.6% in Study 205.105 and thus absolute bioavailability may be between 27 and 33% with the BINEB device (19.5% for dry powder inhalation).

Geometric mean PK parameters and a graphical representation of dose-normalized (to 18 µg) plasma concentration profiles are shown in Table 1 and Figure 1, respectively.