Matrix Metalloproteinases and the Central Nervous System

Satellite Symposium of the Society for Neuroscience

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Neuroscience Center of Excellence
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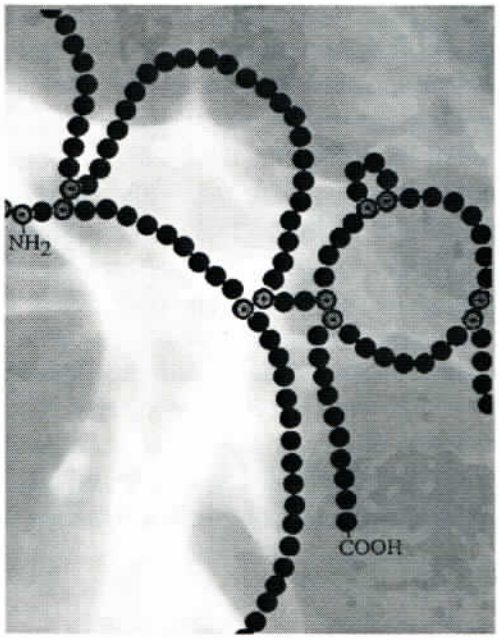
PROGRAM

Thursday, October 30, 1997

2:00 – 2:15 p.m.	Introduction and Welcome NG Bazan
	Session 1: Regulation of MMPs Chair: NG Bazan
2:15 – 2:45 p.m.	*Significance of MMPs in the CNS: Introduction* NG Bazan LSUMC Neuroscience Center of Excellence, New Orleans, LA
2:45 – 3:15 p.m.	*Matrix Metalloproteinases and Their Inhibitors in Epilepsy and Ischemia* M Khrestchatisky INSERM, Paris, France
3:15 – 3:45 p.m.	Coffee Break and Poster Viewing
	Session 2: MMPs in Development and Cell Culture Chair: P Gottschall
3:45 – 4:15 p.m.	*Regulation of Gelatinase Production in Cultured Astrocytes* P Gottschall University of South Florida, Tampa, FL
4:15 – 4:45 p.m.	*Expression of Metalloproteinases and Their Inhibitors in Cultured Rat Astrocytes and C6 Glioma Cells* M Marx-Lukes University of New Mexico, Albuquerque, NM
4:45 – 5:15 p.m.	*MMPs in Development and Axonal Growth* D Muir University of Florida, Gainesville, FL
5:15 – 5:45 p.m.	Questions and Answers Speaker's Panel

Friday, October 31, 1997

8:00 – 8:30 a.m.	Continental Breakfast
	Session 3: MMPs in Neurological Diseases Chair: G Rosenberg
8:30 – 9:00 a.m.	Matrix Metalloproteinase Expression in Alzheimer's Disease and Amyotrophic Lateral Sclerosis Z Tokes University of Southern California, Los Angeles, CA
9:00 – 9:30 a.m.	*Role of MMPs in Human Glioma Progression* J Rao MD Anderson, Houston, TX
9:30 – 10:00 a.m.	*Magnetic Resonance Imaging of C6 Glioma in Rats and Quantitative Comparison with Histomorphology and Immunohistochemistry* A Lukes University of New Mexico, Albuquerque, NM
10:00 – 10:30 a.m.	Coffee Break and Poster
10:30 – 11:00 a.m.	*Targeting Recurrent Glial Tumors: Out of My Head!* S Treasurywala Barrow Neurological Institute, Phoenix, AZ Viewing
11:00 – 11:30 a.m.	*The Role of Matrix Metalloproteinases in Experimental Autoimmune Neuritis* K Miller British Biotech, Oxford, UK
11:30 a.m. – 12:00 p.m.	*Matrix Metalloproteinase mRNA Expression and Protein Distribution in the LPS-Injected Brain* S Mun-Bryce University of New Mexico, Albuquerque, NM
12:00 – 1:00 p.m.	Lunch
1:00 – 1:30 p.m.	*Matrix Metalloproteinase Expression Increases Following Cerebral Focal Ischemia* A Romanic SmithKline Beecham, King of Prussia, PA
1:30 – 2:00 p.m.	*Proteolysis by Gelatinases Increases Blood-Brain Barrier Permeability After Reperfusion in Rat* G Rosenberg University of New Mexico, Albuquerque, NM
2:00 – 2:30 p.m.	Future Directions Open Discussion



