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Cathepsin B in Neurodegeneration of Alzheimer's Disease, Traumatic Brain Injury, and Related Brain Disorders

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Abstract

Investigations of Alzheimer's disease (AD), traumatic brain injury (TBI), and related brain disorders have provided extensive evidence for involvement of cathepsin B, a lysosomal cysteine protease, in mediating the behavioral deficits and neuropathology of these neurodegenerative diseases. This review integrates findings of cathepsin B regulation in clinical biomarker studies, animal model genetic and inhibitor evaluations, structural studies, and lysosomal cell biological mechanisms in AD, TBI, and related brain disorders. The results together indicate the role of cathepsin B in the behavioral deficits and neuropathology of these disorders. Lysosomal leakage occurs in AD and TBI, and related neurodegeneration, which leads to the hypothesis that cathepsin B is redistributed from the lysosome to the cytosol where it initiates cell death and inflammation processes associated with neurodegeneration. These results together implicate cathepsin B as a major contributor to these neuropathological changes and behavioral deficits. These findings support the investigation of cathepsin B as a potential drug target for therapeutic discovery and treatment of AD, TBI, and TBI-related brain disorders.

Keywords

cathepsin B; Alzheimer's disease (AD); traumatic brain injury (TBI); neurodegeneration; brain; memory; cognition; behaviors; pathology; human brain; biomarker; gene knockout; inhibitors; active site binding; lysosomal leakage

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Declaration of interests

The majority of authors (MC, CM, GI, SP, BH, RR, AO) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Neurodegeneration of Alzheimer's disease (AD), traumatic brain injury (TBI), and related dementia (ADRD) disorders displays behavioral deficits in memory, cognition, problem solving, executive function, language, emotion, and related brain functions (1–15). Severe cognitive decline, dementia, occurs in ADRD disorders of Alzheimer's disease (1–3), traumatic brain injury (TBI) (4–6), brain ischemia and stroke (7–9), and related neurodegenerative brain disorders (10–15). These disorders are widespread affecting more than 47 million people worldwide, with high prevalence in the aged population.

There have been tremendous efforts in the field of drug discovery for AD, TBI, and numerous related ADRD disorders, but decades of drug development research have not yet yielded effective therapeutics to attenuate or reverse these neurodegenerative diseases. Advancements in dementia therapeutics strategies will necessitate elucidation of novel molecular mechanisms in ADRD which can be modulated through drug intervention to ameliorate the behavioral dysfunctions and neuropathology of ADRD disorders. Investigation of novel targets of ADRD will advance opportunities for therapeutics development to extend prior efforts for targeting neuropathological amyloid-β (Aβ) (16–20), phospho-tau (21–23), and related mechanisms (24–27).

Cathepsin B, a lysosomal cysteine protease, represents a candidate drug target for AD, TBI, and related brain disorders. Cathepsin B has been shown by numerous studies to participate in AD and TBI cognitive and behavioral deficits, and in neuropathology. This review highlights the multi-disciplinary research of cathepsin B in human clinical studies, and in AD, TBI, and related animal models using cathepsin B gene knockout and chemical inhibition. Findings from these studies support the hypothesis that lysosomal leakage of cathepsin B to the cytosol leads to neurodegeneration and behavioral deficits in AD, TBI, and related brain disorders. These results provide the rationale for investigation of cathepsin B as a mechanistic drug target for therapeutics discovery and development for AD, TBI, and related dementia.

2. Cathepsin B elevation in human clinical AD, TBI, and related neurodegenerative disorders.

Several studies have shown increased levels of cathepsin B protein or activity in plasma, CSF, and amyloid plaques in human AD, TBI, and related neurodegenerative diseases (Table 1) (28–39). Importantly, increased plasma cathepsin B correlates with cognitive dysfunction in AD (29) and severe trauma outcomes (36).

2.1. Human AD in aging and cathepsin B regulation.

In AD patients, cathepsin B levels are elevated in serum compared to age-matched healthy controls (28–30) (Figure 1). Notably, the elevated cathepsin B correlated with MMSE (Mini-Mental State Exam) scores of dementia, cognitive impairment, in AD patients (29) (Figure 1). Elevated plasma cathepsin B occurs in both mild and severe AD compared to healthy controls. In patients with mild cognitive impairment (MCI), plasma cathepsin B levels were

similar to controls (30). These findings show that increases in plasma cathepsin B correlate with AD cognitive status, and occur in mild and severe stages of AD.

Notably, cathepsin B in human AD brain displays abnormal extracellular localization with amyloid plaque pathology (31). Extracellular cathepsin B contrasts with its intracellular location in lysosomes. These findings demonstrate re-distribution of lysosomal cathepsin B to abnormal extracellular locations in AD brain. The extracellular location suggests that cathepsin B levels in CSF may become elevated. Indeed, CSF analyses have demonstrated higher cathepsin B levels in AD patients compared to controls (32)

AD is an age-related neurodegenerative disease occurring at elderly ages. Aging is a main risk factor for AD and numerous neurodegenerative diseases (33, 34). Age-related increases in cathepsin B levels in CSF occur in elderly patients (35).

2.2. TBI and related neurodegenerative diseases exhibit cathepsin B regulation.

In numerous studies of clinical TBI and related conditions, cathepsin B is significantly elevated in plasma and CSF. Severe trauma patients show elevated cathepsin B in plasma associated with organ failure, compared to patients without complications (36, 37). In TBI patients, cathepsin B protein and activity in CSF are elevated compared to controls (38). Furthermore, cathepsin B activity in CSF is elevated in the inflammatory neurological diseases of Guillain-Barre syndrome (GBS), inflammatory demyelinating polyneuropathy (CIDP), and multiple sclerosis (MS) (39). These findings demonstrate elevated cathepsin B in TBI injury and related neurological diseases.

3. Human brain gene expression of cathepsin B at young to adult ages

Analyses of cathepsin B expression in human brain illustrates its abundant expression in hippocampal and cortical regions which are responsible for cognitive and behavioral functions (40). Cathepsin B mRNA expression is illustrated as RNAseq data overlaid on a human brain MRI image (from the Allen human brain atlas, https://human.brain-map.org/) (41, 42) in Figure 2a.

Cathepsin B is one of the most abundant lysosomal cathepsin proteases expressed in human brain compared to the 15 member lysosomal cysteine protease family, consisting of cathepsins B, C, F, H, K, L, O, S, V, W, Z, D, E, A, and G (43) (Figure 2b). Cathepsin B expression levels represents 12–32% of total cathepsin expression composed of 15 cathepsin proteases, in 16 human brain regions (40). The abundant expression of cathepsin B occurs throughout human brain development from infancy to childhood, adolescence, and adult life. These findings indicate the presence of cathepsin B in brain at all ages for normal lysosomal functions in health and in neurological diseases of brain injuries, neurodegeneration, and related dementia and behavioral disorders.

4. Cathepsin B participates in behavioral deficits and neuropathology in animal models of AD, TBI, and related brain disorders.

Numerous investigations of animal models of AD, TBI, and related dementia disorders demonstrate that cathepsin B is elevated during brain dysfunction and pathogenesis (29, 44–74). Furthermore, gene knockout of cathepsin B in these animal models of brain disorders results in amelioration of behavioral deficits and reduction in neuropathology (75–86, 88–91). Compelling evidence from numerous studies demonstrate that chemical inhibition of cathepsin B improves behavioral dysfunctions and neuropathology in animal models of AD, TBI, and related brain disorders (92–114). These findings are discussed in this section.

4.1. Cathepsin B elevation in animal models of AD, TBI, and related brain disorders.

AD models.—In AD animal models, cathepsin B gene expression is elevated in the 5X familial (FAD) AD mouse model which expresses three APP mutations (APP KM670/671NL, Swedish; APP (716V, Florida; APP V171I, London) and human presenilin 1 (PS1) containing two mutations (PSEN1 M146L; PSEN1 L286V (44) (Table 2). Cathepsin B protein levels are increased by 50% in the cortex and hippocampus brain regions of the APPSwe/PS1 mouse model, which expresses human APP with the Swedish (Swe) FAD mutation and PS1 with FAD mutations, relative to controls (29) (Table 2).

TBI models.—In TBI animal models, cathepsin B is significantly elevated in brains subjected to the controlled cortical impact (CCI) TBI mouse model (45), and in the penetrating ballistic-like brain injury (PBBI) TBI model (38). TBI related conditions of trauma (45–50), spinal cord contusion (SCC) (51, 52), surgery (53), hemorrhage (54, 55), and brain aneurysm (56) result in increased levels of brain cathepsin B.

