Journal of Cellular Biochemistry 64:19–26 (1997)

In Vitro and In Vivo Studies of ICE Inhibitors

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Abstract Interleukin-1β-converting enzyme (ICE) is a cysteine protease responsible for proteolytic activation of the biologically inactive interleukin-1β precursor to the proinflammatory cytokine. ICE and homologous proteases also appear to mediate intracellular protein degradation during programmed cell death. Inhibition of ICE is a new antiinflammatory strategy being explored by the design of both reversible inhibitors and irreversible inactivators of the enzyme. Such compounds are capable of blocking release of interleukin-1β from human monocytes. ICE inhibitors that cross react against multiple ICE homologs can also block apoptosis in diverse cell types. ICE inhibitors impart protection in vivo from endotoxin-induced sepsis and collagen-induced polyarthritis in rodent models. Further optimization of the current generation of peptidyl ICE inhibitors will be required to produce agents suitable for administration in chronic inflammatory and neurodegenerative diseases. J. Cell. Biochem. 64:19–26.

Key words: ICE; protease; interleukin-1; cytokine; programmed cell death; apoptosis

The interleukin-1β-converting enzyme (ICE) and its homologs are potential therapeutic intervention targets for the treatment of inflammatory and neurodegenerative diseases [Dinarello and Wolff, 1993]. Interleukin-1β (IL-1β) itself is a principal mediator of inflammation in human arthritis [Lebsack et al., 1993], inflammatory bowel diseases [Ligumsky et al., 1989; Casellas et al., 1995], sepsis syndrome [Fisher et al., 1994], diabetes mellitus [Holden and Mooney, 1995], and pancreatitis [Bamba et al., 1994; Norman et al., 1995]. IL-1β is also the major osteoclast activating factor in the degenerative bone diseases osteoarthritis [Goldring, 1992; van de Loo et al., 1995] and multiple myeloma [Battaile et al., 1992]. Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis and cerebral injury due to trauma and ischemia may be accelerated by release of IL-1β from the microglia [Holden and Mooney, 1995]. These neurodegenerative and ischemic conditions may also be accelerated by programmed cell death (or apoptosis) of affected brain tissue, which requires intracellular proteolysis of apoptotic substrates by ICE and ICE homologs [Miura et al., 1993; Gagliardini et al., 1994]. Programmed cell death of affected brain tissue, which requires intracellular proteolysis of apoptotic substrates by ICE and ICE homologs [Miura et al., 1993; Gagliardini et al., 1994], may also speed these neurodegenerative and ischemic conditions. A broad range of potential therapies may result, therefore, from the design of inhibitors of ICE and its homologs. This paper presents a brief review and commentary on peptidyl ICE inhibitors.

STRUCTURE AND MECHANISM OF ICE

The solution of the structure of ICE by X-ray diffraction methods provided critical information for the rational design of ICE inhibitors for therapeutic use [Walker et al., 1994; Wilson et al., 1994]. A high-resolution view of the active site of ICE reveals it to be a cysteine protease distinct from those of the papain superfamily cysteine proteases such as Cathepsin B. The secondary structure (fold) and quaternary structure (subunit assembly) of ICE do not resemble those of other classes of cysteine or serine proteases. Molecular interactions, notably hydrogen bonds, of peptidyl ICE inhibitors in the ICE enzyme active site residues are similar to the β-sheet-type recognition patterns observed in inhibited complexes of serine proteases such as chymotrypsin [Dolle et al., 1994b; Mullican et al., 1994; Wilson et al., 1994]. In contrast, the active site catalytic machinery of ICE consists of a cysteine-histidine catalytic diad, which is different than the cysteine-histidine-aspara-

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Received 18 March 1996; Accepted 10 May 1996

gine triad of papain and Cathepsin B or the serine-histidine-aspartate catalytic triad of the serine proteases. Participation of the obligate ICE substrate aspartate residue in hydrolysis may also be critical in the enzyme mechanism proposed by Wilson et al. [1994], but conclusive evidence for such substrate assistance is not yet available. However, peptide substrates with alternate P_1 residues such as glutamate or asparagine are hydrolyzed very inefficiently by ICE[1] [Sleath et al., 1990; Howard et al., 1991].

The structure of ICE also provides a framework for understanding substrate selectivity by the enzyme deduced from kinetic studies of small peptide substrates [Sleath et al., 1990; Howard et al., 1991]. Two arginine residues are apposed in the S_1 pocket. These basic residues confer the very high selectivity of ICE for binding peptidyl substrates or inhibitors with aspartate in the P_1 position. Mutation of either of these arginine residues or of the cysteine or histidine catalytic residues in ICE eliminates enzyme activity [Wilson et al., 1994]. In contrast to the S_1 binding pocket of ICE, the S_2 and S_3 pockets are more solvent exposed and thereby tolerant of a wide variety of substrate or inhibitor residues [Thornberry et al., 1992; Mullican et al., 1994; Prasad et al., 1995] or mutations (D. Livingston, unpublished observation). The P_4 pocket includes a number of important substrate or inhibitor contacts, although ICE can accept both hydrophobic and charged S_4 substituents [Ator, 1994].

