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REVIEW ARTICLE

The roles of the Opioid Growth Regulatory System and naltrexone in diabetes: One side make you taller and the other side makes you smaller[a]

Joseph W. Sassani[1*], Ian S. Zagon[2] and Patricia J. McLaughlin[2]

[1]Departments of Ophthalmology and Pathology, The Penn State Hershey Medical Center, Hershey, PA 17033, USA

[2]Department of Neural and Behavioral Science, Penn State University College of Medicine, Hershey, PA 17033, USA

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*jsassani@pennstatehealth.psu.edu

ABSTRACT

This article provides an overview of the inhibitory role of the Opioid Growth Regulatory System, which is characterized by its mediator, Opioid Growth Factor, [Met[5]]-enkephalin, and its specific receptor, Opioid Growth Factor receptor, in the pathobiology of diabetic complications involving the ocular surface. Additionally, involvement of the Opioid Growth Regulatory System in systemic diabetic complications is illustrated by its role in poor diabetic cutaneous wound healing. An overarching theme is the ability of naltrexone to restore normal homeostasis.

The title of this paper is derived from Lewis Carroll's Alice's Adventures in Wonderland in which Alice is invited by the caterpillar to eat either side of a mushroom with powerful growth inducing characteristics. In the context of this review, this metaphor highlights the antagonistic relationship between the Opioid Growth Regulatory System and naltrexone, which blocks the binding of Opioid Growth Factor to the Opioid Growth Factor receptor thereby preventing or reversing the deleterious effects of the Opioid Growth Regulatory System in the pathobiology of diabetic complications including dry eye, keratopathy (delayed corneal epithelial wound healing and decreased corneal sensitivity) and delayed cutaneous wound healing in animal models of type 1 and type 2 diabetes.

The article proceeds in a stepwise fashion to introduce the reader to the components of the Opioid Growth Regulatory System, their relationships, and the impact of naltrexone on the functioning of that system. The roles of the Opioid Growth Regulatory System on normal cellular homeostasis and its dysfunction in diabetic complications are discussed as is the ability of naltrexone to reverse or prevent these complications. The main focus of the review is on the ocular surface complications of diabetes; however, the impact of the Opioid Growth Regulatory System on cutaneous wound healing in diabetes is included to demonstrate the potential systemic implications of the system in the pathogenesis of diabetic complications. Early phase human studies are discussed.

We believe these data support further clinical trials of naltrexone in the treatment of diabetic ocular surface disease and delayed cutaneous wound healing and may have implications for the significance of the Opioid Growth Regulatory System in the pathobiology of other diabetic complications.

1. Introduction.

The World Health Organization states that diabetes is "…a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar) which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves".[1] Diabetes is further subdivided into type 2, which is the most common variety, and usually is associated with adult onset when the body becomes resistant to insulin or under produces it; and type 1 or juvenile (insulin dependent) diabetes, in which the pancreas produces little or no insulin[1]. Both forms of diabetes will be discussed relative, in particular, to their ocular and wound healing complications.

There are two major themes in this paper: 1) the severity of the diabetes epidemic, particularly related to ocular surface and wound healing complications, and 2) the antagonistic relationship between opioid growth factor (OGF) and naltrexone (NTX), and their implications for the Opioid Growth Regulatory System (OGRS). The latter intersection may provide a significant therapeutic opportunity for treating and even preventing diabetic complications. This antagonistic relationship also is the basis for the title of this paper, which paraphrases the conversation between Alice and the caterpillar in Lewis Carroll's Alice's Adventures in Wonderland in which she is offered to take a bite of a mushroom of which "One side will make you grow taller and the other side will make you grow shorter"[2]. In this review we will focus on ocular surface complications of diabetes but also touch upon cutaneous wound healing as evidence for more systemic implications of the dysfunctional OGRS that will be discussed.

2. Scope of the problem.

In the U.S., 38.4 million people have diabetes, which is 11.6% of the U.S. population[3]. Fully 1 in 5 of these individuals are unaware of their disease. Moreover, 1.2 million Americans are newly diagnosed with diabetes every year[4]. In 2022, the care for people with diabetes accounted for 1 in 4 health care dollars in the U.S. of which 61% were attributable directly to diabetes, itself[5]. According to the World Health Organization, 422 million people worldwide have diabetes and the majority of these individuals are living in low-and-middle-income countries[1,6-11]. Unfortunately, there is said to be an increasing disparity regarding the impact of diabetes in rich and poor countries[12]. The economic burden of diabetes only compounds the suffering in these countries[13]. The worldwide cost of diabetes for individuals aged 20 to 79 years was $966 in 2021, which was a 316% increase over the preceding 15 years[14]. Each year 1.5 million deaths are directly attributable to diabetes[1].

3. Ocular surface disease.

Although retinopathy is recognized as a major cause of vision loss in diabetes, ocular surface disease in the forms of dry eye and keratopathy is common and may represent a threat for permanent vision loss[15,16]. Between 17.5% and 65.3 % of diabetics will experience complications secondary to ocular surface disease[12,17]. Keratopathy is represented by delayed epithelial wound healing, reduced corneal nerve density, decreased corneal sensation, and ocular surface discomfort such as burning and dryness[16]. This paper discusses

each of these complications relative to dysregulation of the OGRS and the potential salutary effect of NTX on them. As Bu and associates have stated, "Diabetic complications of the ocular surface need to be addressed as they result in significant visual morbidity and are closely linked to systemic microvascular complications"[12].

4. The Opioid Growth Regulatory System.

There are a number of endogenous opioid systems in the body. All of their functions are receptor dependent. These functions include analgesia, cardiovascular control, respiration, behavior, learning and memory, emotion, and cell division and growth regulation. This paper will focus on the latter role of the OGRS in cell division and growth regulation relative to diabetic complications.

