Oral efficacy of a leukotriene B₄ receptor antagonist in colitic cotton-top tamarins

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Abstract

Leukotriene B₄ (LTB₄) is a potent neutrophil activator and chemotaxin that is present in increased concentrations in the colonic tissue and rectal dialysates of acute ulcerative colitis patients. Cotton-top tamarins (CTTs) with confirmed active colitis were treated with the second generation LTB₄ receptor antagonist, SC-53228 ((+)-(S)-7-[3-(2-cyclopropylmethyl)-3-methoxy-4-[(methylamino)carbonyl]phenoxy]propoxy]-3,4-dihydro-8-propyl-2H-1-benzopyran-2-propanoic acid), 20 mg/kg bodyweight by gavage, twice daily for 56 days. End points were body weights, stool consistency, colonic endoscopy, assay of inflammatory mediators, and haematology and clinical chemistry tests. LTB₄ and prostaglandin E (PGE) values were measured in rectal dialysates at pretreatment, 28 day and 56 day time points. LTB₄ concentrations were reduced from pretreatment mean (SEM) values of 37.3 (0.8) ng/ml to 3.7 (0.8) ng/ml ($p < 0.001$) and 2.3 (0.5) ng/ml ($p < 0.01$) at days 28 and 56, respectively. On the other hand, mucosal protective PGE values remained constant or slightly increased during SC-53228 treatment (pre: 6.9 (2.2) ng/ml; day 28: 6.7 (1.4) ng/ml; day 56: 9.9 (1.6) ng/ml). Furthermore, assessment of a panel of 35 clinical chemistry and haematology parameters throughout the treatment showed there were no significant untoward effects of drug treatment. Six CCTs finished the eight week treatment and five of six gained weight (ranging from 27-121 grams each) while one CTT lost weight (50 g). Stool condition improved in five of six animals while one of six remained unchanged. All CCTs showed dramatic improvement histologically, with no or only minimally active colitis after treatment. The histological changes plus significant weight gains and improvement of stool condition (quality of life parameters) after eight weeks of SC-53228 treatment were remarkable. Furthermore, in follow up biopsies seven months after treatment ceased, three of six CTTs had no active colitis. This is the first time afflicted CTTs have not had recurring colitic exacerbations after a treatment regimen was stopped. It is concluded that in colitic CTTs, SC-53228 has shown both an immediate and a long acting anticolitic activity. It is also concluded that reduced LTB₄ concentrations during treatment

inhibited neutrophil infiltration of the colonic tissue and this, coupled with the maintenance of mucosal protective prostaglandins, contributed to the dramatic anticolitic efficacy. The treatment was safe over eight weeks. A compound such as SC-53228 may be useful in the medical treatment of human inflammatory bowel disease.

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Keywords: colitis, inflammatory bowel disease, cotton-top tamarins, leukotriene B₄, receptor antagonist.

Chronic relapsing inflammatory bowel disease (IBD) is comprised of two major forms, namely, ulcerative colitis, and Crohn's disease, which may or may not represent discrete disease entities.^{1,2} Many models of colonic inflammation in animals have been proposed as mimics of IBD. Colitis induced by such agents as trinitrobenzene sulphonic acid, short chain organic acids, and dextran sulphate deal with some of the aspects of mediator release and cellular trafficking associated with acute, non-specific inflammation.³⁻⁵ Spontaneous colitis in a new world primate, the cotton-top tamarin (CTT) (*Saguinus oedipus*) offers some distinct advantages as an experimental model. With a few identified exceptions,⁵ virtually all of the CTT population in captivity in various colonies throughout the world exhibits a spontaneous colitis characterised by exacerbation and remission cycles. Histologically, this includes an active colitis with varying degrees of neutrophil infiltration of the lamina propria and crypt epithelium, formation of crypt abscesses, and mucin depletion. Most often, this active inflammatory process is superimposed upon an epithelium that has remodelled, often with bizarre formations, after previous active episodes. The lamina propria is often filled with excessive numbers of chronic inflammatory cells (lymphocytes, plasma cells, histiocytes, etc) along with atrophy and lower crypt numbers and depths. Evidence of present or prior active colitic episodes precede the development of high incidences of colonic carcinoma. Other identified changes include the presence of circulating autoantibodies to Mr 40000 (that acts as an autoantigen in human ulcerative colitis),⁶ remission/exacerbation cycles, and lipid mediator release such as high colonic content of leukotriene B₄ (LTB₄).^{7,8}

LTB₄ is a product of arachidonic acid metabolism by the 5-lipoxygenase pathway.

