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(71) Applicant (for all designated States except US): THE UNIVERSITY OF BRISTOL [GB/GB]; Senate House, Tyndall Avenue, Bristol BS8 1TH (GB).

(72) Inventor; and

(75) Inventor/Applicant (for US only): UNEY, James, Brian [GB/GB]; The University of Bristol, Dept. of Medicine, Marlborough Street, Bristol BS2 8H2 (GB).

(74) Agents: TOMBLING, Adrian, George et al.; Withers & Rogers, Goldings House, 2 Hay’s Lane, London SE1 2HW (GB).

(54) Title: TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES

(57) Abstract: Use of a tissue inhibitor of a matrix metalloproteinase (TIMP) in the preparation of a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder in a patient.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES

The present invention relates to the use of tissue inhibitors of matrix metalloproteinases, particularly, in the treatment of stroke and other neurodegenerative conditions.

Matrix Metalloproteinases (MMPs) are a family of zinc endopeptidases that can degrade or remodel most major components of the extra-cellular matrix (ECM). At least 21 MMPs have thus far been identified which can be divided into collagenases, stromelysins, gelatinases, elastase, membrane type MMPs and other MMPs (Nelson, A. R. et al., (2000) J Clin Oncol 18: 1135). While some MMPs show substrate specificity, many act on several classes of ECM substrate. In vivo, the activity of MMPs is regulated at three levels: transcription of the gene, proenzyme secretion and activation and through specific protein interaction with another family of molecules known as the tissue inhibitors of MMPs (TIMPS). Together these molecules regulate the cell matrix interactions that are associated with a variety of physiological processes. A disturbance in this balance can lead to a number of cellular malfunctions and tissue injuries.

A number of different signals, including growth factors, integrins and viruses, can activate transcription of MMPs. However, MMPs are synthesized and secreted from the cell in a proactive or inactive form and require further processing in order to yield active metalloproteinase. Once activated, MMPs can be inhibited by the presence of TIMPs.

To date, four TIMPs have been identified (Docherty, A. J. et al., (1985) Nature 318: 66-69; Stetler-Stevenson, W. G. et al., (1992) Matrix Suppl, 1: 299-306; Uria, J. A. et al., (1994) Cancer Res, 54: 2091-2094; Leco, K. J. et al., (1997) FEBS Lett, 401: 213-217). They are proteins with M, of 22kDa to 30kDa, share 28 to 40% sequence homology and are expressed from distinct genes. Mammalian TIMPs are two-domain molecules, with an N-terminal domain of about 125 amino acids and a C-terminal domain of about 65 residues. The two domains are stabilized by three disulphide bonds (Brew, K. et al., (2000) Biochim Biophys Acta, 1477: 267-283). Like MMPs, their actions tend to be diverse and each may inhibit several classes of MMPs through forming a tight non-covalent bond with MMPs at a one to one ratio, and appear to act via the N-terminal
moiety (Wojtowicz-Praga, S. M. et al., (1997) Invest New Drugs, 15: 61-75). Expression of each of these four TIMP genes shows regional and temporal variation. TIMP-1, TIMP-2 and TIMP-3 are constitutively expressed while TIMP-4 is prominent in the developing brain.


MMPs have also been shown to be involved in some cerebrovascular disorders. In particular, levels of MMP2, MMP3, and MMP9 were altered in infarcted brain (TINS (1999) Trends in Neuroscience) (22, n7) 285-288). Using rat models of stroke, MMPs were shown to mediate the breakdown of the blood brain barrier (BBB). Furthermore, following excitotoxic lesions and ischaemia, the expression levels of MMP and TIMPs were altered (Rivera et al (1997) Journal of Neuroscience 17(11) 4223-42 and Wang et al., 29, 516-520, 1998). Indeed, following this work MMPs and TIMPs are now being
considered as targets for therapeutic intervention in stroke and it is possible that they may influence neuronal survival after stroke. (Rivera et al, supra).

Jaworski et al., (Glia, 30, 199-208, 2000) discloses that on injury to rat brain, levels of TIMP-1 and -2 are increased. This result was considered to indicate that TIMP-1 and -2 may have a role in axon outgrowth and neurite extension.

La Fleur et al., (J. Exp.Med., 194, 2311-2326, 1996) indicates that TIMP-1 is increased in oversized siatic nerves and it is suggested that TIMP-1 may be involved in promoting axonal growth.

