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The effects of the CXCR2 antagonist, MK-7123, on bone marrow functions in healthy subjects

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ABSTRACT

The CXCR2 antagonist MK-7123 causes dose-dependent reductions in absolute neutrophil counts (ANC) and decreases neutrophil tissue responses, but its effects on bone marrow functions are not yet known.

We conducted a double-blind, randomized study in 18 healthy subjects comparing the effects of either MK-7123 (30 mg, po, daily for 28 days) or placebo on peripheral blood counts and bone marrow myeloid cell populations. MK-7123 caused a reversible decrease (approximately 50%) in the ANC as demonstrated on days 1 and 28, the first and last days of the treatment period. Bone marrow aspirate smears and biopsy imprints did not differ in the proportion of mature neutrophils in pretreatment, day 28, day 56 or placebo samples. There were no treatment effects on biopsy or aspirate clot cellularity, myeloid to erythroid or myeloid post-mitotic to mitotic ratios; flow-cytometric analyses of aspirate cells; or bone marrow fat to cell balance as assessed by MRI. MK-7123 was generally well tolerated with neutropenia being the most common adverse event; however, there were no clinical symptoms associated with decreased ANCs.

These findings indicate that the CXCR2 antagonist MK-7123 causes rapidly reversible decrease in the ANC without measurable myelosuppressive effects. The results support the development of CXCR2 antagonists as potentially useful anti-inflammatory agents, primarily interrupting neutrophil trafficking.

1. Introduction

Neutrophil chemotaxis to sites of inflammation is in part driven by ELR-CXC chemokines, chemokines containing the sequence glutamate–leucine–arginine (ELR) immediately preceding the cysteine-x-cysteine (CXC) motif, such as interleukin (IL)-8 and growth-related protein (Gro). Neutrophils express several chemokine receptors on their plasma membranes, including CXCR1, CXCR2, and CXCR4. These receptors respond differentially to the ligands [2,5,13,20,8,24]. In general, binding of CXCR1 stimulates the oxidative burst and release of proteases and phagocytosis, whereas CXCR2 is responsible for neutrophil trafficking. Another receptor, CXCR4, signals for retention of neutrophils in the bone marrow [8,17].

CXCR2 activity in inflammation has been characterized in such tissues as lung [11] and brain [25] and inhibition of neutrophil trafficking via CXCR2 antagonism is a current target of research for the treatment of such diseases as chronic obstructive pulmonary diseases (COPD) [5]. MK-7123 (previously known as SCH 527123), is selective for CXCR2, with an approximately 80-fold higher affinity for CXCR2 than for CXCR1 [10]. MK-7123 has demonstrated efficacy in preclinical and clinical models [4,23,12] and reduced pulmonary neutrophilia and improved lung function in patients with COPD [21].

Human studies with MK-7123 have consistently shown a reversible decline in the absolute neutrophil count (ANC) [21,22] with onset beginning with the first hours of exposure, maximal at 6–8 h and reversed by 48 h. The decline in the ANC may be attributable to reduced egress of neutrophils from the bone

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marrow or a reduction in the number of neutrophils available for release from the bone marrow. The objective of the present study was to determine the effect of MK-7123 on mature (band plus segmented) neutrophils and their precursors in the bone marrow of healthy human subjects. We tested the hypothesis that maturation and composition of hematopoietic cells in the bone marrow would not change after treatment with MK-7123. We also investigated the time course of decline and recovery of neutrophils in the blood, the reversibility of any potential bone marrow and blood effects following cessation of dosing, and the safety and tolerability of MK-7123 during a 4-week period.

2. Methods and materials

2.1. Study design

This was a randomized, double-blind, placebo-controlled, parallel-group study (protocol P04552) in 18 male and female adult healthy volunteers, performed at Phase One Trials A/S, Hvidovre University Hospital, Hvidovre, Denmark between June and December 2006. Subjects were randomized to 30 mg MK-7123 or placebo in a 3:1 ratio. Oral treatment was once daily in the morning at approximately 8 AM, for 28 days. Timing of meals was not limited except for day 28, when samples were taken in fasting subjects for pharmacokinetic analysis. The study was conducted in accordance with principles of Good Clinical Practice and was approved by the appropriate institutional review board.

2.2. Sample size/power

With 16 total subjects, 12 were to be randomized to active comparator and 4 were to be randomized to placebo, in a parallel group design. With this sample size, a relative difference of 37% in the change from baseline in the primary parameter should be detectable with 80% power and two-tailed alpha = 0.05, assuming a standard deviation (SD) of 4.24%.

2.3. Subjects

Healthy men and women between 18 and 65 years of age, who were non-smokers or light smokers (<10 cigarettes per day), and with an ANC of $\geqslant 2.0 \times 10^9$/L, were eligible to enroll in this study. Potential subjects were screened for eligibility up to 28 days prior to Baseline (day-1).

