Calpain inhibition: an overview of its therapeutic potential

Kevin K. W. Wong and Po-Wai Yuen

Increasing evidence now suggests that excessive activation of the Ca²⁺-dependent protease calpain could play a key or co-operative role in the pathiology of a variety of disorders, including cerebral ischaemia, cancer, myocardial ischaemia, muscular dystrophy and atherosclerosis. In this review, Kevin Wong 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 Ca²⁺-signalling in mammalian cells is the Ca²⁺-activated neutral protease calpain. There are two major isoforms: calpain I (or α-calpain) and calpain II (or μ-calpain), which require low and high micro-molar Ca²⁺ concentrations for activation, respectively. Calpain consists of two subunits of about 80kDa and 29kDa (Fig. 1). The larger 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 Cys64 and Cys99 (Fig. 1). Domain III has unknown function and is not homologous to other proteases. Domain IV has four EF-hand Ca²⁺-binding sites. The N-terminal half of the small subunit appears to be important for interaction with phospholipids (for example, phosphorylinositol 4,5-bisphosphate), which lower its Ca²⁺ requirement. The C-terminal half has another four EF-hand structures. It is assumed that these Ca²⁺-binding sites impose a strict Ca²⁺ requirement on the catalytic activity, but the number of occupied Ca²⁺-binding sites required for activity remains debatable.

Following the addition of Ca²⁺, calpain autoinhibits by trancracting an N-terminal portion of domain I and most of domain V. The catalytic activity of this truncated form (56kDa and 14kDa) is still Ca²⁺-dependent. It is uncertain whether this step is reversible before calpain can hydrolyse 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). Interestingly, nCL-1 contains a nuclear translocation-like sequence (Lys-Pro-Lys-Arg-Lys-Arg) in its catalytic domain, while nCL-2 can be alternatively spliced to generate a form that totally lacks the Ca²⁺-binding domain (nCL-2, Fig. 1).

Calpain appears to be a selective for a subset of cellular proteins. 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 sites. 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, Gln, Arg and Lys (from PEST sequences) appear to exist in most calpain substrates and are usually located near the cleavage sites.

Calpain inhibitory agents

Current calpain inhibitors

There have been a number of calpain inhibitors agents described in the literature but 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 against proteases. 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 MDL27370, which belong to the peptide aldehyde class of inhibitors (Table 1). The latter three have demonstrated cell permeability. Unfortunately, this class of compound will also inhibit other cysteine proteases. Enb and Enb-1 are members of the proinflammatory peptides, which are capable of forming irreversible adducts with the cysteine residues of calpain. While Enb does not inhibit serine proteases, it does inhibit papain, carboxypeptidase B and L (Table 1). The membrane permeability of Enb and Enb-1 are relatively poor, but this problem can be overcome by converting the free carboxylic acid group in Enb into an efflux vector (EnbD). Once entering cells, EnbD can be oxidized and detoxified by cell-free extracts to produce the active form EnbD. Other classes of calpain inhibitors included peptide halo- methanes, peptide disulfonamides and peptide halohydrazides. The thioamidates and haloalkylamides (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 polyprotease 3 and kininogen heavy chain analogues 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 haloacetone, diazoketone and epoxy succinyl peptide analogues 3. 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)6CH(OH)CH2CH(OH)CH2Asp-Glu-Leu-H and the peptideyl ketomamide [Z-Leu-Ala-CONH2]-[CH3(CH2)6CH(OH)CH2] showed a 30- and 1250-fold selectivity, respectively 4. As for larger peptides, poor membrane permeability is often a problem with these compounds.

A recent development is the emergence of multipeptide calpain inhibitors (fig. 2). These include an isocongenin derivative 5, 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>
</thead>
<tbody>
<tr>
<td>Synthetic</td>
<td>Whole molecule [89-90]</td>
<td>62</td>
</tr>
<tr>
<td>7-amino capsaicin peptide</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Peptide alkylheterocycles</td>
<td>Leupentin</td>
<td>62</td>
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<tr>
<td>Calpain inhibitor I</td>
<td>Calpain inhibitor II</td>
<td>63</td>
</tr>
<tr>
<td>Calpain inhibitor III</td>
<td>Calpain inhibitor IV</td>
<td>64</td>
</tr>
<tr>
<td>MCL2/170</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Peptide analogues of peptides</td>
<td>pEF-1</td>
<td>9</td>
</tr>
<tr>
<td>pEF-1c</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>pEF-1d</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Peptide homomorphs</td>
<td>Z-Leu-Leu-Leu-ChlF</td>
<td>67</td>
</tr>
<tr>
<td>Peptide diacylamides</td>
<td>Z-Leu-Leu-2CH2Cl</td>
<td>68</td>
</tr>
<tr>
<td>Peptide diacylamides</td>
<td>Z-Leu-Me-CH2Cl</td>
<td>68</td>
</tr>
<tr>
<td>Peptide diacylamides</td>
<td>Leu-Leu-Cys(NHAc)NH2</td>
<td>69</td>
</tr>
<tr>
<td>trypp. 3-lido-lysine, Nps, 5-ido-2-proline-phenyl</td>
<td>69</td>
<td></td>
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Cerebral ischaemia and excitotoxicity

