A Randomized, Double-Blinded, Placebo-Controlled Clinical Trial of LY333013, a Selective Inhibitor of Group II Secretory Phospholipase A_2, in the Treatment of Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To evaluate the efficacy and safety of a selective inhibitor of secretory phospholipase ($sPLA_2$), LY333013, in the treatment of rheumatoid arthritis (RA).

Methods. Two hundred and fifty-one patients with active RA despite treatment with one or more disease modifying antirheumatic drugs (DMARD) received oral doses of LY333013 (50, 250, and 1000 mg) or placebo once daily for 12 weeks. Concomitant low-dose glucocorticoids (≤ 10 mg/day prednisone equivalent) were allowed. Clinical improvement was assessed using the response criteria of the American College of Rheumatology (ACR20), and safety was evaluated with respect to adverse events and laboratory test abnormalities.

Results. The demographic characteristics of the treatment groups were similar. Dose-response relationships were found for ACR20 responses ($p = 0.058$) and reductions in C-reactive protein ($p = 0.058$) at week 1. The proportions of patients with an ACR20 response subsequently increased in all study groups including the placebo group at weeks 4 and 8, and the initial treatment benefit was lost. Adverse events were generally mild in severity and not associated with treatment.

Conclusion. Treatment with LY333013 for 12 weeks was well tolerated but ineffective as an adjunct to DMARD treatment of active RA. (J Rheumatol 2005;32:417–23)

Key Indexing Terms:
RHEUMATOID ARTHRITIS PHOSPHOLIPASE A_2 INHIBITOR

The group II secretory phospholipase ($sPLA_2$) activity present in synovial fluid (SF) from patients with rheumatoid arthritis (RA)[1] has been considered proinflammatory because it releases arachidonic acid, a prostaglandin (PG) and leukotriene precursor, from cell membranes. The activity of both $sPLA_2$ and cytosolic phospholipase ($cPLA_2$), a related enzyme, is increased by inflammatory stimuli such as bacterial lipopolysaccharide, tumor necrosis factor-alpha (TNF-α) and interleukin-1ß (IL-1ß). The secretory and cytosolic PLA_2 enzymes differ in their molecular weights, calcium dependency, and optimum pH[2]. In RA *ex vivo* synovial cell cultures, $sPLA_2$ enhances TNF-α-induction of PG production, in part via increased expression of cyclooxygenase-2 (COX-2) and $cPLA_2$[3]. Mononuclear cells from peripheral blood and SF of RA patients respond to $sPLA_2$ with enhanced release of TNF-α and IL-6[4]. Because transcription of IL-1ß[5], TNF-α[6], and IL-6[7] can be up-regulated by elevated concentrations of leukotrienes and platelet activating factor, $sPLA_2$ could serve as a critical modulator of cytokine-driven inflammation.

In an animal inflammation model $sPLA_2$ incites an inflammatory response when injected into rabbit joints[8]. Transgenic mice over-expressing human $sPLA_2$ and TNF-α develop a more aggressive arthritis than transgenics over-expressing only TNF-α[9], although over-expression of $sPLA_2$ alone does not cause arthritis[10]. Selective inhibition of $sPLA_2$ (sparing $cPLA_2$) has yielded variable results in the adjuvant-induced arthritis model in rats[11].

Correlations have been found in patients with RA between the serum $sPLA_2$ concentration and clinical markers of disease activity, e.g., swollen joints, elevated platelet count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and depressed hemoglobin concentration[12].

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Expression of $sPLA_2$ in RA synovial tissue correlates with histologic features of inflammation, e.g., lining layer thickness[13], and increased $sPLA_2$ activity has been observed in RA SF[1]. The chondrocyte is believed to be another source of SF $sPLA_2$[14,15]. Increased $sPLA_2$ activity also has been observed in gut mucosa in inflammatory bowel disease[16] and in serum in septic shock[17].

In early phase clinical pharmacology studies, LY315920, a highly selective inhibitor of group II $sPLA_2$ was administered intravenously to patients with RA. Three days after a single 6-hour infusion, patients treated with LY315920 experienced significantly greater reduction in swollen and tender joints than a matched placebo infusion group. Based on these results, LY333013, an orally-administered methyl ester pro-drug that is metabolized to LY315920, was utilized in this phase 2 dose-finding study.

MATERIALS AND METHODS

This study evaluated the effect on the signs and symptoms of active RA of 3 doses of LY333013 and a matched placebo administered to parallel groups of patients over 12 weeks. Study treatments were double-blinded and randomly assigned. The study was performed at 27 sites in the US; each site obtained approval for the conduct of the study from its institutional review board, and written informed consent was obtained from each patient prior to study enrollment.

