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Gene Therapy Approach: HSV-Enkephalin Reduces Fibrosis, Inflammation, and Pain

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1. Introduction

Chronic alcohol abuse is a major precipitating cause of pancreatitis along with genetic predisposition. Chronic alcohol and alcohol metabolite exposure can produce profound pancreatic tissue destruction in addition to damaging effects to other organs (Apte et al., 2009; Pezzilli et al., 2009). Pancreatitis causes over 2,000 deaths per year and over 100,000 hospitalizations. Acute initial attacks can be fatal and about 20% of these elevate to chronic conditions which are irreversible. Chronic pancreatitis places patients at 50-60% increased risk of progressing to pancreatic cancer. Severe unmitigated pain is synonymous with pancreatic inflammation and cancer caused by fibrotic blockage of the ductal system, premature trypsin activation, inflammation, perineural damage and nerve exposure.

Pancreatitis is characterized by severe histopathological changes, such as the presence of inflammatory mediators, acinar atrophy, fat necrosis, intraductal hemorrhage, periductal fibrosis and stromal proliferation (Schmidt et al., 1995). Elevated serum α -amylase, lipase, and CRP levels serve as biochemical markers of acute pancreatitis (Merkord et al., 1997; Sparmann et al., 1997). Acute pancreatitis ranges from mild edematous conditions that usually heal without intervention, to severe hemorrhagic necrotizing inflammation that is often fatal over a period of days as patients succumb to abdominal sepsis and multi-organ failure (Schmidt et al., 1992; Vardanyan and Rilo, 2010). The level of pain experienced by these patients is directly linked to decreased pancreatic functioning and increased length of stay during hospitalizations. In patient surveys, 32% of patients in chronic pancreatitis pain report being willing to try any new therapy for relief, and some may resort to suicide for this intractable pain state. Thus, the need to pursue novel pain relief strategies remains high for patients with chronic pancreatitis pain and those with pancreatic cancer who now have increasingly longer survival times.

2. Acute and chronic pancreatitis models: histological features

We have examined several *in vivo* and *in vitro* models of alcohol injury in combination with a gene therapy approach to examine ability to reduce the consequences of alcohol related injury. An acute inflammatory pancreatitis is induced in rats with a noxious chemical, dibutyltin dichloride, used in fertilizers and plastics manufacturing. A chronic pancreatitis is induced by maintenance on a high fat and alcohol (6%) diet. Both the chemical and the diet

induced pancreatitis models produce histologically evident damage to the chymotrypsin producing acinar cells, inflammatory cell invasion and activation, interstitial edema, cell swelling and proliferation of local tissue stellate immune cells within the pancreas (Fig. 1; Lu et al., 2007; Yang et al., 2008).

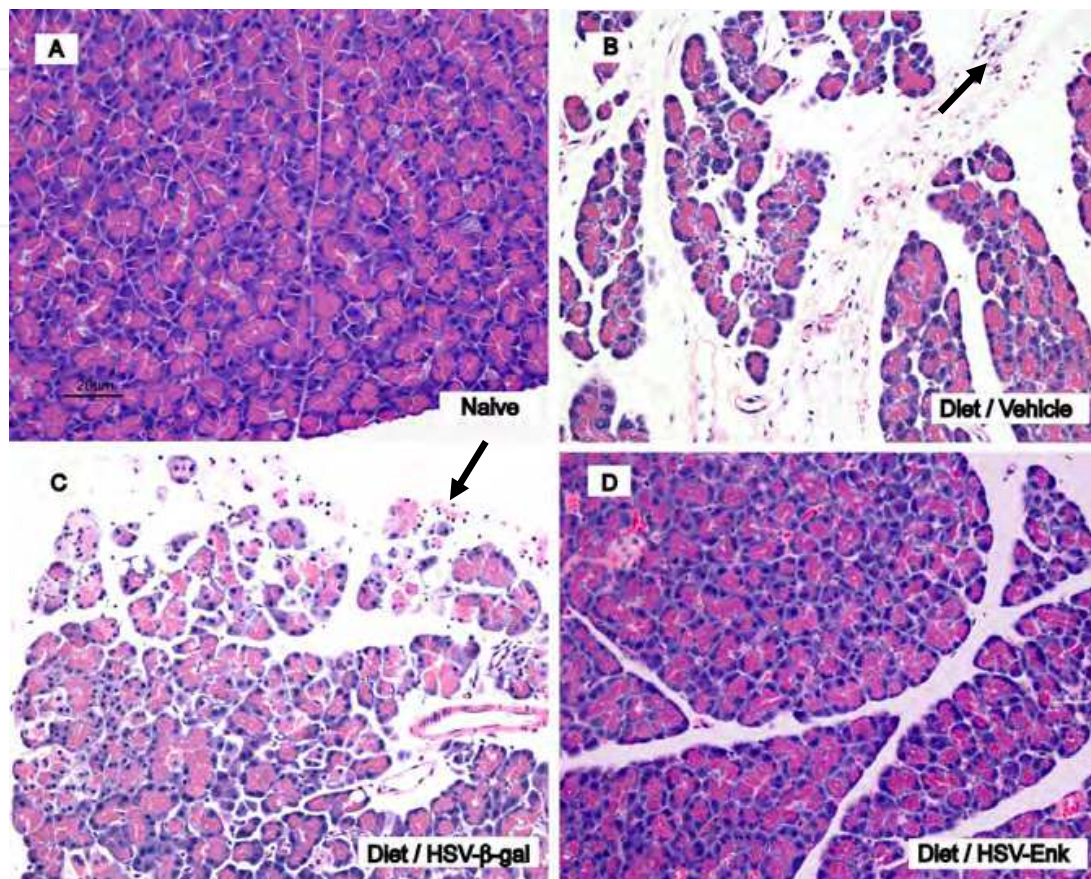
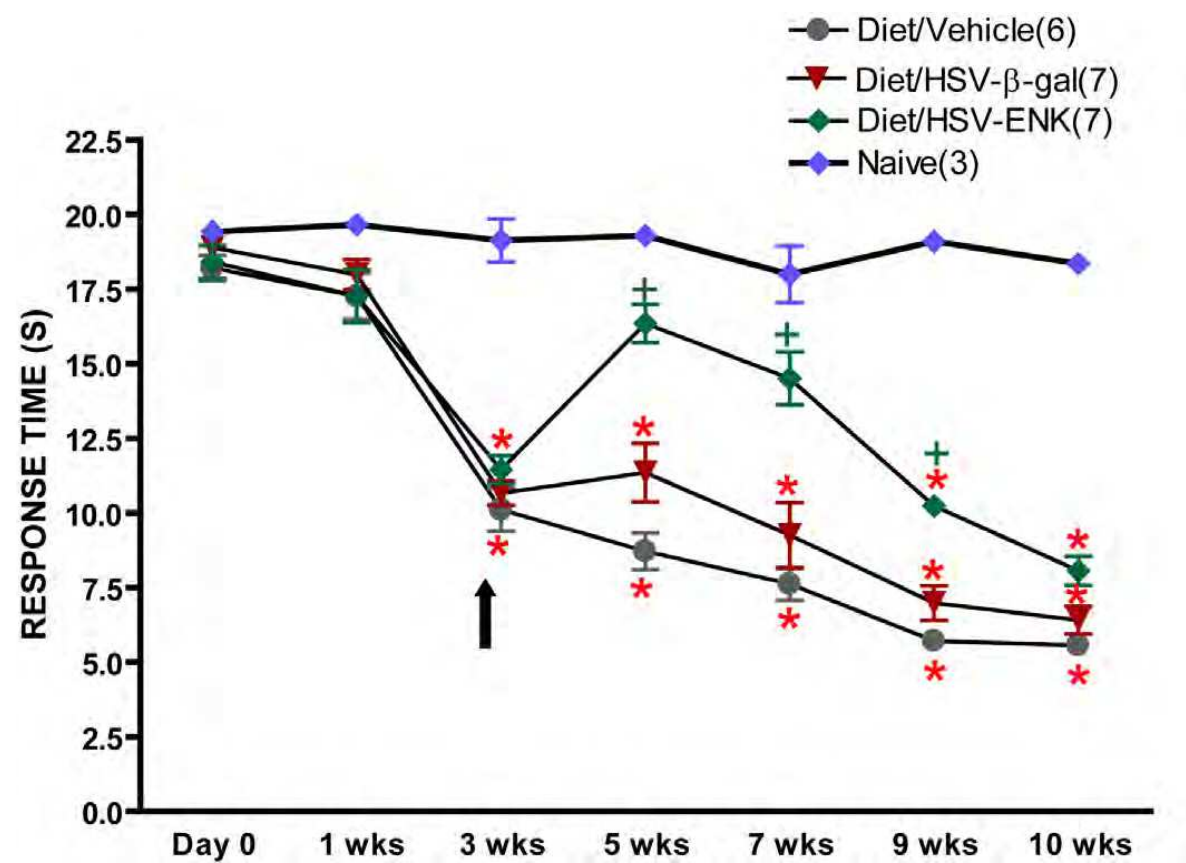


