Section Review

Central & Peripheral Nervous Systems

Therapeutic potential of calpain inhibitors in neurodegenerative disorders

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Pathologic activation of calpain has been linked to a number of degenerative conditions where cellular calcium overloading is involved. Inhibition of this protease would be very attractive from the therapeutic standpoint of minimal side-effects because it exists mainly as the inactive pro-enzyme form under normal physiological conditions. Moreover, the therapeutic window of this approach may be wider compared to ion channel and glutamate receptor antagonism for the treatment of ischaemia since calpain activation is further downstream in the pathogenic cascade. Although calpain was discovered thirty years ago, potent and selective inhibitors have only now become available. This article reviews the recent development of these inhibitors.

Keywords: Ca^{2+}-dependent, calpain, hypoxia, ischaemia, neuroprotection, protease, protease inhibitor, proteolysis


Introduction

Calpain (EC 3.4.22.17) was first discovered as a calcium-activated protease in rat brain and in skeletal muscle independently thirty years ago [1,2]. Two major isoforms (μ-calpain and m-calpain) with different calcium affinities were subsequently isolated and purified in erythrocyte, skeletal muscle and kidney. Both isoforms appear to be heterodimers (80 kDa and 29 kDa) and can be separated by DEAE-chromatography [3,4]. The presence of an endogenous protein inhibitor, calpastatin, was discovered in Murachi’s laboratory [5]. The cloning of the large subunit of m-calpain (chick) and the small subunit (rabbit) was first described by the Suzuki group [6,7]. Murachi’s group also reported the cloning of the calpastatin gene shortly thereafter [8]. To date, at least four mammalian genes have been identified for the large subunit [9,10]. The two genes which encode for the 80 kDa subunit of μ- and m-calpain are located in human chromosomes 11 and 1 [11,12]. A third gene that encodes for the skeletal muscle-specific nCL-1 form (p94) was cloned to the limber-girdle muscular dystrophy type 2A locus on chromosome 15 [13,14]. Another smooth muscle-specific gene was also identified and can produce two alternate spliced forms (nCL-2 and nCL-2‘) [14].

Abbreviations

βA4: β-Amyloid peptide; AD: Alzheimer’s disease;
AMPA: 2-Amino-3-[5-methyl-3-hydroxyisoxazol-4-yl]propionic acid;
APP: Amyloid precursor protein; LTP: Long-term potentiation; MAP2: Microtubule-associated protein 2;
MCAO: Middle cerebral artery occlusion; NMDA: N-Methyl-D-aspartate; SAH: Subarachnoid haemorrhage;
SBDP: Spectrin breakdown product; TBI: Traumatic brain injury; 4-VO: Four-vessel occlusion.

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date, only a single gene (in chromosome 19) has been identified for the small subunit [6,15]. The 29 kDa protein is apparently used by both μ- and m-calpain. The large subunit can be divided into four domains (I - IV) and the small subunit has two regions (V and VI) (Figure 1). Based on sequence comparison, domain II is consistent with a cysteine protease structure [7]. Domain III has no defined function while domain V may be required for interaction with membrane phospholipids. Domains IV and VI each contain four sets of EF-hand calcium-binding structures, similar to those in calmodulin [7]. These calcium-binding structures apparently confer calcium-dependency on the catalytic activity. The large subunit of calpain has been expressed as a functional protein [16], although its activity can be enhanced when co-expressed or recombined with a truncated form of the small subunit [17]. Most recently, μ-calpain has also been successfully expressed in a baculovirus-insect cell system [18].

Calpain activity is tightly regulated by calcium. Although μ-, m-calpain and nCl-1 (p94) [14] require low micromolar, high micromolar and nanomolar concentrations of free Ca\(^{2+}\), respectively, in vitro for full activity, the actual calcium requirement for activation of calpain in vivo may be much lower. For example, acidic phospholipids can activate calpain by increasing its calcium affinity [19-21]. It appears that domain V has a glycine-rich region which might be used to interact with membrane-bound phospholipids [10]. It has also been demonstrated that phosphatidylinositol phosphates are required for calpain activation in intact cells [22,23]. Upon activation by calcium, the proenzyme form of calpain (80 kDa + 29 kDa) undergoes autolysis in domains I and V to produce the fully activated (76 kDa + 18 kDa) form [24]. Apparently, the activated form has a higher affinity for calcium [20,25]. With sustained activation, the large subunit of calpain undergoes further fragmentation, resulting in the loss of enzyme activity [24,26,27]. This could represent a self-regulating mechanism for calpain activity.