Patients. Eligible patients were 18-80 years of age, met the American Rheumatism Association 1987 revised criteria for the classification of RA[18], and were American College of Rheumatology (ACR) functional class I-III[19]. All were receiving one or more of the following disease modifying antirheumatic drugs (DMARD): methotrexate (MTX) ≤ 25 mg/wk, sulfasalazine ≤ 2 g/day, hydroxychloroquine ≤ 400 mg/day, azathioprine ≤ 150 mg/day, and minocycline ≤ 200 mg/day. Patients were allowed to continue systemic glucocorticoids at a dose ≤ 10 mg prednisone-equivalent/day. Nonsteroidal antiinflammatory drugs (NSAID) were allowed at entry to the study, but were discontinued for at least 5 half-lives of the drug before study treatment was given, and throughout the remainder of the patient's participation. Aspirin ≤ 325 mg/day was allowed, but dietary supplements such as omega-3 fatty acids, γ-linoleic acid, glucosamine, and chondroitin sulfate were excluded.

DMARD and glucocorticoid doses were required to be stable for at least 30 days prior to study entry, and doses were maintained during study participation. A minimum baseline level of RA activity was required, as evidenced by at least 3 of the following: ≥ 7 tender joints; ≥ 3 swollen joints; morning stiffness > 45 minutes; and CRP > 1.0 mg/dl. Exclusion criteria included gastrointestinal ulcer within 3 months of study entry; hemoglobin < 9 g/dl; platelet count < 100,000/mm^3; white blood cell count < 3,000/mm^3; serum creatinine > 1.9 mg/dl (> 168 µmol/l), or Cockroft-Gault calculated creatinine clearance < 50 ml/min; alanine (ALT) or aspartate (AST) aminotransferase > 100 IU/l; and pregnant or nursing women. Women of childbearing potential were required to use a reliable method of contraception.

Procedures. Patients meeting the inclusion and exclusion criteria at an initial entry visit were reassessed at their baseline visit to assure that they continued to meet the entry criteria for active RA. The patients completed the Stanford Health Assessment Questionnaire (HAQ)[20], including a 100 mm horizontal visual analog scale (VAS) for pain (0 = no pain; 100 = extreme pain), and assessed the global activity of their arthritis on a 100 mm VAS (0 = very well; 100 = very poorly) at each visit. The investigators were instructed in a standardized 28-joint technique[21] for counting the number of swollen (SJC) and tender joints (TJC) at each visit, and they judged global arthritis activity on a 100 mm VAS (0 = very well; 100 = very poorly).

Safety was assessed on the basis of adverse events reported at each visit (baseline and after 1, 4, 8, and 12 weeks of treatment), findings on physical examination, and laboratory evaluations. Samples for population pharmacokinetics and/or CRP were obtained at baseline and subsequent visits.

Study medications. The study medications were prepared in identical-appearing white tablets containing 0, 50, 125, or 250 mg of LY333013. Patients took 2 tablets twice daily from blister cards that corresponded to assigned dose levels of 0 (placebo), 50, 250, and 1000 mg/day of LY333013. The 50 and 250 mg/day dose groups received active medication only in the morning dose. Randomization to treatment was blocked and stratified by site. Treatment assignments were managed by an interactive telephone voice response system. Treatment identities were provided to the investigators in a sealed envelope, but except for emergencies warranting it, unblinding did not occur until after the last study visit. Unblinded patients were discontinued from study participation unless there was a compelling ethical reason for their continuation in the study. Compliance to the treatment regimen was monitored by counts of medication in the returned blister cards. In the event that the blister cards were lost, medication use was assessed by patient report.

Statistical analysis. The p values for the demographic, RA baseline disease features and medications, and adverse event data (Tables 1-4) were computed for continuous variables from the rank-transformed analysis of variance model with terms for dose and pooled investigative site; p values for categorical variables were computed from the Cochran-Mantel-Haenszel test stratified by pooled investigative site. The primary efficacy evaluation utilized the ACR Definition of Improvement[22] at the 20% level (ACR20). Treatment was deemed a failure if, at the end of 4 weeks of treatment, the patient's condition had worsened by > 20% relative to baseline or, after 8 weeks, there had been < 10% improvement or, after 12 weeks, there had been < 20% improvement. These patients were discontinued from study treatment and were classified as non-responders with respect to any definition of improvement. Efficacy analysis was performed on all randomized patients who received at least one dose of study medication and who had at least one efficacy assessment at the 4, 8, or 12 week visits [intention-to-treat (ITT) group]. Missing data were handled using a last observation carried forward analysis. Adverse event analysis was performed on all patients who received at least one dose of study medication. Assuming that 30% of patients receiving placebo would achieve an ACR20 response compared to 50% of patients receiving LY333013, 1000 mg/day, 59 patients per treatment group would provide 80% probability of detecting a linear dose-response relationship at a one-sided $\alpha = 0.1$ using a logistic regression model with dose as the explanatory variable.

