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Inhibition of *ex vivo* neutrophil activation by oral LY293111, a novel leukotriene B_4 receptor antagonist

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1 The effects of orally administered LY293111 on *ex vivo* neutrophil Mac-1 upregulation were determined in a total of 24 healthy male subjects within three study periods.

2 In the first period, eight volunteers received 60 mg LY293111 or placebo three times daily in 22 total doses over 8 days followed by a 1 week follow-up. The average *ex vivo* Mac-1 response of the LY293111 group was 56% of the pre-dose control (95% confidence interval (CI) 44.3 to 67.9%; $P<0.01$). The inhibitory effect was maximum at the end of dosing and had disappeared by day 14.

3 In the second period, eight subjects received 120 mg LY293111 or placebo three times daily in 22 total doses over 8 days followed by a 1 week follow-up. The average response of the LY293111 group was 70% of the pre-dose control (95% CI 59.7 to 81.0%; $P<0.01$). The inhibitory effect was maximum the day following the initial dose and continued throughout the dosing period.

4 In the third period, eight subjects received 200 mg LY293111 or placebo twice daily in 15 total doses over 8 days followed by a 1 week follow-up. Mac-1 upregulation was 64% of pre-dose levels (95% CI 53.8 to 75.1%; $P<0.01$) over the course of the study period. The inhibition had disappeared 2 days following the final dose. Alternate neutrophil stimulation by fMLP was not inhibited.

5 No statistically significant inhibition was observed for placebo-treated subjects.

6 No statistically significant differences were apparent between the active dose regimens.

7 The results indicate that orally administered LY293111 is pharmacologically active in humans. Results from this study may be useful in determining dose selection for efficacy trials.

Keywords leukotriene B_4 neutrophils macrophage-1 antigen flow cytometry

Introduction

Leukotriene B_4(LTB_4) is a pro-inflammatory, naturally occurring eicosanoid mediator that has been implicated in a variety of human inflammatory diseases [1–5]. LY293111 (2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy] propoxy] phenoxy]benzoic acid) is a potent and selective receptor antagonist for LTB_4 (Figure 1). Previous studies have shown that LY293111 competitively inhibits binding of [^{3}H] – LTB_4 to isolated human neutrophils and selectively blocks LTB_4-induced activation events in neutrophils including: calcium mobilization, superoxide generation, homotypic aggregation, chemiluminescence and upregulation of Macrophage-1 antigen (Mac-1) [6–8]. Furthermore, it has been shown that incubation of LY293111 with whole human blood inhibits LTB_4-induced Mac-1 upregulation on neutrophils *in situ* [7].

Mac-1 is a heterodimeric cell surface adhesion molecule belonging to the integrin superfamily [9, 10].

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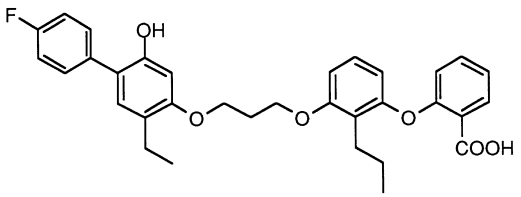

Figure 1 Chemical structure of LY293111.

It is composed of an α_m and β_2 chain and can be immunologically identified by its reactivity with monoclonal antibodies (MAb) specific to the cluster designations (CD) CD11b and CD18.

Cell surface membrane expression of Mac-1 on neutrophils is upregulated in response to various agonists including *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and LTB_4. This upregulation of Mac-1 is closely associated with an increase in neutrophil adhesion as indicated in both *in vitro* studies and in animal models of inflammatory diseases [11–13].

In this, the first human dose studies with LY293111, we measured Mac-1 upregulation on neutrophils taken from subjects receiving the compound orally. The aim of this study centred on collection of these Mac-1 data for use in defining the extent and duration of LY293111's *in vivo* bioactivity on target cells likely to be involved in various inflammatory diseases.

Methods

Subjects

A total of 24 healthy male subjects, ranging in age from 18 to 42 years, and weighing between 57 and 88 kg participated in this study. The study protocol was approved by the Simbec Independent Ethics Committee (Simbec Research Ltd, Merthyr Tydfil Industrial Park, Mid-Glamorgan, UK). Subjects were enrolled into the study after they had been given a full explanation of all procedures and had given written consent to participate.

