Chronic Pain:
The Role of an Inflammatory Protein in the Brain
Disclosure Information

Robert N. Spengler, Ph.D.

• No financial interest that could be considered as conflict of interest.
**Types of Pain**

- **Acute Pain** – elicited by substantial tissue injury and activation of nociceptive transducers at the site of local tissue damage. Healing occurs.

- **Chronic Pain** – elicited by an injury or disease. May be perpetuated by factors other than the cause of the pain.
  - Stress, environmental, and affective factors contribute to the intensity and persistence of chronic pain.
  - People seek health care, but are often not treated effectively.
Chronic Pain

- **Chronic pain:** 97 million cases/year
  $100 billion/year
  *(Brain Facts, Society for Neuroscience, 1997 and The American Academy of Neurobiology Fact Sheets)*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Sufferers</th>
<th>Source (2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Pain</td>
<td>100 million Americans</td>
<td>Institute of Medicine of The National Academies</td>
</tr>
<tr>
<td>Diabetes</td>
<td>25.8 million Americans (diagnosed &amp; estimated undiagnosed)</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>Coronary Heart Disease (heart attack &amp; chest pain) Stroke</td>
<td>16.3 million Americans</td>
<td>American Heart Association</td>
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<td></td>
<td>7.0 million Americans</td>
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<tr>
<td>Cancer</td>
<td>11.9 million Americans</td>
<td>American Cancer Society</td>
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**Main characteristics of chronic pain:**
- Maladaptive
- Persistent → Continuous negative impact on mood/affect and cognitive function

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Neuropathic Pain

- Pain resulting from injury to the nervous system
- Central component
- Characteristic features: Hyperalgesia, Allodynia
Tumor Necrosis Factor-α

**DOUBLE-EDGE SWORD**

**Destructive functions:**
- Lethal tissue injury
- Cachexia (chronic starvation state)
- Brain disorders

**Beneficial roles:**
- Coordination of inflammatory responses
- Influences emotional behavior
- Neuroprotection

**Regulation of norepinephrine (NE) release**
The diagram illustrates the pain signaling pathway. Pain signals travel from peripheral sensory nerve fibers to the spinal cord, then to the dorsal horn, thalamus, and finally to the cortex. The limbic system is involved in processing these signals. Additionally, there is a pathway for descending inhibition that can block pain signals in the spinal cord and dorsal horn.
Neuropathic Pain

SC

TNF

TNF

TNF

SC

Nerve Injury

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Hypothesis

Enhanced levels of TNF in the brain are necessary for expression of chronic pain.
Chronic Constriction Injury (CCI) Model of Neuropathic Pain

- Bennett and Xie (1988)
- 4 loose 4.0 chromic gut ligatures
- Sham procedure - no ligatures applied
Thermal Hyperalgesia

Difference Score (ipsi- contra) (sec)

Days Post-surgery

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Effect of CCI on Levels of TNFα

WC Covey et al., BRAIN RES 859:113-122, 2000
Hippocampus

- Important role in memory formation/behavioral motivation
- Involved in affective/motivational aspects of pain
- Rich in noradrenergic nerve terminals
Immunohistochemistry

- Primary Ab: Rabbit anti-mouse TNF (1:100)
- Secondary Ab: Biotinylated goat anti-rabbit IgG (1:100)
- Enzyme conjugate
- Substrate (DAB)
TNF Immunoreactivity in the Hippocampus

(Magnification: 400X)

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TNF Immunoreactivity in the Hippocampus

(Magnification: 400X)

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Neuropathic Pain

Nerve Injury

SC

↑ TNF

↓ NE

↑ TNF

↑ TNF

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Field Stimulation and Superfusion of Hippocampal Brain Slices
90
60
30
0
30
60
90
0.01
0.03
0.1
0.3
1
3
10
% Inhibition
% Facilitation

[3H-NE Overflow]

[TNF] ng/ml

CCI-8 (4)
CCI-2 (3)
Control (16)
CCI-21 (5)
CCI-16 (9)
CCI-14 (7)
CCI-12 (6)

TA Ignatowski et al., NEUROPHARMACOLOGY 48:448-460, 2005
Neuropathic Pain

Microinfusion of rrTNF into the rat brain
Intracerebroventricular Microinfusion

- Microinfusion of compound into right lateral cerebral ventricle for 14 days

- Compounds Infused:
  Artificial Cerebral Spinal Fluid (aCSF)
  recombinant rat TNFα (1000ng/24hr, rrTNF)
Thermal Hyperalgesia

TA Ignatowski, et al., BRAIN RES 841:70-77, 1999
Thermal Hyperalgesia

CCI (5 -62)
Control w/1000 ng/24 hr rrTNF Infusion (3)