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LTB₄ has been shown to be a potent granulocyte activator and has been proposed as an important mediator of inflammation.⁹ Injected LTB₄ induces neutrophil accumulation in rodent and human skin.¹⁰ LTB₄ is found in increased quantities in the tissue and rectal dialysates of ulcerative colitis patients where it is thought to potentiate the tissue damage seen in this disease.¹¹

SC-53228 is a second generation LTB₄ receptor antagonist with oral, topical, and intracolonic activity.^{12,13} As increased concentrations of LTB₄ have been reported in ulcerative colitis patients,^{11,14} as well as in the CTT,¹⁵ SC-53228 represented a reasonable candidate for an anticolitic agent.

Accordingly, the aims of this study were to (a) confirm with a second generation LTB₄ receptor antagonist, the finding that oral treatment resulted in lowered colonic concentrations of LTB₄,¹⁵ (b) equate lowered LTB₄ values with quality of life parameters, (c) evaluate histologically¹⁶ the effects of longterm drug treatment, and (d) assess SC-53228 safety in CTTs.

Methods

Description of subject population

CTTs (n=6) were housed at the AAALAC-accredited Marmoset Research Center at Oak Ridge, TN. The research protocol was approved and monitored by the IACUC of the University of Tennessee Medical Center, Knoxville.

Assessment of disease activity

Symptoms – stool consistency was followed throughout the study and qualitatively graded on a scale from 1–4 (1=firm: formed stool; 2=loose: semi-formed stool that did not hold its shape after being passed but was not watery; 3=puddly: semi-liquid stool that lost its shape quickly while being passed but could not be described as a solution or suspension; 4=diarrhoea: liquid stool that was watery with no form at all and resembled a dilute suspension).

Endoscopy – the colonoscopy procedure for visualisation and obtaining colonic mucosal samples is described in detail elsewhere.⁷ Briefly, fasted animals were anaesthetised with ketamine hydrochloride (20–25 mg/kg bw) plus butorphanol tartrate (0.15–0.25 mg/kg bw) and examined visually. Two mucosal biopsy specimens were taken at 5, 10, and 15 cm from the anus and fixed in buffered formalin. Any abnormality was described and sampled. Histological tissues were stained with PAS and evaluated for colitic activity.

Disease activity index – histological evaluation of colonic biopsy specimens was performed in a blinded fashion at several laboratories.¹⁷ Mucosal histological score was graded on a scale of 0–5 (0=chronic: no evidence of increased neutrophils (PMN) in lamina propria, crypt epithelium or in the crypt lumen along with increased numbers of chronic inflammatory cells such as plasma

cells, lymphocytes, and histocytes; 1=active mild: increase of a few PMNs in lamina propria; 3=active moderate: PMNs increased in at least two areas (lamina propria and epithelium) with some crypt abscesses; 5=active severe: PMNs heavily infiltrated through mucosa and lamina propria with abscesses in most crypts).

Subject well being – animal body weights were obtained biweekly and chemistry and haematological screens were performed before the experiment and 28 and 56 days after.

Drug treatment

SC-53228 was formulated as a sonicated suspension (5 mg/ml) in 0.5% methylcellulose. Twenty mg/kg bw was given by gavage twice daily at 0800 and 1600.