Calpain prefers substrates which contain Val or Leu in the P2 position and is less stringent at the P1 position [28]. To date, a few dozen proteins have been identified as potential substrates for calpain at least under in vitro conditions [29]. Among these are a significant number of receptor proteins (EGF receptor, IP3 receptor, oestrogen receptor) [30,31], myofibrillar proteins (troponin I and T, myosin heavy chain) [32], cytoskeletal and structural proteins (microtubule-associated protein [MAP2], talin, dystrophin and neurofilaments) [33,34], calmodulin-binding proteins (e.g., α-spectrin, plasma membrane calcium pump, calcineurin and connexins) [27,31,35-37] and transcription factors (AP-1 (c-fos, c-jun), c-myc, AP-2, AP-3 and NFkB) [38-41]. In addition, many enzymes are proteolytically activated by calpain including phospholipase C [42], transglutaminases [43], tyrosine phosphatase IIb [44], tyrosine kinase pp60src [45] and several calmodulin-regulated enzymes [31]. Physiologically, it is quite possible that calpain may have different functions in different cell types. In that regard, its multifunctional capacity could be analogous to protein kinases/phosphatases with the exception that calpain is more likely involved in unidirectional biochemical events. For example, m-calpain apparently is important in mammalian cell mitosis [46] and muscle cell differentiation [47,48]. Calpain has also been implicated in various membrane fusion events through remodelling of the cytoskeletal network [48,49]. Perhaps through a similar mechanism, it might be involved in enhancing glutamatergic synapses and long-term potentiation (LTP) [50]. The calmodulin-binding protein, non-erythroid α-spectrin (280 kDa; α-fodrin) is perhaps one of the most preferred and well-established endogenous substrates for cellular calpain [51,52]. Both μ- and m-calpain attack this protein at two sites near the middle of the molecule in a sequential manner [53,54]. The first site is highly susceptible to cleavage, leading to a spectrin breakdown product (SBDP) of 150 kDa on Western blot. Prolonged exposure to activated calpain next cleaves the SBDP150 at the N-terminus to produce a 145 kDa fragment. These SBDPs are reliable markers for cellular calpain activation. Recently, with the new N-terminal sequence GMPPR as antigen [52], antibodies that specifically detect only the SBDP150 but not the intact protein have also been developed [55].

**Development of Calpain Inhibitors**

Early work on calpain inhibitors was limited to protein inhibitors and other non-selective enzyme inhibitors [51]. The most selective of these is the endogenous inhibitor, calpastatin. The binding of calpastatin to calpain is a calcium-dependent process [56]. Although only one gene (located in chromosome 5) for calpastatin has been identified [57], several mature forms of calpastatin exist. The full length form contains domain I and four repeat inhibitory domains (I - IV) (68 kDa, apparent Mr 107 K on SDS-PAGE) (Figure 1) [8,58]. The presence of several mature forms of calpastatin may be due to alternative splicing in domain L (exon 3 and 5) and perhaps in domain I [59-61]. There is another post-translationally modified form of calpastatin that lacks the first two domains (L and I) (46 kDa, apparent Mr 68 K on SDS-PAGE) found in erythrocytes [62]. Within each repeated domain, there are three conserved regions (A, B, C). Region A and C apparently are helical and interact with the EF-hand calcium binding domains (IV and VI) of calpain, respectively.
Figure 1: Model of calpain and calpastatin.

Top panel: the two major isoforms (m-calpain, μ-calpain) have a distinct homologous large subunit. Domain II is the catalytic domain with the identified catalytic residues (His, Cys, Asn) shown. Domain IV contains four EF-hand calcium-binding motifs. The small subunit (s-calpain) has a glycine-rich region in domain V and four EF-hand motifs that make up domain VI. Initial autolytic cleavages leading to activation are shown by solid arrows.

