Pharmacokinetic and pharmacodynamic profile following oral administration of the phosphodiesterase (PDE)4 inhibitor V11294A in healthy volunteers

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Aims To assess the pharmacokinetic and pharmacodynamic profile of the novel PDE4 inhibitor V11294A (3-(3-cyclopentyloxy-4-methoxybenzyl)-6-ethylamino-8-isopropyl-3H purine hydrochloride) in healthy male volunteers.

Methods This was a double-blind, single dose, randomized crossover study in eight healthy volunteers who received a single oral, fasting dose of V11294A (300 mg) or placebo. Blood samples were taken before and 0.5, 1, 2, 2.5, 3, 4, 6, 9, 12, 18 and 24 h after oral dosing for determination of plasma concentrations of V11294A. Blood samples were also taken before and 3 and 24 h after dosing for the assessment of the effect of V11294A on mononuclear cell proliferation and tumour necrosis factor (TNF) release in whole blood.

Results Following a single oral dose of 300 mg V11294A, plasma concentrations of V11294A and its active metabolite V10332 reached $C_{\max}$ (ng ml⁻¹; mean $\pm$ s.d.; 1398 $\pm$ 298, 1000 $\pm$ 400, respectively) after 2.63 $\pm$ 0.79 and 5.9 $\pm$ 2.3 h, respectively. For V11294A and V10332, $t_{1/2}$ were 9.7 $\pm$ 3.9 and 9.5 $\pm$ 1.7 h, and AUC(0, ∞) were 18100 $\pm$ 6100 and 18600 $\pm$ 8500 ng ml⁻¹ h, respectively. At 3 h dosing, plasma concentrations of V11294A and V10332 (3-(3-cyclopentyloxy-4-methoxy-benzyl)-8-isopropyl-3H-purin-6-ylamine) were 1300 $\pm$ 330 and 860 $\pm$ 300 ng ml⁻¹, 7 and 3 times their *in vitro* IC₅₀s for inhibition of TNF release and proliferation, respectively. Treatment with V11294A resulted in a significant reduction of lipopolysaccharide (LPS)-induced TNF release at 3 h ($P < 0.001$) and at 24 h ($P < 0.05$) post ingestion. The amount of TNF released (pmol ml⁻¹) in response to a submaximal concentration of LPS (4 ng ml⁻¹) was not significantly altered following placebo treatment (before 681 $\pm$ 68 *vs* 3 h postdose 773 $\pm$ 109, $P = 0.27$). In contrast, there was a significant reduction in the amount of TNF released following treatment with V11294A (before 778 $\pm$ 87 *vs* 3 h postdose 566 $\pm$ 72, $P = 0.02$). Phytohaemagglutinin (PHA) stimulated the incorporation of [³H]-thymidine in whole blood prior to drug administration. V11294A inhibited the PHA-induced proliferation at 3 h ($P < 0.05$). No adverse reactions were noted following single oral administration of V11294A.

Conclusions A single oral 300 mg dose of V11294A administered to healthy volunteers results in plasma concentrations adequate to inhibit activation of inflammatory cells *ex vivo*, which persists for at least 24 h without any adverse reactions.

Keywords: lymphocyte proliferation, monocytes, PDE4, pharmacokinetics, thymidine, TNF α , V11294A, whole blood

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Introduction

A variety of pharmacological, biochemical and molecular biological studies have revealed the existence of 11 diverse phosphodiesterase (PDE) families which are comprised of at least 15 gene products with further diversity occurring as a consequence of differential splicing and post-translational processing [1, 2]. Of particular interest is the role of PDE4 in regulating the function of a variety of cells thought to participate in the inflammatory process, and there is considerable interest in the development of PDE4 inhibitors for the treatment of inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD) [3].