Fig. 1. **Histopathology of rat pancreas at week 10.** A. Naïve animals were fed low soy chow and given no treatment. B. Method control animals given the alcohol (6%) and high-fat diet to induce pancreatitis were given pancreatic injection of vehicle (DMEM) only. C. Some animals with alcohol and high-fat diet induced pancreatitis were given pancreatic HSV- β -gal applications, serving as the vector control. Note the steatosis, inflammatory cell infiltration (arrows), acinar cell necrosis, tissue edema, ductal widening and periductal fibrosis seen in the controls, with alcohol and high-fat diet induced pancreatitis given vehicle or the HSV- β -gal applications (B and C). D. Greatly reduced inflammatory cell infiltration and preservation of pancreatic tissue architecture was seen in animals fed the alcohol and high-fat diet but treated with the HSV-ENK vector. The histopathology of the HSV-ENK vector treated animals was similar to that of the naïve animals (A). Hemotoxylin and eosin (H&E) stain. (Reprinted from Yang et al., 2008)

3. Acute and chronic pancreatitis models: pain related behaviors

These pancreatitis models also produce pain related behaviors. The novel open field test box revealed significant reductions in active behavioral measures (exploratory rearing; beam breaks; active time, duration, distance traveled), as well as increased rest time for animals with acute pancreatitis (Fig. 2).



Hotplate test of thermal sensitivity for naïve rats or rats with pancreatitis given either vehicle, HSV-ENK, or HSV-β-gal treatment. * $p \leq 0.05$, vs Day0 (Mann Whitney U test); + $p \leq 0.05$, Diet/HSV-Enk group vs Diet/Vehicle (Mann Whitney U test).

Fig. 2. Hot plate response latency nociceptive behavior measurements. Hot plate response latency measurements are shown for naïve animals and animal groups with alcohol and high-fat induced pancreatitis. Hot plate test was conducted at baseline before induction of pancreatitis and for ten weeks subsequently. Note the significant shortening of hot plate response latencies for rats on the high fat and alcohol diet after week 3, indicating sensitization. The HSV-ENK treatment (arrow) significantly abrogated the shift in response latency for at least four weeks. Four weeks is typical of HSV vector expression. (Reprinted from Yang et al., 2008)

4. Opiate gene therapy studies

In initial fMRI studies using the acute DBTC induced pancreatitis model, we determined levels of neuronal activation in higher brain centers along the visceral pain pathway (Westlund, 2000) finding significant activation in rostral ventrolateral medulla, dorsal raphe, periaqueductal grey, medial thalamus and central amygdala in rats (Westlund et al., 2009).

The pancreatitis induced brain activation was reduced by administration of morphine. Thus, we subsequently studied reduction of histological and behavioral consequences of the Herpes viral vectors (HSV-1) that overexpress the precursor of the endogenous opiate met-enkephalin in both the acute and chronic pancreatitis models. The gene therapy approach was used to overexpress the precursor for the endogenous opiate peptide met-enkephalin and was found to provide histological and behavioral mitigation of the histological (**Fig. 1D**) and behavioral changes (**Fig. 2**) induced by the pancreatitis. Met-enkephalin is an opioid growth factor known to increase wound healing and restore homeostasis in the cornea (Sassani et al., 2003).

The chronic high fat diet and alcohol-induced pancreatitis allowed study of the full time course for enkephalin's effectiveness (6 weeks) after a single inoculation directly into the pancreas (Yang et al., 2008; Westlund, 2009a). Met-enkephalin gene therapy is effective in the chronic alcohol diet induced pancreatitis model, as well as in an acute chemically induced model for reduction of tissue injury, fibrosis, inflammation, and pain-related behaviors (Lu et al., 2007; Yang et al., 2008; Westlund, 2009a). The fibrosis was abundant in animals with chronic pancreatitis and was stainable with picosirius (overnight 0.1% Sirius red) as an addition to hemotoxylin/eosin histology (**Fig. 3**, right). Fibrosis produced by activated stellate cells characterizes the model as a chronic condition since it can lead to ductal stenosis that is one of the primary causes of pain for patients with pancreatitis. Histological data demonstrates that the proenkephalin gene product delivery to pancreas is reparative (**Fig. 1D**).