Days Post-Surgery

TA Ignatowski, et al., BRAIN RES 841:70-77, 1999
Field-Stimulated $^3$H-NE Release

JL Reynolds et al., JPET 310:1216-1225, 2004
Effects of Antidepressants on TNF and Pain

- **TCA**
- **Hippocampus**: Inhibition of Neurodegeneration
- **Brainstem**: Activation of Endogenous Anti-nociceptive Mechanisms
- **NE**: Analgesic Effect
- **5-HT and NE**: Antidepressant Effect
- **TNF**: Sustained input from nerve injury
- **CCI**: POPF Midwest PAIN Expo

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Antidepressant

- Treatment of neuropathic pain and depression
- Reverse hippocampal atrophy
- Antidepressant administration (ZIM, DMI, AMI) decreases TNF expression/inhibits production of TNF
Acute Treatment Paradigm

CCI

AMI

Days Post-CCI
Paw Withdrawal Latency

TA Ignatowski et al., NEUROPHARMACOLOGY 48:448-460, 2005

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TNF Regulation of $^3$H-NE Release

% Inhibition $^3$H-NE Overflow

% Facilitation $^3$H-NE Overflow

[TNF] ng/ml

CCI-8 post-saline (3)
CCI-8 post-amitriptyline (6)

TA Ignatowski et al., NEUROPHARMACOLOGY 48:448-460, 2005
Continuous Treatment Paradigm

CCI

AMI

Days Post-CCI

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Thermal Hyperalgesia

![Graph showing Difference Score (sec) vs. Days Post-Surgery]

- SAL-SHAM (3-5)
- AMT-SHAM (4-10)
- AMT-CCI (4-20)
- SAL-CCI (4-13)

Immunoreactive Staining for TNFα in the Hippocampus


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Alpha$_2$-Adrenergic Receptor Activation in Peritoneal Macrophage Harvested from Rats Microinfused with TNF$\alpha$ or aCSF

![Graph showing % Change in TNF in Supernatants for aCSF and rrTNF infused groups.](image-url)
CCI involves an increase in TNF production in the brain.

Increase in TNF production in the brain mimics CCI induced pain.

- This increased production of TNF in the hippocampus can be prevented by concomitant antidepressant drug administration during CCI.
- Increased production of TNF in the brain directs peripheral macrophage
Translational Relevance
They’re the most powerful painkillers ever invented. And they’re creating the worst addiction crisis America has ever seen.

By Massimo Cafarelli

Morphine & Morphine derivatives

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Clonidine Suppresses Plasma and Cerebrospinal Fluid Concentrations of TNF-α During the Perioperative Period

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The analgesic properties of α2-agonists are well known. In experimental models, tumor necrosis factor (TNF)-α regulates adrenergic responses in the brain. Constitutive TNF-α, in brain regions involved in pain perception, is decreased after the administration of clonidine. We investigated patients undergoing lower-extremity revascularization. Seven patients were clonidine 0.2 mg per os (low), and the other 0.4 mg per os clonidine (high). Eight patients received placebo and were Continuous spinal anesthesia was performed with a pliable catheter into the subarachnoid space. Plasma and cerebrospinal fluid were obtained before injection of local anesthetic agents were analyzed for TNF-α using a Systemic and central release of catecholamines by high-pressure liquid chromatography with electrochemical detection of norepinephrine in plasma and cerebrospinal fluid. Clonidine increased the plasma concentration of norepinephrine by 2- to 4-fold, and the cerebrospinal fluid concentration of norepinephrine was increased by 8- to 10-fold. The analgesic efficacy of clonidine was not observed in this study.

Original Article

Adjuvant Therapy With Intrathecal Clonidine Improves Postoperative Pain in Patients Undergoing Coronary Artery Bypass Graft

Nader D. Nader, MD, PhD, Carlos M. Li, MD, Hasan H. Doshooghi, MD, Tracey A. Ignatowski, PhD, and Robert N. Spengler, PhD

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Background: α2 adrenergic agonists have long been employed as anesthetics and to sedate patients undergoing surgical procedures. In addition, their therapeutic response synergizes with that elicited by opioids. Although this response is well known, the role of α2 agonists, such as clonidine, during various painful surgical procedures remains to be elucidated. The goal of our study was to evaluate the effects of the intrathecal administration of clonidine on postoperative pain control and time to extubation in patients undergoing coronary artery bypass grafting.