PDE inhibitors are currently being developed for the treatment of asthma although side-effects including nausea and vomiting have halted the development of some examples of this class of drug into the clinic. To date, there are a limited number of clinical studies investigating the efficacy of PDE inhibitors in the treatment of asthma. Inhalation of zardaverine was shown to produce a modest bronchodilator effect in patients with asthma, although unacceptable side-effects of nausea and emesis were reported in a significant number of patients [4], while oral administration of cilostazol (PDE3 inhibitor) caused bronchodilation and bronchoprotection against methacholine challenge in healthy subjects at the expense of mild to severe headache [5]. AH-2132 (benzafentrine; a mixed PDE3/4 inhibitor) has also been reported to have significant bronchodilator activity in normal volunteers [6]. The PDE4 inhibitor, ibudilast significantly improved baseline airways responsiveness to spasmogens by 2 fold after 6 months treatment [7] and MKS492 (a PDE3 inhibitor) has been reported to attenuate the early and late asthmatic response in atopic asthmatics [8].

Recently, the orally active PDE4 selective inhibitors, CDP840 [9] and roflumilast [10] have been demonstrated to modestly attenuate the development of the late asthmatic response in mild asthmatics whilst having no effect on the acute response, with no significant side-effects being reported in comparison with placebo. The ability of these novel selective PDE4 inhibitors to inhibit the late asthmatic response was not associated with bronchodilation, suggesting actions of this drug other than smooth muscle relaxation. Furthermore, another PDE4 inhibitor RPR73401, has also been shown to have no significant effect on allergen-induced bronchoconstriction in allergic asthmatic subjects [11], consistent with the suggestion that PDE3 rather than PDE4 may be the important isoenzyme regulating airway smooth muscle tone in asthmatic subjects. However, recent clinical studies with another orally active PDE4 inhibitor, cilomilast have shown that this drug can attenuate bronchoconstriction following exercise in asthmatic subjects [12], an

effect mimicked by 4 weeks of treatment with the selective PDE4 inhibitor roflumilast [13]. Similarly, cilomilast provided significant clinical improvement in patients with asthma and COPD [14].

We have previously reported that V11294A is a novel potent orally active PDE4 inhibitor having a wide range of pharmacological activities including the ability to inhibit the release of TNF from human monocytes and proliferation of human mononuclear cells [15]. Furthermore, in phase I clinical trials in human volunteers, V11294A was shown to be without significant emetic effects consistent with studies in the ferret [15].

In the present study we have assessed the pharmacokinetic and pharmacodynamic profile of a single oral administration of V11294A in healthy volunteers, including the ability of this drug to inhibit TNF release from circulating monocytes as a biomarker.

Methods

Subjects

Eight healthy males between the ages of 18 and 45 years, with weight within the acceptable range for the subjects height according to the 1983 New York Metropolitan Life Insurance Standard Height and Weight Table, were eligible to participate in the study. They were healthy, without evidence of disease, had no recent history of drug or alcohol abuse, were not users of tobacco products, and were negative for hepatitis B, C and HIV. All subjects gave written, informed consent in compliance with King's College Hospital Ethics Committee regulations.

Subjects were excluded from study participation if any of the following criteria were met: history of serious drug hypersensitivity reactions, active or recent substance abuse; healthy without evidence of disease; use of any investigational drug within 4 months of the start of the study or within 3 months of the start of the study for any other drug; use of any other drug including over-the-counter medications during the course and 2 weeks prior to commencement of the study; use of liver enzyme-inducing or liver enzyme-inhibiting drugs during the 3 months before the start of the study; hospitalization for any surgical or medical condition during the 3 months before the start of the study; having undergone dental treatment with a local anaesthetic during the 3 weeks, or a general anaesthetic during the 3 months before the start of the study; blood donation within 4 weeks prior to study.

Study design

This was a double-blind, single dose, randomized cross-over study. Subjects received either placebo (6 × 50 mg)

or V11294A (6×50 mg) capsules following an overnight fast. All doses were administered with 200 ml of water. A low-fat lunch, a snack and a dinner were provided at 4, 10 and 14 h, respectively, after the morning dose. A Holter Monitor was attached to each volunteer to record cardiac rhythm, rate and ST segments overnight until 2 h before dosing. Subjects were confined to our Clinical Trials unit 12 h prior to drug administration and 24 h thereafter during which time blood was collected at various intervals. Strenuous activity during confinement was not permitted.