The discovery of ICE homologs in humans and rodents [Fernandes-Alnemri et al., 1994, 1995a,b; Wang et al., 1994; Faucheu et al., 1995] revealed that the active site catalytic machinery of ICE is completely conserved throughout the family of ICE homologs [Fernandes-Alnemri et al., 1995a]. Unlike the invariant arginine residues of the S_1 pocket, which dictate specificity for aspartate at P_1, other residues in the S_2, S_3, and S_4 binding pockets differ between ICE homologs and can account for differences in their substrate preferences. In vitro, there is redundancy among ICE homologs in processing certain apoptotic protein substrates [Fernandes-Alnemri et al., 1995b]. In similar fashion, small protease inhibitors may inhibit more than one ICE homolog in vitro or in vivo. In fact, few of the peptidyl ICE inhibitors reported to date are very selective for any particular homolog (see next section).

The conserved catalytic machinery of ICE and its homologs also suggests that these proteases use a common catalytic mechanism. Kinetic, structural, and mutagenesis studies demonstrate that the catalytic cysteine residue (Cys285 in ICE) thiolate is an activated nucleophile [Thornberry et al., 1992; Wilson et al., 1994]. Not surprisingly then, all of the ICE inhibitors reported to date incorporate an electrophilic center that is attacked by the Cys285 thiolate nucleophile. This nucleophilic attack by Cys285 can be reversible or irreversible. Reversible inhibition of ICE can be distinguished from irreversible inactivation by analysis of reaction progress curves by using fluorescent peptide aminomethylcoumarin substrates [Thornberry et al., 1992; Thornberry, 1994]. Other moieties of ICE inhibitors also affect the affinity or rate constant for inhibition or inactivation, respectively, for ICE. For example, truncation of the P_4 or P_3 residues of peptide substrates for ICE diminishes reactivity significantly [Sleath et al., 1990; Thornberry et al., 1992]. Similar effects of truncation have been reported for ICE inhibitors [Dolle et al., 1994b, 1995; Graybill et al., 1994; Mullican et al., 1994].

CLASSES OF ICE PROTEASE INHIBITORS

It is useful to summarize the activities of the classes reversible inhibitors and irreversible inactivators that have been designed to block activity of ICE and its homologs. Among the irreversible inactivators of other cysteine proteases are halomethyl ketones (compounds 1 and 2; Fig. 1), which alkylate the active site cysteine residue by attack of the sulfur atom on the methylene carbon of the inhibitor and concomitant elimination of the halogen atom. Rate constants (k_{inact}) for reaction of such inactivators with cysteine proteases such as papain can be fast (5×10^6 M^{-1} mZ s^{-1}). Halomethyl ketone inactivators have been studied in pharmacological models for inhibition of cysteine proteases and have been found to be toxic due to their

[1]The standard terminology for protease substrates and binding pockets is used in this article. The ICE binding pocket for the substrate amino acid residue N-terminal to the scissile peptide bond is referred to as the S_1 pocket. The corresponding substrate residue binding within this pocket is referred to as P_1, and the adjoining substrate residues on the N-terminal side (moving away from the scissile bond) are denoted P_2, P_3, etc. The substrate residues on the C-terminal side of the scissile peptide bond are termed P_1', P_2', etc. For an illustration of these pockets in the ICE active site, see Wilson et al. [1994].