Bottom panel: the full length form of calpastatin is illustrated with four repeats of the inhibitory domain (I - IV). The proposed interaction between calpain and one functional unit of calpastatin is shown. Regions A, B and C (shaded) from calpastatin interact with domains IV, II and VI of calpain, respectively.
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[56,63,64]. Domain B, however, apparently interacts directly with the catalytic site of calpain [65,66] and possibly domain III [67]. Both the 27-mer and the 20-mer peptides based on the core sequence of region B have been found to have inhibitory activity as well [65,66]. However, the large size and resulting low cell permeability of these peptides makes their use rather limited as therapeutic agents. L- and H-kininogens have also been reported as calpain inhibitors [68]. Interestingly, truncation of the kininogens to a key conserved region of both forms (Gln-Val-Val-Ala-Gly-NH₂) also retains irreversible calpain inhibitory properties [69].

Peptidyl aldehydes such as leupeptin (Ac-Leu-Leu-Arg-H) and antipain are also among the earliest reported calpain inactivators [70,71]. Leupeptin inhibits p-calpain and m-calpain with Ki values of 0.32 and 0.43 μM, respectively [72]. Both leupeptin and antipain also inhibit other proteases including papain, trypsin and cathepsin B. Calpain inhibitor I (Ac-Leu-Leu-Nle-H) and calpain inhibitor II (Ac-Leu-Leu-Met-H) developed in Murachi’s laboratory [73] have been widely used in many studies. However, calpain inhibitor I is actually a better inhibitor for cathepsin L (Ki = 0.5 nM) than for calpains. Likewise, calpain inhibitor II can also inactivate cathepsin B (Ki = 100 nM). Both compounds are relatively cell-permeable (Wang, unpublished data). In contrast to the common misconception that calpain inhibitor I is selective for p-calpain and calpain inhibitor II is selective for m-calpain, they are, however, almost equipotent against p-calpain (Ki = 0.1 μM, 0.1 μM) and m-calpain (Ki = 0.2 μM, 1.4 μM, respectively) (Wang et al., unpublished data). Calpeptin (Z-Leu-Nle-H) and MDL28170 (Z-Val-Phe-H) are second generation peptidyl aldehyde inhibitors with improved cell permeability. These compounds have limited therapeutic use due to their tendency to oxidise in vivo [74,75]. Nonetheless, they have been useful in defining the pathological roles of calpain discussed below.

Irreversible inhibitors such as the E64 family have also been studied. While these peptidyl epoxides showed high specificity for cysteine proteases, they are not particularly selective for calpain. In addition, E64 analogues have poor membrane permeability which limited their development [76].

Peptidyl halomethanes and diazomethanes have long been used as protease inhibitors. Potent calpain inhibitors were discovered by modifying the P₁ and P₂ positions with calpain-preferred residues [77]. Selectivity of these inhibitors for calpain over other cysteine proteases has yet to be achieved. The Cephalon group recently published their work on peptidyl fluoromethyl ketone calpain inhibitors [78]. They were able to improve potency slightly by using a tetrahydroiso-quinolyl group as the N-terminal capping group. Their best fluoromethyl ketone, compound 4f (Figure 2), showed only a marginal preference for calpain I (kobs/1 = 27600 M⁻¹ s⁻¹) over cathepsin B (kobs/1 = 7500 M⁻¹ s⁻¹) and cathepsin L (kobs/1 = 72000 M⁻¹ s⁻¹).

Peptidyl hydrazines were reported as cysteine protease inhibitors independently by Graybill et al. (Sanofi Winthrop) and Giordano et al. [79,80]. These compounds showed little selectivity and in some cases have higher reactivity for cathepsin B than calpain.

A compound of interest among the irreversible calpain inhibitors is H-Leu-Leu-Cys(Npys)-NH₂ (compound 3, Sankyo, Figure 2). This compound is much more selective for calpain (IC₅₀ = 0.18 μM) versus other cysteine proteases such as cathepsin B (IC₅₀ = 170 μM) and papain (IC₅₀ = 190 μM). It also does not inhibit serine and aspartic proteases [81].