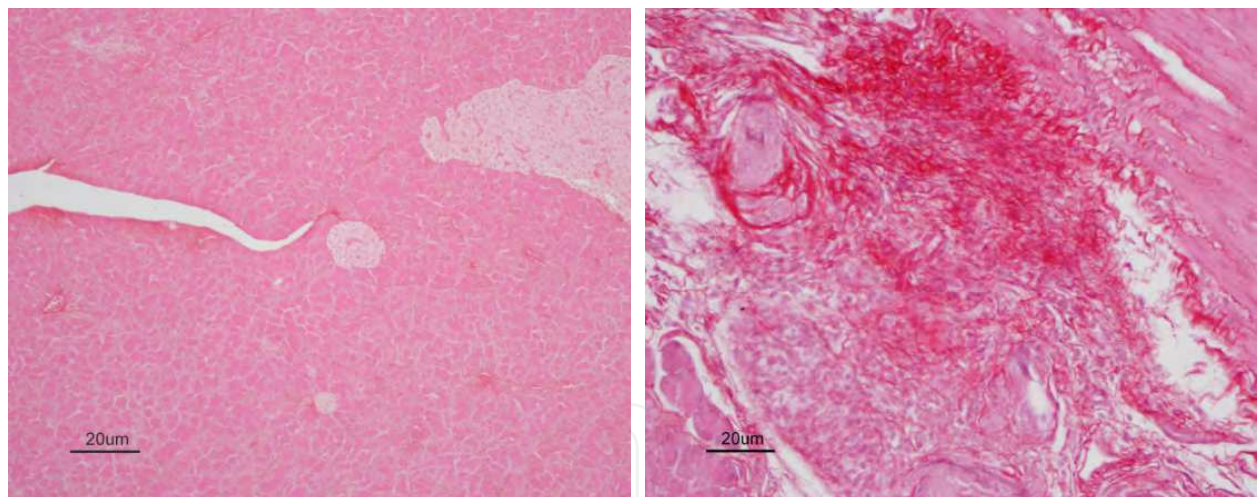


Fig. 3. Alcohol and high fat diet induced pancreatic fibrosis. Histological features evident in control pancreatic tissues (left) were severely disrupted in animals with chronic pancreatitis fed the high fat and alcohol diet (right). Fibrosis evident with Sirius red staining was abundant in the animals with pancreatitis and was less dense in animals given the HSV-1 proenkephalin viral vector.

Staining for met-enkephalin is elevated only in the group treated with the HSV-ENK overexpression vector in chronic alcohol diet fed rats after 10 weeks (**Fig. 4, 5**), as well as in the chemically induced pancreatitis at one week. Immunohistochemical localization of the HSV-1 proenkephalin overexpression product, met-enkephalin, was identified in abundance in both the spinal cord (**Fig. 4D**) and the pancreas (**Fig. 5D**). Levels of met-enkephalin in

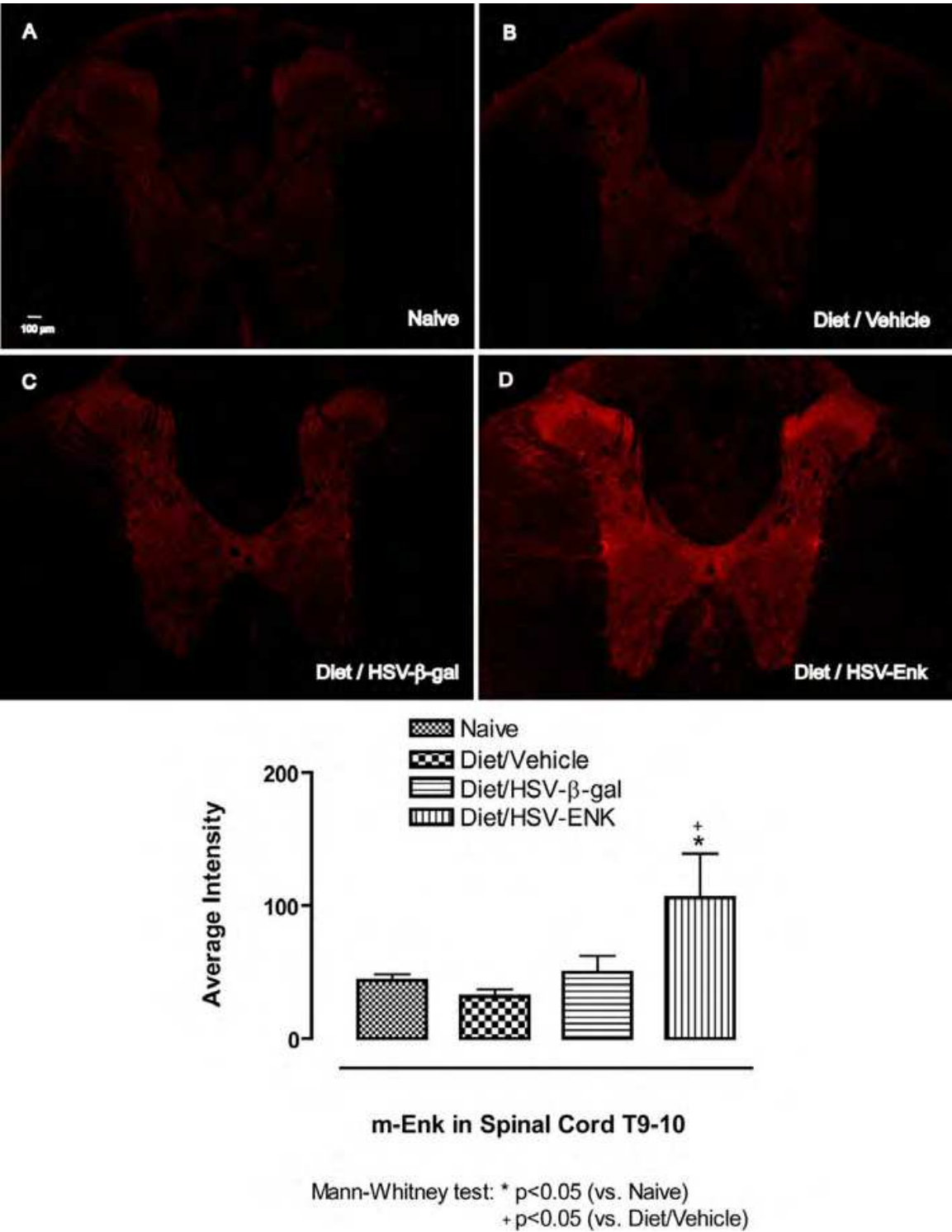


Fig. 4. **met-Enkephalin immunohistochemical staining in spinal cord (T9-10).** A. The spinal cord from a naïve rat is shown for comparison to (B) the spinal cord of animals with diet-induced pancreatitis at week 10. C. The expression of met-ENK after application of vehicle or HSV-β-gal is similar to naïve rats (A). D. Met-ENK expression was significantly increased in HSV-ENK vector-treated animals compared to controls. Met-ENK in the dorsal horn (laminae I-II) of the thoracic spinal cord was increased bilaterally. (Reprinted from Yang et al., 2008)