Current research in the field of neurodegenerative treatment focuses on developing glutamate (NMDA) receptor antagonists to treat stroke patients. These antagonists can prevent nerve cell damage following ischaemia (due to infarct or heart attack) because they prevent extracellular calcium from entering the cell via the NMDA receptors.

Research is also currently aimed at blocking intracellular signaling molecules involved in the programmed cell death (or apoptotic) pathway. Drugs developed via this strategy must be able to enter the cell to exert any beneficial effect (i.e. they do not act on extracellular receptors). This is problematic in that intracellular delivery is generally more difficult to achieve.

The poor neurodegenerative response of adult CNS neurons following injury is caused, in part, by components of the ECM that inhibit axonal outgrowth, such as chondroitin sulphate proteoglycan (CSP).

However, despite the presence of CSP, peripheral nerves regenerate relatively well following injury, suggesting that MMPs and TIMPs may play a role in modulating the normally suppressive environment. Recent work suggests that nerve growth factor (NGF) induced expression of MMP2 is particularly important in mediating this process.
Recently, the inventors have hypothesised that alterations in the expression levels of both MMPs and TIMPs could have beneficial or detrimental effects within the central nervous system.

The inventors have shown that TIMP-1, -2 and -3, produced following the transfection of neurones by an adenoviral vectors are highly neuroprotective. Furthermore, they have also shown that secreted TIMPs protect neurones from excitotoxic damage. It has surprisingly also been found that the TIMPs do not cause their effect by inhibiting MMPs.

Accordingly, a first aspect of the present invention provides a use of a tissue inhibitor of a matrix metalloproteinase (TIMP) in the preparation of a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder in a patient.

A second aspect of the invention provides a pharmaceutical composition for the treatment or prevention or a neurodegenerative disorder in a patient, the pharmaceutical composition comprising a TIMP.

A third aspect of the invention provides a method of treatment or prevention of a neurodegenerative disorder in a patient, comprising supplying to the patient a pharmaceutical composition according to a second aspect of the invention.

Any TIMP can be used in the present invention provided it inhibits the function of a matrix metalloproteinase, including functionally equivalent TIMPs or variants thereof. Preferred TIMPs for us in the present invention include TIMP-1, TIMP-2 and TIMP-3 or a mixture of one or more of the TIMPs. It is further preferred that the TIMP is TIMP-1.

It has been found that one or more TIMPs can be used as a neuroprotectant in order to protect neurological tissue and cells from damage including damage caused by toxins such as free radicals, glutamic acid, glutamate agonists and ischaemic buffers. Furthermore, it has also been found that the TIMPs do not exert their neuroprotective effect through MMPs. Without being bound to any theory concerning the neuroprotective action of the TIMPs, it is suggested that the TIMPs exert their neuroprotective effect by reducing the
level of calcium influx into neurological cells (i.e. neurons) when the cells are contacted with a toxin.

Preferably, the present invention provides the use of a TIMP in the manufacture of a pharmaceutical composition for use as a neuroprotectant.

The neurodegenerative disorder may be any neurodegenerative disorder including stroke, head trauma, Alzheimer’s disease, multiple sclerosis or Parkinson’s disease.

In view of the neuroprotective effects of TIMPs, a TIMP can be used in the treatment of stroke by protecting neurological tissue and cells from damage occurring after the initial seizure due to the subsequent release of toxic concentrations of glutamate and generation of harmful free radicals. The TIMPs can also be used a neuroprotectants to prevent or slow the progression of Alzheimer’s disease, and in the prevention or treatment of head trauma, multiple sclerosis or Parkinson’s disease.

The present invention also provides the use of a nucleic acid molecule encoding a TIMP in the manufacture of a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder.

The present invention also provides a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder in a patient, the pharmaceutical composition comprising a nucleic acid molecule encoding a TIMP.

The invention also provides a method of treatment or prevention of a neurodegenerative disorder in a patient, comprising supplying to the patient a pharmaceutical composition comprising a nucleic acid molecule encoding a TIMP.