Rectal dialysate studies

Rectal dialysis bags were placed in the rectum and descending colons for 1¼ hours under ketamine anaesthesia as previously described.¹⁸ The dialysate solution was composed of sodium chloride, potassium bicarbonate, and fatty acid free albumin adjusted to pH 7.1 with NaOH. Upon removal of the bag, the dialysate was immediately frozen (–70°C) and subsequently analysed for content of lipid mediators of inflammation.

Measurement of lipid mediators

Eicosanoids were measured directly from rectal dialysates as previously reported.¹⁵ Samples were assayed with validated commercial radioimmunoassays for LTB₄ and PGE₂. Values of immunoreactive LTB₄ and PGE₂ were expressed as mean (SEM) in ng/ml rectal dialysate.

Statistical analyses

The Student's *t* test was used to detect statistically significant differences between groups. Data are presented as mean (SEM). A result was considered statistically significant of *p* ≤ 0.05.

Results

During treatment with SC-53228, body weights increased in five of six and decreased in one of six animals at day 56, the last day of treatment (Fig 1A). Furthermore, this trend continued through day 240 with animals either gaining or maintaining their weights. One animal died at day 95 of causes unrelated to treatment (colonic carcinoma). Stool consistency improved in five of six and was the same in one of six animals after 56 days of treatment (Fig 1B). By day 240, four of five remaining animals' stool consistency ratings remained constant while one of five slightly worsened.

The concentration of LTB₄ in rectal dialysates was reduced over 90% from pre-treatment values of 37.3 (0.8) ng/ml to 2.3

Figure 1: Effect of SC-53228 treatment on quality of life parameters in colitic cotton-top tamarins as assessed by body weights (A) and stool consistency (B) (see Methods for grade information).

(0.5) ng/ml ($p < 0.001$) after 56 days of SC-53228 treatment (Fig 2A). These concentrations rose ($p < 0.05$ v day 56) to 8.1 (0.7) ng/ml by day 240 but were still lower ($p < 0.001$) than pretreatment values. In addition, PGE concentrations rose slightly, but non-significantly, from pretreatment values (6.92 (2.2) ng/ml) and remained slightly higher at day 240 (9.01 (2.6) ng/ml) (Fig 2B).

Colonic biopsy inflammatory index dropped in all animals by day 56 of treatment (Fig 3). In addition, at day 240, four of five remaining animals maintained remission while one of five worsened. Pretreatment histological examination was characterised by crypt abscesses, granulocyte infiltration, and mucus depletion, and a diffuse, chronic inflammatory cell infiltrate in the mucosa (Fig 4A). After 56 days of treatment colonic tissue obtained from the same animal, at the same position (10 cm from the anus), showed a dramatic reduction in inflammatory cells along with increased mucus production (Fig 4B). Figure 5 shows the histological changes in the colonic mucosa from the same CTT taken at the same time as Figs 4A and 4B but at 15 cm from the anus. No evidence is seen of active colitis, but increased intercrypt spacing with increased numbers of chronic inflammatory cells are found in the lamina propria. Bizarre crypt distortion has

resulted from mucosal regeneration and repair of previously ulcerated epithelium. Furthermore, treatment was not associated with any untoward findings in haematology/clinical chemistry panel (Table).

Discussion

We have found that oral treatment with a second generation LTB_4 receptor antagonist resulted in a dramatic anticolic effect on CTTs, a disease model for human IBD. Oral treatment results in improved body weights and stool condition, as well as lowered LTB_4 concentrations in rectal dialysates and improved colonic biopsy inflammatory scores. This confirmed earlier preliminary studies with a first generation LTB_4 receptor antagonist in which modest histological improvements were seen along with decreases in LTB_4 concentrations in rectal dialysates.¹⁵ In addition, treatment was accompanied by constant or increased concentrations of PGE in rectal dialysates. This may represent a useful adjunct to the primary pharmacological target (LTB_4 receptor) in that prostaglandins are potent mucosal protective agents.¹⁹

While CTTs cannot directly communicate their feelings of well being, SC-53228's positive

Figure 2: Effect of SC-53228 treatment on leukotriene B_4 (LTB_4) and prostaglandin E concentrations in rectal dialysates of colitic cotton-top tamarins. LTB_4 (mean (SEM) $n = \text{four--six/group}$) were significantly (*) lower at days 28 and 56 of treatment, as well as at day 240, compared with pretreatment values (A). Prostaglandin E concentrations rose slightly over treatment (B).