Drug

The chemical nomenclature (USAN) for LY293111 (Figure 1) is 2-[3-[3-[(5-ethyl-4′-fluoro-2-hydroxy[1,1′-biphenyl]-4-yl)oxy]propoxy]-2-propylphenoxy] benzoic acid. LY293111 sodium salt was produced for clinical use in hard gelatin size No. 2, opaque, white capsules containing 5, 8, 12, and 50 mg of active component. Pre-gelatinized starch, microcrystalline cellulose, and dimethicone were included as inactive constituents. The dimethicone present in all capsules was approximately 1% (by weight) while the microcrystalline cellulose ranged from 18 to 24% and the pregelatinized starch from 60 to 73%. Matching capsules containing these inactive ingredients were used for placebo administration.

Study design

The study was subdivided into three distinct periods, differentiated by the dosage and frequency of LY293111 that was orally administered. In all three periods, subjects were randomized and administered either LY293111 or placebo in a double-blinded manner.

In the first period, volunteers received either 60 mg LY293111 or placebo three times daily for 7 days with a final, single dose on day 8 (22 total doses). Blood samples were collected on day 1, immediately prior to the first dose (pre-dose control) and prior to the 4th (day 2) and 22nd doses (day 8). Additional blood samples were obtained on days 10, 12, and 14, in order to monitor the duration of any effects. All blood samples were challenged *ex vivo* with LTB_4 to study upregulation of cell surface Mac-1 on circulating neutrophils (see detailed procedure below).

The second period was designed similarly to the first except that subjects received either 120 mg LY293111 or placebo three times daily. Blood was drawn at the same intervals as in the first period and again challenged with LTB_4 *ex vivo*. In addition, each blood sample was also challenged *ex vivo* with buffer in order to determine the basal or non-activated CD11b expression levels of the collected neutrophils.

In the third period, volunteers received either 200 mg LY293111 or placebo twice daily for 7 days with a final dose on day 8 (15 total doses). Blood was collected immediately prior to the first (pre-dose control), third (day 2), and 15th (day 8) dose. Additional blood samples were taken 2 h after the 15th dose (day 8) and on days 10, 12 and 14. In this period, each blood sample was treated with either LTB_4, buffer or with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) to measure the selectivity of drug action.

Ex vivo *Mac-1 assay*

A previously described procedure for stimulating and measuring Mac-1 up-regulation on plasma membrane surfaces of human neutrophils was utilized without appreciable modification [7, 14]. Briefly summarized, 5 ml venous blood was collected from subjects into evacuated blood collection tubes containing EDTA as the anticoagulant. Sample processing commenced within 2 h of collection. The buffer used throughout processing was Hanks' Balanced Salt Solution (BioWhittaker, Walkersville, MD, USA) with added 0.1% (w/v) low endotoxin, bovine serum albumin (ICN Biomedicals, Inc., Costa Mesa CA, USA), freshly prepared each day. For most collected samples, 90 µl aliquots of whole blood were added to the base of 12×75 mm polypropylene tubes and mixed by vortex with 10 µl of an LTB_4 solution (10 nM, final concentration), fMLP (50 nM), or buffer and incubated for 30 min in a 37° C water bath. Samples were then rapidly cooled on ice and further incubated with 10 µl of an anti-Mac-1 antibody, coupled to fluorescein (Mo-1-FITC, Coulter Corp., Hialeah, FL, USA) for an additional 30 min at 4° C. To allow for quantitative immunofluorescence comparisons, the same

manufacturer's lot of antibody was used for all samples processed in the study. After antibody incubation, the residing red blood cells were lysed with 2.0 ml FACS® Lysing Solution (Becton Dickinson, San Jose CA, USA) and the resultant white blood cells fixed in a 1% (w/v) paraformaldehyde (Polysciences, Inc., Warrington, PA, USA) solution dissolved in phosphate buffered saline. Processed and fixed samples were stored in the dark at 4° C and analysed within 1 week of collection using a previously described flow cytometric technique [7, 14]. For each sample, the mean fluorescence intensity (MFI) of at least 5000 light scatter-gated neutrophils was determined using a Profile II flow cytometer(Coulter Electronics, Inc., Hialeah FL, USA).