Sample collection and analysis

Plasma V11294A and active metabolite V10332 identities were confirmed by mass fragmentation analysis and comparison with fragmentation patterns of authentic, synthetic standards. Samples were prepared by subjecting plasma to solid phase extraction on mixed function reverse phase/ion exchange cartridges (Varian). V11294 and V10332 were eluted with methylene chloride: isopropanol:ammonium hydroxide, 80:20:2. The eluant containing V11294A and V10332 was subjected to isocratic separation using a Nova-Pak Phenyl column (Waters), with elution by trifluoroacetic acid: acetonitrile:water mobile phase using an Alliance LC/MS system (Waters).

Concentrations of V11294A and the active metabolites V10332 were determined at Purdue Frederick Inc. (Ardsley NY). For quantitation, serial 10 ml venous blood samples were collected for serum V11294A concentrations prior to dosing (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 18 and 24 h postdose. Samples were prepared as described for identification, above. Plasma was subjected to solid phase extraction on mixed function reverse phase/ion exchange cartridges (Varian). V11294 and V10332 were eluted with methylene chloride: isopropanol:ammonium hydroxide, 80:20:2. The eluant containing V11294A and V10332 was subjected to isocratic separation by h.p.l.c. using a Nova-Pak Phenyl column (Waters), with elution of trifluoroacetic acid: acetonitrile:water mobile phase, and detection for quantification by area under the peak at 290 nm at 3 min and 5 min, respectively. Minimum detectable concentration of V11294A and V10332 were 2.5 ng ml^{-1} and 3.0 ng ml^{-1} with coefficients of variation 3% and 4%, respectively ($n = 6$ determinations each).

Ex vivo experiments

Additional blood samples (40 ml) were collected prior to (0 h) and at 3 and 24 h following oral administration of V11294A or placebo. Whole blood was drawn into lithium-heparinized tubes, then transferred to plastic

tubes containing heparin (50 U ml^{-1}) and kept at room temperature for a period no greater than 2 h. Whole blood (200 μl) was pipetted into 96 well plates with 10 μl of LPS ($1\text{--}64 \text{ ng ml}^{-1}$) which were placed into a 37°C incubator in an atmosphere of 5% CO_2 in air for 18 h. Plates were then centrifuged (250 g for 10 min) and aliquots of plasma frozen (-20°C) for assessment of TNF α using standard ELISA techniques. In other experiments, whole blood (100 μl) was pipetted into 96 well plates containing 10 μl of PHA ($1\text{--}100 \text{ }\mu\text{g ml}^{-1}$). Plates were then placed into a 37°C incubator in an atmosphere of 5% CO_2 in air for 4 days. On the third day (24 h prior to harvesting cells), [^3H]-thymidine (0.0185 MBq) was added to the wells and cells were harvested onto filters using a semiautomated cell harvester. The level of thymidine incorporation into cells was determined using liquid scintillation and expressed as disintegration per minute (d min).

Pharmacokinetic analysis and statistical methods

V11294A and V10332 pharmacokinetic parameters (t_{max} , C_{max} , $\text{AUC}(0,24 \text{ h})$, $\text{AUC}(0,\infty)$, and $t_{1/2}$) were estimated using WinNonlin. The effect of V11294A on the concentration response to LPS-induced TNF production and PHA-induced proliferation was evaluated using two-way analysis of variance with repeated measures. *Post hoc* tests to compare means of placebo and V11294 at each concentration of LPS and PHA with predosing (0 h) values were performed using Student's paired *t*-test with Bonferroni correction and considered significant if $P < 0.05$.

Results

Subjects

The mean $\pm$ s.d. age of the eight healthy male volunteers who completed the study was 24.5 ± 4.31 years (range: 19–32). Their mean $\pm$ s.d. weight was 77.3 ± 9.5 kg (range: 62–90.5) and the mean $\pm$ s.d. height was 183.5 ± 4.90 cm (range: 179–194).

Pharmacokinetics

An earlier study in normal male volunteers found that the highest single dose evaluated (300 mg) was without side-effects, and therefore, this dose was chosen for the present study. Following administration of a single oral dose of V11294A (300 mg), plasma V11294A reached C_{max} at approximately 3 h postdose and declined thereafter with a terminal elimination half-life of 9.66 h (Figure 1, Table 1). V10332, the major active metabolite reached C_{max} at approximately 6 h postdose and declined

Figure 1 Plasma concentrations of V11294A (○) and V10332 (■) vs time following single oral administration of V11294A (300 mg). Each point represents the mean and vertical lines represent s.d.