Figure 3: Effect of SC-53228 treatment on inflammatory index in colonic biopsy specimens from colitic cotton-top tamarins. It is noted that by day 56 of treatment five of six subjects had inflammatory indices of 0, that is, no neutrophils present in colonic mucosa.

effect on the life quality parameters such as body weights and stool consistency must translate into overall health and are thus encouraging. Quality of life measurements are a critical part of the therapeutic evaluation of anticolic drugs in humans.²⁰ Indeed, improved life quality parameters are considered an important health outcome in the treatment of human IBD.

Certainly, compounds other than LTB₄ receptor antagonists have shown efficacy in CTTs. The mainstay of human IBD therapy, sulphasalazine, was given to CTTs (50 mg/kg/day) in a randomised 10 week placebo controlled crossover study. Sulphasalazine treatment significantly improved colitis as assessed by histology and body weights.²¹ The 5-LO inhibitor, WY 50,295, given in a similar regimen, was marginally efficacious in preventing relapse. Treatment was not associated with reduced LTB₄ in rectal dialysates in this preliminary study.²² Corticosteroid treatment has yielded mixed results in CTT colitis in at least two different colonies in that some animals have responded while others have not⁵ (N K Clapp, personal unpublished findings). Furthermore, treatment with the tumour necrosis factor alpha humanised antibody, CDP-571, reduced histological scores and maintained an 8% improvement in body weights over one month.²³

Early studies in ulcerative colitis patients with zileuton, a 5-lipoxygenase inhibitor, were encouraging. Improvements in life quality parameters were associated with lower LTB₄ concentrations in rectal dialysates. Dosing four times daily was better than twice daily in achieving 25% remission rates.²⁴ In a phase III study, however, zileuton treatment (600 mg four times daily) was no better than 5-ASA in preventing relapse.²⁵ MK-591, a 5-lipoxygenase activating protein inhibitor, reduced LTB₄ concentrations in rectal dialysates of ulcerative colitis patients with a single 250 mg oral dose; however, clinical efficacy remains to be determined.²⁶ In an open clinical study with an LTB₄ receptor antagonist, SR-2640, 250 mg thrice daily was given orally for six weeks to ulcerative colitis patients with mild to moderate disease. Three of eight patients were improved, three of eight remained unchanged, and two of eight worsened to the point of

requiring corticosteroid intervention.²⁷ In addition, treatment with this sulphidopeptide leukotriene antagonist resulted in remission

Figure 4: Histological sections of colonic mucosa (10 cm from anus in descending colon) from cotton-top tamarin (MO-5490) (A) with active colitis; or (B) after SC-53228 treatment, 20 mg/kg bw, twice daily, 56 days. (A) shows acute colitis with granulocyte infiltration in the lamina propria and crypt epithelium, crypt abscesses, and severe mucin depletion. (B) shows effect of drug treatment: absence of granulocytes, return of mucin production and crypt branching (arrows) from mucosal regeneration and repair after active colitis entered remission (PAS, original magnification $\times 200$).

Figure 5: Photomicrograph of a mucosal biopsy specimen taken at 15 cm from the anus in the same cotton-top tamarin shown in Fig 4 (A) and 4 (B) after SC-53228 treatment, 20 mg/kg bw, twice daily, 56 days. The colonic biopsy specimen shows typical mucosal repair with increased intercrypt spacing and increased numbers of chronic inflammatory cells in the lamina propria, bizarre crypt distortion after mucosal regeneration, and repair of the previously ulcerated epithelium. No granulocytes are seen in the lamina propria, crypt epithelium, or in the crypt lumen (PAS, original magnification $\times 100$).