The time-course of cathepsin B elevation occurs soon after the injury event and the increase is sustained during the course of the injury. In the CCI mouse model of TBI, cathepsin B is elevated at 2 hours and 24 hours post-injury (45). In the severe penetrating ballistic-like brain injury (PBBI) in rats, cathepsin B enzyme levels and activity are increased in the cortex and hippocampus during 3–7 days after PBBI (38). In the weight drop mouse model of brain injury, brain cathepsin B is elevated as early as 6 hours after injury, and peaks at 2 days post-trauma, with sustained elevation for up to one week (57, 58). In a model of TBI in rat, cathepsin B is elevated in the brain within one hour after injury and remains elevated for more than one month; the maximum 3-fold increase of cathepsin B occurs at 8 days post-injury (48). These models of moderate to severe TBI together demonstrate that increased cathepsin B occurs shortly after brain injury and is sustained for weeks to months post-TBI.

ALS, epilepsy, and related neurodegeneration.—In ALS (amyotrophic lateral sclerosis) neurodegeneration, cathepsin B expression is elevated by 3-fold in spinal cord in ALS mouse models compared to controls (59, 60). Cathepsin B is also up-regulated in ALS patients (61). In animal models of epilepsy, which is associated with TBI, cathepsin B protein levels are elevated (62). Furthermore, in excitatory Huntington’s disease, cathepsin B is elevated (63).

Ischemia and inflammatory injury models.—Ischemia and inflammatory brain injuries result in cognitive deficits and display elevated cathepsin B. Ischemia results in increased cathepsin B in the brain, demonstrated in acute and chronic ischemia in rat and monkey animal models (64–67). Meningitis (68), sepsis (69, 70), inflammation (71–73), and inflammatory pain (74) also result in increased cathepsin B.

4.2. Cathepsin B gene knockout improves behavioral deficits and ameliorates neuropathology in animal models of AD, TBI, and related disorders.

Studies of cathepsin B gene knockout in animal models of AD, TBI, infection, and aging in cognitive deficits show that the absence of cathepsin B preserves normal behaviors and ameliorates brain neuropathology and associated biomarkers (summarized in Figure 3).

AD APP/Lon model.—The APP/Lon mouse model of AD expresses the human APP gene with the V717I London mutation, resulting in memory deficits and amyloid plaque brain neuropathology (75). Knockout of the cathepsin B gene in APP/Lon mice results in preservation of memory function (measured by the Morris water maze behavioral assay) to nearly normal function (75) (Figure 3a). Deletion of the cathepsin B gene results in reduction of amyloid plaque load, concomitant with decreases in Aβ(1–40) and Aβ(1–42), and reduced pGlu-Aβ(3–40) and pGlu-Aβ(3–42), in brains of the APP/Lon mice (75–77). The N-truncated and pGlu-modified pGlu-Aβ(3–40) and pGlu-Aβ(3–42) peptides participate in seeding neurotoxic oligomers of Aβ peptides (78, 79). These findings support a role for cathepsin B in memory deficits and amyloid plaque brain pathology in the APP/Lon mouse model of AD.

CCI-TBI model.—TBI results in cognitive deficits (80, 81) and TBI increases the risk for development of AD (26, 82–84) (Figure 3b). Using the CCI (controlled cortical impact) mouse model of TBI, deletion of the cathepsin B gene results in reduced motor dysfunction after CCI compared to sham controls. Notably, the absence of the cathepsin B gene results in recovery to normal function by 7 days post-CCI, compared to the motor deficits displayed by shams (45). Brain tissue lesions resulting from CCI are ameliorated in the cathepsin B knockout mice (45). Cathepsin B knockout blocks the CCI-induced elevation in the pro-apoptotic cell death Bax protein (45). These data provide evidence for participation of cathepsin B in the behavioral dysfunction and brain lesions in the CCI mouse model of TBI.

***Porphyromonas gingivalis* (PgLPS) model.—**Several studies reveal a strong association of peripheral infection by periodontitis with accelerated cognitive decline of AD (85, 86). In a mouse model of periodontitis-induced cognitive dysfunction, middle-aged mice subjected to chronic treatment with lipopolysaccharide from *Porphyromonas gingivalis* (PgLPS) display cathepsin B-dependent learning and memory deficits with Aβ accumulation (72). Cathepsin gene knockout results in improvement of the PgLPS-induced memory deficit, reduction of Aβ42 levels in brain, and reduction of elevated pro-inflammatory IL-1β, TLR2, and TLR4 factors (72) (Figure 3c). These findings demonstrate that cathepsin B participates in PgLPS-induced AD-like cognitive deficits involving elevation of Aβ42 and inflammation.

In addition, recent studies show that the *Porphyromonas gingivalis (P. gingivalis)* gingipain cysteine proteases are present in brains of AD patients, and *P. gingivalis* infection of mice result in neuronal loss in brain combined with increased Aβ42 and TNFα inflammatory factor (87). Thus, cathepsin B-mediated neurodegeneration in *P. gingivalis*-related AD may occur in the presence of bacterial gingipain cysteine proteases.

Aging model.—Advanced age is a major risk factor for dementia of AD and related neurodegenerative diseases (34, 88). Aged mice develop cognitive deficits associated with elevated cathepsin B, neuroinflammation, and ROS (reactive oxygen species) (73). Notably, cathepsin B gene knockout ameliorated cognitive dysfunction in the aged mice. Deletion of cathepsin B also resulted in amelioration of age-dependent elevations in oxidative stress and inflammation (IL-1β and TNFα) (73) (Figure 3d). These findings demonstrate a role for cathepsin B in age-dependent cognitive decline.

4.3. Cathepsin B knockout mice are healthy.

The Cathepsin B knockout mice have been found to be healthy and are indistinguishable from normal animals in behavior, histology, and fertility (89, 90). While there is some decrease in thyroglobulin solubilization, there is no apparent behavioral effect (91). The normal health of mice in the absence of the cathepsin B gene suggests that pharmacological inhibition of cathepsin B will generally be safe.

4.4. Chemical inhibition of cathepsin B alleviates behavioral deficits and reduces neuropathology in AD, TBI, and related animal models

The effectiveness of cathepsin B gene knockout to ameliorate behavioral deficits and neuropathology predicts that chemical inhibitors of cathepsin B can improve deficits of AD, TBI, and related conditions. Indeed, numerous studies show that cathepsin B inhibitors improve cognitive dysfunctions and reduce neuropathology of AD, TBI, and TBI-related animal models (summarized in Table 3).

AD model.—In an AD mouse model, APP/Lon mice, administration of E64d (oral) results in substantial improvement in memory deficits (92, 93), amelioration of amyloid plaque pathology, and reduction of brain levels of Aβ(1–40) and Aβ(1–42) as well as the truncated pGlu-Aβ(3–40) (pGlu, pyroglutamate) and pGlu-Aβ(3–42) peptides (92–94). E64d is a pro-drug which is converted *in vivo* by esterases to the active E64c inhibitor which potently inhibits cathepsin B (95, 96), as well as other cysteine cathepsin proteases (95, 97). It will be desirable to evaluate a selective inhibitor of cathepsin B to assess the role of this protease in the APP/Lon mice.

CA-074 is a potent and selective inhibitor of cathepsin B that can be used to evaluate cathepsin B inhibition in AD mice (APP/Lon) (98–100). This inhibitor is administered as the pro-drug form of CA-074Me, which is converted *in vivo* by esterases to the active CA-074 inhibitor (101). CA-074Me has greater membrane permeability than CA-074 and, therefore, enhanced penetration into cells. When administered (icv) to APP/Lon AD mice, memory deficits were improved, amyloid pathology was reduced, and Aβ(1–40) and Aβ(1–42) peptides in brains were reduced (93). The effectiveness of the CA-074 and E64c inhibitors to

improve AD deficits in an animal model provides proof-of-concept that effective small molecule inhibitors targeting cathepsin B can be developed as candidate therapeutic agents for AD.

TBI models.—Investigation of the CCI model of TBI in mice examined the effectiveness of administering E64d (oral) immediately after CCI injury. E64d reduced the severity of motor dysfunction resulting from CCI and facilitated full recovery to normal motor function more rapidly than vehicle-treated controls (45). E64d reduced brain lesion volume resulting from CCI and reduced neuronal loss. E64d was effective when administered up to 8 hours after CCI, demonstrating a clinically plausible time period for acute therapeutic interventions. TBI trauma-induced deficits in cognitive and motor functions have also been improved by CA-074Me, as well as by the broad cysteine protease inhibitors z-DEVD-FMK and LHVS inhibitors (102, 103).