The nucleic acid molecule can be RNA or DNA. Preferably the nucleic acid molecule is DNA. The nucleic acid can encode any TIMP as defined above. The nucleic acid sequences of TIMPs are well known to those skilled in the art. In particular, the sequence of human TIMP-1 and TIMP-2 is disclosed in Osthues et al., FEBS Lett, 296, 15-20, 1992.
The sequence of rat TIMP-1 is disclosed in Okada et al., Gene, 147, 301-2, 1994, and compared with other TIMPs.

Preferably the nucleic acid molecule of the present invention is in the form of a vector. The vector preferably comprises a promoter and any additional components required to obtain the correct expression of the TIMP. Vectors for expressing proteins are well known to those skilled in the art. The vector may be an integrating vector or an episomal vectors depending on the desired target and on the period of time that the TIMP needs to be expressed.

Preferably the vector is an adenoviral vector expressing a TIMP.

The pharmaceutical compositions of this invention comprise a TIMP or a nucleic acid encoding a TIMP, and any pharmaceutically acceptable carrier, adjuvant or vehicle. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene- polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. Oral administration or administration by injection are preferred. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.
The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or a similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Preferably, the pharmaceutical composition of the present invention is supplied intracerebroventricularly (ICV) or by stereotaxic injection.

Preferably, the pharmaceutical composition of the present invention is supplied so that an effective concentration in the body of the patient can be achieved. It is standard practise for one skilled in the art to determine the required concentration of a pharmaceutical for achieving an effective concentration.
Embellishments of the invention will now be described, by way of example only, and with reference to Figs. 1 to 6 in which:

Figure 1 A to H show hippocampal cultures stained with propidium iodide;

Figure 2 A to C show graphs indicating the MTT readings of hippocampal cultures at various time periods post-exposure to various concentrations of glutamic acid.

Figure 3 shows a graph of MTT readings of hippocampal cultures in conditional media with TIMP-1 post-exposure to glutamic acid.

Figure 4 shows photomicrographs of organotypic cultures (brain slice cultures) for assessing cell death following addition of glutamate using propidium iodide staining; (A) shows β-gal transfected cells (control); (B) shows TIMP-1 transfected cells.

Figure 5 shows calcium influx into hippocampal neurones following the addition of glutamate in a control medium or in a TIMP-1 conditioned medium.

Figure 6 shows MTT levels (+/-SEM) obtained one hour after glutamic acid exposure in hippocampal cultures.

Examples

Construction and purification of Adenoviral vectors
The TIMPs transgenes (TIMP-1, -2 and -3) were first cloned between flanking adenoviral (Ad) sequences in the prokaryotic transfer vector pXCX2. The sequences of TIMP-1, -2 and -3 are described in Ahonen et al., (Adv. Exp. Med. Biol., 451, 69-72, 1998); George et al., (Hum. Gene. Ther., 9, 867-77, 1998; and Baker et al., (J. Clin. Invest., 101, 1478-87, 1998). A plasmid (pJM17) carrying the entire Adenovirus 5 (AD5) genome has been constructed so that the size of the transfer vector inserted in the E1 gene makes the construct too large to package into the virus particle. Vector pXCX2 and plasmid pJM17 are described in Geddes et al., Endocrinology, 137, 5166-5170, 1996; Harding et al., J.
Neurochem., 69, 2630-2624, 1997; Geddes et al., Nature Medicine, 3, 1402-1405, 1997; and Harding et al., Nature Biotechnology, 16, 553-555, 1998. Recombinant virus was generated by homologous recombination between the transfer vector pXCX2 and pJM17 following transfection of 293 HEK 293 cells (a generally available trans-complementing human kidney cell line for E1 function). After recombination, the size of the viral genome was reduced below the packaging limit of the Ad particle and hence only recombinant virus is capable of generating plaques. This Ad suspension was then purified further by caesium chloride density centrifugation (Geddes et al (1997) Nat. Med. 69(6) 2620-3).

Example 1

Assessing the effect of overexpressing TIMPs on neurite regeneration
Previous studies have shown that following axonal injury, TIMP-1 was upregulated. To investigate the involvement of TIMP-1, -2 and -3 in regeneration, Ad vectors were used to overexpress the TIMPs in dorsal root ganglion neurons (DRGs).