Pharmacokinetic measurements. High performance liquid chromatography/mass spectroscopic methods were used to measure the active metabolite of LY333013. Results were evaluated according to the reported time of the last 4 doses of study medication and the assigned dose. A 2-compartment distribution model was used with assumption of first-order elimination. Based on previous studies, it was known that plasma clearance is affected by bioavailability, which in turn is reduced by increasing dose. These factors were included in the model evaluating individual apparent oral clearance and inter-individual variability in this parameter. Systemic exposure was estimated based on dose and apparent oral clearance for all patients with available pharmacokinetics data.

RESULTS

Patient disposition. Of the 319 patients evaluated for participation in the study, 251 met all eligibility criteria and were randomly assigned to treatment with study drug. Most patients (79.2%) completed the protocol; the most common reason for discontinuation of participation was lack of efficacy (11.4%). Protocol violations included use of NSAID (3 patients), initiation of corticosteroids (2 patients), unstable

Table 1. Demographics of ITT population.

LY333013 Daily Dose	50 mg n = 60	250 mg n = 59	1000 mg n = 61	Placebo n = 56	p
Mean Age, yrs (SD)	58.4 (10.7)	55.4 (12.4)	57.3 (11.0)	56.8 (11.7)	0.674
Age Range, yrs	37–78	23–79	41–78	31–80	
< 65 years old, %	63.3	74.6	77.0	71.4	0.404
Female, %	75.0	74.6	78.7	71.4	0.814
Caucasian, %	95.0	84.7	91.8	87.5	0.832
Mean weight, kg (SD)	81.0 (22.1)	74.3 (15.2)	80.8 (16.7)	77.4 (16.5)	0.200

Table 2. RA disease features of ITT population. Values are mean (SD).

LY333013 Daily Dose	50 mg n = 60	250 mg n = 59	1000 mg n = 61	Placebo n = 56	p
Functional class, n, %					0.613
I	2 (3.3)	6 (10.2)	4 (6.6)	1 (1.8)	
II	48 (80.0)	43 (73.9)	46 (75.4)	43 (76.8)	
III	10 (16.7)	10 (16.9)	11 (18.0)	12 (21.4)	
RA Disease Duration					0.245
< 2 yrs	4 (6.7)	7 (11.9)	4 (6.6)	9 (16.1)	
2–5 yrs	14 (23.3)	10 (16.9)	16 (26.2)	7 (12.5)	
> 5 yrs	42 (70.0)	42 (71.2)	41 (67.2)	40 (71.4)	
RF positive, %	85.0	86.4	76.3	81.8	0.478
Tender joint count, No.	17.2 (6.9)	14.4 (6.1)	15.7 (6.7)	16.2 (5.9)	0.125
Swollen joint count, No.	13.9 (6.0)	12.4 (5.4)	14.4 (5.9)	14.2 (5.8)	0.260
Patient pain VAS, mm	62.6 (21.0)	58.2 (21.8)	61.1 (23.5)	60.5 (22.2)	0.672
Patient global, VAS, mm	66.5 (19.3)	64.6 (22.1)	62.6 (22.8)	65.4 (21.5)	0.856
Physician global VAS, mm	60.2 (19.0)	62.2 (16.2)	62.4 (17.4)	64.8 (18.0)	0.578
HAQ, 0–3	1.4 (0.6)	1.4 (0.6)	1.4 (0.6)	1.4 (0.6)	0.842
Morning stiffness, hrs	4.9 (6.8)	3.5 (5.2)	4.4 (6.3)	5.3 (7.3)	0.443
CRP, mg/l	29.5 (36.2)	26.0 (34.4)	21.9 (17.6)	27.0 (34.5)	0.908
ESR, mm/h	31.0 (22.6)	35.1 (22.3)	37.3 (23.1)	36.6 (24.6)	0.571

Table 3. RA medication use entry in the ITT population.