Lectures

SIGNIFICANCE OF MMPs IN THE CNS: INTRODUCTION

N.G. Bazan, A.V. Ershov, and M.A. DeCoster
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Platelet-activating factor (PAF, 1-0-alkyl-2-acetyl-sn-glycerophosphocholine) is a potent bioactive phospholipid that modulates glutamate release, LTP, and memory formation. In addition, PAF is a messenger involved in coupling synaptic events with gene expression. PAF activates AP-1 signaling, NF B and COX-2 gene expression in neural cells. Here, we have tested the hypothesis that PAF modulates plasticity by enhancing neuronal matrix metalloproteinases (MMP) gene expression, which, in turn, participates in the remodeling of the extracellular matrix (ECM). Furthermore, since sustained, enhanced levels of PAF are neurotoxic, MMP expression triggered by this lipid mediator may also be part of cell injury events. We have studied stromelysin -1 and -3, as well as 72 kDa- and 92 kDa-gelatinase, and MT-MMP-1 gene expression using RT-PCR. We have observed that in cortical neurons, NMDA receptor activation, that promotes the generation of PAF, significantly induces only stromelysin-1 expression of the 5 MMPs studied in neurons. NMDA does not activate stromelysin-1 in astrocytes, strongly suggesting that although astrocytes represent about 20% of the neuronal cortical cultures used, the effect was mainly in neurons. The hetrazepine, BN 50730, an intracellular PAF receptor antagonist, completely inhibited NMDA-induced increase of stromelysin-1 expression at 12 and 24 hours. In astrocytes, LPS was a potent inducer of stromelysin-1 and of 92 kDa gelatinase. Whereas BN 50730 was unable to block this induction, a novel nonfluorinated steriod, budesonide epimer R, completely inhibited LPS induction of these MMPs. The neuromessenger, PAF, in the CNS by regulating MMP expression may modulate synaptic plasticity, as well as neuronal migration and, in pathological conditions, promote repair, and gliosis. (Supported by NIH N23002).

MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN EPILEPSY AND ISCHEMIA

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Excitotoxic neuronal death after epilepsy and ischemia involves the activation of glutamate receptors and the increase of intracellular Ca2+ concentrations and is associated with inflammation and tissue remodeling. Neuronal death is thought to result -at least in part- from the alterations of genetic programs involved in the expression of cytotoxic and cytoprotective genes. Those encoding the matrix metalloproteinases (MMPs) and their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), are potential candidates. Indeed, they control the extracellular matrix (ECM) metabolism, critical for cell survival and tissue remodeling and they are implicated in proteinase cascades that lead to the activation of pro-inflammatory cytokines. There is evidence that the activity of MMPs is induced in both the epileptic and ischemic tissue. We have studied by in situ hybridization and immunostaining techniques the expression of TIMP-1 in the brain of epileptic and ischemic rats. We have found that excitotoxic stimuli regulate its expression in a spatio-temporal-, cell- and lesion-dependent manner. Similar results are obtained when studying MMP-9, one of the metalloproteinases expressed in brain. These results support the emerging idea that ECM proteinases and their inhibitors may be instrumental for neurons and astrocytes in coupling early cellular events triggered by seizures and ischemia with the long-lasting changes associated with neuronal death and survival and tissue reorganization.

REGULATION OF GELATINASE PRODUCTION IN CULTURED ASTROCYTES

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Upon activation, astrocytes express a characteristic set of genes not normally expressed, or expressed at lower levels in their "unactivated" counterparts. Although the mechanism of astrocyte activation is unclear, a variety of mainly neuropathological conditions induce the activated state. Activated astrocytes likely participate in an "inflammatory response" in the central nervous system which accompanies repair and regenerative mechanisms that occur after brain injury. We were interested in whether matrix metalloproteinases (MMPs) were important in these repair responses, similar to peripheral wound repair. To this end, the purpose of these experiments was to characterize MMP production, in particular gelatinase (GLase) expression, in cultured astrocytes and to identify agents that regulate MMP production in astrocytes. IL-1ß, TNF-□ and lipopolysaccharide each induced a dose-dependent increase in MMP-9, and to a lesser extent, MMP-2 production in cultured rat astrocytes. ß-amyloid (Aß) peptides, especially Aß(1-40) apparently in its non-fibrillar form, stimulated levels of MMP-9 and MMP-2 in rat astrocyte cultures, in addition to inducing the appearance of a lower molecular weight GLase activity, identified as activated MMP-2. Aß(1-42) was ineffective under any condition in stimulating this activity in rat astrocytes. Both Aß(1-40) and Aß(1-42) stimulated levels of activated MMP-2 in a human astrocytoma line. By immunoblot, an activator of MMP-2, membrane type (MT)-MMP (either MT1 or MT2), was induced by Aß more than 2-fold in the human astrocytoma line. These data indicate that Aß peptides may induce the activation of MMP-2 in astrocytes and suggest a mechanism for the homeostatic control of Aß levels.

