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Pharmacokinetic, Mass Balance and Tissue Distribution of [^{14}C]-BC-3781 in Non-pigmented Rats

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Abstract

Background: BC-3781 is a new antimicrobial agent of the pleuromutilin class. BC-3781 finished successfully four Phase I studies demonstrating a favorable safety and tolerability profile. Current clinical development targets the indications skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP) for the oral and intravenous route of application. BC-3781 exhibits excellent antimicrobial activity against an entire set of different bacterial pathogens often encountered in skin and skin-structure and respiratory tract infections. Target pathogens include among others methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Streptococcus pneumoniae,* atypicals and *Haemophilus influenzae*. In various murine efficacy models BC-3781 demonstrated very good potency characterized by a concentration-dependent killing and a moderate post-antibiotic effect.

Methods: [^{14}C]-labeled BC-3781 was administered to non-pigmented male and female rats as single i.v. dose of 10 mg/kg. The substance-associated radioactivity of whole blood, plasma, expired air, urine, feces, and carcasses was detected using liquid scintillation counting techniques. The tissue distribution was investigated by quantitative whole-body autoradiography (QWBA).

Results: The plasma concentration-time curve of i.v. administered [^{14}C]-BC-3781 described a multi-phasic decline, with a rapid initial distributional phase followed by a terminal elimination phase. Drug concentrations measured in the majority of tissues including skin, soft tissues and lungs were substantially higher compared to plasma levels. Within the investigated time all intra-organ concentrations of BC-3781 approached the lower limit of quantification, being in line with a 96 % total recovery of radioactivity after 7 days. No differences in gender could be determined.

Conclusions: BC-3781 showed a high tissue affinity and a rapid and homogeneous distribution of radioactivity from blood to tissues. The excretion of BC-3781 and/or its metabolites was rapid with 92 % of the material being excreted within 48 h via the urinary (14 %) and fecal route (82 %).

Introduction

The absorption, distribution, metabolism and excretion of BC-3781 was investigated in the rat, using a [^{14}C]-labelled test substance. BC-3781 is in clinical development for intravenous and oral treatment of skin and skin structure infections (SSSI) and community-acquired pneumonia (CAP). The objectives of the study were as follows:

- To determine the rates and routes of excretion of [^{14}C]-BC-3781, and its metabolite(s) after single dose administration to male and female rats
- To determine the tissue distribution of radioactivity after a single dose administration to male and female rats

Methods

Radiochemical purity: The radiochemical purity of [^{14}C]-BC-3781 was >95 % prior to dosing as determined by HPLC analysis. The specific activity of the radiosynthesized BC-3871 was 62.3 µCi/mg.

Animals: The animals used were female and male Sprague Dawley rats Crl:CD(SD) within a weight range of 175–360 g and were 6-13 weeks old at the time of dose administration.

Experimental procedures: All animals were dosed with 10 mg/kg [^{14}C]-BC-3781 into the tail vein. From the first group of animals (five female and five male) blood samples were collected pre-dose and at 5, 15 and 30 minutes and 1, 4, 6, 12, 24 and 48 h to investigate the PK of BC-3781 in whole blood and plasma. A second group of rats was dosed and returned to separate metabolism cages where urine and faeces were collected over a period of 168 h. At the end of the collection period the residuals in the carcasses and the cages were measured as well. Furthermore the radioactivity in the expired air was measured within the first 72 h. The third group of animals was dosed and the tissue distribution of [^{14}C]-BC-3781 was investigated at 5 and 30 minutes and at 6 h, 24 h and 72 h.

Analytical methods: The radioactivity associated with dosing solutions, whole blood, plasma, expired air traps, urine, faeces, carcasses and cage washings was determined using liquid scintillation counting techniques. The tissue distribution was investigated by quantitative whole-body autoradiography (QWBA).