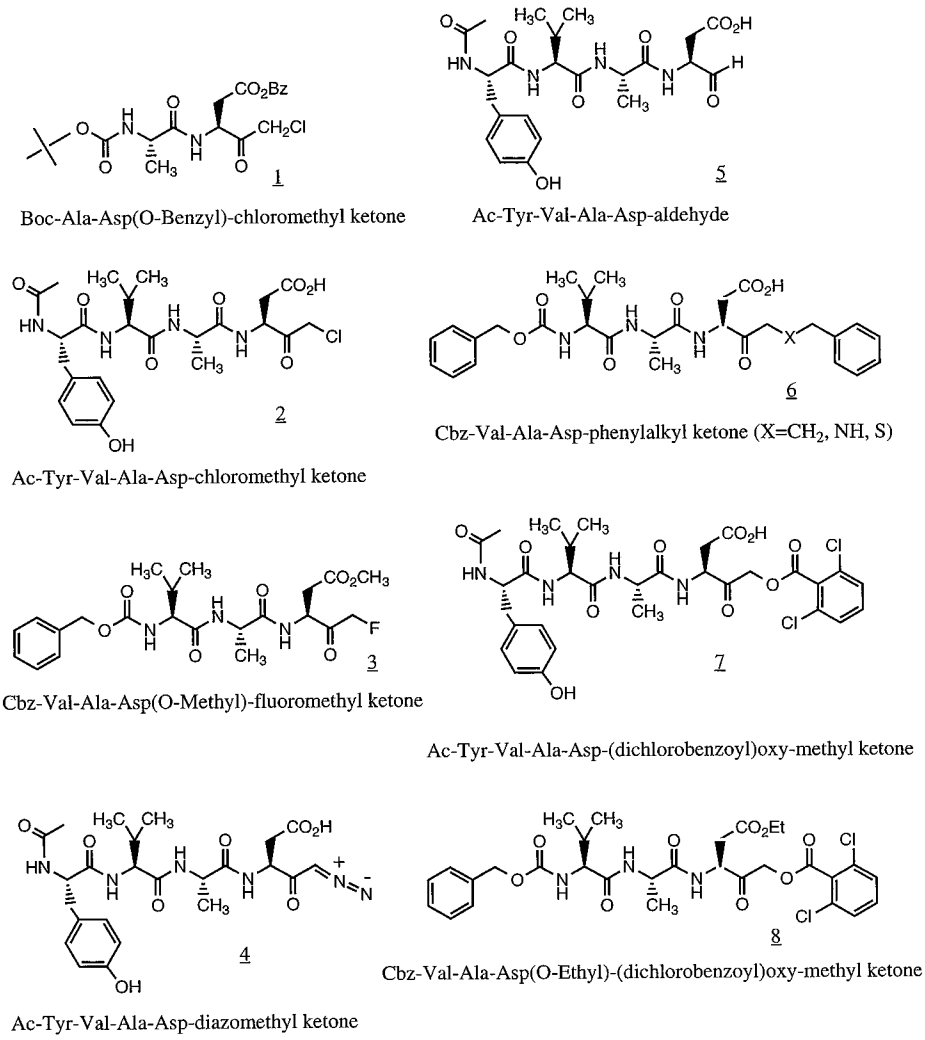

Fig. 1. Peptidyl ICE inhibitors.

nonselective reactions with a variety of serine- and thiol-containing enzymes [Shaw, 1990].

Halomethyl ketone inactivators of ICE, such as Boc-Asp(benzyl)-chloromethyl ketone (*1*), were first reported by Estrov et al. [1995] at Immunex. More specific halomethyl ketone ICE inhibitors are commercially available, including Ac-Tyr-Val-Ala-Asp-chloromethyl ketone (YVAD-CMK, 2, Fig. 1) and the P_1 methyl ester of Z-Val-Ala-Asp-fluoromethyl ketone (ZVAD-FMK, 3, Fig. 1). Although these compounds react with ICE and ICE homologs preferentially to other classes of cysteine or serine proteases, their selectivity among ICE homologs is only now being determined. We have observed, for example, that YVAD-CMK inactivates at least four members of the ICE family (S. Raybuck, unpublished data). In addition, these compounds are unstable in the presence of high concentrations of thiol reagents.

Diazomethyl ketone inactivators of ICE react in an equivalent manner to the halomethyl ketones but are somewhat more selective for cysteine vs. serine proteases [Shaw, 1990]. A diazomethyl ketone affinity reagent (*4*) was used by Thornberry et al. [1992] to identify the active site cysteine residue of ICE (Fig. 1), and the crystal structure of an ICE-adduct of such an inhibitor has been reported [Walker et al., 1994]. The selectivity for such inhibitors against ICE homologs vs. other classes of enzymes containing active site nucleophile residues is higher than that for the halomethyl ketones, although diazomethyl ketone peptidyl inhibitors inactivate ICE inefficiently ($k_{inact} = 1.6 \times 10^4\ M^{-1}$ mZ s^{-1}) [Thornberry et al., 1992]. In addition, diazo-

methyl ketone inhibitors of other cysteine proteases have been found to be embryotoxic [Ambroso and Harris, 1994].

A more stereochemically hindered and selective class of cysteine protease inactivator was first described by Smith et al. [1988] for use as inhibitors of Cathepsin B. The acyloxymethyl ketone inactivators provide a leaving group activated upon binding to the enzyme in the S_1' binding pocket. Such inactivators have been modified for use in design of ICE inhibitors (7, 8, Fig. 1) [Dolle et al., 1994a,b; Revesz et al., 1994; Thornberry et al., 1994]. The rate constant for ICE inactivation by these compounds can be as high as 2×10^6 M^{-1} mZ s^{-1}, and such compounds are quite selective for ICE vs. other classes of cysteine protease [Dolle et al., 1995]. A biotinylated form of a tetrapeptide ICE inactivator [Ac-Tyr-Val-Lys(biotin)-Asp-(acyloxy)-methyl ketone] was used for ultrastructural localization of active ICE in human monocytes to the cell membrane [Singer et al., 1995]; however, the reactivity of this compound with other ICE homologs may complicate interpretation of these data. We have observed similar peptidyl inhibitors to react with ICE homologs at rate constants of $>10^5$ M^{-1} mZ s^{-1} (S. Raybuck, unpublished data).