EXPRESSION OF METALLOPROTEINASES AND THEIR INHIBITORS IN CULTURED RAT ASTROCYTES AND C6 GLIOMA CELLS

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Matrix-degrading metalloproteinases (MMP) play an important role for the remodeling of the extracellular matrix (ECM). The turnover of the ECM is crucial for the invasive growth and angiogenesis of astroglial tumors. The expression and localization of MMPs and their natural inhibitors (TIMPs) have been profiled in neonatal rat astrocytes and C6 glioma cells by immunocytochemistry, confocal laser microscopy and zymography. Under basal culture conditions both cell types constitutively expressed a wide range of MMPs and TIMPs. The quantity of MMP produced and the distribution within the cells varied according to the state of differentiation. Differentiated primary cells tended to express lower levels of MMPs which were localized to contact points with other cells. Immature primary and tumor cells had an even distribution in their cytoplasm. Primary rat astrocytes in long term cultures expressed high levels of MMP-3 and MMP-9 and low levels of MMP-2 and MT-MMP-1. C6 glioma cells expressed all MMPs and TIMPs, but produced higher levels of the activated forms of MMP-2. These results indicate that the expression of MMPs and TIMPs is correlated with the stage of differentiation of astroglial cells and that the increased expression of activated MMPs in C6 cells may contribute to their invasive phenotype seen *in vivo*. Sources of support: Swiss National Science Foundation (MLM), Department of Clinical Investigation, University of Berne (AL), Bernese Cancer Society (AL), and NIH RO1 NS 21164 (GR).

MMPS IN DEVELOPMENT AND AXONAL GROWTH

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Axonal growth requires extensive growth cone motility and invasion of neural target tissues. MMP-2 is expressed by embryonic and adult dorsal root ganglionic neurons (DRGn) *in vivo* and *in vitro* and is transported to the growth cone. NGF was found to induce MMP-2 expression by cultured DRGn. Also, neurite growth and invasion within a 3-dimension basal lamina gel was MMP-dependent and increases by elevated NGF. Thus, combined with its neurotrophic effects, NGF triggered a coordinate mechanism to enhance matrix degradation and growth cone invasion. Promoting and inhibiting components of the extracellular matrix regulates axonal growth. MMP-dependent neuritic growth was examined in a cryoculture model by culturing DRGn on fresh/frozen nerve sections. Cryoculture provided the opportunity to examine neuritic outgrowth independent of proteolysis-dependent neuritic invasion of 3-dimensional matrix barriers. Neuritic growth by DRGn cultured on nerve sections was partially inhibited by MMP inhibitors. Also, the length of neurites was markedly increases if sections were first treated with MMP-2. MMP-2 and chondroitinase had similar and non-additive effects. MMP-2 degraded and inactivated a neurite-inhibiting chondroitin sulfate proteoglycan isolated from nerve. We conclude that MMP-2 can unmask (de-inhibit) the neurite-promoting potential of nerve basal laminae. Thus, MMP-2 may have two interrelated functions in axonal growth, one involving neuritic invasion, and another more selective process of de-inhibiting the neurite-promoting properties of neural substrata by inactivation of inhibitory proteoglycans.

MATRIX METALLOPROTEINASE EXPRESSION IN ALZHEIMER'S DISEASE AND AMYOTROPHIC LATERAL SCLEROSIS

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Two major MMPs are seen in the CNS of patients with AD or ALS. MMP-2 (gelatinase A) is observed predominantly in astrocytes while MMP-9 (gelatinase-B) is produced by neurons. MMP-2 expression remains unchanged in AD, in ALS spinal cord, and is decreased in the motor cortex of ALS patients. The predominant forms of MMP-2 are intracellular and latent. Its aberrant release and activation may lead to the destruction of perivascular matrix. Latent MMP-2 is also detected in the CSF of normal and AD patients. Latent MMP-9, present as several complexed forms (100, 130, and 240-280 kDa), is increased 2-3 fold in both AD and ALS. In ALS, 53% to 60% of motor neurons are MMP-9 positive. If these neurons release the active enzyme at the neuromuscular junction, the synaptic architecture would be destroyed, leading to both neuronal and muscular degeneration. Patches of MMP-9, without detectable TIMP-1 and -2, are observed at the surface of degenerating muscles only in ALS. While coordinated expression of TIMP-2 and MMP-9 is seen only in control specimens, no similar correlation was found in ALS specimens. In AD, if MMP-9 is released from hippocampal neurons and becomes activated, beta-amyloid peptides would be cleaved and their amyloidogenic properties would be destroyed. Thus, an active MMP-9 would reduce the beta-amyloid peptides capacity to accumulate in senile plaques. MMP expression is induced by inflammation, which also increases the synthesis of protease inhibitors that can interfere with MMP activation. Thus, inhibited activation of MMP-9 may contribute to the neuropathogenesis of AD. This testable hypothesis is now under investigation in our laboratory. (ALS Association and RO1-AG05142.)