Related neurodegeneration and brain injury models.—Inhibitors of cathepsin B improve cognitive and behavioral deficits in animal models of various brain neurodegeneration and injuries which include ischemia, cerebral hemorrhage, excitatory Huntington's disease, excitatory epilepsy, inflammatory pain, and meningitis (Table 3). In ischemia models, inhibitors of cathepsin B consisting of E64c, E64d, CA-074, and CA-074Me (administered intraperitoneally (ip) or intravenously (iv)) resulted in decreased neuronal death in the brain (104–109). In trauma of cerebral bleeding, motor deficits were improved with administration of the broad-acting cysteine protease inhibitor CP-1 (iv), accompanied with decreased neuronal cell death (110). In Huntington's disease and excitatory epilepsy, memory and learning deficits are improved with Z-FA-FMK and CA-074Me inhibitors, respectively (111, 112). Pain is ameliorated by CA-074 (administered as CA-074Me), and inflammatory pain is improved with the inhibitor K11777, a cysteine protease inhibitor (74, 113). In addition, meningitis infection is improved by CA-074 assessed by clinical score (68).

Brain penetrance of cathepsin B inhibitors in animal model studies of AD and ischemia.—Information on the penetrance of cathepsin B inhibitors across the blood brain barrier has been demonstrated by inhibition of cathepsin B activity in the brain (92, 106). In AD mouse model studies, oral administration of E64d resulted in 80% inhibition of brain cathepsin B activity at 10 mg/kg E64d which was efficacious for improvement of memory deficits in the APPLon **mice** (92). E64d is a pro-drug which is converted *in vivo* by esterases to the active E64c inhibitor (92). In monkeys subjected to ischemic brain injury, administration of CA-074 or E64c inhibitors (4–5 mg/kg, iv) resulted in significant inhibition of cathepsin B activity in brain by 85–50% in sub-regions of the hippocampus and substantially reduced neuronal loss (106)".

While inhibition of cathepsin B activity in brain by CA-074 or E64c was observed subsequent to peripheral administration (oral or iv) (92, 106), the brain concentrations of these these inhibitors have not been measured. It will be important in future studies to assess the pharmacokinetics of the concentrations of these inhibitors in the brain.

Overall, the findings of these studies indicate that inhibitors of cathepsin B, and inhibitors of cysteine cathepsins, improve behavioral deficits and ameliorate neuropathology in AD, TBI, and related brain disorders. Therefore, development of inhibitors of cathepsin B is a logical strategy for discovery of effective therapeutics for AD, TBI, and related brain dysfunctions.

5. Maturation of preprocathepsin B, member of the cysteine cathepsin protease family.

Cathepsin B is a lysosomal cysteine protease belonging to the clan CA and the papain-like family C1A. The other C1A papain-like cysteine proteases in humans are cathepsins C, F, H, K, L, O, S, V, W, and Z (also known as cathepsin X) (40, 43, 114). The cathepsin family also includes the aspartyl proteases cathepsins D and E, and the serine proteases cathepsins A and G. These cathepsins comprise the 15 protease members of lysosomal cathepsin proteases. Cathepsin B is among the most abundantly expressed cathepsins in the human brain (40).

Cathepsin B is synthesized as an inactive prepro-enzyme which undergoes sequential proteolytic processing for maturation of active cathepsin B (Figure 4a) (115, 116). The preprocathepsin is synthesized from its mRNA and undergoes removal of the NH_2-terminal signal sequence (17 residues) in the rough endoplasmic reticulum (43, 114–116); the resultant procathepsin B is translocated to the Golgi apparatus and routed to lysosomes. Procathepsin B is processed by removal of the propeptide to form the mature, active enzyme (117–120). The cathepsin B enzyme can undergo additional processing to generate heavy and light chains with disulfide linkage.

Activation of procathepsin B occurs by removal of the propeptide to generate the mature active cathepsin B (43, 114–120). Procathepsin B can undergo autoprocessing since the proenzyme has low catalytic activity (117). Activation of procathepsin B has been shown to occur in unimolecular (117–119) and intermolecular mechanisms (120). Removal of the propeptide of procathepsin B occurs in one or several proteolytic steps which can involve dipeptidylpeptidase to generate the mature single-chain cathepsin B (117–121). Procathepsin B can also be activated by exogenous proteases consisting of the lysosomal cathepsin D and cathepsin L proteases (120), as well as by the serine proteases cathepsin G, elastase, and urokinase-type plasminogen activator (122). The mature cathepsin B can be further processed to the heavy chain and light chain (5 kDa) linked by disulfide bonds, which is enzymatically active (43, 114).

Cathepsin B possesses the active site Cys108 residue which mediates proteolysis (shown by asterisk * in Figure 4a). The Cys108 is located at the S1 subsite of the enzyme which interacts with the substrate at the P1-↓P1’ residues for cleavage of the peptide bond. The Schechter-Berger nomenclature (123) designates residues at the N-terminal side of the cleavage site as the non-prime (P1, P2, etc) residues, and prime (P1’, P2’, etc) residues are those located at the C-terminal side of the cleavage site (Figure 4b). Corresponding enzyme non-prime subsites (S1, S2, etc) interact with the non-prime amino acid side chains, and the prime subsites (S1’, S2’, etc) interact with the prime residues designated according to the Schechter-Berger nomenclature.

6. Selective CA-074 inhibitor of cathepsin B

Development of inhibitors of cathepsin B may lead to novel therapeutic agents for the treatment of the devastating AD, TBI, and related cognitive and behavioral deficits. Selectivity of inhibitors for cathepsin B compared to other members of the cysteine cathepsin family is important for targeted inhibition of cathepsin B without disturbing other related proteases and associated functions. Distinct structural features of cathepsin B have resulted in design and synthesis of the highly selective CA-074 inhibitor of cathepsin B, developed by the seminal research of Professor Nobuhiko Katunuma, renowned scientist in the field of proteolysis and their inhibitors (124).

Design and features of CA-074 selective inhibition of cathepsin B.

The CA-074 selective inhibitor of cathepsin B was developed by structural analyses of E64 and E-64c (98, 99, 125) which are irreversible inhibitors of cysteine cathepsins, as well as papain (98, 99, 125, 126,) and calpains (127). Crystal structure analysis of papain with E-64c and derivatives of E-64 (98, 99, 128) suggested that an L-*trans*-epoxy-succinic acid group would lead to a selective inhibitor of cathepsin B, and a series of epoxysuccinyl peptides were designed, synthesized, and evaluated (98). Novel L-trans-epoxysuccinyl peptides were identified as potent and selective inhibitors of cathepsin B, consisting of the inhibitors CA-074 (N-(L-3-trans-propyl-carbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline) and CA-030 (N-(L-3-trans-ethoxycarbonyloxirane-2-carbonyl)-L-isoleucyl-L-proline) (98). CA-074 and CA-030 display potent K_i values of 2–5 nM for inhibition of cathepsin B (98, 99). Both inhibitors were highly selective for cathepsin B, indicated by the high K_i values of these inhibitors for cathepsins L and H at 40 μM to 200 μM (98, 99). *In vivo* studies showed that CA-074 selectively inhibited cathepsin B in rat liver (99); however, CA-030 was not selective in the *in vivo* studies (99).

These results identified CA-074 as a highly selective and potent inhibitor of cathepsin B (98, 99, 129). CA-074 and its methylated form CA-074Me have been extensively utilized to examine the roles of cathepsin B in human disease models in cells and animals, including AD, TBI, and related animal models (Table 3). CA-074Me has improved cellular penetration and is converted by abundant cellular esterases to CA-074 (101). While CA-074Me has been found to inhibit both cathepsin B and cathepsin L, its conversion by CA-074 *in situ* supports the use of this inhibitor to investigate cathepsin B in biological functions.

CA-074 interactions with cathepsin B.

The binding interactions CA-074 for inhibition of cathepsin B has been analyzed by the x-ray crystal structure of the CA-074 and cathepsin B complex (100), illustrated by the Molecular Operating Environment (MOE) platform for visualization and modeling of molecules to protein structures (130, 131) (shown in Figure 5). Cathepsin B (mature) possesses the catalytic active site residue Cys29 which is part of an extended substrate binding groove that is mostly hydrophobic with some polar regions (Figure 5a). Binding of CA-074 to cathepsin B subsites utilizes the Schechter-Berger nomenclature (123) for interactions with the enzyme prime (S1', S2', etc.) and non-prime (S1, S2, etc.) subsites (Figure 4b).