LY333013 Daily Dose, n (%)	50 mg n = 60	250 mg n = 59	1000 mg n = 61	Placebo n = 56	p
Methotrexate	49 (81.7)	49 (83.1)	43 (70.5)	48 (85.7)	0.168
Hydroxychloroquine	13 (21.7)	10 (16.9)	7 (11.5)	8 (14.3)	0.475
Sulfasalazine	8 (13.3)	9 (15.3)	6 (9.8)	8 (14.3)	0.764
Corticosteroids	27 (45.0)	30 (50.8)	30 (49.2)	27 (48.2)	0.933
Minocycline	2 (3.3)	1 (1.7)	5 (8.2)	1 (1.8)	0.205
Folate	15 (25.0)	13 (22.0)	10 (16.4)	14 (25.0)	0.632
Azathioprine	2 (3.3)	2 (3.4)	2 (3.3)	1 (1.8)	0.882
NSAID	10 (16.7)	7 (11.9)	6 (9.8)	10 (17.9)	0.542
NSAID in past 5 years	45 (75.0)	52 (88.1)	41 (67.2)	49 (87.5)	0.003

use of corticosteroids within 30 days of study entry (2 patients), and insufficient evidence of RA activity (2 patients). All patients were included in the safety analyses; efficacy measures were evaluable in 236 patients.

Demographic and baseline characteristics. There were no statistically significant differences among the treatment groups in age, gender, ethnicity, weight (Table 1), marital status, education, or use of cigarettes or alcohol (data not shown) for patients included in the efficacy analysis. The mean age of study participants was 57.0 years, and from 63.3% to 77.0% of the patients in each group were less than 65 years of age. Most were women (75.0%) and Caucasian (89.8%).

The treatment groups did not differ significantly with respect to baseline characteristics, including ACR functional class, HAQ disability score, RA disease duration, percent

Table 4. Adverse events by overall incidence in all randomized patients. Values are No. (%).

LY333013 Daily Dose	50 mg n = 62	250 mg n = 62	1000 mg n = 64	Placebo n = 63	p
Rhinitis	14 (22.6)	15 (24.2)	15 (23.4)	11 (17.5)	0.929
Headache	13 (21.0)	7 (11.3)	15 (23.4)	14 (22.2)	0.259
Cough increased	11 (17.7)	8 (12.9)	10 (15.6)	5 (7.9)	0.458
Diarrhea	8 (12.9)	7 (11.3)	2 (3.1)	6 (9.5)	0.229
Nausea	4 (6.5)	6 (9.7)	7 (10.9)	5 (7.9)	0.809
Fever	4 (6.5)	4 (6.5)	6 (9.4)	7 (11.1)	0.725
Dizziness	3 (4.8)	5 (8.1)	6 (9.4)	2 (3.2)	0.520
Abdominal pain	3 (4.8)	2 (3.2)	8 (12.5)	2 (3.2)	0.101
Sinusitis	3 (4.8)	3 (4.8)	6 (9.4)	3 (4.8)	0.557
Pharyngitis	3 (4.8)	5 (8.1)	3 (4.7)	3 (4.8)	0.826
Chest pain	4 (6.5)	1 (1.6)	4 (6.3)	2 (3.2)	0.453
Dyspepsia	3 (4.8)	2 (3.2)	2 (3.1)	4 (6.3)	0.819
Rash	3 (4.8)	2 (3.2)	4 (6.3)	2 (3.2)	0.783

rheumatoid factor (RF) positive, and components of the ACR response criteria (Table 2). Overall 82.3% of patients were RF positive and from 67.2% to 71.4% in each group had had RA for more than 5 years. Medications taken by greater than 10% of patients at the time of study consent were (in descending order of frequency) MTX, corticosteroids, hydroxychloroquine, NSAID, and sulfasalazine (Table 3). There were no statistically significant differences in use of these drugs among treatment groups, although NSAID use at study entry was somewhat lower in the LY333013 250 and 1000 mg/day groups than in the placebo group. An exploratory analysis showed that the treatment groups did differ significantly (p = 0.003) with respect to NSAID use at some time within the 5 years prior to study entry, with the lowest proportions of NSAID users in the LY333013 50 and 1000 mg/day groups.

Treatment exposure. The mean plasma steady-state concentration of the active metabolite of LY333013 (LY315920) was estimated to be 222, 627, and 1445 ng/ml for the 50, 250, and 1000 mg/day dose groups, respectively. There were no apparent interactions between LY315920 concentrations and co-therapy with MTX, sulfasalazine, and hydroxychloroquine. Ninety-one percent of the efficacy analysis patients took 80-119% of their prescribed doses of study drug.