ROLE OF MMPS IN HUMAN GLIOMA PROGRESSION

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Glioblastomas, the most devastating human primary brain tumors, are highly invasive. The invasive potential of these gliomas is dependent, in part, on the expression of matrix metalloprotease-9 (MMP-2 and MMP-9) apart from other proteolytic enzymes. Our studies by northern blot and in situ hybridization at message level, and immunohistochemistry and gelatin zymography at the protein levels, both showed that MMP-2 and MMP-9 expression was upregulated in glioblastomas, compared to anaplastic astrocytomas, low-grade gliomas and normal brain tissue. Moreover, an antibody against MMP-9 significantly inhibited the invasion of glioblastomas in vitro consolidating the role of MMP-9 in glioma invasion. In a recent study, we showed that induction of MMP-9 by phorbol ester requires an organized cytoskeleton and formation focal adhesion contact. By using cell-shape modulators, we found the MMP-2 (72 kDa gelatinase) is expressed in culture irrespective of cell shape and focal adhesion formation, in contrast to MMP-9 activation. Our results suggest that MMP-2 and MMP-9 have distinct signaling mechanisms that can be modulated by cell shape, in particular, actin cytoskeleton modulators.

MAGNETIC RESONANCE IMAGING OF C6 GLIOMA IN RATS AND QUANTITATIVE COMPARISON WITH HISTOMORPHOLOGY AND IMMUNOHISTOCHEMISTRY

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Oncogenes and other neoplastic factors create in astrocytes an imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). This imbalance leads to breakdown of the extracellular matrix (ECM), promotes the invasive phenotype of astrocytes, and causes disruption of the blood-brain barrier (BBB).

We compared *in vivo* tumor growth, invasion and angiogenesis in the C6 glioma model volumetrically by magnetic resonance imaging (MRI) with gadolinium and histomorphologically by immunohistochemistry. MRI allowed analysis of tumor size and vascularity at different time points in the same animals. Tumor size by histomorphology and vessel count by immunohistochemistry correlated well with MRI measurements of tumor volume.

We conclude that MRI of the rat C6 glial tumor model offers an accurate anatomic and functional tissue characterization *in vivo* to test novel therapeutic strategies to control tumor growth and angiogenesis.
Support: Department of Clinical Investigation, University of Berne (AL), Bernese Cancer Society (AL), Swiss National Science Foundation (MLM), and NIH RO1 NS 21164 (GAR).

TARGETING RECURRENT GLIAL TUMORS: OUT OF MY HEAD!

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Malignant gliomas remain rapidly lethal despite aggressive treatment including surgery, chemotherapy and radiation. In the last 25 years there has been little significant improvement in the median survival for patients with malignant gliomas. It is our contention that the invasive nature of neoplastic astrocytes causes them to be such a clinically refractory disease. It is the motile, invasive cells, permeating into normal brain from the primary tumor, that ultimately give rise to recurrent, lethal lesions. We believe that the invasive glioma cells differ from the primary tumor cells, and that these differences shield the residual glioma cells from effective therapy. We have found that highly migratory glioma cells are less proliferative (*"go versus grow"*). Cells with a protracted G_0 phase of the cell cycle show less response to radiation and chemotherapy. Current treatments, directed at arresting cell proliferation, may induce cells to stop proliferating, which in turn might increase the local invasion. This would potentially give rise to new tumors away from the primary tumor mass. To further explore the paradigm characterizing migration as a central phenotypic trait of gliomas, we hypothesized that in order for glioma cells to move they must first detach from neighboring cells *("let go...let's go")*. A direct inverse relationship was found between the number of gap junctions and migration rates of glioma cells. Glioma cells that have a reduced capacity to connect to each other have an accelerated migration rate. We propose that the best strategy for management of gliomas will be to exploit this migratory activity as a therapeutic target. Such treatment may include haptotaxis and chemotaxis manipulations which would lead the migratory glioma cells to a precise location, preferably, *out of the head.*

THE ROLE OF MATRIX METALLOPROTEINASES IN EXPERIMENTAL AUTOIMMUNE NEURITIS

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There is emerging evidence that matrix metalloproteinases (MMPs), a large family of enzymes involved in the degradation of extracellular matrix proteins, are involved in inflammatory diseases of the central nervous system. Proteolytic activity of MMPs can disrupt the blood-brain barrier, control leukocyte migration, generate immunogenic fragments of myelin basic protein and cause demyelination. MMPs are upregulated in the cerebrospinal fluid and lesions of patients with multiple sclerosis (MS) and inhibitors of MMP activity attenuate disease severity in experimental autoimmune encephalomyelitis, an animal model of MS.