2.4. Sample collections

At baseline and on days 28 and 56 ($\sim$12 h post dose), subjects underwent bone marrow aspiration and biopsy from the iliac crest for analysis of aspirate smears and clots and biopsy sections and imprints. One subject in the MK-7123 group declined the bone marrow examination at day 56. Blood samples (0.5 mL) were collected in EDTA tubes at baseline, days 28 and 56 for complete blood cell counts (CBC). An additional sample for a blood smear and flow cytometry was collected at the time of the bone marrow biopsy. Subjects fasted for approximately 10 h before the final dose of study drug on day 28, and blood samples ($\sim$5 mL) were collected pre-dose and at 0, 1, 2, 4, 6, 8, 12, and 24 h after dosing for CBC and pharmacokinetic analysis.

2.5. Analytical laboratories

Haematopathologic analysis of bone marrow and blood samples were performed at the Department of Pathology, Copenhagen University Hospital, Rigshospitalet, Denmark. Flow cytometry samples were analyzed at the Department of Haematology, Copenhagen University Hospital, Rigshospitalet, Denmark. Pharmacokinetic analysis was performed at Schering-Plough Research Institute, Summit, NJ, USA.

2.6. Blood smears

Smears of blood were fixed in methanol and stained with May–Grünwald–Giemsa. Differential counts, assessment of leukocyte morphology, assessment of erythrocyte shape and size, and assessment of platelet size and number were assessed manually.

2.7. Bone marrow

Smears of bone marrow aspirates and imprints were fixed for 5 min in methanol and stained with May–Grünwald–Giemsa. One smear of bone marrow was fixed for 5 min in methanol and stained for iron (Perls Berlin blue reaction for ferri iron). The clot was formalin fixed and paraffin embedded according to routine procedures. The bone marrow biopsy was formalin fixed, decalcified in formic acid, and paraffin embedded according to routine procedures. Five micrometer sections were stained with/for: HE, iron and reticulin (modified Gordon and Sweet), respectively, and all specimens were evaluated by one haematopathologist (NH). Assessment of apoptosis was made using immune histochemical staining for caspase-3 on bone marrow biopsy. Flow cytometry was performed on samples of bone marrow aspirates using a panel of standard antibodies.

2.8. Magnetic Resonance Imaging (MRI)

MR-scanning was conducted on a 3T Siemens Trio MR-scanner (Siemens, Erlangen, Germany) at baseline, days 28 and 56 for visual assessment of: (1) the bone marrow changes in the hip and pelvis regions; (2) changes in the size of the liver and spleen; and (3) changes of lymph node status in the neck region.

The MRI images of the hip and pelvis regions were acquired in the coronal image plane with the following MR pulse sequences: (1) T1-weighted SE-sequence (TR = 600 ms, TE = 14 ms, pixel size = 0.8 $\times$ 0.8 mm^2, slice thickness = 5 mm); (2) T2-weighted TSE-sequence (TR = 3030 ms, TE = 71 ms, pixel size = 0.8 $\times$ 0.8 mm^2, slice thickness = 5 mm); (3) T2-weighted fat suppressed TIR-sequence (TI = 180 ms, TR = 9480 ms, TE = 13 ms, pixel size = 0.9 $\times$ 0.9 mm^2, slice thickness = 5 mm); (4) T2-weighted fat-suppressed 3D-MEDIC-sequence (TR = 23 ms, TE = 12 ms, FA = 12°, voxel size = 0.6$\times$0.6$\times$0.6 mm^3).

For the liver and spleen assessments, axial images were acquired with the following MR pulse sequences: (1) PSIF-sequence (TR = 4.8 ms, TE = 2.4 ms, FA = 80°, pixel size = 0.7 $\times$ 0.7 mm^2, slice thickness = 8 mm); (2) T2-weighted HASTE-sequence (TR = 1400 ms, TE = 92 ms, pixel size = 0.7 $\times$ 0.7 mm^2, slice thickness = 8 mm); (3) T1-weighted TIR-sequence (TI = 800 ms, TR = 1270 ms, TE = 35 ms, pixel size = 0.7 $\times$ 0.7 mm^2, slice thickness = 8 mm); (4) T1-weighted fat-suppressed STIR-sequence (TI = 180 ms, TR = 1270 ms, TE = 35 ms, pixel size = 0.7 $\times$ 0.7 mm^2, slice thickness = 8 mm); (5) T1-weighted FLASH-sequence with in- and opposed phases echo times (TR = 137 ms, TE = 2.5 ms and 6.1 ms, FA = 70°, pixel size = 0.7 $\times$ 0.7 mm^2, slice thickness = 8 mm).

For the neck and supraclavicular regions, coronal and axial images were acquired with: (1) T1-weighted TSE-sequence (TR = 495 ms, TE = 15 and 17 ms, pixel size = 0.5 $\times$ 0.5 and 0.9 $\times$ 0.9 mm^2, slice thickness = 3 mm); (2) T2-weighted TSE-sequence (TR = 8380 ms, TE = 103 and 92 ms, pixel size = 0.5 $\times$ 0.5 and 0.9 $\times$ 0.9 mm^2, slice thickness = 3 mm). A T2-weighted fat-suppressed 3D-MEDIC-sequence (TR = 23 ms, TE = 12 ms,