Excitatory synaptic levels of glutamate (the major excitatory neurotransmitter in the CNS) are neurotoxic.[1] 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 Ca²⁺ through these ligand-gated ion channels. Na⁺ influx causes depolarization that opens voltage-gated neuronal Ca²⁺ channels resulting in further Ca²⁺ influx. Neuronal Ca²⁺ release high intracellular concentrations, and consequently activates a number of Ca²⁺-dependent systems, including calmodulin, PKC, phosphatase A₂, and calpains. 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 unregulated release of glutamate has been demonstrated to mediate neuronal injury in cerebral ischaemia (for example, in stroke or cardiac arrest). Models of focal or global cerebral ischaemia in rats, agonists of either the NMDA receptor (for example, dizocilpine) or the AMPA receptor (for example, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzofuran (DNQX) have been shown to provide significant neuroprotection.[2,3] However, targeting of ionotropic EAA receptors may not be ideal since psychomimetic side-effects are apparently associated with NMDA receptor antagonists.[4]

Calpain inhibition

As an alternative approach, researchers have suggested that inhibition of the overactivated Ca²⁺-dependent processes could prevent neuronal death. Perhaps the most universal mechanism for calpain activation is the demonstration of spectrin (120kDa) breakdown by calpain, producing the characteristic fragments of 45kDa and 28kDa. In both in vivo (hippocampal slice) and in vivo models of ischaemia, spectrin breakdown products were readily observed.[5,6,7] 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 in dogs that calpain activation promotes cell death has been obtained from studies involving calpain inhibitors in neuronal culture, brain slices and in vivo ischaemic models.[8]

In chick embryonic neuronal cultures, N,N-diCys-4,5-hexapeptide (N,N-diCys-4,5-hexapeptide) blocks calpain activity and protects neurones against excitotoxic injury.[9] In the adult rat, N,N-diCys-4,5-hexapeptide (N,N-diCys-4,5-hexapeptide) blocks calpain activity and protects neurones against excitotoxic injury.[10] However,
a lack of correlation between calpain inhibition (reflected by spectrin breakdown) and neuroprotection has been reported in cultured cerebellar granule cells. 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. MDL28170 also protected Purkinje cells in cerebellar slices against AMPA-toxicity.

3) Administration of leptin in a prolonged ventricular infusion paradigm enhanced hippocampal CA1 neurone survival in gerbils subjected to transient ischaemia. 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. Similarly, calpain inhibitor I reduced neuronal damage of the hippocampal CA1 subfield in global ischaemia in rats. Barros et al. recently reported that cerebral perfusion of AK275, a new calpain inhibitor, reduced infarct size in a rat middle cerebral artery occlusion model.

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 these located in the cytoplasm) 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 neurons 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\(^{2+}\) accumulation in traumatic brain injuries, presumably resulting from the activation of ionotropic EAA receptors, is well documented. Evidence for calpain translocation to the membrane fraction in a rabbit brain trauma model has also been reported and hence it seems reasonable to assume that the excitotoxicity associated with head trauma also involves calpain activation.

Subarachnoid haemorrhage

Anxuroyal subarachnoid haemorrhage (SAH) is known to induce long-lasting cerebral vasospasm. The restricted blood flow then triggers ischaemic events similar to those in stroke. It has been suggested that endothelin released from endothelial cells in response to the invasian of oxyhaemoglobin from the blood clots, and the
subsequent endothelial receptor acquisition in vascular smooth muscle cells leads to the long-lasting spastic response. In a case of SLE model, vasospasm can be induced by successively intracoronary injection of autologous blood near the basilar artery. The resulting blood clot induces a spastic response on the blood vessel within a few days. In this model, topical application of nitrater dilates the exposed spastic basilar artery. Although the mechanism is unclear, the involvement of prototypic activation of PKC by calpain has been suggested since the PKC inhibitor H7 had also 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 SLE (Ref. 29). Cerebral vasospasm has also been shown to cause ischemic stroke, and therefore it appears that calpain inhibition 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. 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 the abnormal processing of the amyloid precursor protein (APP) to produce the β-amyloid peptide, which, by self-aggregation, forms the major component of senile plaques in the brain over decades. Together with other proteins, calpain is capable of fragmenting APP (Fig. 31). It appears to down 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 should produce a fragment that contains the N-terminal 87 residues of APP and the entire 77-residue amyloidogenic C-terminal half. This is also evidence of calpain activation in the brain of Alzheimer's patients, as judged by the increased ratio of the C-terminus fragment to the inactive 80-kDa form of the calpain I large subunit.