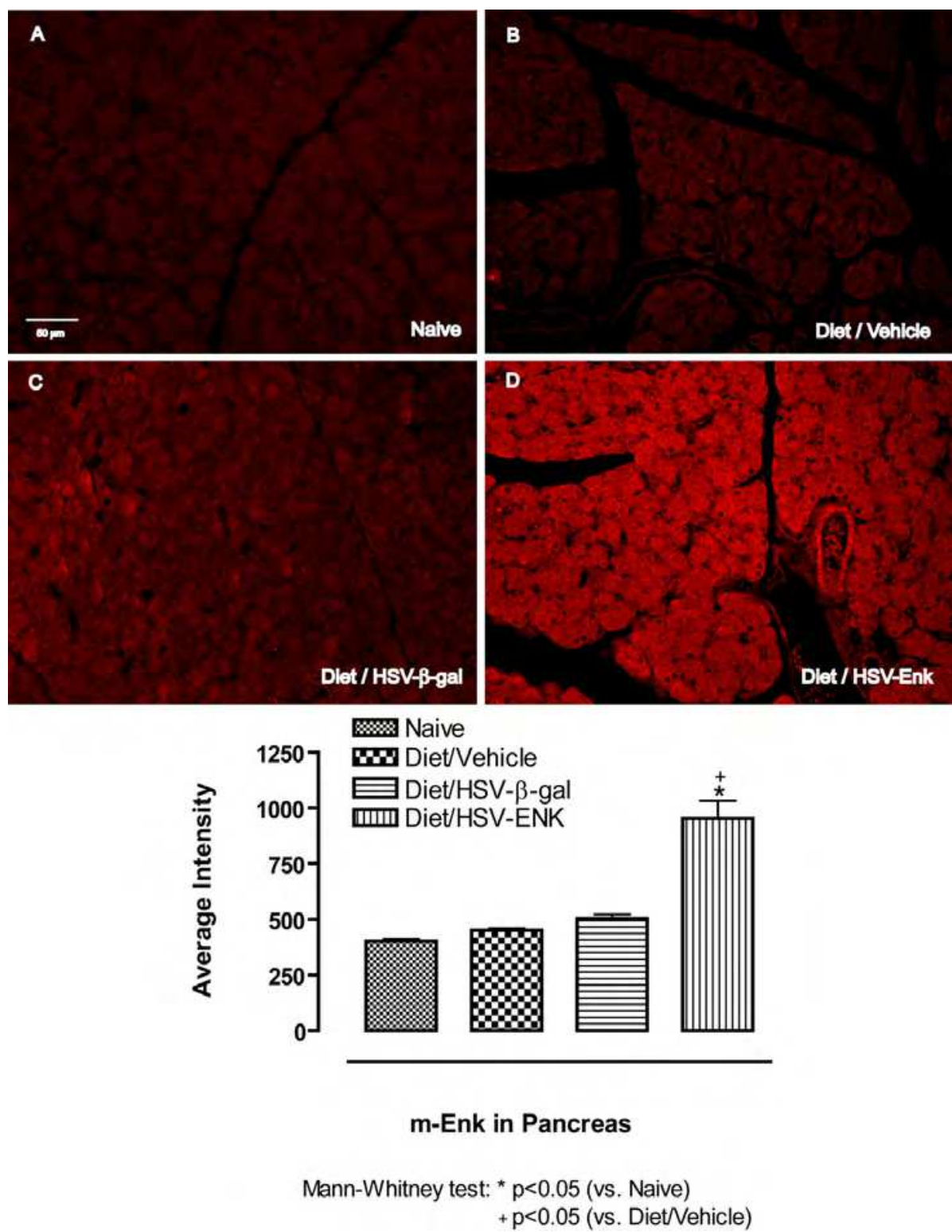


Fig. 5. **met-Enkephalin immunohistochemical staining in pancreas.** Photomicrographs and quantification of immunohistochemical staining for met-enkephalin in pancreas are shown for week 10. Minimal or no staining is seen in pancreas of (A) naïve and (B-C) control animals with alcohol and high-fat diet induced pancreatitis. D. Met-ENK expression was significantly increased in the pancreas of HSV-ENK-treated animals fed the same diet compared to the controls. (Reprinted from Yang et al., 2008)

pancreas were measured in a small number of animals with HPLC after one week of acute DBTC-induced pancreatitis. Levels, barely detectable in control pancreas, increase to about 3500ng/ml after one week of met-enkephalin overexpression. No staining for met-enkephalin was evident in naïve, vehicle or in animals receiving control viral vector (**Fig. 4A-C, 5A-C**).

There is negligible RANTES staining in naïve controls (**Fig 6A**) or animals with pancreatitis after met-enkephalin overexpression (**Fig 6D**) with either model indicating met-enkephalin protects the pancreas from effects of invading inflammatory cells. Staining for the inflammatory mediator RANTES is clearly evident in the pancreas in vehicle- and HSV- β -gal-treated animals with pancreatitis at week 10 (**Fig 6B and 6C**, respectively) at week 10. Both acinar cells and infiltrating inflammatory cells were positive for RANTES and COX-2 at one week (not shown).