Efficacy. At week 1 of treatment LY333013, 1000 mg/day, was superior to placebo in the proportion of patients achieving an ACR20 response (p = 0.064) and a dose-response relationship was observed (p = 0.058) (Figure 1). A statistically significant dose-response relationship was observed for CRP at week 1 (p = 0.046). However, ACR20 response rates increased in all treatment groups at study weeks 4 and 8, with loss of difference from placebo. Exploratory analyses showed a trend towards a dose-dependent relationship for ACR20 response (p = 0.077) among patients older than 65 years. Analyses of secondary endpoints, including time to achieve an ACR20 response, time to treatment failure, and time to achieve a 20% improvement in patient-reported pain or global arthritis status did not differ among treatment groups. An ACR50 response was achieved by 9/56 (16.1%) patients treated with placebo, and by 10/60 (16.7%), 5/59 (8.5%), and 5/61 (8.2%) patients treated with LY333013 50, 250, and 1000 mg/day, respectively.

Changes among the treatment groups in ACR response components were generally small or inconsistent and not statistically significant. TJC increased at weeks 8 and 12 in the LY333013, 250 and 1000 mg/day groups, and SJC did so at week 12. The investigators' global assessment reflected these findings, but the patients' global and pain assessments did not.

Exploratory analyses did not suggest a treatment response in terms of presence of RF, number of DMARD used, or specific DMARD used at study entry. Notably, only 18% of patients were taking NSAID at study entry, in contrast to 48% using low-dose corticosteroids. Corticosteroid use appeared to reduce achievement of ACR20 in the LY333013 1000 mg/day group.

Safety. Of the 251 patients who received at least one dose of study medication, 182 reported at least one adverse event (AE). The most commonly reported AE were: rhinitis (21.9%), headache (19.5%), cough (13.5%), diarrhea (9.2%), nausea (8.8%), fever (8.4%), dizziness (6.4%), abdominal pain (6.0%), sinusitis (6.0%), and pharyngitis (5.6%), with no statistically significant differences across treatment groups for any of these events (Table 4).

In general, the type, severity, and distribution of AE were similar across all treatment groups. The overall proportion of patients with AE was somewhat lower in the placebo group (60.3%) than in the LY333013 50 (69.4%), 250 (79.0%), or 1000 mg/day groups (81.3%, overall p = 0.043), but an analysis of AE by organ system indicated that only the cardiovascular system showed a statistical difference among treatment groups (p = 0.033). Cardiovascular AE did not appear to be dose-related; they occurred in more patients