Ischemic injury

Calpain is capable of degrading myelin sheath proteins and was found co-localized with fragmented myelin in myelinated nerve fibres from rabbit. It is tempting to suggest that calpain may be mediating myelin degradation as seen in demyelinating disorders such as Wallerian degeneration, multiple sclerosis and perivenous necrotic necrosis. In experimental spinal cord injury, calpain 1 accumulates within the injured area, resulting in the activation of calpains and hence contributing to the degeneration of axons and myelin. Leupeptin has been found to reduce axonal damage in an experimental spinal cord injury model.

Cardiac ischaemia

The injury sustained by cardiomyocytes during ischaemic myocardial infarction is likely similar to that observed in neurons in cerebral ischaemia. However, cardiomyocytes appear to suffer from a secondary form of injury during coronary repulsion. The involvement of calpain is suggested from the demonstration that myocardial proteins including myosin heavy chain, tropomyosin 1 and 2, tropomysosin A and actin are susceptible to calpain. 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. Furthermore, spectrin breakdown could be prevented by 10 μmol 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 normal 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. Generally, this reversible insult leads to compromised myocardial function, which can be measured by parameters such as developed pressure. Makrides et al. showed that leupeptin significantly enhanced myocardial functional recovery following stunning. E64 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. The combination of E64 and reperfusion was found to significantly reduce infarct size, but E64 alone did not show significant reduction of infarct size.

The evidence provided by these models indicates that calpain is activated during myocardial ischaemia and several cellular 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 sarcosomes that regulates the stretch-sensitive calpain 3 'back channelling', and is absent in Duchenne muscular dystrophy patients as well as rods seen as a result of a genetic defect. In mdx mice, intracerebral calpain 2 levels in skeletal muscle fibres were found to be significantly raised with a concurrent increase in the rate of protein degradation. The rate of protein degradation could be reduced to normal levels by leupeptin. As noted above, myeloblastar protein turnover, both normal and abnormal, is probably mediated by calpains. It has also been observed that the calpain I level is apparently higher in dystrophic hamster skeletal muscle. Interestingly, mdx mouse myotubes cultured continuously in the presence of leupeptin (0.1 μmol) did not
experience the elevation of intracellular Ca²⁺ levels that is normally seen in red Crusades. Hence, it can be postulated that calfins degrades a sarcosomal protein that leads to further increased opening of the Ca²⁺-loosing channels, and if this hypothesis is correct, calfins inhibition should have the dual benefit of reducing myofibrillar protein degradation and restoring Ca²⁺ 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 is crystallins (α, β, γ, and γ-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 highly elevated cytosolic Ca²⁺ levels. Activated calcinus thaps and precipitates calcinus, 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 calcinus II were found in both lens epithelium and differentiated fibre cells while the levels of calcinus I were considerably lower. When a cataract is chemically induced, the lens cells have been found to contain 680 μg of free Ca²⁺, which should be sufficient to activate calcinus II (Ref. 40). Furthermore, in cultured rat lens treated with calcium ions, sperry breakdown could be observed. Calcinus II can also fragment β-crystallins in vitro causing their eventual insolubilization50. Sequencing of the N-terminals revealed that the in vitro calcinus cleavage sites of β-crystallins matched those found in rats in the in vivo—induced cataract in rats.

Several calcinus inhibitory agents have been examined for their effect on cataract formation: 17-β-estradiol (1 μM) and MDL28170 (50 μM) were effective in reducing opacity while markedly reducing the insolubilization of β-crystallins51. Calcinus inhibitor I and II were not protective, most likely because of their cytotoxicity52. 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 E64 was found to be more potent (10 μM) than E64 in reducing opacity in calcium-treated lens.

The effects of E64 on senile cataract formation in rats were also investigated. Rats were given E64 i.p. twice a day for eight days before the administration of sodium, 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 cataract53. However, even in the E64-treated group, β-crystallin breakdown continued to occur, but at a reduced rate, and hence it was interpreted that the amount of E64 was still sufficient to maintain all calcinus activities. It is not yet clear how these findings in rats can be translated to humans but if calcinus is shown to be involved in the development of human cataract, calcinus inhibitors may be an economical alternative to lens replacement surgery.