A reversible ICE inhibitor, Ac-Tyr-Val-Ala-Asp-aldehyde (YVAD-H, 5, Fig. 1), was used to demonstrate that ICE inhibition in human whole blood can block release of mature IL-1β from monocytes [Thornberry et al., 1992]. Peptide aldehydes bind reversibly to ICE, but because they have affinities for the enzyme approaching 100 pM, their off rates are slow [Thornberry et al., 1992]. N-methylation of the P_1 or P_3 amide nitrogens in YVAD-H results in complete loss of binding to ICE [Mullican et al., 1994].

YVAD-H is one of the most selective ICE inhibitors reported to date, with its affinity for the ICE homolog CPP32 being at least six orders of magnitude lower [Fernandes-Alnemri et al., 1995b]. A related reversible inhibitor of the ICE homolog CPP32, Ac-Asp-Glu-Val-Asp-aldehyde (DEVD-H), has been reported [Nicholson et al., 1995]. DEVD-H inhibits the ICE homolog CPP32 with a K_i of 0.2 nM; however, it is not a specific inhibitor of CPP32. Fernandes-Alnemri et al. [1995b] reported that this compound inhibits the homolog MCH-3 with a K_i of 1.8 nM. This compound also inhibits ICE and other homologs with nanomolar affinity (S. Raybuck, unpublished data). A crystal structure of DEVD-H bound to the ICE active site illustrates the capacity of ICE to form favorable electrostatic interactions with peptidyl substrates having charged acidic residues at P_3 and P_4 (K. Wilson, unpublished observations).

Another interesting class of reversible ICE inhibitors consists of the phenylalkyl ketones (6, Fig. 1) reported by Mjalli et al. [1993]. These inhibitors are capable of binding to the ICE active site reversibly with low nanomolar affinity, although they are not as potent as the corresponding aldehyde inhibitors. The high affinity for the active site of ICE by the phenylalkyl ketones suggests that interactions with the S′ pockets may be important for the observed inhibition properties. This group later reported more activated phenylalkyl ketones with β′-carbon replacements by heteroatoms and electron-withdrawing groups [Mjalli et al., 1994], which are capable of covalently inactivating the enzyme over very slow time scales, producing a covalent adduct that can be observed by X-ray crystallography (K. Wilson, unpublished observations).

CELLULAR STUDIES WITH ICE INHIBITORS

YVAD-H (5, Fig. 1) inhibits the release of mature IL-1β from human whole blood stimulated with heat-killed *S. aureus* with an IC_{50} of approximately 4 μM [Thornberry et al., 1992]. This compound also inhibits IL-1β release from human or mouse whole blood stimulated by endotoxin with an IC_{50} of 0.7 and 2.5 μM, respectively [Fletcher et al., 1995]. However, YVAD-H is a very poor inhibitor of Fas-induced apoptosis in thymocytes or monocytic and osteosarcoma cell lines [Fearnhead et al., 1995; Nicholson et al., 1995; Zhu et al., 1995]. Given the high relative selectivity of this compound for ICE vs. other homologs (S. Raybuck, unpublished data), these observations suggest that ICE alone is not required for the mediation of apoptosis.

Halomethyl ketone inactivators of ICE inhibit release of IL-1β from human peripheral blood leukocytes with an $IC_{50} < 2.5$ μM [Estrov et al., 1995]. These compounds are also capable of inhibiting mature IL-1β release from human myelogenous leukemia cells [Estrov et al., 1995]. Halomethyl ketone ICE inactivators are being widely used in cellular experiments that measure IL-1β release or apoptosis. Compounds such as Z-VAD-FMK (methyl P_1 aspartyl ester)

and YVAD-CMK have been shown to inhibit apoptosis in diverse cell types such thymocytes and THP.1 monocytic or Jurkat cell lines [Enari et al., 1995; Fearnhead et al., 1995; Zhu et al., 1995].

Acyloxymethyl ketone inactivators of ICE are more potent cellular inhibitors of IL-1β release than are halomethyl ketones. For example, the compound [ZVal-Ala-Asp-(dichlorobenzoyloxy)-methyl ketone] inhibits IL-1β release from monocytes with an IC_{50} of 2 μM [Ku et al., in press]. The P_1 ethyl aspartyl ester of this compound [Z-Val-Ala-Asp (O-ethyl)-(dichlorobenzoyloxy)-methyl ketone, 8, Fig. 1] inhibits IL-1β release with an IC_{50} of 0.5 μM [Ku et al., in press]. Unlike the aldehyde ICE inhibitors, inactivators such as [Z-Val-Ala-Asp(O-ethyl)-(dichlorobenzoyloxy)-methyl ketone] are capable of inhibiting apoptosis, with an IC_{50} of ca. 1 μM (M. Harding, unpublished data).