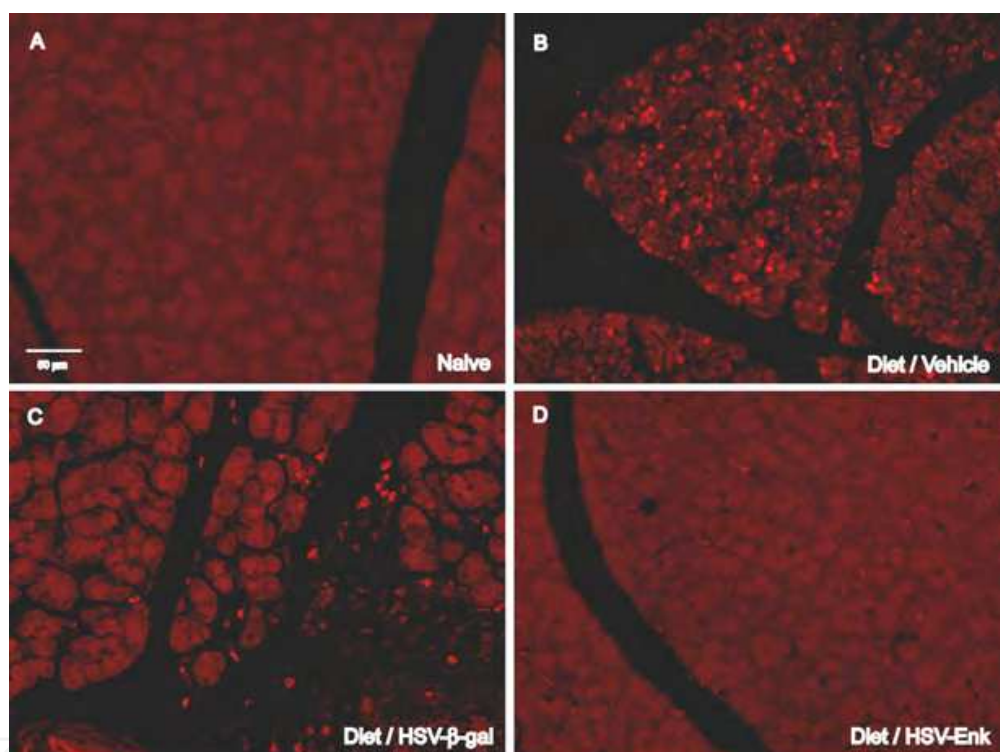


Fig. 6. RANTES immunohistochemical staining in pancreas. Photomicrographs of immunohistochemical staining of RANTES in the pancreas of rats are shown for week 10. A. Naïve rat pancreas. B. Diet-induced pancreatitis and application of vehicle as control. C. Diet-induced pancreatitis and application of the HSV- β -gal control vector. D. Animals given the same diet and application of HSV-ENK. Note the increased RANTES staining in pancreata of animals with alcohol and high-fat diet induced pancreatitis treated with vehicle or HSV- β -gal applications. Little or no staining of RANTES is noted in naïve and HSV-ENK-treated animals. (Reprinted from Yang et al., 2008)

We propose that met-enkephalin is acting as a protective/restorative agent against pancreatic insult. Met-enkephalin acts on μ -opioid receptors that we observed in pancreatic acinar and stellate cells in our chemically induced pancreatitis model (Lu et al., 2007). Increased expression of μ -opioid receptors was detected in the pancreas with the DBTC induced acute

pancreatitis at one week. The μ -opioid receptors induced in pancreas by DBTC pancreatitis are reduced by HSV-enkephalin except in stellate cells (Lu et al., 2007).

In our studies, HSV-ENK injected rats with pancreatitis have normal or nearly normal responses to noxious heat (**Fig. 2**). Opioid peptides generated from three precursor genes, proenkephalin, prodynorphin and POMC, are conserved phylogenetically (Salzet, 2001). The mechanism of action for reduction of pain related behaviors in this and previous studies are likely related to enkephalin's influence directly both on the central and the peripheral opioid receptors by the overexpressed met-enkephalin at those sites (**Figures 4, 5**). Proenkephalin derived opioid peptides are released peripherally after HSV-ENK administration (Yeomans et al., 2006). Their effect would mimic the enhanced endogenous release of opiates from immune cells that invade the region of inflammation and modulate both pain and inflammatory parameters (Stein, 1995). While many of the opiate peptides are delivered by inflammatory cells drawn to sites of inflammation, opioid neuropeptides may be more prominent in influencing nociceptive signaling when delivered at the synaptic endings. Proenkephalin gene product met-enkephalin is expressed in a 3:1 ratio relative to proenkephalin gene product, leu-enkephalin. The factors regulating why some gene products from the opioid families have more efficient translational processing, or are expressed in greater ratios is not completely understood (Danielson and Dore, 1999). The proenkephalin viral construct is chosen for proposed gene therapy pain studies since it is more prominent in influencing nociception.

The opioid-mediated anti-hyperalgesia of HSV-ENK infected animals mimics the effects of endogenous or intrathecally administered enkephalin in our hands. However, enkephalins are very labile and the natural neuronal ending release provided by the HSV-1 viral vector is a superior release method. In a cutaneous inflammation model, HSV-ENK infected animals were no different from controls suggesting that opioids are not tonically released, but are released only when there is a substantial activation of the afferents providing evidence that hyperalgesia can be blocked without altering baseline nociception (Wilson et al., 1999).

Our studies were equally successful with replication conditional or replication defective viral constructs. Both effectively reduce nociceptive behaviors in our models and in somatic pain models in previous studies (Wilson et al., 1999) but have no effect in control animals. Previous studies of HSV-1 transgene therapy have been used successfully to assess the anti-nociceptive effects of transduced opioids in inflammatory models, including experimental models of cutaneous inflammation and polyarthritis (Braz et al., 2001; Wilson et al., 1999; Wilson and Yeomans, 2000; Wilson and Yeomans, 2002). In the previous studies, application of HSV-1 virus vector containing a human proenkephalin gene resulted in transmission by viral spread through primary afferent fibers into the dorsal root ganglia. Subsequent protein expression from the proenkephalin or β -galactosidase gene (control neutral protein) could be visualized in the spinal cord (Wilson et al., 1999). Human proenkephalin-encoding HSV-1 viral vector reduced hyperalgesia by 60% in a polyarthritis model (Braz et al., 2001). Histological and behavioral effects were observed at 4-9 days post-infection (Wilson et al., 1999). The maximal response after hindpaw application of HSV-1 enkephalin-coding viral vector was reported at 14 days (Braz et al., 2001). Both of these studies validate that time is required for incorporation of viral vectors into nerve terminals, for retrograde transport of the viral vector to the DRG, and for subsequent peptide production.