Statistical analysis

To determine the effect of orally administered LY293111 on *ex vivo* neutrophil Mac-1 upregulation, the pre-dose blood sample was utilized to determine the baseline response for each subject. LTB_4 and fMLP-stimulated Mac-1 determinations were made in duplicate and averaged. The net stimulated MFI values were determined by subtracting the basal (buffer-treated) Mac-1 value. Since basal levels were not determined in the 60 mg period, exogenous data sources were used to furnish an average basal value. A mean ($\pm$s.e. mean) basal value was computed from the placebo-treated subjects in the 120 and 200 mg groups ($MFI = 39 \pm 2$, $n = 26$) and utilized for the 60 mg period's data analysis. Calculations of net MFI that lead to negative values were truncated to 0. Hence, the net MFIs of the 60 mg period can be considered *approximations*, and conclusions drawn from these data should be tempered with the recognition that the responses could be biased. The net MFI values were converted to ratios (expressed in %), relative to their respective pre-dosing values. In the case of placebo subject D3 from the 60 mg period (Table 1), the pre-dose response was conspicuously low ($MFI = 55$), and seriously distorted the average of ratios for the placebo group. Consequently, an accommodation technique was applied that simply replaced the original value with the average over all days (81), since responses were quite stable after the original measurement (86.4 ± 4.2 s.d.).

A split-plot analysis of variance (ANOVA) for a repeated-measures design was conducted which incorporated dose groups (0, 60, 120, and 200 mg) and measurement days (2, 8, 10, 12, and 14) as fixed factors. Subjects within dose groups served as the whole-plot error with the residual as the subplot error. *A posteriori* comparisons were made within the ANOVA model through pairwise contrasts. A value of $P \leq 0.05$ (two-tailed) was considered as nominally significant. Due to the multiplicity of statistical tests performed, caution needs to be taken in interpreting statistical significance. With missing observations (9%) in the data, least-square means are tested and reported in the statistical analyses. The statistical software package SAS® (SAS Institute, Cary NC, USA) was used in all tests and computations.

Results

60 mg period

The effect of LY293111 administered at 60 mg three times daily on *ex vivo* neutrophil Mac-1 upregulation is displayed in Table 1 and summarized in Table 4 and Figure 2. Subject D6 dropped out of the study prior to receiving all 22 doses of LY293111 for reasons that were independent of any study issues. All subjects receiving

Table 1 Plasma membrane surface expression of Mac-1 on human neutrophils taken from eight normal subjects receiving 60 mg three times daily LY293111 or placebo

Subject	*Oral dose*[a]	Ex vivo *challenge*	*Day 1*[b]	*Day 2*	*Day 8*	*Day 10*	*Day 12*	*Day 14*
D1	LY293111	LTB_4	89[c]	59	34	76	76	93
			89[d]	61	33	74	73	89
D2	LY293111	LTB_4	130	66	40	108	100	125
			131	57	44	108	98	124
D4	LY293111	LTB_4	75	55	23	68	66	82
			81	49	24	70	63	81
D5	LY293111	LTB_4	89	42	40	84	79	97
			86	51	58	88	76	96
D6	LY293111	LTB_4	81	35	N.A.[e]	N.A.	N.A.	N.A.
			81	53	N.A.	N.A.	N.A.	N.A.
D8	LY293111	LTB_4	102	59	27	51	77	N.A.
			93	62	27	54	78	N.A.
D3	Placebo	LTB_4	54	79	94	85	83	90
			56	87	87	86	83	90
D7	Placebo	LTB_4	81	89	76	74	76	96
			80	89	78	76	73	95

[a]22 oral doses of LY293111 (60 mg) or placebo; [b]pre-dose period; [c]mean fluorescence intensity (MFI) of at least 5,000 light scatter-gated neutrophils; [d]processed and measured in duplicate; [e]sample not analysed.