Thrombogenic platelet aggregation

There are high concentrations at both calcinus 1 and 2 in platelets, and a number of platelet proteins are degraded by a-calcin in platelet aggregation54. 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 (aggrecin) is somehow responsible for the latency of the fibrinogen receptor. Wism platelets are stimulated with, for example, fibrinogen concentrations of intracellular free Ca²⁺ rise to 1-5 μM, which trigger calcinus activation. Calcinus then hydrolyses aggrecin, and hence exposes the fibrinogen receptors to the platelet surface55. This shows fibrinogen to bind to the platelets, which promotes aggregation. In support of this pathway, it has been shown that calcinus inhibitors (e.g., antipar and Phe-Glu-Val-Val-Cys(OMe)-gly-gly-NH2, blocked aggregin breakdown and platelet aggregation56.)
Table 2: Therapeutic areas in which calcineurin overactivation is implicated

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Proposed mechanism</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke</td>
<td>Degradation of cytoskeletal proteins and other cellular proteins leading to neuronal cell death and permanent tissue damage</td>
<td>10-26</td>
</tr>
<tr>
<td>Brain injury</td>
<td>Serosal disruption</td>
<td>28</td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>Calcineurin phosphorylation is an essential protein kinase C-related to maintenance of cerebral blood flow</td>
<td>29,29</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>Abnormal processing of amyloid precursor protein by calpain</td>
<td>71-32</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>Degradation of myelin proteins</td>
<td>56-36</td>
</tr>
<tr>
<td>Traumatic/traumatic</td>
<td>Calcineurin-mediated breakdown of myelin protein causing cellular/extracellular cell wall and tissue damage</td>
<td>59-42</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>In Duchenne muscular dystrophy, genetic deletion of the dystrophin gene leads to compromised sarcolemma, affecting calcium to break down myofibril proteins</td>
<td>44-47</td>
</tr>
<tr>
<td>Cataract</td>
<td>Lens profile (crystallizzation) breakdown by calpain in aging lens leads to precipitation that causes lens opacity</td>
<td>49-52</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>In rheumatoid arthritis, calcineurin-mediated pro-inflammatory effects lead to sarcolemmal disruption and activation of cellular functions</td>
<td>53,53</td>
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<tr>
<td>Renal failure</td>
<td>Renal remodeling after angioplasty causes cell damage in the blood vessels</td>
<td>57,58</td>
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<tr>
<td>Arthritis</td>
<td>Calcineurin found in synovial fluid and is thought of breaking down cartilage in the cartilaginous matrix component of proteoglycan</td>
<td>59-67</td>
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</table>

Redothesis

Percutaneous transluminal coronary angioplasty is a widely accepted medical procedure to expand the inner diameter of occluded arteries in patients with athero-

clastic coronary artery disease. However, the resistance of this procedure is dampened by spontaneous stenosis and slow remolding of the arteries. Recently, it has been shown that calcineurin (100μg) and calcineurin inactivated (100μg) inhibited cell cycle at the G2-M phase and prevented the proliferation of cultured vascular smooth muscle cells. The same agents were shown to inhibit angiogenesis in vitro. It is likely that the calcineurin inhibitors inhibit overactivation by controlling proliferative and migration of smooth muscle cells to the neoinflammatory layer of the blood vessels.

Joint inflammation and arthritis

Both calcineurin I and II were found extracellularly in the synovial fluid of the knee joint, especially in patients with rheumatoid arthritis. Furthermore, the major cartilage matrix component, proteoglycan, is reported to be a calcineurin substrate. It is hypothesized that externalized calcineurin damages the extracellular matrix and contributes to the inflammatory process. Together with other proteases and metalloproteases, however, it is not yet known if calcineurin inhibitors have any beneficial effects when applied to inflamed joints.

Perspectives

In summary, it is evident that calcineurin overactivation may be a key component in a number of disorders (Table 2) and the common theme for most of these disorders is cellular Ca2+ overload. The physiological roles of calcineurins are not yet defined, but they are thought to be involved in systemic unidirectional cellular processes (such as mitosis and membrane fusion). Since a sustained increase in intracellular Ca2+ concentrations is required for calcineurin to be activated, calcineurin largely exists in a latent form in resting cells. Therefore, we would expect that calcineurin is an ideal pharmacological target since this protein is not active at the time of pathology. Furthermore, acute side-effects are not anticipated as a result of calcineurin inhibition since calcineurin is rarely activated under physiological conditions. This will particularly be the case if a selective calcineurin inhibitor can be identified.

Selected references