rates similar to sulphasalazine. New therapeutic regimens are certainly required given the failure of low dose, oral cyclosporine to either improve symptoms or reduce the requirements of standard treatment.²⁸ Also, cyclosporine was not efficacious when given as an enema (350 mg/day) to patients with mildly to moderately active ulcerative colitis.²⁹

In summary, oral treatment with the LTB₄ receptor antagonist, SC-53228, was associated with lower LTB₄ concentrations in rectal dialysates coupled with fewer neutrophils infiltrating colonic mucosa. These results correlate with improved life quality parameters, which resulted in a significant impact upon relapse in

this animal model. Relapse is among the most serious sequelae of human ulcerative colitis, second only to the diagnosis of colonic carcinoma. During the relapse cycle there seems to be an association between the signs and symptoms of active disease and the presence of neutrophils. While our studies do not prove a mechanism of action, lower LTB₄ concentrations resulting in reduced neutrophil recruitment would seem to play a part. Whether efficacy in the CTT is a useful bridge to human studies awaits the outcome of clinical trials where the true role(s) of LTB₄ in IBD can be evaluated.³⁰

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Clinical chemistry values of cotton-top tamarins treated orally with SC-53228 (20 mg/kg bw, thrice daily for eight weeks)

Clinical chemistry test	Mean (SEM)			Units
	Before treatment	Day 28	Day 56	
Glucose	208.3 (28.0)	174.5 (17.3)	173.5 (35.2)	mg/dl
BUN	15.3 (2.0)	12.0 (6.1)	12.0 (1.0)	mg/dl
Creatinine	0.5 (0.0)	0.7 (0.1)	0.66 (0.1)	mg/dl
BUN/creatinine ratio	28.9 (3.0)	14.5 (4.8)	21.7 (1.8)	ratio
Sodium	157 (9.5)	153.0 (1.2)	154.7 (1.4)	mEq/l
Potassium	4.4 (0.2)	4.5 (0.5)	4.8 (0.4)	mEq/l
Chloride	114.7 (2.5)	106.3 (1.1)	108.0 (2.6)	mEq/l
Total protein	6.9 (0.3)	7.1 (0.1)	6.9 (0.3)	gm/dl
Albumin	3.4 (0.3)	3.5 (0.3)	3.7 (0.4)	g/dl
Bilirubin total	0.4 (0.0)	0.5 (0.1)	0.4 (0.0)	mg/dl
Alkaline phosphatase	271.0 (45.0)	415.5 (61.2)	432.7 (109.4)	U/l
ALT	57.3 (7.4)	73.8 (20.1)	47.5 (6.9)	U/l
LDH	1250.0 (203.2)	1150.0 (108.3)	1041.7 (158.0)	U/l
AST	386.3 (67.1)	569.8 (236.9)	322.3 (96.2)	U/l
CPK	1688.2 (852.2)	1928.5 (797.1)	936.7 (421.1)	U/l
Calcium	8.8 (0.3)	8.4 (0.2)	8.7 (0.4)	mg/dl
Phosphorus	3.4 (0.3)	5.1 (0.5)	5.5 (0.9)	mg/dl
Cholesterol	93.0 (16.1)	91.8 (13.4)	87.5 (16.4)	mg/dl
Carbon dioxide	9.3 (0.5)	10.5 (0.8)	12.5 (2.6)	mEq/l
Uric acid	0.4 (0.0)	0.7 (0.1)	0.9 (0.3)	mg/dl
Triglycerides	56.0 (8.3)	65.0 (19.4)	54.7 (9.2)	mg/dl
Anion gap	33.0 (2.4)	36.2 (1.4)	40.7 (6.7)	mEq/l
Osmolality	318.1 (2.4)	307.6 (2.7)	310.6 (2.5)	mOsm/kg
GGT	41.7 (4.1)	38.3 (2.7)	38.5 (5.2)	U/l

BUN=blood urea nitrogen, ALT=alanine aminotransferase, LDH=lactate dehydrogenase, AST=aspartate aminotransferase, CPK=creatinine phosphokinase, GGT=γ-glutamyltransferase.

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