The CA-074 inhibitor is oriented in the substrate active site binding groove of cathepsin B, extending from the S2 to S2' pockets of the enzyme (Figure 5a, b). CA-074 is covalently bound by its C6 oxirane atom to the Cys-29 active site residue (S1 subsite) of cathepsin B for irreversible inhibition (100). The S2 site of the enzyme interacts with the propyl chain of CA-074 and involves Glu245 at the S2 subsite. The Ile-Pro-OH at the C-terminus of CA-074, important for selective inhibition, binds to the S1' and S2' subsites. The C-terminal proline of CA-074 interacts with His110 and Hs111 of the occluding loop of cathepsin B, which participates in specific inhibition of cathepsin B. Overall, CA-074 covalently binds to the active site Cys-29, with the inhibitor situated at S2 to S2' sites near the occluding loop of the enzyme.

7. Regulation of cathepsin B proteolysis by its distinct occluding loop

Cathepsin B uniquely possesses the occluding loop compared to other cysteine cathepsins (132–137). The occluding loop is located near the enzyme's substrate binding pocket containing the active site Cys-29 residue (100, 132) (Figure 5) which catalyzes endopeptidase and dipeptidyl carboxypeptidase activities. Thus, these activities are influenced by the occluding situated near the primed subsites which favor binding of peptide substrates with two residues carboxy-terminal to the cleaved scissile peptide bond. The His-110 and His-111 residues of the occluding loop provide positive charged anchors for the C-terminal carboxylate group of peptide substrates, which explains the dipeptidyl carboxypeptidase activity of cathepsin B. The occluding loop blocks substrate binding to the Sn' ($n \geq 3$) subsites of the protease.

The role of the occluding loop for endopeptidase compared to exopeptidase activities of cathepsin B has been assessed by deletion mutagenesis of a central 12-residue domain of the loop (133). The deletion mutant lacked exopeptidase activity (monitored with dansyl-Phe-Arg-Phe(NO_2)-Leu substrate). The mutant proenzyme was autoprocessed to yield the mature enzyme with endopeptidase activity (monitored with Z-Phe-Arg-AMC substrate) comparable to wild-type cathepsin B. These findings demonstrate that the occluding loop restricts access to the active site and endows the dipeptidyl carboxypeptidase activity of cathepsin B.

Further evidence for regulation of endopeptidase activity by the occluding loop was illustrated by site-directed mutagenesis of selected residues within the loop. Such mutations resulted in increased endopeptidase activity (134). The triple mutant D22A/H110A/R116A resulted in the greatest increase in endopeptidase activity of 600-fold increase compared to WT cathepsin B (endopeptidase Abz-AFRSAAQ-Eddnp substrate was used). These findings show that the increase in endopeptidase activity of the mutant may reflect an increase in loop flexibility when an endopeptidase substrate binds to the enzyme.

The occluding loop regulates the pH dependence of propeptide inhibition of cathepsin B endopeptidase activity (135). At pH 6.0, propeptide inhibition had a K_i of 3.7 nM, and at pH 4.0 the propeptide was less effective with a K_i of 82 nM. The pH dependence of inhibition was eliminated by deletion mutagenesis of a segment of the occluding loop, and by the His110Ala mutation in the loop which increased the potency of propeptide inhibition to K_i

of 2 nM at pH 4.0, similar to the nM K_i for propeptide inhibition at pH 6.0. These findings indicate that the occluding loop regulates the pH dependence of propeptide inhibition of cathepsin B (132, 134–136).

The occluding loop of cathepsin B can be displaced by the stefin protein inhibitors (137). The crystal structure of the complex of human cathepsin B and human stefin A showed that the occluding loop residues were displaced. These findings indicate that the occluding loop can exist in different positions and may become displaced by stefin or other endogenous protein inhibitors.

Clearly, the occluding loop distinguishes cathepsin B from other cysteine cathepsins. These structurally distinct features of the occluding loop provides the rationale for development of selective inhibitors of cathepsin B, as shown by CA-074 selective inhibition of cathepsin B.

8. Lysosomal leakage of cathepsin B to the cytosol in cell death and inflammation of AD, TBI, and related brain disorders.

Abnormal lysosomal leakage of cathepsin B into the cytosol of cells occurs as an integral mechanism for cell death and inflammation in AD, TBI, and related brain disorders. Cathepsin B and cathepsin proteases are normally sequestered within lysosome organelles for homeostasis by degradation cellular proteins into amino acids (138, 139). In AD, TBI, and related brain disorders, lysosomal membranes lose their integrity to result in lysosomal leakage and redistribution of cathepsin B to the cytosol (illustrated in Figure 6) (140–142). Notably, cytosolic cathepsin B activates cell death (143–147) and inflammatory (148–152) pathways which participate in AD, TBI, and related disorders (153–159). Cytosolic cathepsin B directs caspase-dependent cell death through cleavage of bid and anti-apoptotic Bcl-2 homologues, and removal of apoptosis-preventing Bcl-xl (143, 144). Furthermore, cytosolic cathepsin B increases inflammatory IL-1β cytokine production via activation of the neuroinflammasome (148–152). Lysosomal leakage results in pathogenic, cytosolic of cathepsin B in neurodegeneration of AD, TBI, and related brain disorders.

AD and lysosomal leakage.

In AD, oligomeric Aβ42 induces lysosomal leakage and relocation of lysosomal enzymes, including cathepsin B, to the cytosol to result in cell death of neuronal cells (160–163). Oligomers of pGlu-Aβ(3–42) also induce lysosomal leakage and apoptosis (162). The N-truncated and modified pGlu-Aβ(3–42) peptide increases in the aging AD brain and facilitates seeding of oligomeric Aβ peptide neurotoxicity (78, 79).

The Aβ42 induction of lysosomal leakage and cell death is exacerbated by ApoE4 (apolipoprotein E4), the major genetic risk factor for AD (164, 165). In ApoE4 transgenic mice, elevation of Aβ brain levels, achieved by inhibition of the Aβ-degrading enzyme neprilysin, results in cognitive deficits (165). These findings link oligomeric Aβ with lysosomal leakage and behavioral cognitive dysfunction.

TBI and TBI-related lysosomal leakage.

Numerous studies demonstrate that TBI and related neurodegenerative disorders result in lysosomal leakage of cathepsin B leading to cell death and neuronal damage (166–176). TBI modeled by the central fluid percussion injury (CFPI) model of TBI (in rats) results in elevation of intracranial pressure (ICP) (166). During increased ICP, brain neurons display a redistribution of cathepsin B from the lysosome to the cytosol, with mechanoporation of neurons, observed at 6 hours after CFPI. Mechanical injury to neurons in culture results in immediate cathepsin B release from the lysosome and increased cell death accompanied by increased tBid and release of cytochrome c from mitochondria (167); inhibition of cathepsin B improved cell viability.

TBI results in vascular disruption and brain ischemia. Lysosomal membrane permeabilization occurs in ischemia, indicated by cathepsin B release from lysosomes at 2 hours after ischemia in brain hippocampus (ischemia induced by oxygen-glucose deprivation) (168). Ischemia produced by middle cerebral artery occlusion results in lysosomal leakage of cathepsin B to the cytosol in 30–45% of neurons (coronal sections) at 1–4 hours after ischemia (169). The cytosolic cathepsin B was co-localized with caspase-3, tBid, and cytochrome-c of the apoptotic pathway.

Parkinson’s disease (PD) neurodegeneration can be modeled in mice and cells by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment (170–172). The MPTP-treated mice display lysosomal membrane permeability and leakage of cathepsins to the cytoplasm, triggering caspase-dependent apoptotic cell death (172). PD brains accumulate neurotoxic α-synuclein aggregates in Lewy bodies (173, 174), and such a-synuclein aggregates induce lysosomal rupture combined with induction of cathepsin B-dependent increase in reactive oxygen species (ROS) and inflammation (175). It is hypothesized that the pathogenesis of PD involves α-synuclein and lysosomal dysfunction in neuronal cell death (176).

9. Hypothesis for pathogenic, cytosolic cathepsin B in AD and TBI-related neurodegeneration.

Translocation of lysosomal cathepsin B to the cytosol in neurodegeneration.

Lysosomal leakage results in the pathogenic redistribution of cathepsin B from the lysosome to the cytosol in neurodegeneration, in contrast to the normal intra-lysosomal location of cathepsin B for protein degradation and homeostasis, which leads to the hypothesis that abnormal cytosolic cathepsin B participates in AD and TBI-related dysfunctions (illustrated in Figure 6). The key question is what are the cytosolic substrates of cathepsin B that lead to neurodegeneration? The profiles of cytosolic substrates of cathepsin B in brain have not yet been determined. However, it is known that cytosolic cathepsin B cleaves Bid to generate tBid which leads to mitochondrial cytochrome c release and apoptosis. Also important is the role of cytosolic cathepsin B in regulating the neuroinflammasome for activation of IL-1β production in inflammation. Neurodegeneration resulting from cell death and inflammation leads to deficits in cognition, memory, thinking, executive functions, and related behaviors.