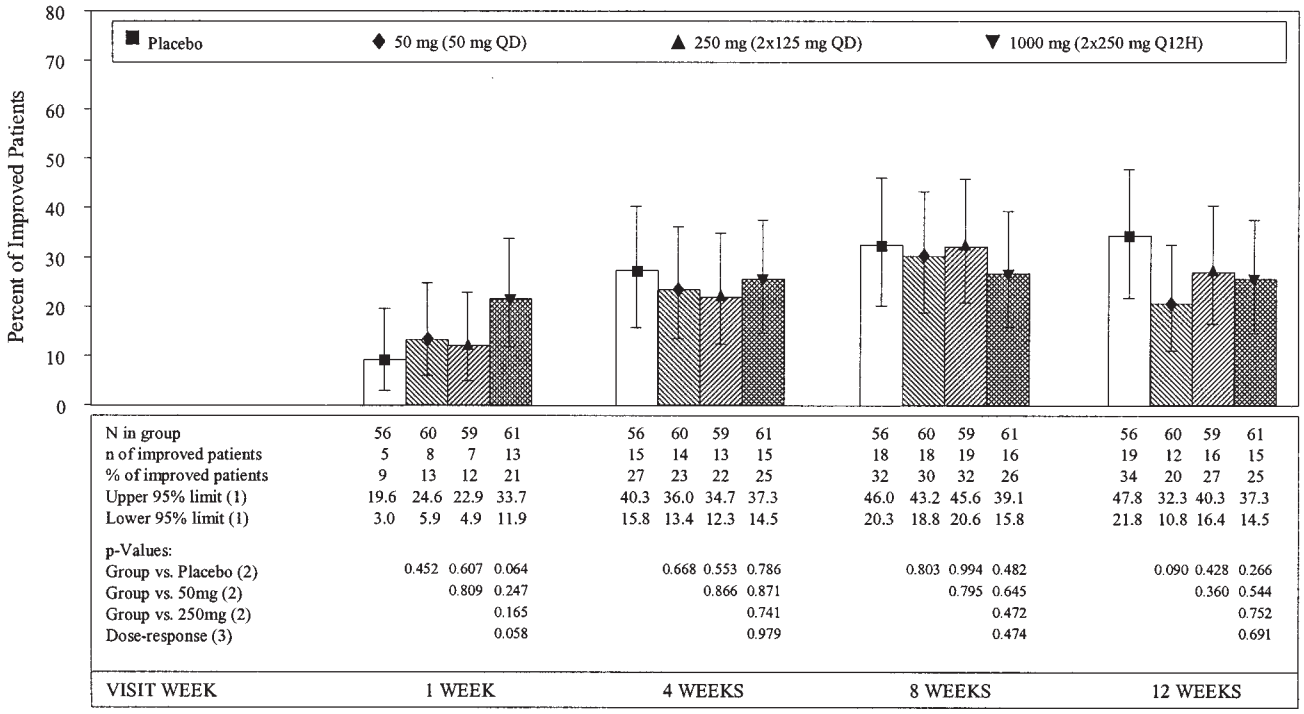

Figure 1. Percentages of patients with RA who met the American College of Rheumatology criteria for 20% improvement (ACR20) during daily oral treatment with LY333013 or placebo. (1): Based on exact methods (Clopper-Pearson method); (2): Test for equality of proportions between 2 treatment groups (chi-square test); (3): test for a linear dose-response relationship (logistic regression model).

in the LY333013 50 and 1000 mg/day treatment groups (16.1%; 15.6%, respectively) than in the LY333013 250 mg/day (3.2%) and placebo groups (4.3%). Upper respiratory tract infections were more common in LY333013-treated patients, whereas urinary tract infections were more common among patients receiving placebo. These differences were not thought to be clinically significant.

Six patients with non-serious AE discontinued study treatment: all were receiving LY333013 (Table 5). These AE were vertigo (50 mg); dyspepsia, nausea, rash (250 mg); asthenia and somnolence (1000 mg). Serious AE were observed in 3 placebo- and 4 LY333013-treated patients; these included septic arthritis, pneumonia, and stroke in the placebo group, and chest pain (50, 250, 1000 mg groups) and perforated colonic diverticulum (50 mg) in the LY333013 groups. The latter patient had a history of diverticulitis, and all 3 patients with chest pain had prior diagnoses of cardiovascular disease. One patient (250 mg group) underwent coronary angioplasty. Study treatment was discontinued in patients with serious AE, i.e., pneumonia and colonic perforation. Only the latter serious AE was assessed by the investigator to be possibly related to study treatment. No patients died during study participation.

There were no clinically relevant changes in laboratory variables, vital signs, or electrocardiogram recordings. Using the National Cancer Institute Common Toxicity Criteria (CTC) grades, only serum AST and calcium differed between placebo and LY333013 ($p = 0.049$). No patient had

Table 5. Reasons for study discontinuation in the ITT population. Values are No. (%).

LY333013 Daily Dose	50 mg n = 60	250 mg n = 59	1000 mg n = 61	Placebo n = 56
Protocol complete	42 (70.0)	50 (84.7)	49 (80.3)	46 (82.1)
Discontinued				
Adverse event	2 (3.3)	1 (1.7)	1 (1.6)	0 (0)
Lack of efficacy	10 (16.7)	5 (8.5)	5 (8.2)	7 (12.5)
Unable to contact patient/lost followup	1 (1.7)	0 (0)	0 (0)	0 (0)
Protocol entry criteria not met	1 (1.7)	0 (0)	1 (1.6)	1 (1.8)
Protocol violation	2 (3.3)	2 (3.4)	3 (4.9)	2 (3.6)
Patient decision	2 (3.3)	1 (1.7)	2 (3.3)	0 (0)

a CTC grade 4 abnormality of any analyte; the highest CTC grade for serum calcium was 2. Serum AST was more frequently elevated in the LY333013 1000 mg/day group; the highest value in this group was 122 IU.

DISCUSSION

Our study did not find a significant sustained benefit from administration of a potent inhibitor of $sPLA_2$, which achieved high plasma concentrations. A dose-dependent response observed at week 1 was subsequently overtaken by the clinical improvement of the placebo control group. The transient response may be attributed to inhibition of the preformed $sPLA_2$ stored in and released from granules in platelets, mast cells, synoviocytes, and hepatocytes, perhaps followed by induction of accelerated synthesis of $sPLA_2$. The synthesis and release of $sPLA_2$ is stimulated by IL-1ß, TNF-α and IL-6[23]. Inhibition of $sPLA_2$ by LY311727, a selective $sPLA_2$ inhibitor, did not inhibit amplification of TNF-α and IL-6 induced by addition of $sPLA_2$[3]. Furthermore, a mutant enzymatically-inactive $sPLA_2$ has been shown to up-regulate cyclooxygenase-2[24], and intraarticular injection of enzymatically-inactive human $sPLA_2$ into rabbits induces inflammation, albeit to a lesser degree than the active enzyme[7].