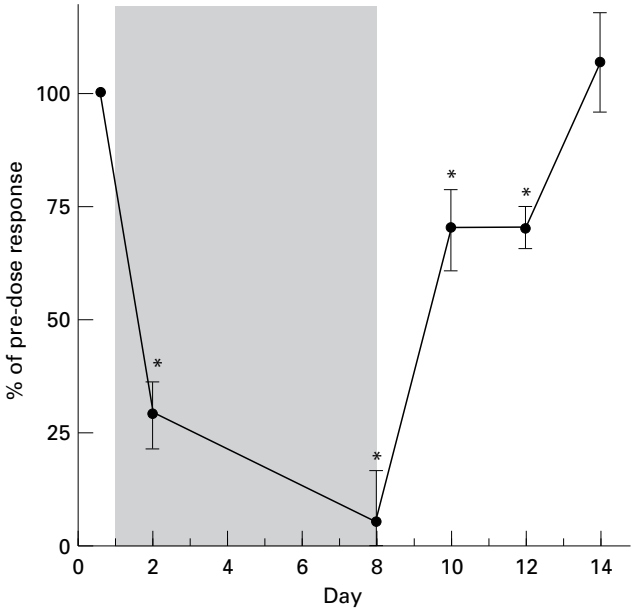

Figure 2 Effect of LY293111 (60 mg three times daily) on LTB_4-induced Mac-1 expression. Normal human subjects received 60 mg LY293111 three times daily orally and whole blood was collected and challenged *ex vivo* with LTB_4 and reacted with anti-Mac-1 antibody. The mean fluorescence intensity of the neutrophils in each sample was determined by flow cytometry. The ratio (expressed in % ± s.e. mean) to the pre-dose response is displayed. Asterisks indicate $P \leqslant 0.05$ (compared with pre-dose responses). The hatched area represents the active dosing period.

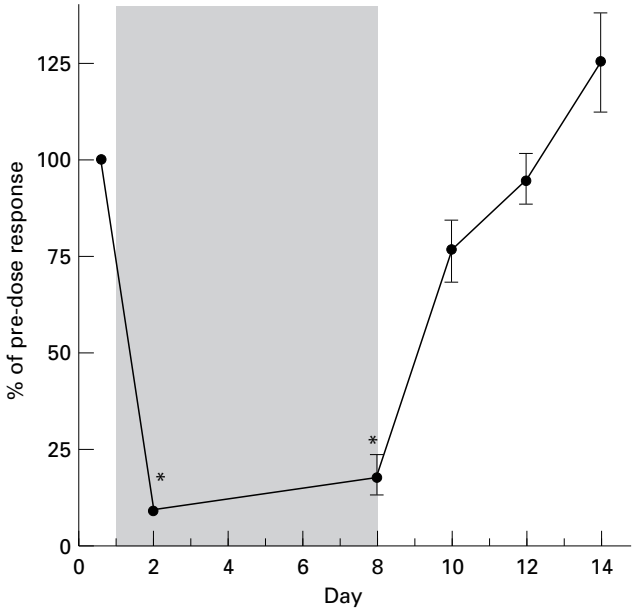

Figure 3 Effect of LY293111 (120 mg three times daily) on LTB_4-induced Mac-1 expression. Normal human subjects received 120 mg LY293111 three times daily orally and whole blood was collected and challenged *ex vivo* with LTB_4 and reacted with anti-Mac-1 antibody. The mean fluorescence intensity of the neutrophils in each sample was determined by flow cytometry. The ratio (expressed in % ± s.e. mean) to the pre-dose response is displayed. Asterisks indicate $P \leqslant 0.05$ (compared with pre-dose responses). The hatched area represents the active dosing period.

LY293111 displayed inhibited Mac-1 responses by the first measurement period after receiving the drug (Table 1). The average ratio for this time period was 31% (of pre-dose values) and was computed to be a statistically significant ($P < 0.01$) inhibition. The inhibitory effect was maximum on day 8 and gradually waned after the cessation of dosing (Figure 2). No suppression of Mac-1 responses were observed in the placebo-dosed (D3 and D7) subjects (Table 1).

120 mg period

A similar inhibitory profile was observed for subjects receiving the 120 mg dose of LY293111. As observed in the 60 mg period, all subjects receiving LY293111 displayed inhibited Mac-1 responses by the first measurement period after receiving drug. This first measurement point (day 2) was in fact the period of maximum inhibition for this period (Figure 3). The Mac-1 response remained significantly suppressed through day 8. The response became less inhibited through the remainder of the sampling periods. Responses from placebo-treated blood samples (Table 2, subject D10) were virtually unchanged from pre-dose levels.