The potency of these compounds in apoptosis inhibition probably arises from their potential to inactivate multiple ICE homologs. It is unlikely that any single ICE homolog [Nicholson et al., 1995] is required for the mediation of apoptosis in all cell types [Kumar and Harvey, 1995]. Instead, multiple ICE homologs are activated during apoptosis, possibly different homologs in different tissues, and their proteolytic functions during apoptosis may be redundant and/or part of a proteolytic cascade [Fernandes-Alnemri et al., 1995a,b; Kumar and Harvey, 1995]. The irreversible inhibitors described to date are unlikely to be useful tools for assigning cellular antiapoptotic effects to any specific ICE homolog, unless supporting data can be generated that demonstrate expression and activation of a restricted subset of ICE homologs in the cell type under investigation.

An unexpected property of certain ICE inhibitors is their suppression of IL-1α release from the cell. The compound [Z-Val-Ala-Asp-(dichlorobenzoyloxy)-methyl ketone] inhibits IL-1a release from human adherent monocytes with an IC_{50} of 10 μM [Ku et al., in press]. The P_1 ethyl aspartyl ester of this compound [Z-Val-Ala-Asp(O-Ethyl)-(dichlorobenzoyloxy)-methyl ketone] inhibits IL-1α release from these cells with an IC_{50} of 0.3 μM [Ku et al., in press]. A similar suppression of IL-1α release is observed in splenocytes derived from transgenic mice with an ICE gene disruption [Kuida et al., 1995; Li et al., 1995]. The mechanism of this suppression of IL-1α release is not understood. The mechanism may be due to a feedback effect on inhibition of IL-1β release. Alternatively, ICE or associated proteins may constitute part of the export apparatus for IL-1α. It is noteworthy that inhibition of IL-1a release is not a property of all ICE inhibitors. For example, YVAD-H (*5*) does not inhibit release of IL-1a at concentrations of up to 100 μM, two orders of magnitude over the IC_{50} for IL-1β release inhibition (M. Harding, unpublished data). The differential effects of different ICE inhibitors on IL-1α release is a subject of current investigation.

PHARMACOLOGICAL STUDIES WITH ICE INHIBITORS

Very limited data have been reported on in vivo studies with ICE inhibitors. We have used acyloxymethyl ketone inactivators of ICE in rodent models of sepsis, acute inflammation, and chronic arthritis to explore therapeutic uses of ICE inhibitors. When the acyloxymethyl ketone ICE inactivator Z-Val-Ala-Asp(O-ethyl)-(dichlorobenzoyloxy)-methyl ketone (*8*) is administered intraperitoneally 1 h after mice are challenged with LPS, a suppression of serum IL-1β induction is observed at 7 h after LPS challenge [Ku et al., in press]. Intraperitoneal injection of Z-Val-Ala-Asp-(dichlorobenzoyloxy)-methyl ketone in mice can also suppress zymosan-induced production of mature IL-1β in a subcutaneously implanted chamber [Miller et al., 1995].

Fletcher et al. [1995] reported similar data by using YVAD-H (*5*). Mice were presensitized with an injection of *Pseudomonas acnes* prior to stimulation of IL-1β release with LPS. YVAD-H was injected intraperitoneally 90 min after LPS challenge, and IL-1β levels were measured 90 min later. The ED_{50} for YVAD-H was reported to be 2 mg/kg. ICE-deficient transgenic animals are also resistant to sepsis induced by high-dose LPS, due to suppression of secreted IL-1β and IL-1α [Li et al., 1995].

We have administered the ICE inactivator Z-Val-Ala-Asp(O-ethyl)-(dichlorobenzoyloxy)-methyl ketone intraperitoneally in a type II collagen-induced chronic arthritis murine model developed by Geiger et al. [1993]. In this model, anti-IL-1β antibodies are capable of inhibiting the inflammatory swelling of the paws [Geiger et al., 1993]. We observed similar effects with the ICE inactivator administered at 50 mg/kg once per day over a 14–28-day period [Ku et al.,

in press]. This compound was capable of alleviating inflammation when administered either in a prophylactic mode (concomitant with collagen boost) or in a therapeutic mode (10 days after collagen boost). In addition, the compound was able to inhibit joint destruction as measured by protection of proteolglycan degradation by using histopathological scoring [Ku et al., in press]. Recent studies with ICE-deficient transgenic mice are also resistant in the collagen-induced arthritis model (M. Harding, unpublished data).

FUTURE PROSPECTS

The vast literature on the chemistry and biochemistry of protease inhibitors belies the fact that very few proteases have been successfully targeted for human therapy. The majority of protease inhibitors reported in the literature are too unselective or metabolically unstable to be used effectively for human therapy. Protease inhibitors are currently in use for clinical treatment of high blood pressure (angiotensin-converting enzyme) and for HIV infection (HIV aspartyl protease). Other therapeutic protease targets such as the cysteine proteases calpain and Cathepsins B, L, and S have been the subject of intensive medicinal chemistry efforts, but widely used drugs have not emerged from the clinic.