Corticosteroid therapy has been noted to increase $sPLA_2$ activity in bronchoalveolar lavage fluid obtained from patients with stable asthma shortly after inhaled allergen challenges, possibly indicating an enhancement of acute $sPLA_2$ release (Bowton DL, personal communication). Relative refractoriness to $sPLA_2$ inhibitor therapy was noted in the corticosteroid-treated RA patients in this study.

The concentrations of $sPLA_2$ in RA SF greatly exceed those in plasma[25]. It is possible that concentrations of LY315920 achieved in the RA synovial tissue and RA SF were insufficient to achieve adequate inhibition of $sPLA_2$. Such pharmacokinetic and pharmacodynamic measurements were not performed. However, the tissue distribution of LY315920 in animals is broad, and the volume of distribution in humans approximates the extracellular fluid volume (data on file, Eli Lilly & Co.).

In comparison to its ability to inhibit group II $sPLA_2$, LY315920 is 5- to 10-fold less active against $sPLA_2$ groups V and X, and is 40-fold less active against group Ib $sPLA_2$ (data on file, Eli Lilly & Co.). It is possible that one or more of these other phospholipases is important in RA inflammation, and was not adequately inhibited[26]. Our study did not include measurement of down-stream eicosanoid products, which might support or refute this possibility.

In cultured RA synovial fibroblasts, $cPLA_2$ has been proposed as the predominant enzyme mediating cytokine-induced PG production[27]. Its relative importance in the adjuvant-induced arthritis model has been shown[28]. LY333013 and LY315920 have essentially no activity against $cPLA_2$ and would be expected to be ineffective if $cPLA_2$ is an important mediator of inflammation in RA.

Despite the clinical evidence of RA disease activity, with moderately high SJC and TJC, patients in our study had modest elevations of CRP. The small proportion of patients taking NSAID at entry but who subsequently discontinued contributed a modest flare with respect to the ACR response criteria components of investigator-assessed SJC, TJC, and global status, and patient-assessed pain and global status. Perhaps the acute inflammatory component of the RA disease process was largely held in check by the DMARD and low-dose corticosteroid therapy, thus minimizing the impact of inhibition of $sPLA_2$. A study design utilizing a NSAID-withdrawal flare might have yielded a different result. For example, hydroxychloroquine has been shown to inhibit activation of phospholipases in macrophages, and down-regulate induction of IL-1ß and TNF-α[29]. A longer duration of followup than we performed may be required to observe this type of effect. Nevertheless, the results of this study cast substantial doubt on the clinical importance of group II $sPLA_2$ in partially-treated RA.

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REFERENCES

1. Sielhamer J, Pruzanski W, Vadas P, et al. Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. J Biol Chem 1989;264:5335-8.
2. Kudo I, Murakami M, Hara S, et al. Mammalian non-pancreatic phospholipases A2. Biochim Biophys Acta 1993;1170:217-31.
3. Bidgood MJ, Jamal OS, Cunningham AM, et al. Type IIA secretory phospholipase A2 up-regulates cyclooxygenase-2 and amplifies cytokine mediated prostaglandin production in human rheumatoid synoviocytes. J Immunol 2000;165:2790-7.
4. Triggiani M, Granata F, Oriente A, et al. Secretory phospholipases A2 induce cytokine release from blood and synovial fluid monocytes. Eur J Immunol 2002;32:67-76.
5. Rola-Pleszczynski M, Lemaire I. Leukotrienes augment interleukin 1 production by human monocytes. J Immunol 1985;135:3958-61.
6. Poubelle PE, Gingras D, Demers C, et al. Platelet-activating factor (PAF-acether) enhances the concomitant production of tumor necrosis factor α and interleukin 1 production by subsets of human monocytes. Immunology 1991;72:181-7.
7. Brach MA, De Vos S, Arnold C, Gruss HJ, Mertelsmann R, Herrmann F. Leukotriene B4 transcriptionally activates interleukin-6 expression involving NF-kB and NF-IL6. Eur J Immunol 1992;22:2705-11.
8. Bomalaski JS, Lawton P, Browning JL. Human extracellular recombinant phospholipase A2 induces an inflammatory response in rabbit joints. J Immunol 1991;146:3904-10.
9. Chapdelaine JM, Ciofalo VB, Grass DS, et al. Human extracellular