200 mg period

As displayed in Figure 4, the *ex vivo* Mac-1 responses of subjects receiving 200 mg LY293111, twice daily were

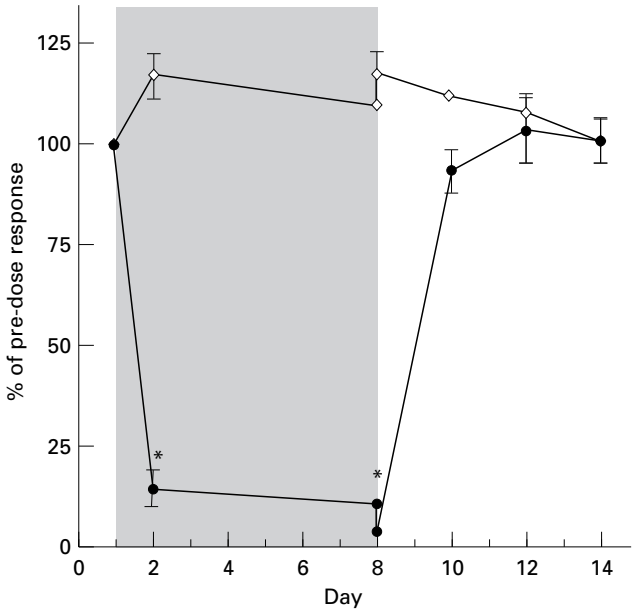

Figure 4 Effect of LY293111 (200 mg twice daily) on LTB_4-induced Mac-1 expression. Normal human subjects received 200 mg LY293111 three times daily orally and whole blood was collected and challenged *ex vivo* with LTB_4 (●) or fMLP (◇) and reacted with anti-Mac-1 antibody. The mean fluorescence intensity of the neutrophils in each sample was determined by flow cytometry. The ratio (expressed in % ± s.e. mean) to the pre-dose response is displayed. Asterisks indicate $P \leqslant 0.05$ (compared with pre-dose responses). The hatched area represents the active dosing period.

Table 2 Plasma membrane surface expression of Mac-1 on human neutrophils taken from eight normal subjects receiving 120 mg three times daily LY293111 or placebo

Subject	*Oral dose*[a]	Ex vivo *challenge*	*Day 1*[b]	*Day 2*	*Day 8*	*Day 10*	*Day 12*	*Day 14*
D9	LY293111	LTB_4	72[c]	25	44	57	79	81
D9	LY293111	LTB_4	68[d]	26	44	55	78	80
D9	LY293111	buffer	8	22	44	22	29	41
D11	LY293111	LTB_4	97	34	35	79	98	99
D11	LY293111	LTB_4	91	33	36	81	92	98
D11	LY293111	buffer	41	27	28	40	41	32
D12	LY293111	LTB_4	89	25	48	80	86	93
D12	LY293111	LTB_4	60	27	44	84	87	96
D12	LY293111	buffer	43	22	34	42	49	28
D13	LY293111	LTB_4	63	22	31	62	76	72
D13	LY293111	LTB_4	58	22	33	63	74	72
D13	LY293111	buffer	22	16	21	38	45	23
D15	LY293111	LTB_4	64	23	24	61	65	69
D15	LY293111	LTB_4	37	24	26	61	79	70
D15	LY293111	buffer	14	21	22	22	32	20
D16	LY293111	LTB_4	66	25	47	79	80	82
D16	LY293111	LTB_4	49	27	48	81	65	80
D16	LY293111	buffer	22	23	38	48	32	28
D10	Placebo	LTB_4	79	74	80	74	78	85
D10	Placebo	LTB_4	77	72	80	73	78	81
D10	Placebo	buffer	35	21	23	29	34	45
D14	Placebo	LTB_4	81	N.A.[e]	N.A.	N.A.	N.A.	N.A.
D14	Placebo	LTB_4	64	N.A.	N.A.	N.A.	N.A.	N.A.
D14	Placebo	buffer	34	N.A.	N.A.	N.A.	N.A.	N.A.

[a]22 oral doses of LY293111 (120 mg) or placebo; [b]pre-dose period; [c]mean fluorescence intensity (MFI) of at least 5000 light scatter-gated neutrophils; [d]LTB_4-stimulated samples processed and measured in duplicate; [e]sample not analysed.

significantly inhibited in their ability to respond to an LTB_4 challenge but not inhibited in their response to fMLP, an alternate neutrophil agonist. As observed in the other periods (60 mg and 120 mg), the response to LTB_4 was once again significantly depressed by the first sampling period (day 2) and remained suppressed through day 8. For this period, an additional blood sample was taken 2 h after the final dose (day 8). This sample yielded the maximum point of inhibition of the LTB_4-challenged responses (Figure 4). No suppression of the fMLP-induced responses were observed, throughout the sampling periods of this segment. Furthermore, subjects receiving placebo (Table 3, subjects D19 and D24) did not display suppressed responses to either LTB_4 or fMLP.

