

Efficacy of a Novel Antifolate CH-4051 in a Rat Collagen-Induced Arthritis Model

Abstract #SAT0137

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Introduction

The classic and most commonly used antifolate is methotrexate (MTX), which has been used for the treatment of cancer for nearly 5 decades^{1,2,3}. MTX is utilized today in the treatment of a number of other indications, including rheumatoid arthritis (RA), lupus, psoriasis, Crohn's disease, psoriatic arthritis, and Reiter's syndrome.

MTX enters cells via the Reduced Folate Carrier (RFC) system. Once inside cells, it is converted enzymatically to polyglutamylated derivatives. These metabolites cannot be readily effluxed and are retained in tissues^{4,5}. The accumulation of polyglutamyl metabolites of MTX for prolonged periods may play a significant role in both the efficacy and the toxicity of this compound. MTX in its parent form only has activity against DHFR. Its polyglutamylated derivatives also have significant activity against other enzymes (e.g. TS). In addition to polyglutamylation metabolism, MTX is also hydroxylated in the liver to a metabolite known as 7-hydroxymethotrexate, which is also subject to polyglutamylation and cell retention. This metabolite has been associated with liver and kidney toxicity of the parent compound, while contributing no role in efficacy^{6,7,8,9}.

Chelsea has been developing CH-4051, a novel, metabolically inert, antifolate that we hypothesize may be safer, better tolerated and more efficacious than MTX. CH-4051 has been shown in vitro to be a nonpolyglutamylatable and nonhydroxylatable antifolate that is more efficiently taken up into cells by the RFC system than is MTX¹⁰ (Table 1). CH-4051 has significant activity on DHFR without the need for polyglutamylation. The lack of hydroxylation potentially leads to enhanced levels of the active drug in the cell. Furthermore, the glutamyl moiety is not susceptible to being cleaved by carboxypeptidase. Thus CH-4051 may be referred to as a metabolically stable antifolate. The hypothesis is that CH-4051 in the clinical setting will demonstrate the efficacy of classical antifolates (via folate enzyme inhibition), but will be devoid of the toxicity secondary to the formation of the polyglutamylated and hydroxylated metabolites, providing a significantly improved therapeutic index compared to classical antifolates, such as MTX. The current study explored the efficacy and safety of CH-4051 in a rat model.

Table 1: In vitro Properties of CH-4051 and Related Non-Metabolizable Antifolates

Inhibitor	DHFR IC ₅₀ (nM)	RFC IC ₅₀ (nM)	CRF-CEM Growth Inhibition
L-MTX	1.1 (0)	9 (1)	14.7 (0.5)
D-MTX	ND	49 (1)	ND
CH-1504	2.6 (0.2)	1.8 (0.1)	8.3 (0.4)
CH-4051	1.3 (0.1)	1.1 (0)	5.2 (0.7)

Methods

Toxicology: Male and female SD rats were used (n=10 - 15/grp). Animals were treated with daily oral doses of 0, 2.5, 5 or 10mg/kg for 13 weeks. Blood was drawn at 0, 6 and 13 weeks for evaluation of standard hematology and clinical chemistry parameters and at 13 weeks end organs were processed for histological examination.

CIA Model: Lewis rats (n=10/grp) were injected SC with bovine collagen in IFA on day 0 and 6. Animals were treated, (day 0 to 16) with various dose regimens of CH-4051, vehicle or MTX as a control. Body weight and ankle diameter were measured daily from the start of disease onset until the end of the study. On termination, affected joints (knees and ankles) were processed for histological evaluation of joint status. Plasma samples were taken on day 16 for the determination of the blood levels of CH-4051.

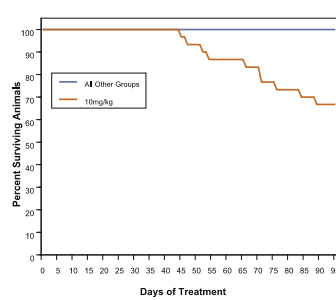


Figure 1: Mortality Observed in 3 Month Toxicology Study

Animals treated with 10 mg/kg of CH-4051 displayed significant morbidity, beginning around 6 weeks of treatment. Clinical observations included paleness of extremities, hunched posture, piloerection and furstaining. Morbidity and mortality were not seen in animals dosed with 5 mg/kg during this 13 week study.

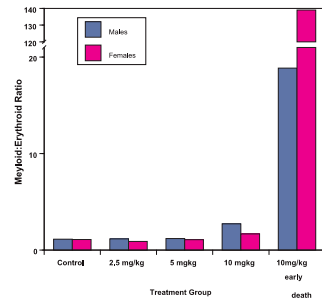


Figure 3: Bone Marrow Depletion of Cells in the Erythroblast Lineage

Myeloid to erythroid cell ratios were modestly affected in animals dosed with 10 mg/kg of CH-4051 who survived through the 13 weeks of treatment. In contrast, animals in the top dose group who died prematurely exhibited extreme decreases in the number of cells of the erythroid lineage. This appeared to be the dose-limiting toxicity for high doses of CH-4051.

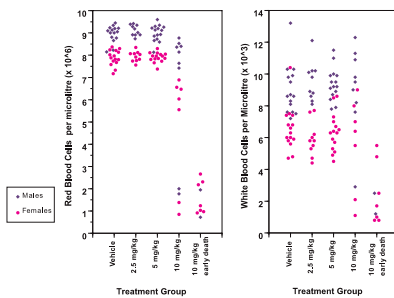


Figure 2: High Dose CH-4051 Suppresses Numbers of Circulating Blood Cells

Thirteen weeks of treatment with CH-4051 at 10 mg/kg induced suppression of the number of circulating red blood cells and, to a lesser extent, white blood cells in most animals. The effect was most pronounced in animals that died during the study, with most of these animals suffering from severe anemia at time of euthanization.