Key Words: coronary revascularization, postoperative pain, respiration, spinal, clonidine


α2 adrenergic agonists have been used alone and in combination with opioids to provide sedation and analgesia for patients undergoing various procedures. They enhance
Neuropathic Pain & Clonidine

THE $\alpha_2$-ADRENERGIC RECEPTOR

• Key role in pain modulation
• Inhibits brain NE release in association with TNF and is increased during neuropathic pain
• Agonists widely used as analgesics in clinical settings. CLONIDINE
Immediate Neurological Recovery Following Perispinal Etanercept Years After Brain Injury

Edward Tobinick · Helen Rodriguez-Romanace · Arthur Levine · Tracey A. Ignatowski · Robert N. Spengler

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Abstract. Positron emission tomographic brain imaging and pathological examination have revealed that a chronic, intracerebral neuroinflammatory response lasting for years after a single brain injury may occur in humans. Evidence suggests that the immune signaling molecule, tumor necrosis factor (TNF), is centrally involved in this pathology through its modulation of microglial activation, role in synaptic dysfunction, and induction of depressive symptoms and neuropathic pain. Etanercept is a recombinant TNF receptor fusion protein and potent TNF inhibitor that has been found to reduce microglial activation and neuropathic pain in multiple experimental models. We report that a single dose of perispinal etanercept produced an immediate, profound, and sustained improvement in expressive aphasia, speech apraxia, and left hemiparesis in a patient with chronic, intractable, debilitating neurological dysfunction present for injury. These results induced a pathologic leptinergic target that can result in rapid administration and producing immediate neurological dysfunction.

Key Points
- Acute brain injury
- Intracerebral neuron activation
- Excess tumor necrosis factor
- Etanercept in addition to microglial activation
- Perispinal administration producing rapid functional recovery

Electronic supplementary material. The online version of this article (doi:10.1007/s40261-014-0186-1) contains supplementary material, which is available to authorized users.

Perispinal Etanercept for Post-Stroke Neurological and Cognitive Dysfunction: Scientific Rationale and Current Evidence

Tracey A. Ignatowski · Robert N. Spengler · Krishnan M. Dhamdaphani · Hely Fokkerma · Roger F. Butterworth · Edward Tobinick

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Abstract. There is increasing recognition of the involvement of the immune signaling molecule, tumor necrosis factor (TNF), in the pathophysiology of stroke and chronic brain dysfunction. Etanercept plays an important role both in modulating synaptic function and in the pathogenesis of neuropathic pain. Etanercept is a recombinant therapeutic that neutralizes pathologic levels of TNF. Brain imaging has demonstrated chronic intracerebral microglial activation and neuroinflammation following stroke and other forms of acute brain injury. Activated microglial release TNF, which mediates neurotoxicity in the stroke penumbra. Recent observational studies have reported rapid and sustained improvement in chronic post-stroke neurological and cognitive dysfunction following perispinal administration of etanercept. The biological plausibility of these results is supported by independent evidence demonstrating reduction in cognitive dysfunction, neuropathic pain, and microglial activation following the use of etanercept, as well as multiple studies reporting improvement in stroke outcome and cognitive impairment following therapeutic strategies designed to inhibit TNF. The causal association between etanercept treatment and reduction in post-stroke disability satisfies all of the Bradford Hill Criteria: strength of the association; consistency; specificity; temporality; biological gradient; biological plausibility; coherence; experimental evidence; and analogy. Recognition that chronic microglial activation and pathologic TNF concentration are targets that may be therapeutically addressed for years following stroke and other forms of acute brain injury provides an exciting new direction for research and treatment.

Key Points
- Accumulating evidence suggests that chronic post-stroke intracerebral microglial activation and
Dr. Edward Tobinick treats stroke victim Anna Alfaro. He has a patented method for delivering the anti-inflammatory medicine etanercept to the brain.
Rapid intracerebroventricular delivery of Cu-DOTA-etanercept after peripheral administration demonstrated by PET imaging

Edward L Tobinick*, Kai Chen and Xiaoyuan Chen

Published: 27 February 2009
BMC Research Notes 2009, 2:28

This article is available from: http://www.biomedcentral.com/1756-0500/2/28
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Fig.1. PET image transverse section of a living rat brain following perispinal extrathecal administration of $^{64}$Cu-DOTA- etanercept, imaged 5-10 minutes after administration.
Conclusions

• Chronic pain is controlled by an inflammatory protein (TNF) that is produced in the brain.

• TNF regulates NE release in the brain.

• Basic science discoveries translate to clinical efficacy of TNF blockade in the brain.
Acknowledgements

Collaborators
Tracey A. Ignatowski, PhD
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Jessica Reynolds, PhD
Amy Renauld, PhD

Funding Support
NIH 5R01NS41352 (RNS, PRK, TAI)
NIH R03 (RNS)
Spinal Cord Research Fdn (TAI, RNS)
Paralyzed Veterans of America (RNS)
Arthritis Foundation (RNS)
American Heart (RNS)

Technical Assistance
Wade Sigurdson, PhD

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