The medicinal chemistry challenges are particular demanding for delivery of protease inhibitors to intracellular targets, where high enzyme affinity and selectivity do not guarantee that such compounds will efficiently penetrate the plasma membrane or localize to the desired subcellular compartment. An example of such an inhibitor is DEVD-H, which despite its low nM K_i values against multiple ICE homologs, is inactive in blocking apoptosis at concentrations below 100 μM. A safe and effective therapeutic delivery of a protease inhibitor at correspondingly high blood concentration is unlikely. Furthermore, for treatment of neurodegenerative diseases such as Alzheimer's disease, blood–brain barrier penetration is necessary and will impose another significant hurdle to successful drug design.

The ICE inhibitors reported to date are peptidyl compounds with short circulatory half-lives and poor selectivity vs. other ICE homologs. For chronic clinical applications, ICE inhibitors must be designed with higher selectivity, metabolic stability, and oral bioavailability. Initial clinical applications may involve diseases where short courses of drug administration might offer beneficial antiinflammatory effects. If stability to oral administration can be combined with high cellular potency, improved compounds would be useful in chronic disease settings. In the interim, we can look forward to reports on the effects of ICE inhibitors in different animal models of inflammation and neurodegenerative disease.

ACKNOWLEDGMENT

I thank my colleagues, Dr. Matthew Harding, Dr. Scott Raybuck, and Dr. Keith Wilson, on the ICE project at Vertex Pharmaceuticals for their suggestions on the manuscript and for allowing me to cite their unpublished data.

REFERENCES

Ambro JL, Harris C (1994): In vitro embryotoxicity of the cysteine proteinase inhibitors benzyloxycarbonyl-phenylalanine-alanine-diazomethane (Z-Phe-Ala-CHN_2) and benzyl oxycarbonyl-phenylalanine-phenylalanine-diazomethane (Z-Phe-Phe-CHN_2). Teratology 50:214–228.

Ator M (1994): Peptide and non-peptide inhibitors of interleukin-1β converting enzyme. Paper presented at Inflammatory Cytokine Antagonists—Targets, Strategies and Indications. Cambridge Healthtech Institute (Waltham, MA), Philadelphia, October.

Bamba T, Yoshioka U, Inoue H, Iwasaki Y, Hosoda S (1994): Serum levels of interleukin-1β and interleukin-6 in patients with chronic pancreatitis. J Gastroenterol 29:314–319.

Bataille R, Chappard D, Klein B (1992): The critical role of interleukin-6, interleukin-1β and macrophage colony-stimulating factor in the pathogenesis of bone lesions in multiple myeloma. Int J Clin Lab Res 21:283–287.

Casellas F, Papo M, Guarner F, Antolin M, Segura RM, Armengol JR, Malagelda J-R (1995): Intracolonic release in vivo of interleukin-1β in chronic ulcerative colitis. Clin Sci 89:521–526.

Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van NK, Greenstreet TA, March CJ, Kronheim SR, Druck T, Cannizzaro LA, Huebner K, Black RA (1992): Molecular cloning of the interleukin-1β converting enzyme. Science 256:97–100.

Dinarello CA, Wolff SM (1993): The role of interleukin-1 in disease. N Engl J Med 328:106–113.

Dolle RE, Hoyer D, Prasad CVC, Schmidt SJ, Helaszek CT, Miller RE, Ator MA (1994a): Aspartate-based peptide α-((2,6-dichlorobenzoyl)oxy)methyl ketones as potent time-dependent inhibitors of interleukin-1β converting enzyme. J Med Chem 37:563–564.

Dolle RE, Singh J, Rinker J, Hoyer D, Prasad CVC, Graybill TL, Salvino JM, Helaszek CT, Miller RE, Ator MA (1994b): Aspartyl α((1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl)oxy)methyl ketones as interleukin-1β converting enzyme inhibitors. Significance of the P_1 and P_3 amido nitrogens for enzyme-peptide inhibitor binding. J Med Chem 37:3863–3865.

Dolle RE, Singh J, Whipple D, Osifo IK, Speier G, Graybill TL, Gregory JS, Harris AL, Helaszek CT, Miller RE, Ator MA (1995): Aspartyl α((diphenylphosphinyl)-xy)methyl ketones as novel inhibitors of interleukin-1β converting enzyme: Utility of the diphenylphosphinic acid leaving group for the inhibition of cysteine proteases. J Med Chem 38:220–222.

Enari M, Hug H, Nagata S (1995): Involvement of an ICE-like protease in Fas-mediated apoptosis. Nature 375: 78–80.

Estrov Z, Black RA, Sleath PR, Harris D, Van Q, LaPushin R, Estey EH, Talpaz M (1995): Effect of interluekin-1β converting enzyme inhibitor on acute myelogenous leukemia progenitor proliferation. Blood 86:4594–4602.

Faucheu C, Diu A, Chan AWE, Blanchet A-M, Miossec C, Herve F, Collard-Dutilleul V, Gu Y, Aldape R, Lippke J, Rocher C, Su MS-S, Livingston DJ, Hercend T, Lalanne J-L (1995): A novel human protease similar to the interleukin-1β converting enzyme induces apoptosis in transfected cells. EMBO J 9:914–1922.