Results

Blood levels of [^{14}C]-BC-3781 in Sprague Dawley Rats

[^{14}C]-labeled BC-3781 was administered as a single bolus application (10 mg/kg) to two groups of five male and female Sprague Dawley rats each. Radioactivity concentrations were measured in blood and plasma samples obtained pre-dose and 5, 15 and 30 min and 1, 4, 6, 12, 24, and 48 h post-dose and are expressed as µg equivalent/g. The plasma-concentration time curve and respective pharmacokinetic parameters (Table 1) showed no statistically significant difference between female and male animals. After 12 h radioactivity levels dropped below the detection limit. Mean ratio of whole blood and plasma concentrations was comparable in both genders

Table 1: Pharmacokinetic parameters measured in plasma collected from male and female Spraque Dawley rats following a single i.v. dose of [^{14}C]-BC-3781 at a dose level of 10 mg/kg

PK-Parameter	10 mg/kg	10 mg/kg
Sex	male	female
Route of Administration	i.v.	i.v.
Number of animals per group	5	5
Vehicle	saline	saline
C_0 ± SD [µg equivalents/g]	2.63 ± 0.40	2.45 ± 0.57
$AUC_{0\text{-inf}}$ [µg equivalents·h/g]	2.48	3.36
Terminal $t_{1/2}$ [h]	2.95	3.43
Mean whole blood/plasma ratio	1.45	1.35

Quantitative Whole-body Autoradiography of [^{14}C]-BC-3781 in Sprague Dawley Rats Following i.v. Bolus Administration

The mean radioactive tissue concentration-time data of tissues of therapeutic interest and excretion organs are provided in Table 2. Already at early distribution phase (five minutes after administration) most organ and tissue concentrations of BC-3781 are higher compared to the corresponding BC-3781 blood levels. Tissue:blood ratios were greater than 1 in most tissues already at five and 30 minutes after administration indicating rapid distribution from blood into tissues. Concentrations in the brain and spinal cord were lower than those in blood at early sampling times indicating that drug-related material had not crossed the blood/brain barrier. By six hours, tissue:blood ratios were greater than 1 in all tissues analyzed with exception of brain/spinal cord and bone. In the terminal elimination phase of the BC-3781 PK profile (24 h to 72 h) all investigated organs and tissues showed a rapid decrease in radioactivity, indicating that no intra organ accumulation of BC-3781 and/or its metabolites occurs. Highest concentrations of radioactivity were mainly observed in the gastrointestinal tract content, indicating a major excretion *via* the bile and/or the mucosa. An overview of the qualitative distribution pattern for male animals (5 min and 30 min) is shown in Figure 1. Figure 2 shows the distribution in a male and in a female rat 6 h after application.

Figure 1: Whole-body autoradiography of male rats 5 min (left image) and 30 min (right image) after an i.v. dose of 10 mg/kg [^{14}C]-BC-3781

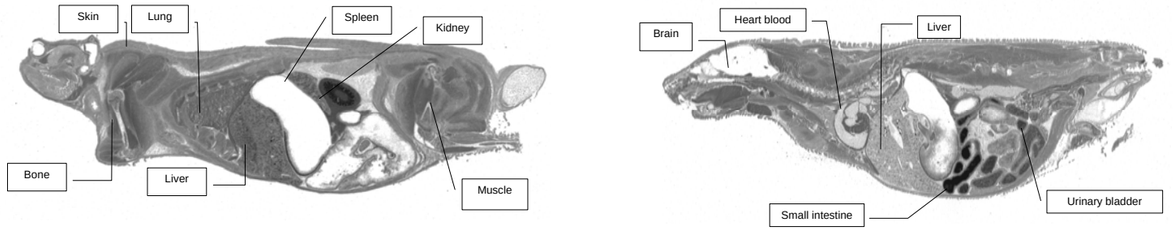

Figure 2: Whole-body autoradiography of a male (right) and a female (left) rat 6 hours after an i.v. dose of 10 mg/kg [^{14}C]-BC-3781

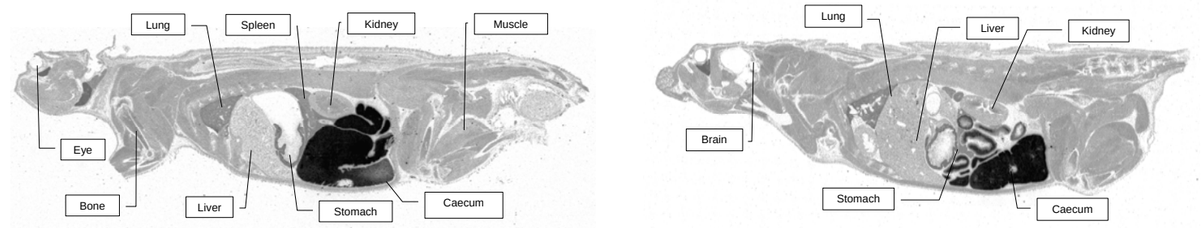

Table 2: Concentrations of radioactivity in the tissues of male and female Sprague Dawley rats following a single i.v. administration of [^{14}C]-BC-3781 at a nominal dose level of 10 mg/kg