Table 1 V11294A and V10332 pharmacokinetic parameters (mean $\pm$ s.d., $n = 12$) after single oral administration with V11294A (300 mg).

Parameter	V11294A	V10332
$t_{\max}$ (h)	2.63 ± 0.79	5.88 ± 2.32
$C_{\max}$ (ng ml $^{-1}$)	1398 ± 298	1038 ± 415
$t_{1/2}$ (h)	9.66 ± 3.93	9.5 ± 1.7
AUC(0,24 h) (ng ml $^{-1}$ h)	14691 ± 4555	14603 ± 6339
AUC(0, ∞) (ng ml $^{-1}$ h)	18181 ± 6085	18648 ± 8537

thereafter with a terminal elimination half-life of 9.5 h (Figure 1, Table 1). At $C_{\max}$, the plasma concentration of V11294A and V10332 was 1398 ± 298 and 1038 ± 415 ng ml $^{-1}$, respectively, 7 and 3 times their *in vitro* IC $_{50}$ s for inhibition of PDE4 of 212 ± 14 and 305 ± 36 ng ml $^{-1}$, respectively.

Ex vivo experiments

TNF release

LPS induced a concentration-dependent increase in TNF in whole blood (Figure 2a). V11294A administered to healthy volunteers, inhibited TNF release stimulated by LPS in whole blood (Figure 3). The TNF release in response to low but not high concentrations of LPS (1–4 ng ml $^{-1}$) was significantly inhibited following oral administration of V11294A but not placebo at 3 h ($P < 0.0001$, ANOVA) and 24 h ($P = 0.018$, ANOVA), the magnitude of the effect being greater at 3 h. The mean difference in TNF concentrations (pmol ml $^{-1}$) induced by

Figure 2 (a) TNF concentrations (pmol ml $^{-1}$) and (b) proliferative response ([3 H]-thymidine incorporation; d min $^{-1}$) in whole blood following stimulation with LPS and PHA, respectively. Each point represents the mean and vertical lines represent s.e. mean.

LPS (1 ng ml $^{-1}$: 176 [95% CI, 98, 245]; 2 ng ml $^{-1}$: 179 [29, 330] and 4 ng ml $^{-1}$: 428 [70, 785]) was significantly lower 3 h following treatment with V11294A than placebo ($P < 0.05$). Similarly, the mean difference in TNF concentrations (pmol ml $^{-1}$) induced by LPS (2 ng ml $^{-1}$: 195 [32, 359]; and 4 ng ml $^{-1}$: 342 [56, 627]) was significantly lower 24 h following treatment with V11294A than placebo ($P < 0.05$).

The amount of TNF released (pmol ml $^{-1}$) in response to a submaximal concentration of LPS (4 ng ml $^{-1}$) was 681 ± 68 before and 773 ± 109 , 3 h after placebo ($P = 0.27$). In contrast, there was a significant reduction in the amount of TNF released following treatment with V11294A (before 778 ± 87 vs 3 h, 566 ± 72 , $P = 0.02$). The data from individual subjects are shown in Figure 4.

Proliferation

PHA induced a concentration-dependent increase in proliferation of mononuclear cells in whole blood and like all lectins, PHA causes nonspecific activation of lym-

Figure 3 Concentrations of TNF (pmol ml^{-1} ; change from predosing values) in whole blood following stimulation with LPS 3 h (a) and 24 h (b) following single oral administration of V11294A. Vertical lines represent s.d. Post-test comparison of the means of TNF production in placebo-treated (open bars) and V11294A-treated (closed bars) subjects revealed significant differences at the LPS concentrations indicated (* $P < 0.05$).

phocytes. The efficiency of cross-linking is reduced at high concentrations of lectin, and hence, a bell shaped dose-response curve is observed (Figure 2b). The proliferation of mononuclear cells in whole blood induced by PHA was significantly reduced in subjects following oral administration of V11294 compared with placebo at 3 h ($P < 0.05$, ANOVA) which was still modestly evident at 24 h postdose ($P < 0.05$, ANOVA, Figure 5).

Adverse events

Most treatment emergent adverse events in this study were rated as mild. A rash was observed around the blood sampling site, the trunk and the head of an individual who was administered placebo and V11294A. A stuffy nose was also reported by an individual taking placebo and V11294A. Nausea was recorded in one individual 10 min following administration of placebo that remained for 10 min.