Additional findings in the experimental polyarthritis model reported by Braz and colleagues (2001) was clear radiographic evidence that the animals receiving the HSV enkephalin gene encoding viral vectors also sustained significantly less joint destruction than the control animals after CFA injection. Both studies demonstrated that the opioid receptor antagonist, naloxone, delivered subcutaneously or intrathecally, could partially or completely restore sensitization in the HSV-1 enkephalin encoding vector infected animals but had no effect on HSV-1- β -galactosidase encoding vector or mock infected animals. This suggests opioid mediation of observed anti-nociceptive effects and suggests that the effect was in part due to spinal release of opioids. Naloxone methiodide administration increased hyperalgesia in animals infected with HSV-ENK when administered for three days (Braz et al., 2001). In another model, HSV-ENK delivery by intradermal application was as effective in controlling pain related measures as intrathecal administration of enkephalin (Wilson et al., 1999). These and our studies are compelling in that HSV-1 opioid gene delivery potentially offers a significantly sustained response (up to 7 weeks) in the experimental rodent models. **Thus, analgesia and restorative effects of HSV-Enk gene therapy will likely be effective in both somatic and visceral clinical pain.**

5. Viral vectors: analgesic and anti-inflammatory potential

Few studies to date report effective decreases in ongoing visceral pain with pharmacological treatments other than with opiates. Activation of opiate receptors leads to potent analgesia (Schafer et al., 1998), and opiates remain the primary therapeutic agent despite significant side effects and development of tolerance. Gene therapy is a novel drug delivery system capable of bringing over-expressed opiates directly to pancreatic tissues. The site specific delivery of HSV-1 viral vectors, which have an affinity for uptake by primary sensory neuronal endings (neurotropic), provided the preferable gene delivery construct for visceral anti-nociception. Wild type HSV-1 is a 154 Kb neurotropic double stranded DNA virus, containing 84 essential and nonessential viral genes. After natural primary cutaneous or mucosal inoculation, viral particles enter sensory axon terminals innervating the affected area. They are carried by retrograde axonal transport from the periphery to DRG, where the virus may establish a life-long latent state (Burton et al., 2001; Steiner et al., 1990). For gene therapy applications, the modified HSV-1 nucleocapsid and tegument can also use the same transport mechanism for successful passage to DRG. Modified viruses have been constructed and used successfully in many pre-clinical studies and Phase I/II clinical trials. Results of trials are published at www.oxtl.com. In one strategy, replication defective viral constructs have deletions of essential immediate early genes from the HSV-1 genome. In another strategy, replication conditional viruses are generated by insertion of the desired gene into the HSV-1 genes required for productive infections (i.e. thymidine kinase gene).

A recent Phase I clinical trial administered a replication defective HSV vector similar to the one given in our studies to upregulate proenkephalin (Wolfe et al., 2009b). The clinical report indicates that the vector provided significant pain relief for 12–24 patients with intractable focal pain from terminal cancer.

There are several inherent advantages to using HSV based viral constructs for foreign gene delivery in certain clinical settings, rather than other viral delivery models under study. The inserted genes are under the control of a strong constitutive human cytomegalovirus

promoter (hCMV), which **allows expression of inserted gene product at an intracellular locale, in the absence of productive HSV-1 infection and without integration into the host genome** (Wilson et al., 1999). The recombinant viral vectors designed for gene therapy rendered replication conditional or defective do not produce productive viral infection *in vivo*, but can persist for months despite negligible viral protein synthesis. These viral constructs establish a quiescent state similar to natural viral latency but **cannot reactivate to cause active infection in neuronal cells *in vivo*** (Goins et al., 2001; Wilson et al., 1999; Wilson and Yeomans, 2002). Lentiviruses as vectors offer very efficient infection, gene expression in activated cells and genome integration into host DNA for gene replacement therapy. However, current lentiviral constructs result in systemic infection and cannot be contained and the site of lentiviral insertion into the host genome cannot be controlled. They are considered a potential causal agent of cancers, autoimmune diseases and acquired immune deficiency syndrome (AIDS). Potential concerns exist regarding development of helper phenonoma for unwanted expressions and genetic recombination with other lentiviruses, including those already integrated in the host genome. Viral construct design for adenoviruses has also yielded promising results, but adenoviruses may not be advantageous since they can also involve the central nervous system.

HSV-1 viral constructs may offer unique advantages in peripheral inflammation, as they selectively infect primary sensory neurons but do not integrate into the host genome. Thus, they will be an independent “minipump” source for protein synthesis in the neuronal cytoplasm. As a DNA-based viral construct, (1) the rate of mutation and recombination in these HSV-1 constructs will be minimal to nonexistent since (2) these constructs do not enter into productive infection or latency phases. Further, preliminary data suggest that HSV replication deficient viral constructs do not generate proteins that would induce an amnestic response from the host, activating latent prior HSV-1 infection. These are very important properties of HSV-1 based viral vectors, as 70-90% of the human adult population has evidence of prior HSV-1 infection. This limited potential to generate a host response also improves the potential for using repeated dosings of HSV-based viral constructs. Adenoviruses cause productive infection, induce host inflammation above the inflammation already generated in target tissues and increase potential for extended injury and host innate immune response to the virus making repeated doses problematic (Minter et al., 2001).

Local injection of HSV-1 viral construct with human proenkephalin gene insert results in targeted tissue expression of opioid protein (Yeomans et al., 2006), whereas systemic administration of some neural agents have undesirable or intolerable side effects (Goss et al., 2002). HSV-1 gene delivery and local opioid expression may potentiate exogenously administered morphine, lowering doses needed and delaying morphine tolerance (Stein et al., 1996). This has been demonstrated in other HSV-based gene therapy models, where HSV-viral vector infections potentiated chemotherapy agents in lung cancer (Toyoizumi et al., 1999) and breast cancer (Thomas and Fraser, 2003). Attenuated herpes simplex viruses have already been successfully used in Phase I/II trials for treatment of CNS glioblastoma without apparent nonspecific toxic effects (Papanastassiou et al., 2002). The efficacy and potency of peripheral opioid effects are generally enhanced when drugs are administered during active inflammatory conditions (Antonijevic et al., 1995; Lamigeon et al., 2001; Schafer et al., 1998; Stein, 1995; Walker, 2003).