Fearnhead HO, Dinsdale D, Cohen GM (1995): An interleukin-1β-converting enzyme-like protease is a common mediator of apoptosis in thymocytes. FEBS Lett 375:283–288.

Fernandes-Alnemri T, Litwack G, Alnemri ES (1994): CPP32, a novel human apopototic protein with homology to *Caenorhabditis elegans* cell death protein Ced-3 and mammalian interleukin-1 converting enzyme. J Biol Chem 269:30761–30764.

Fernandes-Alnemri T, Litwack G, Alnemri ES (1995a): Mch2, a new member of the apoptotic Ced-3/Ice cysteine protease family. Cancer Res 55:2737–2742.

Fernandes-Alnemri T, Takahashi A, Armstrong R, Krebs J, Fritz L, Tomaselli KJ, Wang L, Yu Z, Croce CM, Salveson G, Earnshaw WC, Litwack G, Alnemri ES (1995b): Mch3, a novel apoptotic cysteine protease highly related to CPP32. Cancer Res 55:6045–6052.

Fisher CJJ, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, Iberti TH, Rackow EC, Shapiro MJ, Greenman RL (1994): Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double blind, placebo-controlled trial. JAMA 271:1836–1843.

Fletcher DS, Agarwal L, Chapman KT, Chin J, Egger LA, Limjuco G, Luell S, Macintyre DE, Peterson EP, Thornberry NA, Kostura MJ (1995): A synthetic inhibitor of interleukin-1β-converting enzyme prevents endotoxin-induced interleukin-1β production in vitro and in vivo. J Interferon Cytokine Res 15:243–248.

Gagliardini V, Fernandez PA, Lee RK, Drexier HC, Rotello RJ, Fishman MC, Yuan J (1994): Prevention of vertebrate neuronal death by the *crmA* gene. Science 263:826–828.

Geiger T, Towbin H, Cosenti-Vargas A, Zingle O, Arnold J, Rordorf C, Glatt M, Vosbeck K (1993): Neutralization of interleukin-1β activity in vivo with a monoclonal antibody alleviates collagen-induced arthritis in DBA/1 mice and prevents the associated acute-phase response. Clin Exp Rheum 11:515–522.

Graybill TL, Dolle RE, Helaszek CT, Miller RE, Ator MA (1994): Preparation and evaluation of peptidic aspartyl hemiacetals as reversible inhibitors of interleukin-1 beta converting enzyme (ICE). Int J Pept Protein Res: 44: 173–82.

Goldring M (1992): Degradation of articular cartilage in culture: Regulatory factors. In Woessner JF Jr, Howell DS (eds): "Joint Cartilage Degradation: Basic and Clinical Aspects." New York: Marcel Dekker, pp 281–345.

Holden RJ, Mooney PA (1995): Interleukin-1β: A common cause of Alzheimer's disease and diabetes mellitus. Med Hypoth 45:559–571.

Howard AD, Kostura MJ, Thornberry NA, Ding, GJ, Limjuco G, Weidner J, Salley JP, Hogguist KA, Chaplin DD, Mumford RA, Schmidt JA, Tocci MJ (1991): IL-1 converting enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31-kDa IL-1a. J Immunol 147:2964–2969.

Ku G, Faust T, Lauffer LL, Livingston DJ, Harding MW (1996): Irreversible inhibitors of interleukin-1β converting enzyme block IL-1β and IL-1α release and progression of type II collagen-induced arthritis in mice. Cytokine 8:377–386.

Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS-S, Flavell RA (1995): Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. Science 267:2000–2003.

Kumar S, Harvey NL (1995): Role of multiple cellular proteases in the execution of programmed cell death. FEBS Lett 375:169–173.

Lebsack ME, Paul CC, Martindale JJ, Catalano MA (1993): A dose- and regimen-ranging study of IL-1 receptor antagonist in patients with rheumatoid arthritis. Arthrit Rhemat 36:S39.

Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J, Towne E, Tracey D, Wardell S, Wei F-Y, Wong W, Kamen R, Seshadri T (1995): Mice deficient in interleukin-1β converting enzyme are defective in production of mature IL-1β and resistant to endotoxic shock. Cell 80:401–411.

Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D (1989): Role of interleukin 1 in inflammatory bowel disease—Enhanced production during active disease. Gut 31:686–689.

Miller BE, Krasney PA, Gauvin DM, Holbrook KB, Koonz DJ, Abruzzese RV, Miller RE, Pagani KA, Dolle RE, Ator MA, Gilman SC (1995): Inhibition of mature IL-1β production in murine macrophages and a murine model of inflammation by WIN 67694, an inhibitor of IL-1β converting enzyme. J Immunol 154:1331–1338.

Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J (1993): Induction of apoptosis in fibroblasts by IL-1β-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. Cell 75:653–660.

Mjalli AMM, Chapman KT, MacCoss M, Thornberry NA (1993): Phenylalkyl ketones as potent reversible inhibitors of interleukin-1β converting enzyme. Bioorg Med Chem Lett 3:2639–2692.

