Calpain inhibition: an overview of its therapeutic potential

Kevin K. W. Wang and Po-Wai Yuen

Increasing evidence now suggests that excessive activation of the Ca$^{2+}$-dependent protease calpain could play a key or contributory role in the pathology of a variety of disorders, including cerebral ischaemia, cataract, myocardial ischaemia, muscular dystrophy and platelet aggregation. In this review, Kevin Wang and Po-Wai Yuen discuss the evidence linking these disorders to calpain overactivation. At present, it is difficult to confirm the exact role of calpain in these disorders because of the lack of potent, selective and cell-permeable calpain inhibitors. However, given the multiple therapeutic indications for calpain, it appears that achievement of selective calpain inhibition is an important pharmacological goal.

One of the many cellular proteins involved in Ca$^{2+}$ signalling in mammalian cells is the Ca$^{2+}$-activated neutral protease calpain$^{1,2}$. There are two major isoforms: calpain I (or µ-calpain) and calpain II (or m-calpain), which require low and high micromolar Ca$^{2+}$ concentrations for activation, respectively. Calpain consists of two subunits of about 80 kDa and 29 kDa (Fig. 1). The large subunit can be further divided into four domains. Domain II is the catalytic domain homologous to other cysteine proteases, especially in regions that cover the two catalytic residues (His and Cys) (Fig. 1). Domain III has unknown function and is not homologous to other proteins. Domain IV has four EF-hand Ca$^{2+}$-binding sites. The N-terminal half of the small subunit appears to be important for interaction with phospholipids [for example, phosphatidylinositol (4,5)-bisphosphate], which lower its Ca$^{2+}$ requirement. The C-terminal half has another four EF-hand structures. It is assumed that these Ca$^{2+}$-binding sites impose a strict Ca$^{2+}$ requirement on the catalytic activity, but the number of occupied Ca$^{2+}$-binding sites required for activity remains debatable.

Following the addition of Ca$^{2+}$, calpain autolyzes by truncating an N-terminal portion of domain I and most of domain V. The catalytic activity of this truncated form (76 kDa and 18 kDa) is still Ca$^{2+}$-dependent. It is uncertain whether this step is essential before calpain can hydrolyze other substrates. Recently, cDNA clones for a skeletal muscle- and a stomach-specific form of the large subunit were identified (nCL-1 and nCL-2, respectively). Interest-

ingly, nCL-1 contains a nuclear translocation-like sequence (Lys-Lys-Lys-Lys-X-Pro) in its catalytic domain, while nCL-2 can be alternatively spliced to generate a form that totally lacks the Ca$^{2+}$-binding domain$^2$ (nCL-2'; Fig. 1).

Calpain appears to be selective for a subset of cellular proteins$^{2,3}$. These include cytoskeletal proteins (for example, spectrin), membrane receptors (for example, epidermal growth factor receptor), calmodulin-binding proteins, G proteins, enzymes involved in signal transduction [such as protein kinase C (PKC)] and many transcription factors. Calpain prefers Leu or Val as the second residue on the N-terminal side of the cleavage site$^4$. However, this rule is not strictly followed when the cleavage sites of endogenous protein substrates are examined. It was also proposed that a secondary recognition sequence may come into play. Hydrophilic sequences enriched in Pro, Glu, Asp, Ser and Thr (or PEST sequences) appear to exist in most calpain substrates and are usually located near the cleavage sites$^5$.

Calpain inhibitory agents

Current calpain inhibitors

There have been a number of calpain inhibitory agents described in the literature that are commercially available (Table 1). The most potent of all is the naturally occurring endogenous calpain inhibitor protein, calpastatin. It inhibits both calpain I and II specifically, but not other proteases such as cathepsin B or papain. A 27 amino acid peptide based on a repeated region of calpastatin also retained the calpain inhibitory activity (Table 1).