DRGs were then dissected and plated onto polyornithine coated dishes (Sigma). They were then transfected with recombinant Ad vectors expressing TIMP-1, -2 and -3. Control plates were incubated in the absence of the neurotoxic agent, glutamate and with Ad vectors expressing the marker enhanced green fluorescent protein EGFP and β-galactosidase. Cultures were incubated for 24 hours and then re-plated on laminin coated dishes. The cells were then fixed at various time points (6, 12, 18 & 24 hours) for 10 minutes in 4% paraformaldehyde and then washed in 3 x medium. The longest neurite outgrowth and the surface area of individual DRGs were then measured and recorded.

A typical experiment gave the following results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>The average lengths of neurites at the end of 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. control minus virus</td>
<td>300 micrometers</td>
</tr>
<tr>
<td>2. control + Ad+β-gal</td>
<td>297 micrometers</td>
</tr>
<tr>
<td>3. DRG expressing TIMP-1</td>
<td>246 micrometers</td>
</tr>
</tbody>
</table>
4. DRG expressing TIMP-2 312 micrometers
5. DRG expressing TIMP-3 307 micrometers

These results show that TIMP-1-expressing Ad vectors retard neurite outgrowth while TIMP-2 and TIMP-3 do not significantly alter the rate of neurite regeneration. The rate of neurite regeneration in each group remained constant when monitored over the different time points.

Example 2

Assessing the neuroprotective effect of TIMPs on primary hippocampal cultures exposed to glutamate

Following the induction of seizures in the brain it was observed that TIMP-1 was differentially expressed in neurons and glial cells. To investigate whether the upregulation in neurons may be protective, TIMP-expressing Ad vectors were used to transfect hippocampal neurons and the transfected hippocampal neurons were then exposed to cytotoxic concentrations of glutamate.

Hippocampi were dissected from (E16-18) rat embryos. The hippocampi were then placed in Hank's balanced salt solution (HBSS) (Sigma) and then disaggregated by incubation in Hanks in the absence of Ca\(^{2+}\) and Mg\(^{2+}\) containing 0.1% trypsin, for 30 min at 37°C. The cells were then washed three times in Hanks with Ca\(^{2+}\) and Mg\(^{2+}\) and triturated in 500μl of Dulbeccos modified eagles medium (DMEM) (Sigma) supplemented with 100mM (Sigma) glutamine, 1μl/ml antibiotic/antimycotic (Sigma) (penicillin and streptomycin were used), and containing 5% foetal calf serum (FCS) (Gibco) (this medium plus supplements will be referred to as DMEMS). After trituration, 2mls of DMEMS was added and 50μl of the suspension aliquoted onto glass cover slips coated with 0.1mg/ml poly-L-lysine positioned in multiwell (24) dishes. The culture suspension was incubated for 40 minutes at 37°C in a CO\(_2\) (5%) incubator and after this period a further 0.5ml of DMEMS was added. 10μM cytosine arabinoside was also added to suppress glial cell proliferation.
Once established, the hippocampal neurons were transfected with Ad vectors expressing TIMP-1, -2 and -3. 48 to 72 hours after transfection, cells were exposed to 400μM glutamate. Conditioned media containing TIMP-1 and TIMP-2 peptide (supernatant taken from hippocampal cultures transfected with AdTIMP-1 and AdTIMP-2 48 hours earlier) was added to untransfected control cells 12 hours before exposure to glutamate. Cellular viability was assessed using MTT assays, to give an indication of mitochondrial activity and by staining with propidium iodide to detect dead cells. All experiments were conducted in triplicate and conducted at least three times.

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) assay (Sigma):

5mg/ml MTT solution was added to each culture well at a volume equal to one tenth the original culture media volume and incubated at 37°C for three to four hours. At the end of the incubation period the medium was removed and the converted dye was solubilized with acidified isopropanol (0.04-0.1 N HCl in absolute isopropanol). Absorbency of the converted dye was measured at 595 nm using a BIO-RAD micro plate reader. Measurement of MTT formation is the standard method of assessing cell toxicity/viability. It measures mitochondrial activity, and the higher the MTT value the healthier the cell (see Figures 1, 2 & 3). Morphological assessment was carried out as follows: Monolayer cells were examined under a phase contrast microscope and neurons scored as viable if the cell bodies appeared round or oval with smooth outline and the neurites were smooth with a ‘shiny outline’. In degenerated neurons, neurites were fragmented and beaded, and the cell bodies were rough and irregular to shape. To further facilitate counting of dead cells, nuclei of dead cells were labeled with propidium iodide (Uney et al (1993) J. Neurochem 60(2) 639-665). Random fields were chosen and ~200 cells examined under an inverted Leica fluorescent microscope. Low power images (20x) were captured on an inverted Leica fluorescent microscope and image capture system.