With respect to the mechanism of lysosomal leakage, studies have demonstrated involvement of the calpain cysteine protease which cleaves carbonylated Hsp70.1 to result in lysosomal permeabilization and release of lysosomal cathepsin proteases (141, 177–179). The HSP70.1 chaperone functions as a guardian of lysosomal integrity. The role of calpain in lysosomal rupture and the redistribution of cathepsin proteases suggests the dual participation of 'calpain-cathepsin' in neurodegeneration (141, 177–179). The consequence of lysosomal permeabilization is the translocation of lysosomal cathepsin B to the cytosol to initiate cell death and inflammation occurring in neurodegeneration of AD, TBI, and related brain injuries.

Cathepsin B is active at the neutral pH of the cytosol.

The redistribution of cathepsin B to the cytosol changes its normal acidic pH environment of lysosomes (pH 4.6) (180–183) to the neutral pH of the cytosol (pH 7.2) (184–186). Despite the large change in proton concentration at cytosolic pH 7.2 compared to lysosomal pH 4.6, cathepsin B retains proteolytic activity at neutral pH (187–189). Furthermore, the stability of cathepsin B at alkaline pH can be enhanced by binding to heparin of membranes to potentiate the endopeptidase activity at neutral pH (189).

The large 400-fold difference in proton concentration from lysosomal pH 4.6 to cytosolic pH 7.2 results in an altered charge state of cathepsin B (Table 4). Mature cathepsin B has estimated charge states of +9 at pH 4.6, and −10.3 at pH 7.2 (calculated using protein calculator 3.4, http://protcalc.sourceforge.net/). These distinct charge states can result in different protein-substrate interactions with cathepsin B at the cytosol compared to lysosomal pH.

Furthermore, the occluding loop may possess very different charge states at lysosomal compared to cytosolic pH. The occluding loop has an estimated charge state of +2.2 at lysosomal pH 4.6, which contrasts with a predicted charge state of −0.9 at cytosolic pH 7.2. These different charge states of the occluding loop can influence substrate binding to the active site pocket at Sn' sites located near the occluding loop and modulate proteolysis.

Cytosolic cathepsin B and cell death.

Lysosomal leakage and release of cathepsin B to the cytosol initiates apoptosis by proteolysis of Bcl-2 family members Bcl-xl, Bax, and Bid (143), resulting in apoptosis-inducing Bid and removes apoptosis-preventing Bcl-Xl. Furthermore, apoptosis-promoting Bax is elevated through cathepsin B proteolysis of a Bax-degrading protease.

The E-64d inhibitor of cathepsin B, as well as cysteine proteases, prevents apoptosis, Bid cleavage, and degradation of the anti-apoptotic Bcl-2, BCl-xL, and Mcl-1 (144). A pan caspase inhibitor (N-benzyloxycarbonyl-Val-Ala-Asp9OMe)fluoromethyl ketone) prevents apoptosis, but has no effects on cathepsin B cleavage of Bid to tBid, indicating that caspase-mediated apoptosis occurs downstream of cathepsin B. Production of tBid leads to its mitochondrial binding to BAK to release cytochrome c in the apoptotic pathway (146). These findings illustrate the role of cathepsin B in the regulation of anti-apoptotic Bcl-2 members and Bid to trigger the mitochondrial pathway to apoptosis.

Cytosolic cathepsin B and inflammation.

Cathepsin B in the cytosol activates the neuroinflammasome to elevate IL-1β production (68, 71, 73,148–152, 190, 191). The NLRP3 inflammasome in microglia participates in AD and related neurodegenerative diseases (148–152). Oligomeric Aβ (16–20) and oxidative stress (150, 192–195) of AD and neurodegeneration, induce cathepsin B-mediated activation of the NLRP3 inflammasome to initiate processing of pro-caspase-1 to caspase-1, and subsequent caspase-1 conversion of pro-IL-1β to active IL-1β inflammatory factor (71, 73, 150). The detailed mechanism of how cathepsin B mediates activation of the NLRP3 inflammasome complex to activate IL-1β production has not yet been defined.

10. Conclusions and future perspectives

The hypothesis that lysosomal leakage of pathogenic cathepsin B to the cytosol results in neurodegeneration and behavioral deficits of AD, TBI, and related brain disorders (Figure 6) is supported by ample evidence from clinical and animal model studies of AD, TBI, and related brain disorders. This leads to the question of what are the cytosolic substrates of cathepsin B that lead to neurodegeneration? Current data implicate cathepsin B substrates involved in cell death and inflammatory pathways. It will be advantageous to advance understanding of the distinct functions of cytosolic cathepsin B contributing to neurodegeneration and brain dysfunctions of AD, TBI, and related disorders. Such findings may lead to novel targets for drug discovery to address the unmet need for therapeutics to treat these neurodegenerative diseases.

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V. Hook organized manuscript for participation by coauthors and wrote the manuscript. MY, CM, and AJO conducted the MOE representation of the cathepsin B structure complexed with the CA-074 inhibitors, and conducted literature analyses for this manuscript. SP conducted the analyses of cathepsin B and lysosomal proteases in human brain using the Allen Brain Atlas resource. GI contributed to extensive literature searches for this manuscript. BH and RR contributed current knowledge of AD and TBI research in the field. GH contributed to the outline of topics for inclusion in this manuscript and their integration for this manuscript. V. Hook and G. Hook have equity positions at American Life Science Pharmaceuticals, Inc.

V. Hook’s conflict has been disclosed and is managed by her employer, the University of California, San Diego. The other authors have no conflicts of interest.

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Highlights

- Cathepsin B is elevated in human Alzheimer's disease (AD) and traumatic brain injury (TBI) patients, and correlates with behavioral and injury outcomes
- Cathepsin B gene expression in human AD brains is prevalent in numerous regions throughout young to adult ages
- Elevated cathepsin B occurs in animal models of AD, TBI, and related brain disorders
- Inhibition of cathepsin B in AD and TBI animal models results in alleviation of cognitive and behavioral deficits with improvement in neuropathology
- Preprocathepsin B undergoes processing to generate the active, mature cathepsin B
- Selective inhibition of cathepsin B by CA-074 via irreversible linkage to the active site of cathepsin B
- The occluding loop of cathepsin B regulates dipeptidylcarboxypeptidase and endopeptidase activities of cathepsin B
- Lysosomal leakage of cathepsin B to the cytosol in cell death and inflammation of AD, TBI, and related brain disorders

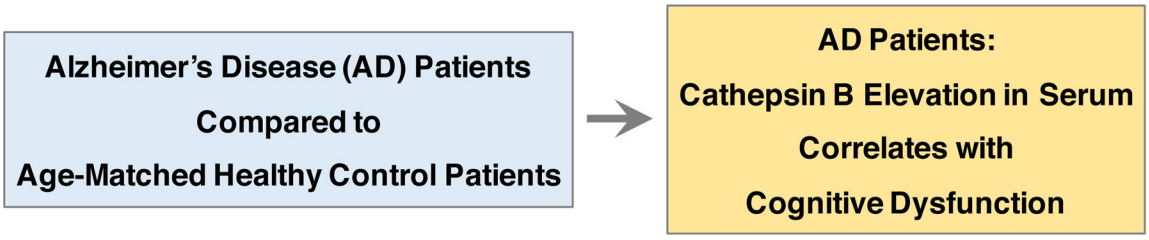

Figure 1. Elevation of cathepsin B in serum correlates with cognitive dysfunction in Alzheimer's disease patients.

Cathepsin B levels were significantly elevated by ~50% in serum from AD patients compared to age-matched control patients, shown by studies of Sun et al., 2015 (29). Furthermore, the increased cathepsin B in serum was significantly correlated with cognitive dysfunction, measured the Mini-Mental State Exam which tests for cognitive functions of memory, attention, language, and orientation (29).