Therapeutic Indications

In recent years there has been intense interest in determining the roles that pathological activation of calpains may play in disease states. Calpain overactivation is usually a consequence of calcium overload when the cells are under compromised conditions. Since calpain can potentially act on a large number of cellular proteins, unchecked activation of calpain would have detrimental effects on these downstream pathways. Besides the neurological disorders that will be covered below, several non-neuronal disorders have been linked to calpain overactivation [51,82]: muscular dystrophy [83], cataracts [84,85], thrombotic platelet aggregation, restenosis and arthritis [86], vasospasm [87] and cardiac ischaemia [88].

Cerebral ischaemia

In strokes, as a result of compromised blood flow, neurons in the affected area are subjected to hypoxia/ischaemia [89]. It is now well established that excessive synaptic glutamate build-up triggers events which ultimately result in neuronal injury (excitotoxicity) [89-92]. Overactivation of ionotropic glutamate receptors in the postsynaptic membrane leads to sustained influx of Na⁺ and Ca²⁺. Na⁺ influx-induced membrane depolarisation in turn activates voltage-gated neuronal Ca²⁺ channels furthering Ca²⁺ influx. Sustained elevation of neuronal Ca²⁺ to high nanomolar concentrations results. Among other calcium-dependent systems, calpain was found to be activated under these conditions, as illustrated in many in vivo and in vitro models [93]. For example, SBBDPs and MAP2 breakdown can be readily observed [33,94]. It has been hypothesised that calpain activation can lead to cytoskeleton/plasma membrane damage followed
Figure 2: Recent calpain inhibitors in development.
by a general loss of cell function and the eventual death of neurones [51,82]. Evidence supporting the contributory role of calpain in neuronal loss came from studies of the following three categories based on biological complexity.

**Neuronal cultures**
Primary CNS neuronal cultures have been found to be susceptible to either direct excitotoxic insults (e.g., glutamate, NMDA, AMPA) or hypoxia/hypoglycaemia [95,96]. Evidence of SBDP in many of these models has been documented [95-97].

Leupeptin and calpain inhibitor I were found to protect chick embryonic neuronal cultures against hypoxia [98]. Similarly, E64, MDL28170 (10 μM each) and leupeptin (100 μM) protected cerebellar Purkinje cells from AMPA-induced toxicity [99]. Even delayed application of MDL28170 (10 μM) and E64 (10 μM) still protected cerebellar Purkinje neurones against kainate induced toxicity [97]. The recently identified non-peptide calpain inhibitor also protected foetal rat neocortical neurones from hypoxic/hypoglycaemic damage and reduced the amount of associated spectrin breakdown products [53].

**Brain slices**
Hippocampal slices are perhaps one of the most widely used in vitro models of excitotoxicity and ischaemia [100,101]. For instance, exposure of hippocampal slices to a brief period of hypoxia would produce irreversible neuronal loss, especially in the CA1 and CA3 regions. In fact, the functional loss in the CA1 neurones can be monitored by measuring the evoked post-synaptic potential (EPSP). The decrease or loss of this response indicates neuronal damage in the CA1 region. Using this model, several groups have demonstrated that cell-permeable calpain inhibitor I and MDL28170 improved the functional recovery (measured by EPSP) of the CA1 neurones in the hippocampal slices after hypoxic challenges [102-104]. Again the associated spectrin breakdown was attenuated by calpain inhibitor I [100]. Similarly, synaptic transmission in gerbil neocortical slices after hypoxia was improved by MDL28170 (50 μM) [105]. Cerebellar slices can also be challenged with AMPA, and the Purkinje neurones contained there will die within a few hours [106]. Both MDL28170 and the Parke-Davis calcium binding site targeting inhibitor, PD150606 (vide infra) (Figure 2) are neuroprotective in this model [53,106].