Comparison of treatment effects

The average effects on LTB_4-stimulated Mac-1 responses (post dose) over the course of the studies are displayed in Table 5. All three treatment protocols resulted in significantly ($P<0.01$) inhibited LTB_4-stimulated responses as compared with both pre-dose levels and placebo-treated controls (Table 5). Although some differences in daily effects were observed for the three dosing regimens (Table 4), the treatment protocols were not significantly different in their average effects with respect to LTB_4 induced Mac-1 upregulation (Table 6).

Discussion

A role for LTB_4 in inflammatory disease has been suggested for some time [1–5, 15–21]. The evidence for its importance in inflammation has led to the development of several potent and selective receptor antagonists [22]. While many of these potential drugs have been evaluated in *in vitro* systems, only a few of them have been evaluated in humans [23]. In this manuscript, we present data collected during the first clinical study of LY293111 in humans.

The study described in this manuscript was primarily

Table 3 Plasma membrane surface expression of Mac-1 on human neutrophils taken from eight normal subjects receiving 200 mg twice daily LY293111 or placebo

Subject	*Oral dose*[a]	Ex vivo *challenge*	*Day 1*[b]	*Day 2*	*Day 8*	*Day 8.1*	*Day 10*	*Day 12*	*Day 14*
D17	LY293111	LTB_4	116[c]	50	51	49	131	117	124
D17	LY293111	LTB_4	125[d]	51	56	53	132	117	127
D17	LY293111	fMLP	172[d]	181	180	182	186	174	173
D17	LY293111	fMLP	177	178	183	178	179	173	168
D17	LY293111	buffer	49	39	47	49	49	66	58
D18	LY293111	LTB_4	104	42	44	33	93	94	103
D18	LY293111	LTB_4	106	43	45	36	89	96	97
D18	LY293111	fMLP	160	161	163	161	160	152	159
D18	LY293111	fMLP	157	158	167	157	163	151	155
D18	LY293111	buffer	36	38	43	31	33	31	38
D20	LY293111	LTB_4	114	36	37	25	107	107	111
D20	LY293111	LTB_4	115	40	37	26	110	106	124
D20	LY293111	fMLP	173	178	183	175	173	159	172
D20	LY293111	fMLP	175	179	179	173	168	159	171
D20	LY293111	buffer	41	32	32	27	35	26	51
D21	LY293111	LTB_4	107	45	46	26	74	82	100
D21	LY293111	LTB_4	110	44	43	26	72	82	95
D21	LY293111	fMLP	144	159	147	158	150	137	138
D21	LY293111	fMLP	151	157	151	156	154	141	142
D21	LY293111	buffer	51	25	32	23	30	26	45
D22	LY293111	LTB_4	122	50	66	39	97	107	115
D22	LY293111	LTB_4	126	51	61	36	99	100	115
D22	LY293111	fMLP	156	155	158	153	152	136	157
D22	LY293111	fMLP	154	153	155	155	148	141	159
D22	LY293111	buffer	61	36	51	34	41	25	39
D23	LY293111	LTB_4	135	45	62	43	116	129	151
D23	LY293111	LTB_4	136	46	63	43	120	126	141
D23	LY293111	fMLP	182	191	187	188	184	180	174
D23	LY293111	fMLP	179	191	187	188	182	182	181
D23	LY293111	buffer	55	43	57	41	43	31	53
D19	Placebo	LTB_4	94	93	100	109	102	93	98
D19	Placebo	LTB_4	97	93	101	103	99	93	101
D19	Placebo	fMLP	146	152	153	146	150	145	150
D19	Placebo	fMLP	144	146	158	151	149	145	152
D19	Placebo	buffer	49	35	38	36	39	39	43
D24	Placebo	LTB_4	114	95	109	120	109	102	119
D24	Placebo	LTB_4	114	94	104	114	111	102	117
D24	Placebo	fMLP	164	172	165	173	171	158	173
D24	Placebo	fMLP	167	169	164	173	169	158	166
D24	Placebo	buffer	51	31	44	31	34	37	42

[a]15 oral doses of LY293111 (200 mg) or placebo; [b]pre-dose period; [c]mean fluorescence intensity (MFI) of at least 5,000 light scatter-gated neutrophils; [d]LTB_4 and fMLP-stimulated samples processed and measured in duplicate.

designed as a safety study. With this in mind, all measures appropriate to such a study were undertaken including the measurement of plasma drug levels and profiles of the subjects' well being. It should be noted (data not shown) that the results of this study indicated that LY293111 was present in the subject's plasma and no significant toxic events were noted for any subject at any dose administered.