Figure 4 Whole blood concentrations of TNF (pmol ml^{-1}) in each healthy volunteer before and 3 h following oral administration of V11294A or placebo.

Figure 5 The proliferation of mononuclear cells (d min^{-1} ; change in $[\text{³H}]$ -thymidine incorporation from pre dosing values) in whole blood following stimulation with PHA 3 h (a) and 24 h (b) following single oral administration of V11294A. Vertical lines represent s.d.. Post-test comparison of the means of proliferation in samples from placebo-treated (open bars) and V11294A-treated (closed bars) subjects revealed significant differences at the PHA concentrations indicated (* $P < 0.05$).

Discussion

The present study was designed to evaluate the pharmacokinetic and pharmacodynamic profile of the PDE4 inhibitor, V11294A in healthy male volunteers. Following a single oral administration of V11294A, the peak plasma concentration ($3 \mu\text{M}$) was achieved 2.6 h postdosing. The plasma half-life was 9.6 h and concentrations had not returned to predose values after 24 h. The plasma concentrations achieved at 3 h and to a lesser extent at 24 h were sufficient to significantly attenuate *ex vivo* biomarkers, namely TNF release and mononuclear cell proliferation in whole blood. Thus, a single oral 300 mg dose of V11294A administered to healthy volunteers results in plasma concentrations adequate to inhibit activation of inflammatory cells *ex vivo*, which persists for at least 24 h without any adverse reactions including nausea and vomiting.

The major metabolite, V10332 reached peak plasma concentrations ($2.2 \mu\text{M}$) at 5.8 h following single oral administration of V11294A. V10332 is an active metabolite with a potency identical to the parent V11294A and its C_{max} was three fold greater than the IC_{50} values against mononuclear cell proliferation and TNF release in whole blood.

V11294A is a potent PDE4 inhibitor which has been demonstrated to inhibit a number of inflammatory cell functions including mononuclear cell proliferation, TNF release from monocytes, tracheal smooth muscle relaxation and recruitment of eosinophils to the airways following antigen challenge in immunized guinea-pigs and rabbits [15]. The effect of V11294A on cellular function is similar to that previously reported for other PDE4 inhibitors [16]. However, many of the studies investigating the effect of PDE inhibitors usually involve isolation and purification of the cell under investigation. We have demonstrated that exogenous administration of V11294A can inhibit mononuclear cell proliferation and TNF release from whole blood consistent with findings observed with isolated cells.

Using these biomarkers of cell activation there was a significant inhibition of mononuclear cell proliferation and TNF release in whole blood 3 h following single oral administration of V11294A. The plasma concentrations of V11294A at this time point were seven fold greater than the IC_{50} value for V11294A against these cellular effects and show for the first time that the plasma concentration of V11294A achieved following oral administration can inhibit inflammatory cell function, a feature consistent with the recent findings obtained clinically with another PDE4 inhibitor roflumilast [10]. Furthermore, the plasma concentrations achieved were not associated with nausea or vomiting indicating that V11294A is a safe and well tolerated PDE4 inhibitor in healthy

volunteers. An interesting finding was that there was some degree of inhibition of cellular function 24 h after single oral administration of V11294A and suggests the possibility of providing anti-inflammatory activity for a prolonged period. It remains to be established whether V11294A will inhibit inflammatory cell function in asthmatic subjects, although this may be anticipated since the plasma concentrations achieved following single oral administration of V11294A in healthy volunteers is sufficient to inhibit inflammatory cell activity and other recent clinical findings suggest that PDE4 inhibitors may have nonbronchodilator actions in subjects with airway disease [9, 10, 14].

Whereas a number of clinical studies have shown a small but positive effect of PDE inhibitors with regard to their ability to inhibit the late asthmatic response, bronchoprotection and/or bronchial hyperresponsiveness [4–9, 11, 14], concerns regarding side-effects including headache, nausea and vomiting have often necessitated the use of doses which may be suboptimal. However, we have clearly demonstrated that it is possible to achieve sufficient plasma concentrations of a PDE4 inhibitor, V11294A to have a significant effect on inflammatory cell activation without side-effects that have previously been associated with earlier PDE4 inhibitors.