6. Anti-inflammatory effects and tissue protection by HSV-Enk treatment

It is well known that long term administration of morphine reduces immune function through reduced hypothalamic-pituitary-adrenal axis activation. Opioid receptors are constitutively expressed in non-neuronal sites including vascular endothelial cells (Cadet et al., 2000 and Saeed et al., 2000), on immune cells such as macrophages and lymphocytes (Gavériaux et al., 1995), and keratinocytes (Bigliardi et al., 2002). A role for neuronal opiates has also been shown for reduction of inflammation (for review see Machelska and Stein, 2003). While the role/s of specific extra-neuronal peripheral opioid receptors has not been fully established, mu (morphine), kappa (U50488H) and delta-2 (deltorphan II), but not delta-1 (DPDPE) opioid agonists have been shown to produce dose-dependent immunosuppressive effects on a plaque-forming assay, effects that were blocked by respective selective opioid antagonists (Rahim et al., 2001). It is also well established that the spinal cord can regulate peripheral inflammation through a variety of dorsal horn receptor mechanisms and retrograde primary afferent activity (Rees et al., 1994; Ren and Dubner, 1999; Sluka et al., 1993; Sluka et al., 1994; Sluka and Westlund, 1993; Sorkin et al., 2003). This includes glutamate, GABA, substance P and adenosine receptor mechanisms likely influenced by opiates (Boyle et al., 2002). Much less well studied is the report that mu opiates can directly reduce plasma extravasation ((24-36%) Joris et al., 1990; Binder et al., 2001; Green and Levine, 1992; Barber et al., 1993; Taylor et al., 2000). These effects are induced by activation of opioid receptors located in the central and peripheral nervous systems. In the periphery, opioid receptors are expressed on a significant proportion of capsaicin sensitive sensory fibers and sympathetic postganglionic terminals, where they may participate in the modulation of nociceptive information under certain pathological conditions (Zhou et al., 1998). The reversibility of the effects induced by the opioid receptor agonists (edema and extravasation) has been established after the administration of antagonists (i.p.) (Romero et al., 2005). The work of Lei and Rogers (1999) in intact respiratory tissue suggests that opioid receptors located on sensory fibers and immune cells are selectively activated by low (neuronal) or high (non-neuronal) doses of mu- and delta-opioid receptor agonists. The local administration of mu- and delta-opioid receptor agonists, at doses that show no systemic effect, has been shown to decrease plasma extravasation during peripheral inflammation (Hong and Abbott, 1995). This suggests a clinical application for low dose morphine or met-enk as a pre-emptive treatment to avoid endoscopic procedure induced pancreatitis. It is likely that a similar mechanism is reducing inflammatory signs in the HSV-ENK treated animals releasing enkephalin directly to the pancreas.

7. Development of opiate tolerance?

In our study, hyperalgesia in the control HSV- β -gal and vehicle treated animals with alcohol and high fat diet induced pancreatitis was maintained through seven weeks. The overexpression of the proenkephalin products in the HSV-ENK treated animals was sufficient to abrogate the effects of the pancreatitis for an extended period of time **without tolerance** demonstrating that this is an adequate model for treatment in clinical studies. The important issue of desensitization and tolerance to the enkephalin generated by the HSV-ENK construct, appears to be a non-issue in the time frame of study. We propose that it is the release of met-enkephalin directly onto receptors at nerve terminals both centrally and

peripherally that provides additive effectiveness for reducing hyperalgesia and tissue protection from inflammatory responses without tolerance. In this site directed manner, enkephalin can affect receptors on neuronal endings that receive information about noxious conditions in the pancreas and have reparative ability as an apparent added benefit. Standard therapies relying on higher and higher levels of circulating opiates, on the other hand, frequently result in intolerable side-effects and development of tolerance. Gene therapy using HSV vectors for gene product delivery may be clinically preferred in patients with prospects of longer life spans and functionality, or in intractable chronic nonmalignant pancreatitis and generally indicated in patients with longer life spans. **HSV-1-based viral vector infections may offer a novel, effective, well-tolerated and advantageous approach for treatment of chronic pancreatic pain in patients. This approach might also be expanded to deliver additional human gene products that could impact the pain, inflammation, structural integrity and repair of the pancreas in patients.**

8. Testing safety and efficacy issues

Safety and efficacy of replication defective HSV vectors have already been demonstrated for other purposes in Phase 1 clinical trials (Todo, 2002; Shah 2003; Yu et al, 2004; Satoh, 2005; Sawai et al., 2001; Wolfe et al., 2009, a,b). For our studies we examined the spinal cord for evidence of HSV-1 infection after 12 weeks of study. While we expected and have demonstrated HSV-1 in the dorsal root ganglia sensory neurons (Yang et al., 2008), the preferred host cell for HSV, no central nervous system infection was found. This has not been tested in spinal cord previously with these viral vectors. The replication deficient virus has already been used safely when injected directly into the brain as a treatment for glioblastoma (Papanastassiou, et al., 2002). The efficacy and clinical relevance of the direct injection into the pancreas was successfully tested in our pre-clinical studies by examining behavioral, inflammatory and cellular activation responses, such as FOS protein expression in spinal neurons and phospho-p38 in DRG. Effects of met-enkephalin have also been tested in *in vitro* pancreatic cell models. We have infected DRG and pancreas cells in cultures (PANC-1) with HSV vectors to assess the longevity of enkephalin, inflammatory mediator release, re-activation and other safety issues in PANC-1 cells, clonal human pancreatic tumor-derived tubular epithelia equivalent to the cells lining the pancreatic ducts. The PANC-1 cells have been used previously by Eisenberg et al. (2005) to assess increased efficacy of HSV vectors used as an anti-cancer adjuvant to chemotherapeutic agents. In our studies the proenkephalin overexpression significantly reduced cytokine expression (unpublished data).

The phase I clinical trial using a related replication defective viral vector reported successful reduction of pain levels assessed using a numeric rating scale (NRS), the Short Form McGill Pain Questionnaire (SF-MPQ) and concurrent reduction of opiate usage in the terminal cancer patients initially with intractable pain (Wolfe et al., 2009b). The gene therapy studies provide compelling new insights in support of the effectiveness of enkephalin for reduction of tissue injury, fibrosis, inflammation, and pain related behaviors. The HSV viral system offers great potential for simultaneous delivery of multiple other gene products, as the HSV vector cassette is very large. Further improvement of vector system design may provide other anti-inflammatory gene products to protect and restore functional integrity of damaged tissues.