The novel aspect of this study was the ability to measure LY293111's pharmacological activity in a study designed primarily for safety evaluations. We accomplished this by measuring the levels of Mac-1

Table 4 Assessment of treatment effects by day

Dose	*Day*	*Average ratio (%)*	*Test* P-*value* vs *Pre-dose*	vs *Placebo*	vs *60 mg*	vs *120 mg*
Placebo	2	114	0.10			
	8	116	0.06			
	10	111	0.21			
	12	102	0.79			
	14	118	0.04			
60 mg	2	31	<0.01	<0.01		
LY293111	8	5	<0.01	<0.01		
	10	69	<0.01	<0.01		
	12	70	<0.01	<0.01		
	14	105	0.56	0.34		
120 mg	2	10	<0.01	<0.01	0.08	
LY293111	8	19	<0.01	<0.01	0.21	
	10	86	0.08	0.04	0.14	
	12	100	0.96	0.87	<0.01	
	14	135	<0.01	0.12	0.02	
200 mg	2	15	<0.01	<0.01	0.18	0.66
LY293111	8	11	<0.01	<0.01	0.58	0.45
	10	93	0.37	0.13	0.04	0.54
	12	103	0.74	0.98	<0.01	0.84
	14	100	0.96	0.14	0.68	<0.01

Table 5 Assessment of treatment effects averaged over days

Dose	*Average ratio (%)*	*Standard error*	*95% CI*	*Test* P-*value* vs *pre-dose*	vs *placebo*
Placebo	112	5.6	100.5–123.8	0.04	
60 mg LY293111	56	5.6	44.3–67.9	<.01	<.01
120 mg LY293111	70	5.1	59.7–81.0	<.01	<.01
200 mg LY293111	64	5.1	53.8–75.1	<.01	<.01

Table 6 Comparison between active treatments

Contrast	*Mean difference*	*Standard error*	*95% CI*	*Contrast* P-*value*
60 mg *vs* 120 mg	−14.2	7.6	−30.1–1.6	0.08
60 mg *vs* 200 mg	−8.3	7.6	−24.2–7.5	0.28
120 mg *vs* 200 mg	5.9	7.2	−9.2–1.6	21.0

adhesion molecules on neutrophils in small blood samples taken throughout each period. The data indicate that LY293111 is orally absorbed in humans and has a pharmacological effect on blood neutrophils. The neutrophils from LY293111-treated subjects were selectively inhibited in their ability to react to exogenous LTB_4.

Three doses (60, 120, 200 mg) of LY293111 were evaluated in this study. All were effective in producing the Mac-1 inhibitory effect. There were no inhibitory effects of placebo observed in this study. It should be noted that *all* subjects that received LY293111 displayed an LTB_4-specific blockade of neutrophil activation. Furthermore, this inhibitory effect was observed immediately (during the first sampling period) after dosing. LTB_4-induced neutrophil activation remained inhibited throughout the dosing periods and gradually returned to pre-dose levels 2–6 days after the cessation of dosing. Neutrophils were not blocked in their response to fMLP, indicating a selectivity of the pharmacological effect. Although there were slight differences in activity of the doses on specific days, no statistically significant differences were apparent when the dose regimens were evaluated in total. Among possible explanations for this lack of dose/action differentiation might be that all three doses were above the threshold for producing the maximal inhibitory effect and only doses below those

used in this study would be capable of producing less inhibition. Furthermore, given the limited number of subjects enrolled, the statistical power is not optimal for discriminating slight effects.

Taken collectively, these data document a pharmacological activity of LY293111 in humans. We believe that data from this study may be helpful in determining the appropriate dose of LY293111 that could be efficacious in treating inflammatory disease.

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