Almost all of the calpain inhibitors reported in the literature are active-site-targeted peptide inhibitors. Many of these compounds were used in studies to determine the role of calpain in a disorder. The caveat is that none of them are highly selective for calpain, making data interpretation sometimes difficult. The most widely used are leupeptin, calpeptin, calpain inhibitor I and MDL28170, which belong to the peptide aldehyde class of inhibitors (Table 1). The latter three have demonstrated cell permeability$^4$. Unfortunately, this class of compound will also inhibit other cysteine proteases. E64 and E64c are members of the epoxy-succinyl peptides, which are capable of forming irreversible sulphide linkage with the active-site Cys thiol. While E64 does not inhibit serine proteases, it does inhibit papain, cathepsin B and L (Table 1). The membrane permeability of E64 and E64c are relatively poor, but this problem can be overcome by converting the free carboxylic acid group in E64c into an ethyl ester (E64d). Once entering cells, E64d can be readily hydrolyzed by cellular esterases back to the more active precursor E64c. Other classes of calpain inhibitors included peptide halo-methanes, peptide diazomethanes and peptide halo-hydrazides. The diazomethanes and halo-hydrazides (Table 1) were claimed to be more selective for inhibiting cysteine proteases over serine proteases, but are not commercially available.
**Future calpain inhibitors**

Most of the chemical structures being claimed as calpain inhibitors in the patent literature are peptide analogues (Fig. 2). These compounds range from large peptides, such as human calpastatin-like polypeptide and kinogen heavy chain analogue to modified dipeptides containing standard protease-inhibiting elements. They can also be classified into two categories: reversible inhibitors, such as aldehydes and ketones, and irreversible inhibitors, such as haloketones and epoxysuccinyl peptide analogues. Most of these small peptide analogues have submicromolar activity against calpain, but they generally have similar affinity toward inhibiting cathepsin B. However, two examples showed selectivity for calpain over cathepsin B: CH3(CH2)9CH(OH)CH2CONH-Asn-Gln-Leu-H and the peptidyl ketoamide \[Z\text{-Leu-Abu-CONH-(CH2)8CH3}\] showed a 30- and 1250-fold selectivity, respectively. As for larger peptides, poor membrane permeability is often a problem with these compounds.

A recent development is the emergence of nonpeptide calpain inhibitors (Fig. 2). These include an isocoumarin derivative, which has only low affinity for calpain.

### Table 1. Currently used calpain inhibitors

<table>
<thead>
<tr>
<th>Class</th>
<th>Inhibitor</th>
<th>Refs</th>
</tr>
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<tbody>
<tr>
<td>Calpastatin</td>
<td>Whole molecule (50–90 kDa)</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>27-mer calpastatin peptide</td>
<td></td>
</tr>
<tr>
<td>Peptide aldehydes</td>
<td>Leupeptin</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Calpain inhibitor I</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Calpain inhibitor II</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Calpeptin</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>MDL28170</td>
<td>65</td>
</tr>
<tr>
<td>Epoxysuccinyl peptides</td>
<td>E64</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>E64c</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>E64d</td>
<td>66</td>
</tr>
<tr>
<td>Peptide halomethanes</td>
<td>Z-Leu-Leu-Tyr-CH2F</td>
<td>67</td>
</tr>
<tr>
<td>Peptide diazomethanes</td>
<td>Z-Leu-Leu-Tyr-CHN2</td>
<td>67</td>
</tr>
<tr>
<td>Peptide halohydrazides</td>
<td>Z-Tyr(III)-NHNHCOCH2I</td>
<td>68</td>
</tr>
<tr>
<td>Peptide disulphides</td>
<td>Leu-Leu-Cys(Npys)-NH2</td>
<td>69</td>
</tr>
</tbody>
</table>

Tyrl), 3-iodo-l-tryosine; Npys, 3-nitro-2-pyridinesulphenyl.
(calpain I IC_{50}=10 \mu M; calpain II IC_{50}=120 \mu M) and it is also known to potently inhibit serine proteases.