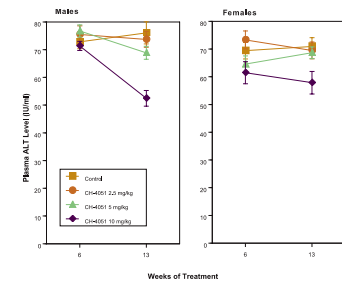


Figure 4: The Liver was not Affected by Chronic Treatment with CH-4051

Serum levels of liver enzymes (ALT and AST) were not elevated by 13 weeks of daily oral dosing up to 10 mg/kg. At the top dose of 10 mg/kg, serum levels of ALT and AST were significantly lower in animals dying prematurely than in animals surviving the entire study (inset). This likely reflects decreased liver metabolism as a morbidity related change rather than a direct effect of the drug.

Results

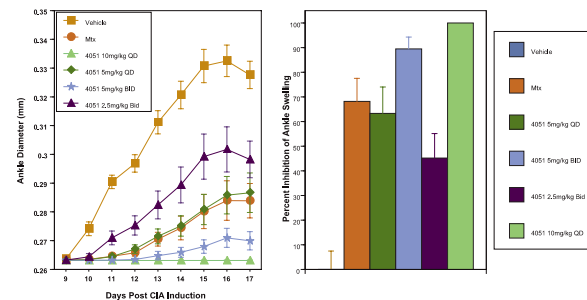


Figure 5: Daily Treatment with CH-4051 Inhibits the Development of CIA in the Rat

Arthritis was induced in rats (n=10/group) as described in methods and beginning on day 0, animals were treated orally with either vehicle, 0.25 mg/kg of MTX twice weekly, daily with 5 or 10 mg/kg of CH-4051, or twice daily with 2.5 or 5 mg/kg of CH-4051. A) Control animals developed progressive swelling of their ankles from day 9 through 16 of the study. Treatment with CH-4051 significantly decreased swelling at all doses. CH-4051 at 10 mg/kg daily completely suppressed swelling of the ankles in all animals. Daily treatment with 5 mg/kg of CH-4051 or twice weekly treatment with MTX resulted in roughly equivalent suppression of ankle swelling. B) Twice daily treatment with 5 mg/kg of CH-4051 resulted in a significant increase in the percent inhibition of ankle swelling relative to control when compared to that seen with once daily treatment or Mtx treatment (p<0.05).

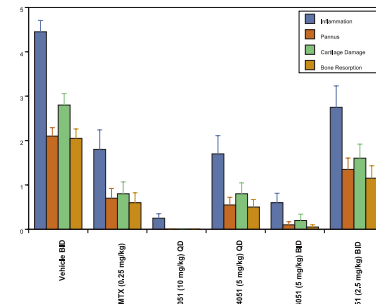


Figure 6: Effect of CH-4051 Treatment on Histopathological Changes Induced Ankle Joints by CIA Induction

Induction of CIA caused significant inflammation, pannus formation, cartilage destruction and bone resorption in vehicle treated animals. In contrast, all treatments with CH-4051 significantly decreased the degree of histological damage observed in the ankle joint. Similar results were observed in the knee joints (data not shown). As was observed for ankle swelling, daily treatment with 10 mg/kg of CH-4051 almost completely blocked histological damage to the joints, with only a few animals showing mild inflammatory infiltrates of the joint. Daily treatment with 5 mg/kg of CH-4051 or twice weekly treatment with Mtx, showed similar levels of suppression of joint destruction, while twice daily treatment with 5 mg/kg of CH-4051 resulted in nearly complete suppression of cartilage damage and bone resorption (mild effects observed in 1/10 animals).

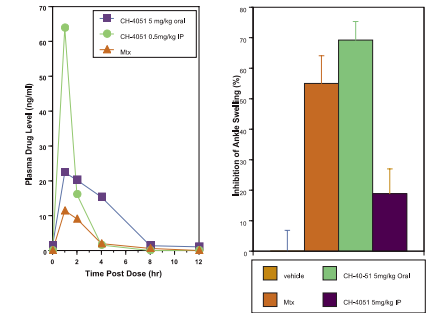


Figure 7: Effect of CH-4051 Dosing Route on the Efficacy of CIA Therapy

Arthritis was induced in rats (n=15/group) as described in methods and beginning on day 0, animals were treated once daily with vehicle, 5 mg/kg of CH-4051 administered orally, 0.5 mg/kg of CH-4051 administered by interperitoneal injection (ip) or 0.06 mg/kg of MTX orally. Although, ip injection resulted in high levels of CH-4051 in the blood (C_{max}), the drug was rapidly cleared and overall exposure was less than that observed following oral administration of the drug (A). Suppression of ankle swelling by once daily oral treatment with 5 mg/kg of CH-4051 was similar to that observed in the previous study. In contrast, daily treatment by ip injection resulted in significantly more ankle swelling (p<0.01; figure 7B). Further, although oral treatment with CH-4051 resulted in suppression of histological measures of joint destruction relative to control animals, suppression of joint destruction was not observed following ip treatment with CH-4051 (data not shown).

Conclusions

CH-4051 was demonstrated to be safe and well tolerated at doses associated with significant activity in the rat CIA model of inflammation. It would appear that AUC is a more meaningful parameter than C_{max} in predicting anti-inflammatory efficacy of this molecule. Finally, we conclude that these data support the continued development of CH-4051 as an oral DMARD therapy for RA.

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