**In vivo cerebral ischaemia models**
In vivo models for cerebral ischaemia generally belong to one of the two major types: irreversible focal ischaemia (e.g., middle cerebral artery occlusion; MCAO) and transient global ischaemia (e.g., four-vessel occlusion; 4-VO). Spectrin breakdown was observed in the hippocampus in both the global [102,107] and the focal ischaemia models [108]. Leupeptin, when administrated in a prolonged infusion paradigm, enhanced hippocampal CA1 neurone survival in gerbils subjected to transient global ischaemia [103]. Similarly, in administration of MDL28170 (30 - 60 mg/kg) significantly reduced the infarct size in a rat focal ischaemia model [108]. Calpain inhibitor I also reduced neuronal damage of the hippocampal CA1 subfield in global ischaemia [98]. Supraconical perfusion of AK275 also markedly reduced infarct size in a rat MCAO model. A related compound AK295 also reduced infarct size in a rat MCAO model when infused intra-arterially [109,110].

It is now well established that, in both in vitro and in vivo models of cerebral ischaemia, selective glutamate receptor antagonists of either the NMDA receptor (e.g., dizocilpine; MK-801) or the AMPA/kainate receptor (e.g., 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[3quinoxaline; NBQX) can provide significant neuroprotection [89,91]. However, these antagonists may also elicit adverse side-effects considering the critical neurotransmitter role glutamate plays in the CNS [111]. In this regard, calpain inhibitors may be strategically superior since most calpain exists in a latent non-activated pro-enzyme form. It is likely that only a subpopulation of calpain (e.g., subplasma membrane-located) could be activated in signal transduction pathways, while the majority remains inactive. Therefore, calpain inhibition may have less significant CNS side-effects than neurotransmitter antagonists.

**Traumatic brain injury**
Traumatic brain injury (TBI) refers to impact-induced acute neurodegeneration. Glutamate receptor overactivation and excessive intracellular Ca²⁺ accumulation have been identified in TBI models [112,113]. Evidence of calpain translocation to the membrane fraction [114], together with the breakdown of MAP2 and neurofilament H and L, has been reported in an animal model of TBI [115,116]. Since the tissue damage in TBI is mediated through glutamate excitotoxicity, the inhibition of calpain activation predictably reduces the amount of neuronal damage as well as enhancing functional recovery [117,118]; Hays personal communication.

**Subarachnoid haemorrhage/vasospasm**
Subarachnoid haemorrhage (SAH), as a result of a rupture of a saccular intracranial aneurysm, can induce long-lasting cerebral vasospasm by restricting local blood flow [119]. It was proposed that endothelin
referred from endothelial cells leads to endothelin receptor activation in vascular smooth muscle cells which produces a long-lasting spastic response. L-type calcium channel blockers (e.g., nimodipine) have been used successfully in SAH, suggesting the involvement of calcium influx into the vascular smooth muscle cells [120]. In a canine SAH model, vasospasm is induced by intracisternal injection of autologous blood near the basilar artery [121]. Topical application of calpeptin (1 µM) dilated the exposed spastic basilar artery [122]. Possible proteolytic activation of PKC by calpain was suggested since the PKC inhibitor, 1-(5-isouquinolinylsulfonyl)-2-methylpiperazone (H7), had a similar vasodilating effect [122]. Calpain involvement was further supported by the observed increase of spectrin breakdown products in the smooth muscle cells of spastic basilar artery in a rabbit model of SAH [122].

Demyelination disorders

Calpain may mediate myelin degradation in demyelination disorders such as Wallerian degeneration, multiple sclerosis and peripheral neuropathies [130]. Calpain was found to co-localise with fragmented myelin in myelinated nerve fibres in rabbit [131]. In addition, in Wallerian degeneration, influx of calcium into the axon distal to the site of injury has been reported [132,133]. m-Calpain can localise in axons of neurons [134] and can degrade axonal proteins, such as neurofilaments H, M and L in vitro [132,135,136]. Thus, it may participate directly in the axonal degeneration process in demyelination disorders.

Spinal cord injury

The pathology of spinal cord injury also appears to involve excitotoxins, much like traumatic brain injury [89]. In experimental spinal cord injury, Ca<sup>2+</sup> accumulates within the injured axons [137]. Calpain is thus activated and hence contributes to the degeneration of axons and myelins [138,139]. The calpain inhibitor, leupeptin, was found to reduce axonal damage in experimental spinal cord injury [140].