### Cerebral ischaemia and excitotoxicity

Excessive synaptic levels of glutamate (the major excitatory neurotransmitter in the CNS) are neurotoxic\(^{11,12}\). This phenomenon (excitotoxicity) is thought to involve overactivation of ionotropic excitatory amino acid (EAA) receptors (NMDA, AMPA and kainate receptors) in the postsynaptic membrane, resulting in sustained influx of Na\(^+\) and Ca\(^{2+}\) through these ligand-gated ion channels. Na\(^+\) influx causes depolarization that opens voltage-gated neuronal Ca\(^{2+}\) channels, resulting in further Ca\(^{2+}\) influx. Neuronal Ca\(^{2+}\) reaches high nanomolar concentrations, and consequently activates a number of Ca\(^{2+}\)-dependent systems, including calmodulin, PKC, phospholipase A\(_2\) and calpain. Uncontrolled activation of one or perhaps all of these processes is thought to kill the neurones in a delayed fashion (Fig. 3).

In the past decade, excitotoxicity (resulting from excessive presynaptic release and impaired reuptake of glutamate\(^{11,12}\)) has been demonstrated to mediate neuronal injuries in cerebral ischaemia (for example, in stroke or cardiac arrest). In models of focal or global cerebral ischaemia \textit{in vivo}, antagonists of either the NMDA receptor (for example, dizocilpine) or the AMPA receptor [for example, 2,3-hydroxy-6-nitro-7-sulphamoyl-benzo(F)-quinoxaline (NBQX)] have been shown to provide significant neuroprotection\(^{11,12}\). However, targeting at ionotropic EAA receptors may not be ideal since psychotomimetic side-effects are apparently associated with NMDA receptor antagonism\(^3\).

### Calpain inhibition

As an alternative approach, researchers have suggested that inhibition of the overactivated Ca\(^{2+}\)-dependent processes could prevent neuronal death. Perhaps the most universal marker for calpain activation is the demonstration of spectrin (220 kDa) breakdown by calpain, producing the characteristic fragments of 150 kDa and 145 kDa. In both \textit{in vitro} (hippocampal slices) and \textit{in vivo} models of ischaemia, spectrin breakdown products were readily observed\(^{14,15}\). Other cytoskeletal proteins, such as microtubule-associated protein 2 (MAP-2) and neurofilament proteins, are also susceptible to calpain. Extensive cytoskeleton and plasma membrane damage by calpain can translate to increased membrane permeability to ions or even macromolecules and the eventual death of neurones (Fig. 3). Evidence to demonstrate that calpain activation promotes cell death has been obtained from studies involving calpain inhibitors in neuronal culture, brain slices and \textit{in vivo} ischaemia models:

(1) In chick embryonic neuronal cultures, NaCN-induced hypoxic damage was partially blocked by leupeptin and calpain inhibitor I (Ref. 16). E64 (10 \mu M) MDL28170 (10 \mu M) and leupeptin (100 \mu M) also protected cerebellar Purkinje cells from AMPA toxicity\(^{16}\). However,
a lack of correlation between calpain inhibition (reflected by spectrin breakdown) and neuroprotection has been reported in cultured cerebellar granule cells\textsuperscript{18,19}. It is conceivable that the granule cells have adopted a calpain-independent cell-death pathway.

(2) Cell-permeable calpain inhibitor I and MDL28170 were found to improve functional recovery of both hippocampal slices and gerbil neocortical slices from hypoxia\textsuperscript{20,21}. MDL28170 also protected Purkinje cells in cerebellar slices against AMPA-toxicity\textsuperscript{22}.

(3) Administration of leupeptin in a prolonged ventricular infusion paradigm enhanced hippocampal CA1 neurone survival in gerbils subjected to transient ischaemia\textsuperscript{23}. At the same time, spectrin breakdown was also reduced. These results suggest that in spite of poor cell permeability, neuroprotection can be achieved when enough compound is accumulated. More recently, i.v. administration of MDL28170 significantly reduced infarct size in a rat focal ischaemia model\textsuperscript{24}. Similarly, calpain inhibitor I reduced neuronal damage of the hippocampal CA1 subfield in global ischaemia in rats\textsuperscript{25}. Bartus \textit{et al.} recently reported that cerebral perfusion of AK275, a new calpain inhibitor, reduced infarct size in a rat middle cerebral artery occlusion model\textsuperscript{26}.