We have investigated the role of MMPs in the pathogenesis of acute inflammation of the peripheral nervous system. Quantitative PCR and immunohistochemical techniques have been used to profile the expression of MMPs and the pro-inflammatory cytokine, TNF, throughout the course of experimental autoimmune neuritis, a demyelinating rat model of Guillain-Barré syndrome. We have tested combined inhibitors of matrix metalloproteinase activity and TNF precursor processing in this model, and have shown a reduction in disease severity, conduction deficit and weight loss associated with the disease. Histological analysis showed that these compounds also inhibited macrophage and T cell infiltration into the nerves and reduced demyelination. Inhibitors of MMP activity and TNF processing may therefore be beneficial in the treatment of demyelinating diseases of the peripheral nervous system.

MATRIX METALLOPROTEINASE mRNA EXPRESSION AND PROTEIN DISTRIBUTION IN THE LPS-INJECTED BRAIN

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Induction of inflammatory cytokines and matrix metalloproteinases (MMPs) has been associated with several neuroinflammatory-related diseases. An intracerebral injection of lipopolysaccharide (LPS) causes a significant rise in gelatinase B (MMP-9) production, and increases blood-brain barrier (B^3) permeability to small and large molecules. We studied the distribution of MMPs at 8 hrs after LPS injection into the caudate, using immunohistochemistry (IHC). In addition to the presence of MMP-9 protein, we found positive staining for matrilysin (MMP-7), and stromelysin (MMP-3) at the LPS-injection site. Each of these MMPs showed intense peroxidase staining around blood vessels near the lesion site but not in the uninjected hemisphere. Low levels of constitutively expressed gelatinase A (MMP-2) and MMP-7 proteins were apparent in normal brain tissue. RT-PCR of MMP-2, -3, -7, -9 and -13 mRNA was detected at 6 hrs, and showed increased levels at 24 hrs. Upregulation of MMP transcripts and protein in response to LPS stimulation suggests that several MMPs are involved in altered B^3 permeability during neuroinflammation.
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MATRIX METALLOPROTEINASE EXPRESSION INCREASES FOLLOWING CEREBRAL FOCAL ISCHEMIA

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Matrix metalloproteinases (MMPs), a family of proteolytic enzymes that degrade the extracellular matrix, are implicated in pathological conditions including atherosclerosis, inflammation, tumor growth and metastasis. Following focal stroke produced by permanent middle cerebral artery occlusion (MCAO) in the rat, MMP protein expression was measured by western blot and zymogram analysis over a time-course ranging from 6 h to 30 d. Immunohistochemistry was also utilized to characterize the expression of several MMPs and related proteins following stroke, including their cellular source. Increased MMP-9 expression was detected within 24 h following stroke and persisted for 5 d, returning to basal level by 15 d. MMP-9 was localized with endothelial cells and neutrophils identified both within and at the periphery of the infarct 24 h after injury. After 5 d, MMP-9 was identified with macrophages in the infarct. MMP-2 was identified 5 d following MCAO and was localized with macrophages within the infarcted region. Unlike MMP-9 and MMP-2, tissue inhibitor of metalloproteinase-1 (TIMP-1) was identified in both control and ischemic tissue following MCAO and was localized not only in the cortical region of the brain but also in the nerve tracks located within the white matter and was co-expressed with a marker for neurofilament. MMP-1 and MMP-3 were not detected in the brain following focal stroke. The results indicate that MMPs are up-regulated following stroke and contribute to the breakdown of the blood-brain barrier and the influx of inflammatory cells into the infarct. Further, the expression of TIMP-1 provides an endogenous means of protecting the nerve tracks from structural damage due to MMP proteolysis.

PROTEOLYSIS BY GELATINASES INCREASES BLOOD-BRAIN BARRIER PERMEABILITY AFTER REPERFUSION IN RAT

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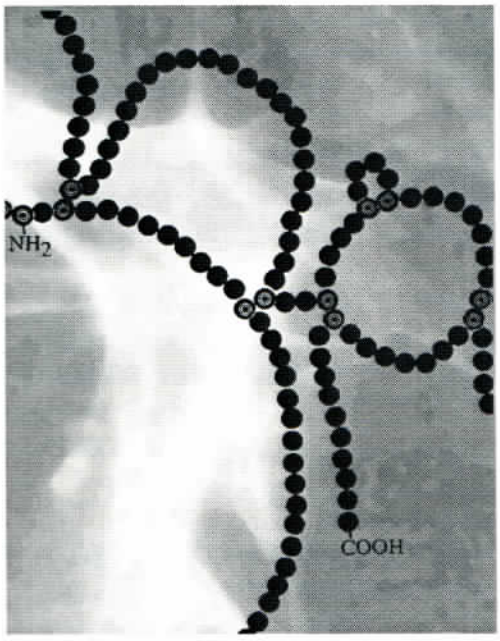
BACKGROUND: During reperfusion injury, the blood-brain barrier (BBB) opens, causing cerebral edema and hemorrhage. Gelatinases are matrix metalloproteinases (MMP), which increase capillary permeability. They are up-regulated in permanent middle cerebral artery occlusion. Therefore, we studied MMP and tissue inhibitors to metalloproteinases (TIMP) in an animal model of ischemia/reperfusion. We compared BBB permeability and edema with MMP and TIMP levels , and tested the effect of a MMP inhibitor.