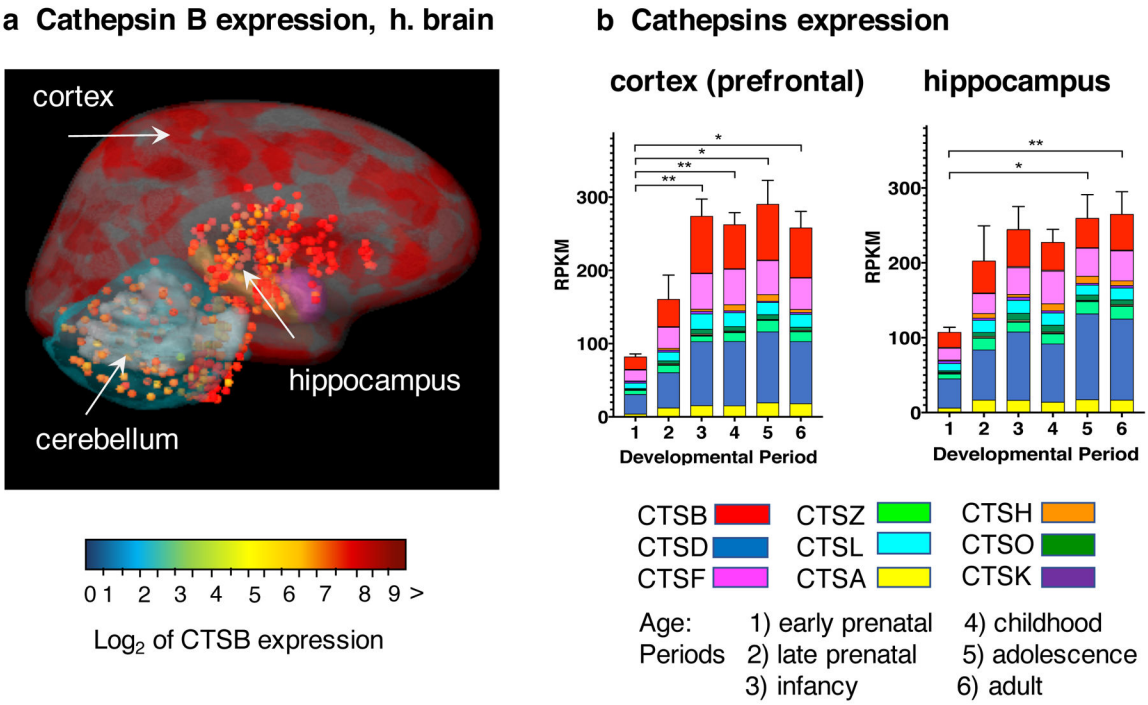

Figure 2. Expression of cathepsin B and lysosomal cathepsins in young and adult human brains.

a. Cathepsin B expression in human adult brain. Cathepsin B mRNA expression levels were determined by RNAseq analyses by the Allen Human Brain Atlas (https://human.brain-map.org/). The $\log_2$ normalized expression values of cathepsin B mRNA levels are illustrated overlaid on an MRI image of adult human brain, in color-coded ranges of high (dark red), medium (yellow), and lower (green-blue) levels of expression. The cortex regions display very high levels of cathepsin B expression and the hippocampus shows high levels of expression.

b. Expression of cathepsin B and lysosomal cathepsin proteases in human brain regions at young through adult ages. Gene expression levels of cathepsin B and the majority of the lysosomal cathepsin proteases. Cathepsins D, F, Z, L, A, H, O, and K, representing the most abundantly expressed among 15 cathepsin proteases (40) are illustrated. Gene expression data are assessed as reads per kilobase million (RPKM) for six age periods of early prenatal, late prenatal, infancy, childhood, adolescence, and adult. Bar graphs show the total cathepsin gene expression as the average RPKM + s.e.m., with statistical significance (*$p < 0.05$, **$p<0.01$). Cathepsins B, F, and D are the most abundantly expressing cathepsins.

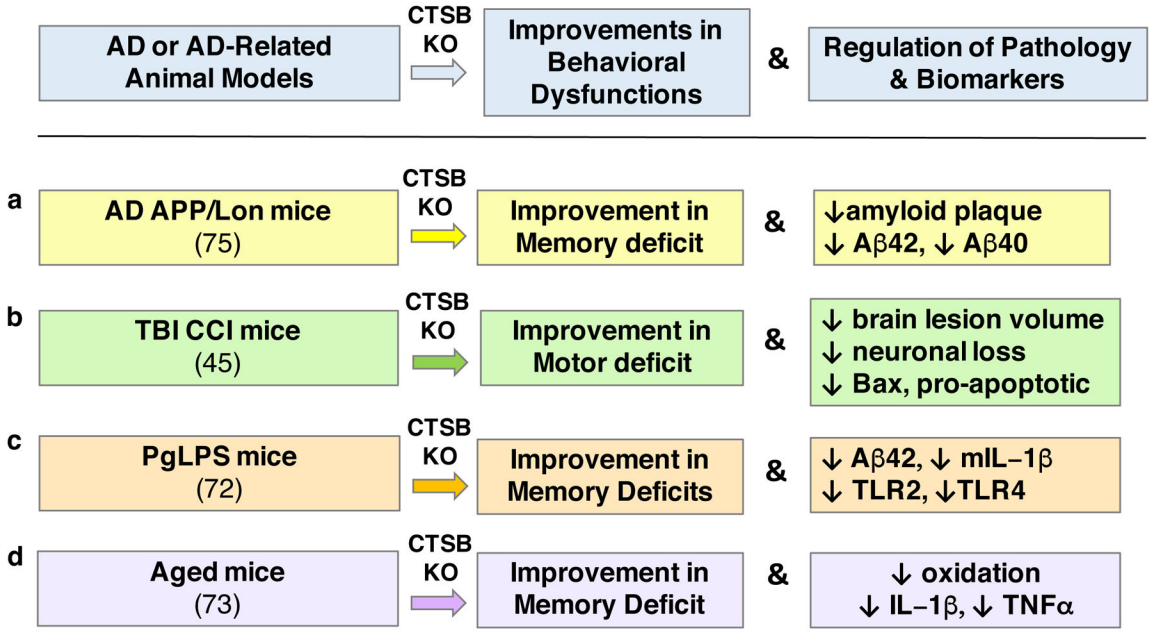

Figure 3. Cathepsin B gene knockout in animal models of AD, TBI, PgLPS, and aging.

Knockout (KO) of the cathepsin B gene (CTSB) results in alleviation of memory deficits in animal models of AD (75), PgLPS (72), and aging (73), and improvement in motor dysfunction in the CCI-TBI animal model (45), shown in panels a, b, c, and d, respectively. These improvements are accompanied by reduced neuropathology and reductions in biomarkers associated with the behavioral deficits. (a) In the APP/Lon mouse model of AD, CTSB KO results in substantial improvements in memory deficit measured by the Morris water maze test, and reduces amyloid plaque pathology with reduced brain levels of Aβ(1–40) and Aβ(1–42) (75), as well as reductions in pGlu-Aβ(3–40) and pGlu-Aβ(3–42) (77). (b) In the controlled cortical impact (CCI) model of traumatic brain injury (TBI), CTSB KO results in amelioration of motor deficits and allevation of brain tissue lesion pathology, combined with reduced neuronal loss and decreased pro-apoptotic Bax levels (45). (c) In mice suffering from cognitive decline due to infection by lipopolysaccharide from *Porphyromonas gingivalis* (PgLPS), CTSB KO results in improvement in memory deficit and reductions in Aβ42 and pro-inflammatory IL-1β, TLR2, and TLR4 (72). (d) CTSB KO in aged mice improves cognitive dysfunction, and ameliorates age-dependent increases in oxidation, IL-1β, and TNFα (73).