Calpain inhibitors may be strategically superior to antagonists of EAA receptors since calpains exist normally in a latent form. It is likely that only a very small fraction of calpains (such as those located in the cytosol just below the plasma membrane) could be activated as a signal transduction pathway, while the majority of them remain inactive, as evidenced by the lack of spectrin breakdown in neurones stimulated with non-toxic concentrations of NMDA or AMPA. Thus, it is reasonable to assume that inhibition of calpains would not lead to profound CNS side-effects.

**Traumatic brain injury**

Excessive intracellular Ca\textsuperscript{2+} accumulation in traumatic brain injuries, presumably resulting from the activation of ionotropic EAA receptors, is well documented\textsuperscript{12}. Evidence for calpain translocation to the membrane fraction in a rabbit brain trauma model has also been reported\textsuperscript{26}, and hence it seems reasonable to assume that the excitotoxicity associated with head trauma also involves calpain activation.

**Subarachnoid haemorrhage**

Aneurysmal subarachnoid haemorrhage (SAH) is known to induce long-lasting cerebral vasospasm. The restricted blood flow thus triggers ischaemic events similar to those in stroke\textsuperscript{27}. It has been suggested that endothelin is released from endothelial cells in response to the invasion of oxyhaemoglobin from the blood clots, and the
subsequent endothelin receptor activation in vascular smooth muscle cells leads to the long-lasting spastic response.

In a canine SAH model, vasospasm can be induced by successive intracisternal injection of autologous blood near the basilar artery. The resulting blood clot induces a spastic response on the blood vessel within a few days\(^\text{28}\). In this model, topical application of calpeptin dilated the exposed spastic basilar artery\(^\text{28}\). Although the mechanism is unclear, the involvement of proteolytic activation of PKC by calpain has been suggested since the PKC inhibitor H7 also had the same vasodilating effect. The involvement of calpain was further supported by the observed increase of spectrin breakdown in the smooth muscle of spastic basilar artery in a rabbit model of SAH (Ref. 29). Cerebral vasospasm has also been shown to cause ischaemic stroke\(^{30}\), and therefore it appears that calpain inhibitors could further provide beneficial effects at the vasculature level.

Other neurological disorders

Chronic neurodegeneration
In several chronic neurodegenerative disorders such as Huntington’s disease, Parkinson’s disease and amyotrophic lateral sclerosis, there is evidence to suggest that glutamate toxicity contributes at least in part to the neurodegeneration\(^\text{11,12}\). It is conceivable that calpain inhibitors may ameliorate the accompanying excitotoxicity in these disorders.

Alzheimer’s disease
The pathogenesis of Alzheimer’s disease has been linked to an abnormal processing of the amyloid precursor protein (APP) to produce the \(\beta\)-amyloid peptide, which, by self-aggregation, forms the major component of senile plaques in the brain over decades. Together with other proteases, calpain is capable of fragmenting APP (Ref. 31). It appears to cleave APP at three different sites. Since all cleavages are located extracellularly, if calpain were to attack APP, it is likely that internalized APP has been cleaved. The cleavage that occurs nearest to the C-terminus would produce a fragment that contains the whole of the \(\beta\)-amyloid peptide (amyloidogen) if it is further processed by another protease. Incidentally, calpain immunoreactivity was also found in senile plaques\(^{32}\). There is also evidence of calpain activation in the brain of Alzheimer’s patients, as judged by the increased ratio of the activated 76 kDa form to the inactive 80 kDa form of the calpain I large subunit\(^{33}\).

Spinal cord injury
Calpain is capable of degrading myelin sheath proteins\(^{34}\) and was found co-localized with fragmented myelin in myelinated nerve fibres from rabbit\(^{35}\). It is tempting to suggest that calpain may be mediating myelin degradation as seen in demyelination disorders such as Wallerian degeneration, multiple sclerosis and peripheral nervous neuropathy. In experimental spinal cord injury, \(\text{Ca}^{2+}\) accumulates within the injured axons, resulting in the activation of calpain and hence contributing to the degeneration of axons and myelins\(^{36}\). Leupeptin has also been found to reduce axonal damage in an experimental spinal cord injury model\(^{37}\).