Peripheral nerve injury

Calpain activation may also be involved in peripheral nerve injury. Administration of leupeptin improved functional recovery after peripheral nerve repair in a monkey model [141,142]. Interestingly, the authors noted that administration of leupeptin improved for months did not appear to produce any adverse effects.

Neuronal apoptosis

Apoptosis, or programmed cell death, is a form of cell death that is controlled by gene expression and a cascade of biochemical events. Apoptosis is characterised by the presence of chromatin condensation, DNA cleavages at the nucleosome linkage regions, membrane blebbing and cell shrinkage [143]. There is an abundant body of literature tying the contribution of neuronal apoptosis to neurological disorders such as cerebral ischaemia, Huntington’s disease, Parkinson’s disease and Alzheimer’s disease [144-146]. Calpain activation was first identified in apoptosis in thymocytes and lymphocytes [147-149]. Subsequently, calpain activation in neuronal apoptosis in cultured neurons was confirmed by the presence of calpain autolysis as well as calpain-specific α-spectrin breakdown products [150]. Interestingly, calpain appears to work in parallel with ICE-like protease on α-spectrin breakdown which possibly leads to cytoskeletal rearrangement. Addition of calpain inhibitors could apparently inhibit apoptosis in certain immune cells and neuronal cells [147,149,150]. Hence, calpain inhibitors

Other chronic neurodegenerations

Chronic neurodegenerative disorders such as Huntington’s disease, Parkinson’s disease and amyotrophic lateral sclerosis are linked in part to excitotoxicity [89,91]. Calpain inhibitors may have the potential to alleviate the accompanied glutamate-induced neurological deficits in these disorders.
may have utility in suppressing unscheduled apoptosis.

### Specific Compounds Under Development

**AK275**

AK275 (Alkermes; Figure 2) is a dipeptide α-ketamide calpain inhibitor. It is the L,L-diastereomer of CX275 which was developed by Cortex. AK275 inhibits μ-calpain with a $K_i$ of 77 nM [151], although it was found to be protective in the rat MCAO model when chronically perfused directly into the cortical surface [109]. However, its poor aqueous solubility (0.08 mg/ml) renders it unlikely to be a clinical candidate.

**AK295**

AK295 (Alkermes; Figure 2) is a structural analogue of AK275 with a C-terminal amide modification. With a morpholinopropyl group replacing the ethyl group of AK275, AK297 is found to be more soluble in aqueous media without compromising potency and selectivity. In the rat focal cerebral ischaemia model with continuous intra-arterial infusion at a dose of 3 mg/kg/h (for 20 h), AK297 provided a 32% reduction in infarct volume [110]. This compound is still in preclinical development.

**WIN-68100 and WIN-69211**

WIN-68100 and WIN-69211 (Sanofi Winthrop; Figure 2) are two peptidic calpain inhibitors isolated from the fermentation broth of Streptomyces griseus (SC480) [152]. WIN-68100 is a diketopiperazine which inhibits human μ-calpain with an IC$_{50}$ value of 0.8 μM. The tetrapeptide WIN-69211 has an IC$_{50}$ of 1.2 μM against μ-calpain. It is not known at this time whether these two inhibitors will inhibit other proteases besides calpain. Results of further in situ and in vivo studies of these two compounds have yet to be published.

### Compound 8

Sanofi Winthrop reported a series of quinolincarboxamide based calpain inhibitors. Compound 8 in the series exhibits a 50-fold selectivity for μ-calpain (IC$_{50}$ = 0.5 μM) over both cathepsin B (IC$_{50}$ = 25 μM) and cathepsin L (IC$_{50}$ = 22 μM). These quinolincarboxamides are found to be non-active site targeting reversible calpain inhibitors [153]. No further data are available on this series of compounds.

### Compound 69

Sanofi Winthrop has also developed a series of peptidyl acyloxyethyl ketones as calpain inhibitors. Compound 69 in the series (Figure 2), a tripeptide 2,6-difluorobenzoyloxyethyl ketone, displays greater than 100-fold selectivity for μ-calpain ($K_{obs/II}$) = 31000 M$^{-1}$s$^{-1}$ over both cathepsin B ($K_{obs/II}$ = 100 M$^{-1}$s$^{-1}$) and cathepsin L ($K_{obs/II}$ = 300 M$^{-1}$s$^{-1}$). There is evidence suggesting that the leaving group structure can override the calpain P$_1$ - P$_2$ specificity in determining the potency of these inhibitors [154]. No further data are available on this series of compounds.