METHODS: Adult rats (n= 55) had the middle cerebral artery occluded for 2 hrs by a suture. At 3, 6, 15, 24, 48, 120, or 336 hrs after reperfusion, they were injected with ^{14}C-sucrose to measure brain uptake. Tissue levels of MMP and TIMP were measured by zymography and reverse zymography. Twenty-seven rats had water and electrolytes measurements at 3, 24 or 48 hrs after reperfusion, and were compared with 25 rats given BB-1101 (30mg/kg i.p.;British Biotechnology)

RESULTS: Capillary permeability to sucrose was increased significantly at 3 and 48 hrs ($p<0.05$). A marked increase in gelatinase B (MMP-9) was seen at 48 hrs. Gelatinase A (MMP-2) increased significantly at 48 and 120 hrs. TIMP-1 was also increased at 48 hrs, while TIMP-2, was unaffected. The synthetic MMP inhibitor significantly reduced BBB permeability in the ischemic hemisphere at 3 hrs and lowered brain water at 24 hrs after reperfusion.

CONCLUSIONS: An imbalance between MMP and TIMP, resulting in excessive proteolysis, was found after reperfusion. An inhibitor to MMP reduced the early BBB opening and cerebral edema, but not the later stages. Our data suggest that proteolytic disruption of the BBB contributes to reperfusion injury, but in a complex manner.



Posters

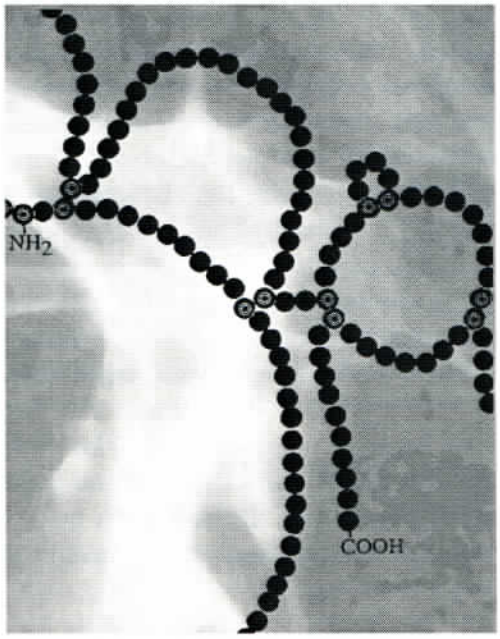
1. **THE APPEARANCE OF ACTIVATED MATRIX METALLOPROTEINASE-2 (MMP-2) IN RESPONSE TO ß-AMYLOID (Aß) IN RAT ASTROCYTES MAY BE DUE TO INCREASED MEMBRANE-TYPE MATRIX METALLOPROTEINASE (MT-MMP) EXPRESSION.** S. Deb and P. E. Gottschall. University of South Florida, Department of Pharmacology and Therapeutics, Tampa, FL

2. **ASSOCIATIVE INDUCTION OF GELATINASE ACTIVITY AND GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) LEVELS IN RAT BRAIN FOLLOWING KAINIC ACID TREATMENT.** W. Zhang and P. E. Gottschall. University of South Florida, Department of Pharmacology and Therapeutics, Tampa, FL.

3. **MMP EXPRESSION IN AN EXPERIMENTALLY-INDUCED DTH RESPONSE IN THE RAT CNS.** D.C. Anthony, K.M. Miller, G.M.A. Wells, J.M. Clements, A.J.H. Gearing, and V.H. Perry. Department of Pharmacology, University of Oxford, Oxford, U.K.

4. **MATRIX METALLOPROTEINASE (MMP) INHIBITORS GALARDIN (GM 6001) AND THE NOVEL DIBENZOFURAN PD 157341 INHIBIT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN PL/J x SJL/J(F1) MICE.** Robichaud LJ, Bugajski JM, Dasovic D, Kupina N, Johnson L, Hupe D, Roth BD, White A, Colbry Nl, and Boxer PA. Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI.

5. **DOWNREGULATION OF MMP-2 EXPRESSION IN ASTROGLIOMA CELLS BY TUMOR NECROSIS FACTOR-α AND INTERFERON-γ.** Hongwei Qin and Etty N. Benveniste. Department of Cell Biology, University of Alabama at Birmingham, AL.