9. Clinical significance

As pancreatitis patient survival is becoming a fortunate reality, consideration of route of administration for viral constructs should be a part of investigative pre-clinical studies. While celiac plexus neurolysis is a currently accepted clinical treatment for severe intractable abdominal pain in abdominal malignancies and chronic pancreatitis, it is usually reserved for patients who have failed other treatment modalities. The reported year 1 success rate for pain relief is reportedly between 57-100%, depending on the approach and ethanol concentration (Okuyama et al., 2002; Vranken et al, 2002; Schmulewitz and Hawes, 2003; Klapman and Chang, 2005). Celiac plexus neurolysis requires surgical or anesthetic specialization and is usually performed at tertiary referral centers. Some procedural approaches require an epidural block for anesthesia. Although generally well-tolerated, reported complications include back pain, hypotension, and diarrhea (Chan, 1996; Kulke, 2002). Celiac plexus neurolysis essentially scleroses the nerve and blocks the afferent and efferent transmission of all neurochemicals. Neural blockade of a significant part of the enteric sympathetic nervous system in an otherwise functioning enteric system may lead to pancreatic or enteric pathologies, as the sympathetic nervous system plays a vital role in modulating intestinal secretory and absorptive processes. This may become more of an issue with the improved cancer treatments and longer lifespan for pancreatic malignancies. Viral vectors could be administered to coeliac ganglia in patients by anesthesiologists. However, it is not entirely clear that HSV-Enk would have sufficient uptake efficiency from the axons themselves as the primary afferents pass through the sympathetic ganglia and do not terminate there. We will compare sympathetic ganglia injections to administration at the pancreatic terminal endings which already appears to be quite robust. Our studies imply that chronic infusions of HSV vectors directly onto the pancreas surface from implantable, transcutaneously refillable pumps would provide relief from chronic pain. This potential treatment would also be nerve plexus sparing and avoid the severe complications of pancreatic duct disruption encountered by endoscopists. The sparing of neural connections would decrease the potential for pancreatic insufficiency and enteric damage.

Pancreatic cancer and chronic pancreatitis are among those syndromes characterized as causing the most severe pain states. Pancreatic cancer is the 4th leading cause of cancer deaths in the US, and over 70% of pancreatic cancer patients have significant debilitating abdominal pain upon clinical presentation. Over 50% of patients with idiopathic and alcoholic pancreatitis report chronic pain. The current treatment of pain in pancreatic cancer and chronic pancreatitis includes parenteral narcotic agents, surgical intervention at the level of pancreas or neural pathways and complementary therapies. Narcotic agents are not optimal as there are risks of tolerance, addiction and intolerable side effects of sedation and constipation and nausea. Surgical intervention with total pancreatectomy and islet autotransplantation provides pain relief for a considerable number of patients (Blondet et al., 2007; Hildebrand et al., 2011). However, surgery is invasive and can result in transient or suboptimal relief of pain, postoperative diabetes, and maldigestion. Surgery may be therapeutically or fiscally inappropriate based on the clinical status of some patients. Chronic abdominal pain from the pancreas has a significant negative long term impact on patient quality of life and mortality as it profoundly decreases appetite and leads to weight loss. When patients lose $\geq 20\%$ of lean body mass, host immunocompetence is profoundly

impaired. Therefore, effective management of pain is imperative, not just for the legitimate concern for pain's sake, but for improvement in physical functioning, general health, patient survival, quality of life, and functional independence. Effective management of chronic pain would lift the economic burden of pain-induced debilitation on the individual, their support system and public health.

Pain is a serious public health problem, costing the US about 100 billion dollars/yr (JAHCO report). Pain is the single greatest cause of disability, decreased physical function, decreased work productivity, absenteeism, reactive depression and lower quality of life (QOL, i.e. SF36) scores. In fact, 40-60% of patients with chronic pain report it has significantly and negatively impacted their personal relationships, work productivity and daily routines of living. Considerable improvements have been made in opioid medications, in term of bioavailability, half-life, transdermal delivery, breakthrough opioid combinations and usage with enhancing drugs, such as tricyclic antidepressants. However, side effects and potential for opioid addiction remain a concern to the patient, health care providers and the community. About 60% of patients with chronic pain have expressed fears regarding narcotic medication side effects and fear of addiction to current narcotic regimens. In a Partners Against Pain Survey of 1000 patients in chronic pain, 50% of the patients reported difficulty >1 yr in getting their pain under control and 78% of the patients reported that they would be willing to try new treatments.

Innovative Aspects of the Gene Therapy Studies

Unique advantages offered by HSV-1 viral vector delivery of proenkephalin expression products by the peripheral nerves include:

- Potential for simultaneous delivery of analgesic peptides to both peripheral and central sites, neuronal ending sites, optimizing the effects without tolerance
- More effective and prolonged abrogation of nociceptive responses
- Normalization of pain related behavior to near baseline levels as shown in the published studies
- Potential for positive impact on pancreatic inflammation as shown in the published studies
- HSV-1 viral constructs to be used are replication deficient/ defective
- HSV-1 viral constructs will not incorporate into the host genome or become lytic
- Focused delivery to the target organ allows a much lower viral titer (5-10X lower viral titer than in skin)
- Potential for reduction/elimination of the use of narcotic drugs
- Novel palliative strategy for alleviating the unremitting pain of pancreatic cancer and chronic pancreatitis

10. Conclusion

In summary, our studies indicate that the proenkephalin gene product delivery to pancreas is reparative and significantly reduce pain related behaviors in rodent pancreatitis models. Gene therapeutic approaches that promote the endogenous opiate enkephalin, particularly

delivery by the neuronal innervation, may have significant clinical relevance for reducing inflammation, pain-related behavior and tissue destruction.

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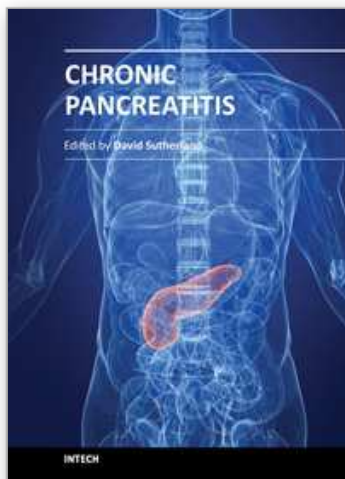
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Chronic pancreatitis is a disease of diverse etiologies in which pain can be devastating, severely impairing quality of life, and treatment is a challenge. This book covers cutting edge basic science research and clinical diagnosis and treatment issues in chronic pancreatitis. Basic science chapters include studies on amelioration of chronic pancreatitis in rats by bone marrow derived mesenchymal cells; on gene therapy using HSV-Enkephalin to reduce fibrosis, inflammation and pain in a rats; and on pancreatic acinar and island neogenesis according to vascular and matrix dynamics of human and animal tissue. In regard to the clinical aspects, the role of endoscopic ultrasound in detecting the changes of chronic pancreatitis are addressed as well as the endoscopic treatment via duct drainage procedures or stone removal. Finally, the surgical options for chronic pancreatitis (there are well over 20 procedures) are extensively discussed, with a final chapter on total pancreatectomy and islet autotransplant to definitively remove the root cause of the pain with preservation of endocrine function. This book will be valued by basic scientists and clinicians striving to understand the mechanisms of pain in chronic pancreatitis and the treatment options in patients so afflicted.

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