Cardiac ischaemia
The injury sustained by cardiomyocytes during ischaemic myocardial infarction is likely similar to that observed in neurones in cerebral ischaemia. However, cardiomyocytes appear to suffer from a secondary form of injury during coronary reperfusion. The involvement of calpain is suggested from the demonstration that myofibrillar proteins including myosin heavy chain, troponin T and I, tropomyosin a and \(\alpha\)-actinin are susceptible to calpain\(^{38}\). In freshly isolated neonatal rat myocytes, calpain activity has been shown to be elevated during hypoxia, as evidenced by the presence of spectrin breakdown products\(^{39,40}\). Furthermore, spectrin breakdown could be prevented by 10 \(\mu\)M of calpain inhibitor I or E64, and while hypoxia alone caused about 60% cell death in six hours, the presence of either inhibitor reduced cell death to slightly above the normoxic level.

The isolated heart can be subjected to a temporary (for example, 15 min) global ischaemia followed by reperfusion; this is known as myocardial stunning\(^{41}\). Generally, this reversible insult leads to compromised mechanical myocardial functions, which can be measured by parameters such as developed pressure. Matsumura et al.\(^{41}\) showed that leupeptin significantly enhanced myocardium functional recovery following stunning. E64c was used as a potential protective agent in a dog model where acute myocardial infarction was induced by occlusion of the left anterior descending artery\(^{42}\). The combination of E64c and reperfusion was found to significantly reduce infarct size but E64c alone did not show significant reduction of infarct size.

The evidence provided by these models indicate that calpain is activated during myocardial ischaemia and several cysteine protease inhibitors that inhibit calpain showed beneficial effects. However, this area is still in its infancy.

Muscular dystrophy
Dystrophin is a protein located in the sarcolemma that regulates the stretch-sensitive \(\text{Ca}^{2+}\) ‘leak’ channels\(^{43}\), and is absent in Duchenne muscular dystrophy patients as well as mdx mice as a result of a genetic defect. In mdx mice, intracellular \(\text{Ca}^{2+}\) levels in skeletal muscle fibres were found to be significantly raised with a concomitant increase in the rate of protein degradation\(^{44}\). The rate of protein degradation could be reduced to normal levels by leupeptin\(^{45}\). As noted above, myofibrillar protein turnover, both normal and abnormal, is probably mediated by calpain\(^{36}\). It has also been observed that the calpain II level is apparently higher in dystrophic hamster skeletal muscles\(^{47}\). Interestingly, mdx mouse myotubes cultured continuously in the presence of leupeptin (50 \(\mu\)M) did not
experience the elevation of intracellular Ca\(^{2+}\) levels that is normally seen in mdx muscles\(^{45}\). Hence, it can be postulated that calpain degrades a sarcosomal protein that leads to further increased opening of the Ca\(^{2+}\) leak channels, and if this hypothesis is correct, calpain inhibition should have the dual benefit of reducing myofibrillar protein degradation and restoring Ca\(^{2+}\) homeostasis.

**Cataract**

The mammalian lens is a layer of growing epithelial cells covering layers of densely packed terminally differentiated fibre cells. These cells contain a very high concentration of proteins and approximately 30% of the total protein mass are crystallins (\(\alpha\)-, \(\beta\)-, \(\gamma\)- and \(\delta\)-crystallin). Over its life time, the lens may be damaged by ultraviolet radiation, hereditary disorders, hyperglycaemia (diabetes), exposure to toxic chemicals or drugs such as steroids, resulting in membrane protein and/or lipid oxidation, and consequently the formation of lens opacity or cataract. It is currently believed that oxidative damage increases lens membrane permeability, which leads to elevated cytosolic Ca\(^{2+}\) levels. Activated calpain thus fragments and precipitates crystallins, to produce lens opacity (Fig. 4). To date, no therapeutic agents can significantly prevent, slow down or block cataract formation. The only medical intervention is lens replacement surgery.