Poster Abstracts

THE APPEARANCE OF ACTIVATED MATRIX METALLOPROTEINASE-2 (MMP-2) IN RESPONSE TO ß-AMYLOID (Aß) IN RAT ASTROCYTES MAY BE DUE TO INCREASED MEMBRANE-TYPE MATRIX METALLOPROTEINASE (MT-MMP) EXPRESSION

S. Deb and P. E. Gottschall
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Aggregated Aß is associated with greater neurotoxicity, whereas the non-aggregated form induces neurite outgrowth and interacts with the extracellular matrix (ECM). The neuritic plaque consists of deposited Aß, components of ECM, along with ECM-degrading enzymes. We have shown that Aß(1-40) induces MMP-9, MMP-2, proenzyme and activated form, and MMP-3 production in rat primary cultures. Pro-MMP-2 is activated by a unique cell membrane-mediated process that involves MT-MMP. The aim of this study was to elucidate the mechanism by which Aß peptides increase the production of activated MMP-2. Astrocyte cultures were treated with aged and unaged Aß(1-40) and Aß(1-42) for 72 h. Both aged peptides were neurotoxic to hNT neurons (at 10 μM) and showed positive Congo red staining under polarized light. Zymography of the conditioned medium revealed that unaged Aß(1-40) and Aß(1-42) induced MMP-9 and MMP-2 activity. However, only Aß(1-40) (40 μM) stimulated the appearance of an activated form of MMP-2. Both forms of the aged peptide failed to induce MMP-2 or the activated form of MMP-2. In an effort to determine whether MT-MMP expression was involved in inducing the appearance of activated MMP-2, we estimated MT-MMP mRNA expression levels by RT-PCR. Unaged Aß(1-40) significantly induced the expression of MT-MMP mRNA levels as compared to RNA from untreated astrocytes. Treatment with Aß(1-42) failed to elicit an increase in MT-MMP mRNA as measured by RT-PCR. These results suggest that increased expression of MT-MMP may be responsible for the elevated levels of the activated MMP-2 following Aß (1-40) treatment in rat astrocytes. (Supported by NIH AG12160).

POSTER 1

ASSOCIATIVE INDUCTION OF GELATINASE ACTIVITY AND GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) LEVELS IN RAT BRAIN FOLLOWING KAINIC ACID TREATMENT

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Previously, we have shown that gelatinase A and B activity is differentially induced in regional brain areas only from convulsive rats, and not from non-convulsive rats, following kainate treatment. The objective of the present study was to determine whether the induction of MMP activity correlated with the well-characterized increase in astrocytic GFAP levels that occur after neuronal cell death. Neuronal degeneration was induced in Sprague-Dawley rats by systemic administration of Kainate and rats were divided into convulsive and non-convulsive groups. Animals were sacrificed 6, 12, 24, 72, and 168 h after injection of kainate. Gelatinase A and B were extracted from brain regions in buffer containing 1% Triton-X-100 and purified with gelatin-Sepharose. GFAP levels in correspondent regions were measured using sandwich ELISA. Gelatinase B levels in convulsive rats were induced 8-fold and 6-fold in hippocampus and frontal cortex, respectively, but were induced to a lesser degree in cerebellum. Three days after kainate treatment, gelatinase A activity and GFAP levels were significantly induced in hippocampus and cortex, but both remained unchanged in cerebellum derived from convulsive rats. Neither gelatinase activity nor the levels of GFAP were altered in non-convulsive rats receiving kainate. These results imply that the induction of MMP-2 activity is associated with the activation of astrocytes in areas of neuronal degeneration. (Supported by NIH AG12160).

POSTER 2

MMP EXPRESSION IN AN EXPERIMENTALLY-INDUCED DTH RESPONSE IN THE RAT CNS

D.C. Anthony, K.M. Miller, G.M.A. Wells, J.M. Clements, A.J.H. Gearing, and V.H. Perry
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In an experimentally induced model of MS the rat CNS, a broad-spectrum inhibitor of the MMPs, and TNF converting-enzyme, (BB-1101) reduced leukocyte recruitment, the degree of BBB breakdown, and the extent of tissue damage. This finding supports a role for MMP activity in the pathogenesis of this model, but provided no information concerning the contribution of individual MMPs. In the present study, we examined mRNA expression, by a qRT-PCR method, and protein expression, by IHC, of a range of MMPs and also TNFa in the DTH lesions and control tissue. In control tissue, mRNA for all of the MMPs examined was detectable, but at a low level. Of the MMPs examined by RT-PCR in the DTH lesions, significant increases in the level of mRNA expression were observed for MMP-7, MMP-12, and TNFa. A small increase in MMP-9 mRNA expression was also found. In normal rat brain, none of the MMPs investigated, except MMP-2, could be detected by IHC. However, in DTH lesions, where expression of MMP mRNA increased, there was a corresponding increase in protein expression detected by IHC. MMP-7, MMP-8, MMP-12, and TNFa, were all found to be strongly expressed by Mϕs in the DTH lesions. Strong staining for MMP-13 was also observed within the lesions without concomitant upregulation of the level of mRNA – possible reflecting changes in post-transcriptional events in the lesions. To determine whether the upregulated MMPs could invoke destructive events in the CNS, highly purified activated MMP-2, MMP-7, MMP-8, and MMP-9, were stereotaxically injected into the brain parenchyma. All provoked recruitment of leukocytes. In addition, MMP-9 induced marked BBB breakdown and, in places, hemorrhages. In conclusion, specific MMPs are upregulated in DTH lesions in the CNS; for the most part, upregulation of mRNA, as measured by RT-PCR, was a useful predictor of increased protein expression. From our injections of purified MMPs into the CNS, it is clear that the upregulated MMPs in the DTH lesions could participate in the disruption of the BBB, leukocyte recruitment, and tissue damage.