The results showed that overexpression of TIMPs protected hippocampal neurons from glutamate toxicity. In hippocampal neurons overexpressing TIMP-1 and -2 and exposed to glutamate for 24 hours, MTT assay values remained almost identical (85-95%) to control
cultures not exposed to glutamate. To investigate whether the TIMPs were exerting their protective effects intracellularly or extracellularly, conditioned medium (containing the TIMP peptides) was added to the media of neurons 12 hours prior to exposing them to glutamate. These experiments showed that MTT values remained significantly higher than those of the control neuron cultures.

The results clearly show that TIMPs are protective.

Example 3

Photomicrographs of Organotypic Cultures

Organotypic slice cultures were prepared according to the method of Stoppini et al., J. Neuroscience Methods, 27(2), 173-182, (1991). For these experiments 10-12 day old Sprague-Dawley or Wistar rats were used. Following the careful removal of the brain and microdissection the hippocampal is transferred to a 50ml petri dish containing 40°C Hanks balanced salts solution (Gibco), supplemented with 2% (v/v) glucose (Sigma, Dorset UK). The hippocampus is then placed on melonex strips (Agar Scientific, UK) onto the stage of a McIlwain chopper (Mickle Engineering, UK) and the hippocampus cut perpendicular to the long axis to give transverse slices. A thickness of 400μM was used. The slices are then returned to 40°C Hanks balanced salt solution and separated using a paddle pasteur pipette. The slices are then placed onto millicell, 0.4μM culture plate inserts, consisting of a microporous transmembrane biopore membrane (4 per membrane) in 6 well plates (Nalge Nunc International). In each well, 1.2 ml of medium consisting of 50% (v/v) minimal Hanks, 25% (v/v) Horse serum, 5mg/ml glucose, 1 ml glutamine and 3 ml Amphoterin B was added.

The slices favoured were from the septal half, as these contained the dentate gyrus with an angular apex. These restricted the loss of the CA1 during glutamate experiments. The 6 well plates were placed in an incubator at 37°C/95% saturated air and medium. The medium was changed every 3/4 days and the cultures cultivated such for 14 days. At the end of this period the hippocampal slices had stabilised and thinned out to about 150μM.
Following the same methods as described above, fluorescent photomicrographs of organotypic cultures (brain slice cultures) prepared from rat brain culture were stained with propidium iodide (a fluorescent marker of cell death) after exposure to highly toxic dose of glutamate (1mM). Figure 4A shows propidium iodide staining in a hippocampal organotypic culture that had been transfected with a control adenovirus (expressing β-gal) construct. Figure 4B shows propidium iodide staining in a hippocampal organotypic culture that had been transfected with an adenovirus expressing TIMP-1. The results show there is far less cell death as a consequence of exposure to glutamate in cultures transfected with TIMP-1. To allow the results to be statistically analysed (9 experiments) an image capture system was used and TIMP-1 treated cultures were compared to controls by T-test analysis (P<0.0092).

Example 4

Calcium Influx into Hippocampal Neurons

Hippocampal cultures were incubated in a physiological salt solution (PSS: 127mM NaCl, 1.8mM CaCl₂, 5mM KCl, 2mM MgCl₂, 0.5mM NaH₂PO₄, 5mM NaHCO₃, 10mM glucose and 10 mM HEPES, pH7.4) containing 2mM fura-2 acetoxyethyl ester (fura-2/AM) for 30 minutes at 37°C, 5% CO₂. The cultures were then placed into an incubation chamber and the extracellular dye removed by washing with PSS. Medium changes were achieved by pipetting 4-5ml of solution into the incubation chamber in which the volume was maintained at about 500 ml by means of an aspirator. Cells were stimulated with PSS containing 50 mM KA, 25mM APV and 0.5mM tetrodotoxin (TTX). Dynamic video imaging was performed as previously described using MagiCal hardware and Tardis software (McArdle et al., Mol. Cell Endocrinol, 87, 95-103, 1992). The cells were excited alternately at 340 and 380nm and emitted light was collected at 510nm averaging the data from 16 video frames and subtracting background values before rationing. The ratio of fluorescence at 340 and 380 nm was calculated on a pixel-by-pixel basis and used to determine Ca²⁺ concentration.
Fura-2 measurements of calcium influx were carried out on hippocampal neurones. The results are shown in Figure 5, are the means of 10 experiments and clearly show that calcium influx following the addition of TIMP-1 is significantly reduced.