type II phospholipase A2 (PLA2) transgenic mice provide a tool to determine the role of PLA2 in inflammatory conditions [abstract]. Arthritis Rheum 1996;38 Suppl:S293.
10. Grass DS, Felkner RH, Chiang MY, et al. Expression of human group II PLA2 in transgenic mice results in epidermal hyperplasia in the absence of inflammatory infiltrate. J Clin Invest 1996;97:2233.
11. Garcia Pastor P, De Rosa S, De Giulio A, Paya M, Alcaraz MJ. Modulation of acute and chronic inflammatory processes by cacospongionolide B, a novel inhibitor of human synovial phospholipase A2. Br J Pharmacol 1999;126:301-11.
12. Lin MK, Farewell V, Vadas P, Bookman AA, Keystone EC, Pruzanski W. Secretory phospholipase A2 as an index of disease activity in rheumatoid arthritis. Prospective double blind study of 212 patients. J Rheumatol 1996;23:1162-6.
13. Jamal OS, Conaghan PG, Cunningham AM, Brooks PM, Munro VF, Scott KF. Increased expression of human type IIa secretory phospholipase A2 antigen in arthritic synovium. Ann Rheum Dis 1996;57:550-8.
14. Nevalainen TJ, Marki F, Kortesuok PT, Grutter MG, Di Marco S, Schmitz A. Synovial type (group II) phospholipase A2 in cartilage. J Rheumatol 1993;20:325-30.
15. Pruzanski W, Bogoch E, Katz A, et al. Induction of release of secretory nonpancreatic phospholipase A2 from human articular chondrocytes. J Rheumatol 1995;22:2114-9.
16. Minami T, Tojo H, Shinomura Y, Matsuzawa Y, Okamoto M. Increased group II phospholipase A2 in colonic mucosa of patients with Crohn's disease and ulcerative colitis. Gut 1994;35:1593-8.
17. Green J-A, Smith GM, Buchta R, et al. Circulating phospholipase A2 activity associated with sepsis and septic shock is indistinguishable from that associated with rheumatoid arthritis. Inflammation 1991;15:355-67.
18. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
19. Hochberg MC, Chang RW, Dwosk I, et al. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum 1992;35:498-502.
20. Fries JF, Spitz, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980;23:137-45.
21. Prevoo ML, Van Riel PL, Van 'T Hof MA, et al. Validity and reliability of joint indices. A longitudinal study in patients with recent onset rheumatoid arthritis. Br J Rheumatol 1993;32:589-94.
22. Felson DT, Anderson JJ, Boers M. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727-35.
23. Vadas P, Browning J, Edelson J, et al. Extracellular phospholipase A2 expression and inflammation: the relationship with associated disease states. J Lipid Mediat 1993;8:1-30.
24. Tada K, Murakami M, Kambe T, Kudo I. Introduction of cyclooxygenase-2 by secretory phospholipase A2 in nerve growth factor-stimulated rat serosal mast cells is facilitated by interaction with fibroblasts and mediated by a mechanism independent of their enzymatic functions. J Immunol 1998;161:5008-15.
25. Pruzanski W, Vadas P, Stefanski E, Urowitz MB. Phospholipase A2 activity in sera and synovial fluids in rheumatoid arthritis and osteoarthritis. Its possible role as a proinflammatory enzyme. J Rheumatol 1985;12:211-6.
26. Kim KP, Rafter JD, Bittova L, et al. Mechanism of human group V phospholipase A2 (PLA2)-induced leukotriene biosynthesis in human neutrophils. J Biol Chem 2001;276:11126-34.
27. Roshak A, Mochan E, Marshall LA. Suppression of human synovial fibroblast 85 KDa phospholipase A2 by antisense reduces interleukin-1ß induced prostaglandin E2. J Rheumatol 1996;23:420-7.
28. Amandi-Burgermeister E, Tibes U, Kaiser BM, Friebe WG, Scheuer WV. Suppression of cytokine synthesis, integrin expression and chronic inflammation by inhibitors of cytosolic phospholipase A2. Eur J Pharmacol 1997;326:237-50.
29. Bondeson J, Sundler R. Antimalarial drugs inhibit phospholipase A2 activation and induction of interleukin 1 beta and tumor necrosis factor alpha in macrophages: implications for their mode of action in rheumatoid arthritis. Gen Pharmacol 1998;30:357-66.