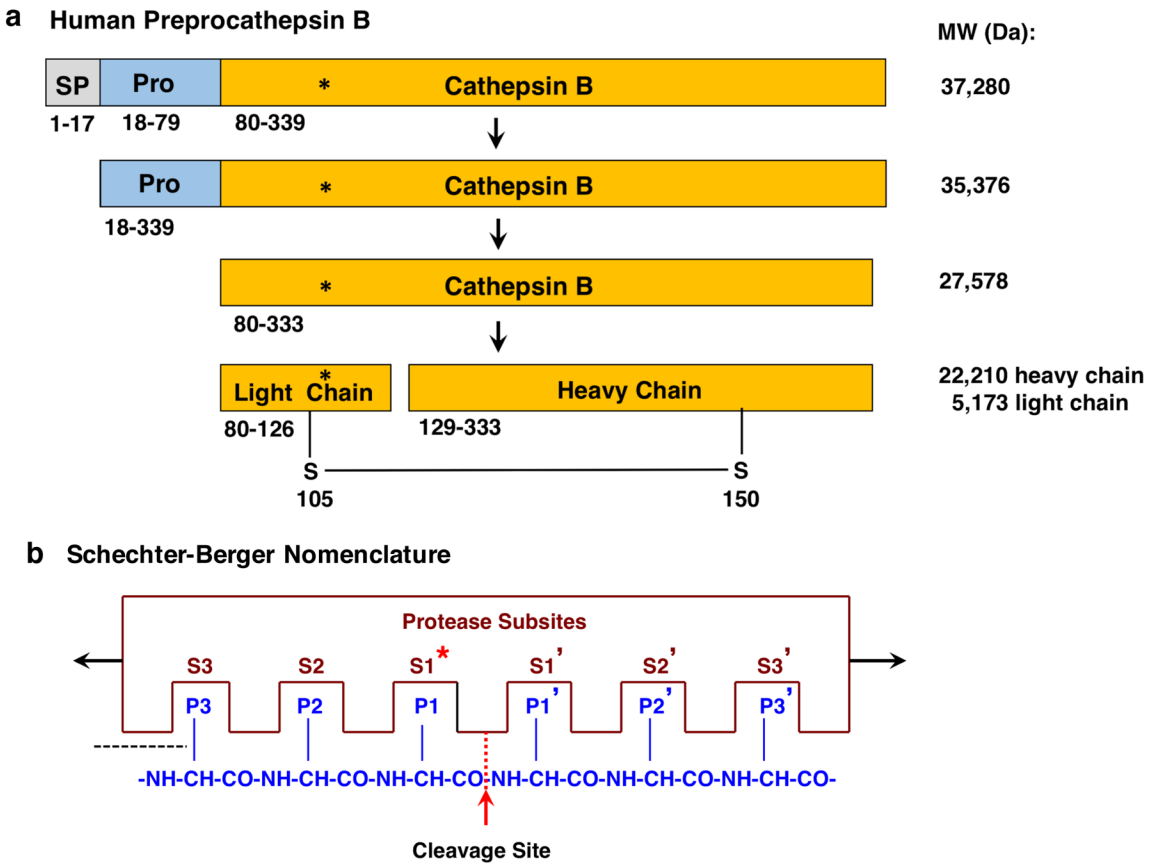

Figure 4. Processing of preprocathepsin B zymogen to active cathepsin B.

a. Preprocathepsin B processing to active protease. Preprocathepsin B (339 residues) (116) is translated from its mRNA at the rough endoplasmic reticulum (RER). The N-terminal signal sequence (SP, 17 residues) is removed at the RER by signal peptidase. The resultant procathepsin B (residues 18–333) is routed through the Golgi apparatus to lysosomes. The procathepsin B undergoes autoprocessing to remove the propeptide (Pro) to generate the active, mature cathepsin B (residues 80–333). Cathepsin B can undergo further processing into light and heavy chains linked by a disulfide bond. The active site Cys108 is indicated by the asterisk (*). Sequences of these forms of cathepsin B were obtained from the NCBI (196) and UniProt (197) protein sequence databases. It is noted that the NH_2-terminal amino acid of mature cathepsin B (residue 80 of preprocathepsin B) may be referred as residue 1, with active site Cys29.

b. Schechter-Berger Nomenclature for protease and peptide interactions at the substrate cleavage site. According to the Schechter-Berger nomenclature (123), protease and substrate interactions are defined by the enzyme (i) non-prime subsites of S1, S2, etc. which are located at the N-terminal side of the cleaved scissile bond, and the (ii) prime subsites of S1', S2', etc. which are located at the C-terminal side of the cleaved peptide bond. Amino acid residues located at the cleaved scissile bond (P1-↓P1') are represented by P1, P2, and Pn residues located at the C-terminal side of the cleavage site, and by P1', P2', and Pn' residues located at the N-terminal side of the scissile bond. The active site Cys108, shown by the asterisk (*), resides in the S1 subsite of the enzyme.

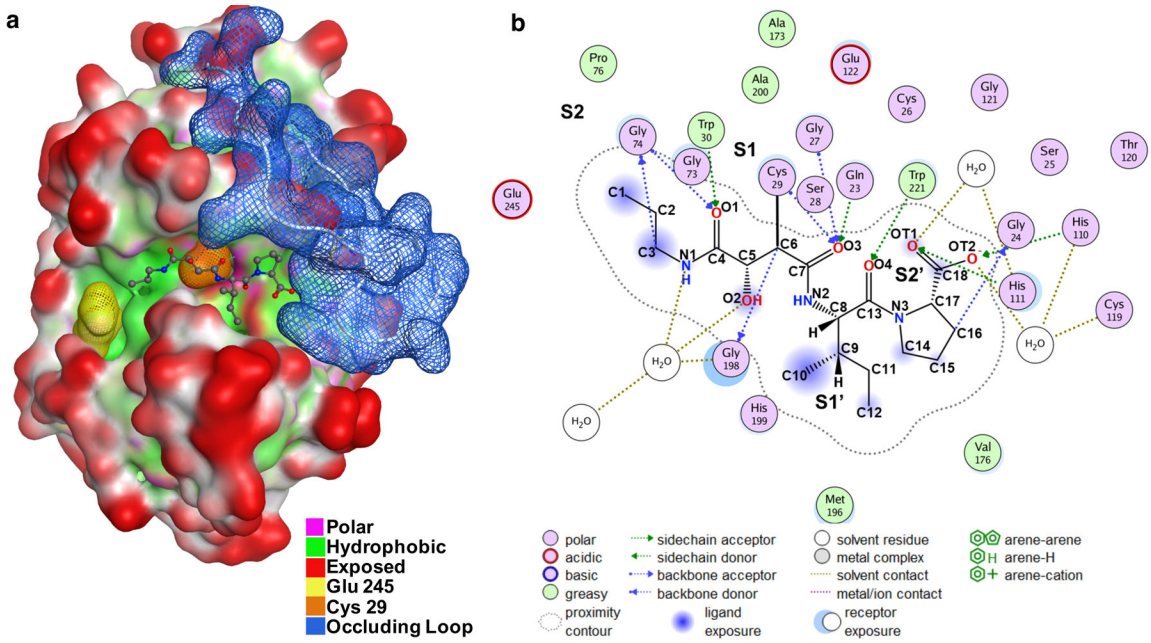

Figure 5. Interaction of the CA-074 inhibitor to the active site pocket of cathepsin B.

a. Structure of the cathepsin B/CA-074 complex. The structure of bovine cathepsin B complexed with CA-074 has been determined by x-ray crystallography by Yamamoto et al., 2000 (100); the PDB ID of this structural data of cathepsin B complexed with CA-074 is ID 1QdQ. This structural data was utilized by us with the Molecular Operating Environment (MOE) (130, 131) for visualization of the interacting features of CA-074 with the cathepsin B structure. The catalytic residue Cys29 (orange) is part of an extended substrate binding groove that is mostly hydrophobic (green) with some polar regions (pink). Using the Schechter and Berger nomenclature (123), Glu245 (yellow) sits in the S2 pocket and the occluding loop blue is orientated close to the S2′ pocket. The CA-074 inhibitor is orientated in the substrate binding groove and shown to extend from the S2 to S2′ pockets.

b. Illustration of two-dimensional (2D) CA-074 inhibitor and cathepsin B interactions. Interactions of CA-074 with cathepsin B (bovine) were determined from their crystal structure (100); CA-074 interacts with S2’ to S2 subsites of the enzyme. The S2’ subsite interacts with the C-terminal Pro of CA-074 with His110 and His111 of the occluding loop by hydrogen bonding; the S2’ subsite involves Gln23, Gly24, His110, His111 of the occluding loop domain of cathepsin B. The occluding loop is located in manner to block substrate accessibility to Sn’ subsites (n > 3). The S1’ subsite interacts with the Ile of the Ile-Pro C-terminal region of CA-074; the S1’ site is composed of a hydrophobic core of Val176, Leu181, Met196, His199, and Trp221 which holds the Ile side chain of CA-074 through hydrogen bonding with Trp221. The S1 subsite contains the active site Cys29 which forms a covalent bond with the C6 oxirane carbon of CA-074; the S1 pocket is composed of Cys29, Gly27, Gly74, and Gly198. The S2 subsite consists of Glu245, Pro76, Ala173, and Ala200 which form a hydrophobic pocket which accommodates the propyl chain of CA-074. These features of CA-074 covalent linkage to enzyme and interactions with the substrate binding pocket in the vicinity of the occluding loop provide the basis for selective CA-074 inhibition of cathepsin B.