Example 5

Neuroprotective Effect of TIMP-1 Not Mediated Through MMPs

Broad spectrum synthetic MMP inhibitors, BB94 and MMPI-1 (Calbiochem), were used in these experiments, and were added to the hippocampal cultures for 18 hours at final concentrations of 1 μM and 200 μM respectively. Data represents the MTT readings +/- SEM, one hour after glutamic acid exposure (300μM for one hour).

No significant neuroprotective effect was seen in treated cultures.

The results given in Figure 6 clearly demonstrate that adenoviral mediated TIMP-1 expression and TIMP-1 containing conditioned media conferred very considerable protection to hippocampal cells exposed a wide range of glutamic acid concentrations (100-600μM). However, broad-spectrum synthetic MMP inhibitors did not confer any neuroprotection to hippocampal cultures. These findings have major implications for the understanding of the physiological role of TIMPs. Furthermore, TIMP-1 is a secreted protein that mediates its neuroprotective effects via the extracellular matrix, and provides a novel therapeutic target whose modulation can be used to prevent neuronal damage following global of focal ischemia or other damage in patients.

To examine the neuroprotective effect of TIMP-1 on excitotoxicity, we used a recombinant adenoviral vector to overexpress TIMP-1 in hippocampal cells. The results given in Figure 6 clearly demonstrate that Ad mediated TIMP-1 expression confers very considerable protection to hippocampal cells exposed a wide range of glutamic acid concentrations (100-600μM). TIMP-1 containing conditioned media also protected neurons, strongly suggesting this protection was mediated via an action of extracellular TIMP-1 following binding to a cell surface target. However, broad spectrum synthetic MMP inhibitors (BB-94 and MMPI-1) did not confer any neuroprotection to hippocampal cultures against
excitotoxins. The results show that TIMP-1 is not mediating its effects through MMPs.

All documents cited herein are hereby incorporated by reference.
Claims

1. Use of a tissue inhibitor of a matrix metalloproteinase (TIMP) in the preparation of a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder or condition in a patient.

2. A pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder or condition in a patient, the pharmaceutical composition comprising a TIMP.

3. A method of treatment or prevention of a neurodegenerative disorder or condition in a patient, comprising supplying to the patient, a composition according to claim 2.

4. The use according to claim 1, the pharmaceutical according to claim 2 or the method according claim 3, wherein the TIMP is TIMP-1, TIMP-2 or TIMP-3.

5. The use according to claim 1, a pharmaceutical composition according to claim 2 or a method according to claim 3, wherein the neurodegenerative disorder or condition is selected from stroke, head trauma, Alzheimer's disease and Parkinson's disease.

6. Use of a nucleic acid molecule encoding a TIMP in the manufacture of a pharmaceutical composition for the treatment or prevention of a neurodegenerative disorder.

7. A pharmaceutical composition for the treatment or prevention or a neurodegenerative disorder in a patient, the pharmaceutical composition comprising a nucleic acid molecule encoding a TIMP.

8. A method of treatment or prevention of a neurodegenerative disorder in a patient, comprising supplying to the patient a pharmaceutical composition comprising a nucleic acid molecule encoding a TIMP.

9. The use according to claim 6, pharmaceutical composition according to claim 7, or the method according to claim 8, wherein the nucleic acid molecule encodes TIMP-1, TIMP-2 or TIMP-3.
10. The use according to claim 6, pharmaceutical composition according to claim 7, or the method according to claim 8, wherein the nucleic acid molecule is a vector.

11. The use according to claim 6, pharmaceutical composition according to claim 7, or the method according to claim 8, wherein the nucleic acid molecule is an adenoviral vector expressing a TIMP.

12. The method of claim 3 or claim 8, wherein the composition is supplied to the patient intracerebroventricularly (ICV).

13. The method of claim 3 or claim 8, wherein the composition is supplied to the patient by stereotaxic injection.
FIG. 4

A, beta-gal transfected control

B, TIMP-1 transfected