**PD150606**

PD150606 (Figure 2) is the first calcium binding site targeting calpain inhibitor developed by Parke-Davis. It is an α-mercaptopropanoic acid derivative which inhibits μ-calpain and m-calpain uncompetitively with inhibition constants ($K_i$) of 0.21 μM and 0.37 μM, respectively. PD150606 is extremely selective against calpain over other proteases such as cathepsin B ($K_i$ = 128 μM), papain ($K_i$ > 500 μM), trypsin ($K_i$ > 500 μM) and thermolysin ($K_i$ = 204 μM). This compound has shown neuroprotective effects in both hypoxic/hypoglycaemic induced injury of cultured cerebrocortical neurones and AMPA induced excitotoxic injury of Purkinje neurones in cerebellar slices [53].

**PD151746**

PD151746 (Parke-Davis; Figure 2) is a structural analogue of PD150606 with the 4iodophenyl group replaced by a 5-fluorodindole ring. Like PD150606, it is a selective inhibitor of calpain. Moreover, PD151746 also showed a 20-fold selectivity between μ-calpain ($K_i$ = 0.26 μM) and m-calpain ($K_i$ = 5.33 μM) [53].

### Perspectives and Prospectives

Calpain inhibitors represent a novel therapeutic approach to neurological disorders related to cerebral ischaemia and excitotoxicity, as they target intracellular mechanisms. The possibility of effective delayed application and the lack of adverse CNS side-effects point to the potential advantages of this strategy. Calpain inhibitors are also likely to have therapeutic value in additional non-CNS diseases such as cataracts, cardiac ischaemia and muscular dystrophy. Lastly, we are currently on the threshold of seeing the birth of a sizeable number of non-peptide and more selective calpain inhibitors. Although most of these compounds are still in the early preclinical development stage, they will certainly provide valuable information on the pathological roles of calpain in acute and chronic diseases.
References to Primary Literature

Papers of special note have been highlighted as:

• of interest
** of considerable interest


One of the first papers describing the discovery of calpain.


One of the first papers describing the discovery of calpain.


Good calpain purification method.


First paper on the cloning of calpain.


First genetic disorder linkage to a calpain gene.


Documentation of functional recombinant m-calpain.


Documentation of functional recombinant μ-calpain by a baculovirus system.


Excellent study on calpain substrate site preferences.


Providing insights on secondary recognition sequences (PEST) in calpain targets and calmodulin-binding proteins as selective targets for calpain.


Good overview on calpain inhibitor development.


It forms the basis for SBP150-specific antibodies (see [55]).


Description of first calcium binding site, non-peptide calpain-specific inhibitors.


Clear demonstration that each repeated domain of calpastatin is a functional inhibitory unit.


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Detailed study on the protective effects of calpain inhibitors in a hippocampal slice model of hypoxia.


Useful alternative model to the hippocampal model (see [103]).


Demonstration of neuroprotective action of calpain inhibitors in vivo.


Another demonstration of neuroprotective action of calpain inhibitors in vivo.


Demonstration of neuroprotective action of calpain inhibitors against TBI-induced neurofilament loss.


Demonstration of neuroprotective action of calpain inhibitors in an animal model of TBI.


121. FOLEY PJ, KASSELL NF, HUDSON SB, LEE KS: Hemoglobin penetration in the wall of the rabbit basilar artery after


Important study linking calpain to Alzheimer’s disease.


It shows that myelin can be degraded by endogenous calpain.


This paper notes that lack of observable side-effects of prolonged administration of leupeptin.


The first published study on the presence of apoptosis in cerebral ischemia.


Convincing demonstration of calpain activation in T-cell apoptosis.


First demonstration that calpain is involved in neuronal apoptosis.

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This paper demonstrates that leaving group structure can influence potency and selectivity of irreversible calpain inhibitor.

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