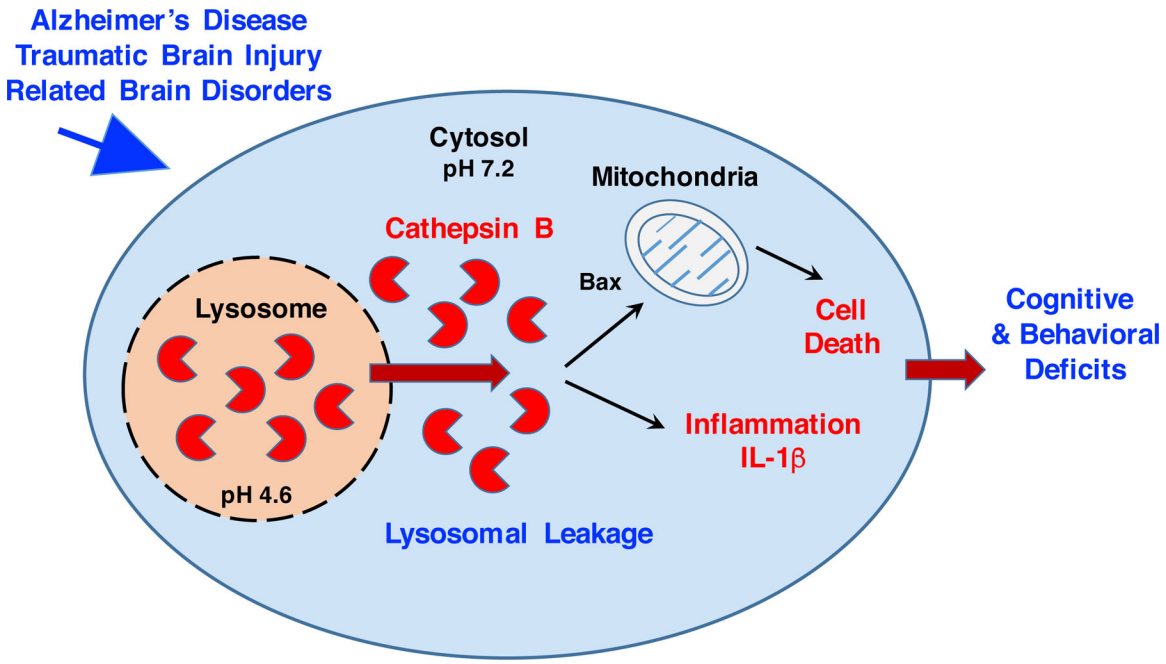

Figure 6. Lysosomal leakage results in cytosolic cathepsin B and activation of cell death neurodegeneration and inflammation in AD, TBI, and related.

Lysosomal leakage occurs in AD (160–163), TBI (166, 167), and related brain disorders (168–176). Lysosomal leakage results from membrane permeabilization, resulting in relocation of cathepsin B from the lysosome to the cytosol. Cathepsin B is active at the neutral pH 7.2 of the cytosol (184–186), which differs from the acidic pH 4.6 within lysosomes (180–183). Cathepsin B proteolysis of cytosolic substrates initiates apoptotic cell death (143–146) and activation of IL-1β production in inflammation (148–155).

Table 1.

Cathepsin B elevation in AD, TBI, and related dementia patients

Clinical Condition	Biofluid or Tissue	Cathepsin B Regulation	Features	Reference
Alzheimer's disease (AD)	plasma	↑	AD patients had 49% increase in CTSB protein above controls	(28)
AD	serum	↑	AD patients had ~45% increase in CTSB protein vs. controls ↑ CTSB correlated with MMSE * cognitive function scores indicating dementia	(29)
AD	plasma	↑	elevated CTSB protein in mild and severe AD by 50–80% above controls	(30)
AD	CSF	↑	CTSB protein was elevated by 1.9-fold in AD compared to control	(31)
AD	brain amyloid plaque	abnormal localization at amyloid plaques of AD brains	high CTSB activity and protein were abnormally localized in senile plaques of AD brains	(32)
Aging	CSF	↑	increased CTSB protein correlated positively with age	(35)
Severe Trauma	plasma	↑	CTSB activity was elevated 5–6 fold in severe trauma leading to organ failure	(36)
Multiple Trauma	plasma	↑	elevated CTSB associated with trauma and sepsis	(37)
Traumatic Brain Injury (TBI)	CSF	↑	elevated CTSB protein by 2-fold increase compared to controls	(38)
Inflammatory Neurological Diseases	CSF	↑	elevated CTSB activity in Guilllain-Barrer syndrome (GBS), chronic inflammatory demyelinating polyneuroopathy (CIDP), multiple sclerosis (MS), and meningitis	(39)

* Mini-mental state examination scores are utilized to monitor cognitive dysfunction, dementia (29).

Table 2.

Regulation of cathepsin B in animal models of AD, TBI, and related brain disorders

Animal Model	Species	Cathepsin B Regulation	mRNA, Protein, or Activity of Cathepsin B	Tissue	Refs.
AD, 5XFAD model	mouse	↑	elevated gene expression in brain	brain	(44)
AD, APPSwe/PS1	mouse	↑	increased protein	brain	(29)
TBI Trauma	mouse rat	↑	elevated mRNA, protein, and proteolytic activity	brain	(45–50)
Trauma SCC	rat	↑	increased mRNA, protein, and activity	brain	(51, 52)
Trauma surgery	mouse	↑	elevated activity in extracellular matrix	intestine trauma model	(53)
Subarachnoid hemorrhage	rat	↑	increased protein	brain	(54, 55)
Brain aneurysm	rat	↑	elevated mRNA and activity	cerebral aneurysm walls	(56)
ALS (amyotrophic lateral sclerosis)	mouse	↑	increased mRNA	spinal cord motoneurons	(59, 60)
Excitatory epilepsy	rat	↑	increased protein	brain and spinal cord	(62)
Excitatory Huntington's disease	rat	↑	elevated protein	brain	(63)
Ischemia, acute	rat	↑	elevated protein and activity	brain	(64)
Ischemia	monkey rat	↑	increased protein and activity	brain	(64–67)
Meningitis brain infection	mouse	↑	increased proteolytic activity	human THP-1 cells	(68)
Sepsis infection	rat	↑	increased proteolytic activity	skeletal muscle	(69, 70)
Inflammation, aging	mouse	↑	increased mRNA and protein	brain	(71–73)
Inflammatory pain	mouse	↑	elevated protein	spinal cord	(74)

Table 3.

Chemical inhibition of cathepsin B improves behavioral dysfunction and neuropathology in AD, TBI, and related animal models

Disease Model	Inhibitor (route)	Behavior	Pathology	Biomarkers	Refs.
AD APPLon	E64d (oral) E64d (icv) CA-074Me (icv)	↓ memory deficits	↓ amyloid plaque	↓ Aβ(1–40/42) ↓ pGluAβ(3–40/42)	(92–94)
TBI Trauma	E64d (oral)	↓ motor deficits	↓ brain lesion	↓ CTSB, ↓ Bax	(45)
	CA-07Me (icv)	↓ memory deficits	↓ brain lesion	↓ CTSB, t-Bid, Bax, cyt.c, caspase 3	(47)
	z-DEVD-FMK (icv)	↓ motor & cognitive deficits	↓ brain lesion	↓ calpain, ↓ spectrin degradation	(102)
	LHVS (icv)	↓ grip strength	↓ brain edema	↓ IL-1β,↓ TNFα	(103)
Ischemia	E64c (ip, iv) E64d (ip) CA-074 (iv) CA-074Me (ip)	nd	↓ neuronal death, brain	↓ CTSB ↓ MAP2 degrad. ↓ caspase 3	(104–109)
Trauma cerebral bleeding	CP-1 (iv)	↓ motor deficits	↓ neuronal death, brain	↑ synaptophysin, brain	(110)
Excitatory Huntington's disease	Z-FA-FMK (is)	nd	↓ brain lesion	nd	(111)
Excitatory epilepsy	CA-074Me (ip)	↑ learning ↑ neurolog. scores	nd	↓ CTSB ↓ LC3, ↓ beclin-1 ↓ bcl-2, ↓ PRG-1	(112)
Pain	CA-074Me (it)	↓ inflammatory pain	nd	↓ IL-1β, IL-18	(113)
Inflammatory pain, edema	K11777 (ip)	↓ inflammatory pain	↓ edema ↓ necrosis	↓ CTSB, CTSL, & CTSS ↓ c-fos	(114)
Meningitis infection	CA-074Me (ip)	nd	↑ clinical score	↓ IL-1β	(68)

Refs. indicate Reference citations

nd: not determined.

LTP, long-term potentiation; MS, multiple sclerosis; EAE, experimental atuoimmune encephalomyelitis; SCC, spinal cord contusion

Table 4.

Changes in cathepsin B charge states at cytoslic compared to lysosomal pH conditons

Enzyme Domain	Charge State:	
	pH 4.6 (lysosome)	pH 7.2 (cytosol)
Cathepsin B, mature	+ 9.5	− 10.3
Occluding loop	+ 2.2	− 0.9

Estimated charge states for human cathepsin B and its occluding loop domain were calculated using Protein Calculator 3.4 (http://protcalc.sourceforge.net/)