Matrix	Sex	Concentrations Expressed as µg equivalents/g				
		5 min	30 min	6 h	24 h	72 h
Blood (heart)	Male	3.44	0.763	0.104	BLQ	BLQ
	Female	4.73	0.93	0.135	BLQ	BLQ
	Combined	**4.09**	**0.85**	**0.120**	**BLQ**	**BLQ**
Muscle	Male	12.0	8.64	1.26	BLQ	BLQ
	Female	8.92	9.94	1.91	BLQ	BLQ
	Combined	**10.5**	**9.29**	**1.59**	**BLQ**	**BLQ**
Skin	Male	4.54	2.89	0.736	0.059	BLQ
	Female	3.58	3.15	0.745	0.087	BLQ
	Combined	**4.06**	**3.02**	**0.741**	**0.073**	**BLQ**
Lung	Male	14.3	5.76	3.33	0.096	0.066
	Female	28.4	9.81	4.81	0.133	BLQ
	Combined	**21.4**	**7.79**	**4.07**	**0.115**	**0.066**
Kidney	Male	60.8	18.7	1.47	0.129	0.128
	Female	41.7	9.52	1.62	0.133	0.095
	Combined	**51.3**	**14.1**	**1.55**	**0.131**	**0.112**
Liver	Male	18.4	3.15	0.633	0.115	0.095
	Female	26.5	8.95	1.19	0.113	0.068
	Combined	**22.5**	**6.05**	**0.912**	**0.114**	**0.082**

Mass Balance

After 168 h all excretion samples and carcasses of all animals were analyzed for radioactivity and recovery was calculated as a percentage of administered drug. In Table 3 the mean equivalent concentrations of BC-3781 and/or its metabolites excreted in urine, feces, and air and the recovery expressed as percentage of dose are presented. The mean (± SD) total recovery of BC-3781 and/or its metabolites was 92 % within the first two days (48 h) and 96.00 % ± 2.25 within 7 days (168 h) indicating a total elimination of the drug. From this total amount ± SD, 13.56 % ± 1.59 was recovered in the urine, 81.61 % ± 3.21 in the feces, approximately 1 % in the cage rinse and residual carcasses, and no radioactivity was detected in the expired air. The excretion data indicate that BC-3781 and/or its metabolites were excreted *via* both routes, urine and feces, but clearly identifying the fecal route as the primary route of elimination.

Table 3: Mass balance results in rats following a single i.v. bolus injection of [^{14}C]-BC-3781

Sex	Dose [mg/kg]	Matrix	Sampling Time [h]	Recovery [%]	Total Recovery ± SD [%]
Male	10	Urine	0-168 h	12.76	96.44 ± 2.58
		Feces		82.79	
Female	10	Urine	0-168 h	14.37	95.56 ± 2.05
		Feces		80.43	
Combined	10	Urine	0-168 h	13.56	96.00 ± 2.25
		Feces		81.61	

Conclusions

- The concentration-time curve of intravenously administered [^{14}C]-BC-3781 showed a multi-phasic decline, with a rapid distributional phase and a prolonged terminal phase
- QWBA showed a rapid distribution into tissues and organs demonstrating
  - higher concentrations in tissues compared with blood
  - good penetration into tissues of relevance for therapeutic indications of interest, SSSI and CAP
  - no radioactivity crossing the blood brain barrier
- A total elimination of the drug and/or its metabolites indicated
  - all intra-organ radioactivities approaching the lower limit of quantification within 72 h (QWBA)
  - a total recovery of 96 %, mainly via feces (82 %) and urine (14 %), as determined by mass balance
  - fecal excretion being the most important route of elimination for BC-3781