POSTER 3

MATRIX METALLOPROTEINASE (MMP) INHIBITORS GALARDIN (GM 6001) AND THE NOVEL DIBENZOFURAN PD 157341 INHIBIT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN PL/J x SJL/J(F1) MICE

Robichaud LJ, Bugajski JM, Dasovic D, Kupina N, Johnson L, Hupe D, Roth BD, White A, Colbry NL, and Boxer PA

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Mice sensitized with myelin basic protein fragment developed severe EAE by day 11 with remissions by day 21. Daily i.p. treatment with 200 mg/kg of GM 6001 or 50 mg/kg of PD 157341 (4-dibenzofuran-2-yl-4-hydroxyimino-butyric acid) dramatically inhibited EAE incidence, onset, weight loss, and both daily and peak severity. GM 6001 was a potent inhibitor (IC50) of full-length enzymes MMP-2 gelatinase A (5.8 nM), MMP-9 gelatinase B (25 nM), and MMP-1 collagenase I (3.5 nM). PD 157341 was a selective inhibitor of gelatinase A (1.3 EM) compared to gelatinase B or collagenase-1 (>100 EM). PD 157341 plasma levels were above those necessary to inhibit gelatinase A. These results suggest that MMPs, particularly MMP-2, may play a role in EAE and that inhibitors may have clinical utility in CNS autoimmune diseases.

POSTER 4

DOWNREGULATION OF MMP-2 EXPRESSION IN ASTROGLIOMA CELLS BY TUMOR NECROSIS FACTOR-α AND INTERFERON-γ

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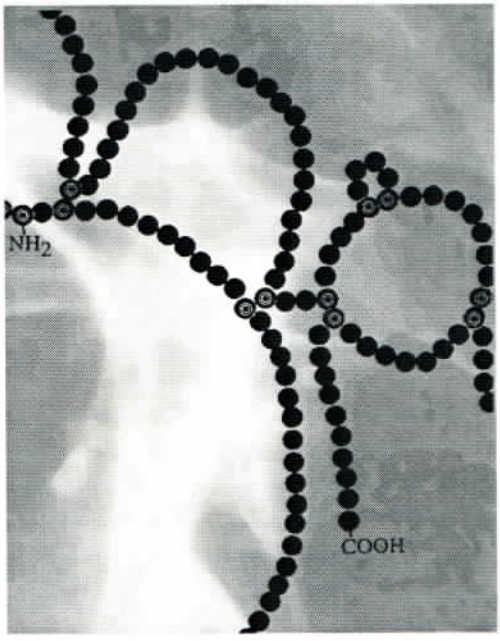
The invasion of primary brain tumors is accompanied by elevated expression of matrix metalloproteinases (MMPs). MMPs are a family of zinc-dependent endopeptidases, and expression is associated with normal tissue remodeling processes. However, MMPs also contribute to pathological conditions associated with progressive matrix degradation such as inflammation and tumor invasion. MMP expression is regulated at the transcriptional level by various cytokines acting through regulatory elements of MMP promoters. Gelatinase A (MMP-2) degrades subendothelial basement membrane constituents, and has been proposed to potentiate the invasion and metastasis of malignant tumors. We investigated the effects of two cytokines, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) on MMP-2 production in the human astroglioma lines U251 and CRT. Our results indicate that both cell lines constitutively express high levels of MMP-2 mRNA, protein, and bioactivity, as assessed by ribonuclease protection assay, western blotting, and zymography assays, respectively. TNF-α and IFN-γ individually can inhibit up to 50% of constitutive MMP-2 expression, and synergize for near complete inhibition of MMP-2 expression. The inhibitory effect of both TNF-α and IFN-γ requires the continuous presence of the cytokines and *de novo* protein synthesis. Inhibition of MMP-2 mRNA levels by IFN-γ and TNF-α is not mediated by destabilization of the MMP-2 message, suggesting an inhibitory effect on MMP-2 gene transcription. The inhibition of MMP-2 expression by TNF-α and IFN-γ raises the possibility that these cytokines may have beneficial effects in attenuating astroglioma invasive properties.

POSTER 5



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