High concentrations of calpain II were found in both lens epithelium and differentiated fibre cells while the levels of calpain I were considerably lower. When a cataract is chemically induced, the lens cells have been found to accumulate as much as 600 \(\mu\)M of free Ca\(^{2+}\), which should be sufficient to activate calpain II (Ref. 48). Furthermore, in cultured rat lens treated with calcimycin, spectrin breakdown could be observed\(^{49}\). Calpain II can also fragment \(\beta\)-crystallins in *vivo* causing their eventual insolubilization\(^{50}\). Sequencing of the N-terminus revealed that the *in vitro* calpain cleavage sites of \(\beta\)-crystallins matched those found *in vivo* in the selenite-induced cataract in rats\(^{50}\).

Several calpain inhibitory agents have been examined for their effect on cataract formation: E64 (5 \(\mu\)M–1 mM) and MDL28170 (500 \(\mu\)M) were effective in reducing opacity while markedly reducing the insolubilization of \(\beta\)-crystallins\(^{51,52}\). Calpain inhibitor I and II were not protective, most likely because of their cytotoxicity\(^{51}\). High concentrations of these inhibitors were required to ensure that a sufficient amount could penetrate deep into the nuclear region of the lens. Subsequently, the membrane-permeable E64d was found to be more potent (EC\(_{50}\) 71 \(\mu\)M) than E64 in reducing opacity in calcimycin-treated lens\(^{53}\).

The effects of E64 on selenite cataract formation *in vitro* were also investigated. Rats were given E64 i.p. two hours before the administration of selenite, and a daily dose of E64 was continued for five days. E64 was shown to reduce the frequency of the most severe stage of cataract, nuclear cataract\(^{45}\). However, even in the E64-treated group, \(\beta\)-crystallin breakdown continued to occur, but at a reduced rate, and hence it was interpreted that the amount of E64 was still not sufficient to neutralize all calpain activities.

It is not yet clear how these findings in rats can be translated to humans but if calpain is shown to be involved in the development of human cataract, calpain inhibitors may be an economical alternative to lens replacement surgery.

**Thrombotic platelet aggregation**

There are high concentrations of both calpain I and II in platelets, and a number of platelet proteins are degraded by calpain during platelet activation\(^{53}\). The key event resulting in platelet aggregation is the binding of fibrinogen to fibrinogen receptors on the platelet surface following activation of platelets by stimuli such as ADP, thrombin or plasmin. The current consensus is that the membrane-bound ADP receptor (aggregin) is somehow responsible for the latency of the fibrinogen receptor. When platelets are stimulated with, for example, thrombin, concentrations of intracellular free Ca\(^{2+}\) rise to 1–5 \(\mu\)M, which trigger calpain activation. Calpain then hydrolyses aggregin, and hence exposes the fibrinogen receptors to the platelet surface\(^{54,55}\). This allows fibrinogen to bind to the platelets, which promotes aggregation. In support of this pathway, it has been shown that calpain inhibitors leupeptin, antipain and Phe-Gln-Val-Val-Cys(3-nitro-2-thiopyridine)-Gly-NH\(_2\) blocked aggregin breakdown and platelet aggregation\(^{55}\).
Table 2. Therapeutic areas in which calpain overactivation is implicated