In conclusion, we have demonstrated that a single oral administration of V11294A is well tolerated in healthy volunteers, achieving plasma concentrations which were sufficient to attenuate various biomarkers over a 24 h period.

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References

- 1 Torphy TJ. Phosphodiesterase isozymes: molecular targets for novel antiasthma agents. *Am J Respir Crit Care Med* 1998; **157**: 351–370.
- 2 Soderling SH, Beavo JA. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Opin Cell Biol* 2000; **12**: 174–179.
- 3 Torphy TJ, Page C. Phosphodiesterases: the journey toward therapeutics. *Trends Pharmacol Sci* 2000; **21**: 157–159.
- 4 Brunnee T, Engelstatter R, Steinijans VW, Kunkel G. Bronchodilatory effect of inhaled zardaverine, a phosphodiesterase III and IV inhibitor, in patients with asthma. *Eur Respir J* 1992; **5**: 982–985.
- 5 Fujimura M, Kamio Y, Saito M, Hashimoto T, Matsuda T. Bronchodilator and bronchoprotective effects of cilostazol in humans *in vivo*. *Am J Respir Crit Care Med* 1995; **151**: 222–225.
- 6 Foster RW, Rakshi K, Carpenter JR, Small RC. Trials of the bronchodilator activity of the isoenzyme-selective phosphodiesterase inhibitor AH 21–132 in healthy volunteers during a methacholine challenge test. *Br J Clin Pharmacol* 1992; **34**: 527–534.

- 7 Kawasaki A, Hoshino K, Osaki R, Mizushima Y, Yano S. Effect of ibudilast: a novel antiasthmatic agent, on airway hypersensitivity in bronchial asthma. *J Asthma* 1992; **29**: 245–252.
- 8 Bardin PG, Dorward MA, Lampe FC, Franke B, Holgate ST. Effect of selective phosphodiesterase 3 inhibition on the early and late asthmatic responses to inhaled allergen. *Br J Clin Pharmacol* 1998; **45**: 387–391.
- 9 Harbinson PL, MacLeod D, Hawksworth R, et al. The effect of a novel orally active selective PDE4 isoenzyme inhibitor (CDP840) on allergen-induced responses in asthmatic subjects. *Eur Respir J* 1997; **10**: 1008–1014.
- 10 Nell H, Louw C, Leichtl S, Rathgeb F, et al. Acute anti-inflammatory effect of the novel phosphodiesterase 4 inhibitor roflumilast on allergen challenge in asthmatics after a single dose. *Am J Respir Crit Care Med* 2000; **161**: A200.
- 11 Jonker GJ, Tijhuis GJ de Monchey JGR. RP 73401 (a phosphodiesterase IV inhibitor) single does not prevent allergen induced bronchoconstriction during the early phase reaction in asthmatics. *Eur Respir J* 1996; **9**: 82s.
- 12 Nieman RB, Fisher BD, Amit O, Dockhorn RJ. SB 207499 (Ariflo™), a second-generation, selective oral phosphodiesterase type 4 (PDE4) inhibitor, attenuates exercise induced bronchoconstriction in patients with asthma. *Am J Respir Crit Care Med* 1998; **157**: A413.
- 13 Timmer W, Leclerc V, Birraux G, et al. Safety and efficacy of the new PDE4 inhibitor roflumilast administered to patients with exercise-induced asthma over 4 weeks. *Am J Respir Crit Care Med* 2000; **161**: A505.
- 14 Torphy TJ, Barnette MS, Underwood DC, et al. Ariflo™ (SB 207499), a second generation phosphodiesterase 4 inhibitor for the treatment of asthma and COPD. from concept to clinic. *Pulm Pharmacol Ther* 1999; **12**: 131–135.
- 15 Gale DD, Hofer P, Spina D, et al. The pharmacology of a novel phosphodiesterase type 4 inhibitor, V11294A. *Pulm Pharmacol Ther* 2002; (in press).
- 16 Spina D, Landells LJ, Page CP. The role of phosphodiesterase isoenzymes in health and in atopic disease. In *Advances in Pharmacology*, ed. August T. San Diego: Academic Press Inc, 1998: 33–89.