Mjalli AMM, Chapman KT, MacCoss M, Thornberry NA, Peterson EP (1994): Activated ketones as potent reversible inhibitors of interleukin-1β converting enzyme. Bioorg Med Chem Lett 4:1965–1968.

Mullican MD, Lauffer DJ, Gillespie RJ, Matharu SS, Kay D, Porritt GM, Evans PL, Golec JMC, Murcko MA, Loung Y-P, Raybuck SA, Livingston DJ (1994): The synthesis and evaluation of peptidyl aspartyl aldehydes as inhibitors of ICE. Bioorg Med Chem Lett 4:2359–2364.

Nicholson, DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle M, Lazebnik YA, Munday NA, Raju SM, Smulson ME, Yamin T-T, Yu VL, Miller DK (1995): Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 376:37–43.

Norman JG, Franz MG, Fink GS, Messina J, Fabri PJ, Gower WR, Carrey LC (1995): Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg 221:625–634.

Prasad CVC, Prouty CP, Hoyer D, Ross TM, Salvino JM, Awad M, Graybill TL, Schmidt SJ, Osifo IK, Dolle RE, Helaszek CT, Miller RE, Ator MA (1995): Structural and stereochemical requirements of the time-dependent inactivators of the interleukin-1β converting enzyme. Bioorg Med Chem Lett 5:315–318.

Revesz L, Briswalter C, Heng R, Leutwiler A, Mueller R, Wuethrich H-J (1994): Synthesis of P1 aspartate-based peptide acyloxymethyl and fluoromethyl ketones as inhibitors of interleukin-1β converting enzyme. Tet Lett 35:9693–9696.

Shaw E (1990): Cysteinyl proteinases and their selective inactivation. Adv Enz 63:271–347.

Singer II, Scott S, Chin J, Bayne EK, Limjuco G, Weidner J, Miller DK, Chapman K, Kostura MJ (1995): The interleukin-1 beta-converting enzyme (ICE) is localized on the external cell surface membranes and in the cytoplasmic ground substance of human monocytes by immunoelectron microscopy. J Exp Med 182:1447-1459.

Sleath PR, Hendrickson RC, Kronheim SR, March CJ, Black RA (1990): Substrate specificity of the protease that processes human interleukin-1β. J Biol Chem 265: 14526–14528.

Smith RA, Copp LJ, Coles PJ, Pauls HW, Robinson VJ, Spencer RW, Heard SB, Krantz A (1988): New inhibitors of cysteine proteinases. Peptidyl acyloxymethyl ketones and the quiescent nucleofuge strategy. J Am Chem Soc 110:4429–4431.

Thornberry NA (1994): Interleukin-1β converting enzyme. Methods Enz 244:615–631.

Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J, Elliston KO, Ayala JM, Casano FJ, Chin J, Ding GJ-F, Egger LA, Gaffney EP, Limjuco G, Palyha OC, Faju SM, Rolando AM, Salley JP, Yamin T-T, Lee TD, Shively JE, MacCross M, Mumford RA, Schmidt JA, Tocci MJ (1992): A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. Nature 356:768–774.

Thornberry NA, Peterson EP, Zhao JJ, Howard AD, Griffin PR, Chapman KT (1994): Inactivation of interleukin-1β converting enzyme by peptide (acyloxy)methyl ketones. Biochemistry 33:3934–3940.

van de Loo FAJ, Joosten LAB, van Lent PLEM, Arntz OJ, van den Berg WB (1995): Role of interleukin-1, tumor necrosis factor α, and interleukin-6 in cartilage proteoglycan metabolism and destruction. Arthrit Rheum 38:164–172.

Walker NPC, Talanian RV, Brady KD, Dang LC, Bump NJ, Ferenz CR, Franklin S, Ghayur T, Hackett MC, Hammill LD, Herzog L, Hugunin M, Houy W, Mankovich JA, McGuiness L, Orlewicz E, Paskind M, Pratt CA, Reis P, Summani A, Terranova M, Welch JP, Xiong L, Moller A, Tracey DE, Kamen R, Wong WW (1994): Crystal structure of the cysteine protease interleukin-1β converting enzyme: A $(p20/p10)_2$ homodimer. Cell 78:343–352.

Wang L, Miura M, Bergeron L, Zhu H, Yuan J (1994): Ich-1, an Ice/Ced-3 related gene, encodes both positive and negative regulators of programmed cell death. Cell 78: 739–750.

Wilson KP, Black JF, Thomson JA, Kim EE, Griffith JP, Navia MA, Murcko MA, Chambers SP, Aldape RA, Raybuck SA, Livingston DJ (1994): Structure and mechanism of interleukin-1β converting enzyme. Nature 370: 270–253.

Zhu H, Fearnhead HO, Cohen GM (1995): An ICE-like protease is a common mediator of apoptosis induced by diverse stimuli in human monocytic THP.1 cells. FEBS Lett 374:303–308.