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Proposed mechanism</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke</td>
<td>Degradation of cytoskeletal proteins and other cellular proteins lead to neuronal cell death and permanent tissue damage</td>
<td>16-25</td>
</tr>
<tr>
<td>Brain trauma</td>
<td>Similar mechanism to stroke</td>
<td>26</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage</td>
<td>Calpain proteolytically activates protein kinase C, leading to sustained cerebral vasospasm</td>
<td>28-29</td>
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<tr>
<td>Alzheimer’s disease</td>
<td>Abnormal processing of amyloid precursor protein by calpain</td>
<td>31-33</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>Degradation of myelin proteins</td>
<td>34-37</td>
</tr>
<tr>
<td>Cardiac ischaemia</td>
<td>Calpain activation leads to breakdown of myofibril proteins causing cardiac myocyte shrinkage, cell death and tissue damage</td>
<td>39-42</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>In Duchenne muscular dystrophy, genetic deletion of the dystrophin gene leads to compromised Ca^{2+} homeostasis allowing calpain to break down myofibril proteins</td>
<td>44-47</td>
</tr>
<tr>
<td>Cataract</td>
<td>Lens protein (crystallins) breakdown by calpain in ageing lens leads to precipitation that causes lens opacity</td>
<td>48-5</td>
</tr>
<tr>
<td>Thrombotic platelet aggregation</td>
<td>Thrombin increases intracellular Ca^{2+} levels in platelets, thus activating calpain-mediated proteolysis of aggregin and promoting platelet aggregation</td>
<td>54,55</td>
</tr>
<tr>
<td>Renarrowing of blood vessel after angioplasty owing to calpain-mediated proliferation and migration of smooth muscle cells</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>Calpain is found in synovial fluid and is capable of breaking down cartilage and the extracellular matrix component proteoglycan</td>
<td>59-61</td>
</tr>
</tbody>
</table>

Restenosis

Percutaneous transluminal coronary angioplasty is a widely accepted medical procedure to expand the inner diameter of clotted arteries in patients with atherosclerotic coronary artery disease. However, the success rate of this procedure is dampened by the spontaneous, slow renarrowing of the arteries (restenosis)\(^5\). Recently, it has been shown that calpeptin (100 \(\mu\)M) and calpain inhibitor I (50 \(\mu\)M) blocked the cell cycle at the G2-M phase and prevented the proliferation of cultured aortic smooth muscle cells\(^5\). The same agents were shown to inhibit angioplastic restenosis\(^5\). It is likely that the calpain inhibitors retard restenosis by controlling proliferation and migration of smooth muscle cells to the neointimal layer of the blood vessels.

Joint inflammation and arthritis

Both calpain I and II were found extracellularly in the synovial fluid of the knee joint, especially in patients with rheumatoid arthritis\(^5,6\). Furthermore, the major cartilage matrix component, proteoglycan, is reported to be a calpain substrate\(^6\). It is hypothesized that externalized calpain damages the extracellular matrix and contributes to the inflammation process, together with other cytoste proteins and metalloproteinases. However, it is not yet known if calpain inhibitors have any beneficial effects when applied to inflamed joints.

Perspectives

In summary, it is evident that calpain overactivation may be a key component in a number of disorders (Table 2), and the common theme for most of these disorders is cellular Ca^{2+} overload. The physiological roles of calpains are not yet defined, but they are thought to be involved in specific unidirectional cellular processes (such as mitosis and membrane fusion). Since a sustained increase of intracellular Ca^{2+} concentrations is required for calpain to be activated, calpain largely exists in a latent form in resting cells. Therefore, it would appear that calpain is an ideal pharmacological target since this protease is most active at the time of pathology. Furthermore, acute side-effects are not anticipated as a result of calpain inhibition since calpain is rarely activated under physiological conditions. This will particularly be the case if a selective calpain inhibitor can be identified.

Selected references
26 Heng, S. C. et al. (1994) Stroke 25, 663-669

**Chemical names**

**AK275**: Z-Leu-Abu-CONHCH₃CH₃

**E64**: trans-epoxyasuccinyl-l-leucylamido-4-guanidino-3-butyrate

**E64c**: (2S,3S)-trans-epoxyasuccinyl-l-leucylamido-3-methylbutyrate

**E64d**: (2S,3S)-trans-epoxyasuccinyl-l-leucylamido-3-methylbutyrate ethyl ester

**H7**: 1-(5-isoquinolylsulphonyl)-2-methylpiperazine

**MDL28170**: Z-Val-Phe-H

**MK801**: (5S,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[α,β]cyclohepten-5,10-imine