4.1 COMPONENTS OF THE OPIOID GROWTH REGULATORY SYSTEM VS NALTREXONE.

The OGRS also is termed the OGF-OGFr axis. It has two major components, Opioid Growth Factor (OGF), itself, and its receptor, OGFr. Expression of OGF and OGFr in the OGRS is a phylogenically old system, that is found in prokaryotes and eukaryotes[18]. Moreover, it is conserved in the cornea in a wide variety of the classes of the phylum Chordata, including mammalia, aves, reptilia, amphibia, and osteichthyes[19]. The OGRS has been shown to function in ontogeny and in the proliferation of animal and human cancers[20-23]. It regulates the growth of developing neoplastic, renewing, and healing tissues. It does not alter apoptosis, necrosis, differentiation, or cell migration.

OGF, chemically, [Met^5]-enkephalin, is a pentapeptide, which has the structure Tyr-Gly-Gly-Phe-Met. It is a negative growth factor that suppresses cell division. It is the only endogenous opioid with an effect on cell division. It is potent, reversible, and species and tissue nonspecific. OGF is tonically produced in an autocrine manner by the cells that will be modulated by it or by their neighbors[24].

OGFr has no molecular resemblance to classical opioid receptors and has been cloned and sequenced in humans, rats, and mice[25,26]. OGF is the specific ligand for OGFr. Figure 1 illustrates the interrelationships between the components of the OGRS.

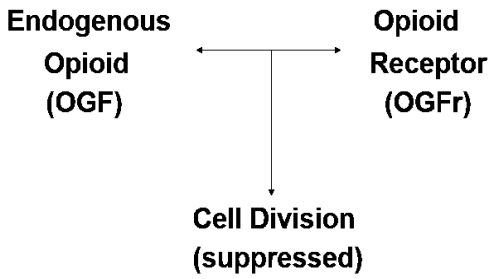

Figure 1. Diagram of the actions of the OGRS components

The third major element is NTX. It is a long-acting opioid antagonist, which is FDA approved for systemic administration for the treatment of opioid or alcohol dependence[27-31]. The systemic dose that is approved by the FDA is 10,000 times the amount that we evaluated for topical use.

4.2 NALTREXONE MODULATION OF THE OPIOID GROWTH REGULATORY SYSTEM.

Fundamentally, the way the OGRS functions is that OGF binds to OGFr on the nuclear envelope and down-regulates cell division. In a state of homeostasis, OGF is neither maximized nor minimized. Therefore,

changing the ratio of OGF to its receptor can up or downregulate cell division. There are multiple strategies that may result in modulation of the OGRS, see Figure 2. For example, if one increases the amount of OGF, one decreases cell division. If OGFr expression is increased, the cell replication is depressed. Conversely, decreasing the amount of OGF increases cell replication, and decreasing the amount of OGFr increases cell replication. We achieved the same end of restoring homeostatic levels of OGF and reversing diabetic complications by blocking the interaction of the OGF with the OGFr using the opioid antagonist NTX thereby increasing corneal cell replication and restoring other aspects of ocular surface homeostasis[32,33].

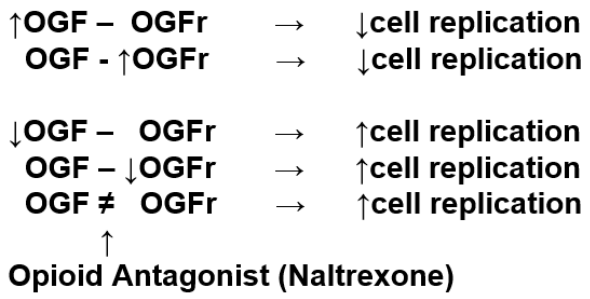

Figure 2. Illustrates ways to modify relationships within the OGRS to impact cell division. Increasing the concentration of OGF or the OGFr decreases the rate cell division. Conversely, decreasing the concentration of OGF or OGFr, or blocking the OGFr with NTX increases the rate of cell division.

4.3 NEED FOR COMPLETE OPIOID GROWTH FACTOR RECEPTOR BLOCKADE.

For the purposes of this review, complete blockade of OGFr with NTX is employed in order to restore homeostasis in the diabetic scenarios that will be discussed because, as will be demonstrated, overproduction of OGF and OGFr contributes to diabetic ocular surface complications. It is vitally important that the opioid receptor is blocked continuously for 24 hours daily in order to achieve the desired results. Blocking it for a shorter period of time with a lower dose of NTX (low dose naltrexone therapy) or a weaker receptor blocking agent, such as naloxone, has the opposite effect. Thus, low dose NTX therapy would be a disaster for what we are trying to accomplish in the treatment of ocular surface complications associated with diabetes. Nevertheless, this treatment actually is used effectively in the treatment of multiple sclerosis, inflammatory bowel disease and cancer[34,35].

5. The Opioid Growth Regulatory System and normal animal corneal epithelium.

Using confocal and immunoelectron microscopy, OGF and OGFr have been localized to the corneal and conjunctival epithelium[36]. Moreover, the presence of OGF and its receptor have been demonstrated in the corneal epithelium of multiple animals, including mammals, birds, reptiles, amphibians, and fish[37]. Thus, the OGRS may have originated at least 300 million years ago. The production of OGF within the ocular surface is in an autocrine manner as evidenced by the finding of the preproenkephalin mRNA, which encodes OGF located in basal and suprabasal cells of the central and peripheral cornea, limbus, and conjunctiva[24].

5.1 FUNCTIONALITY OF THE OPIOID GROWTH REGULATORY SYSTEM ON OCULAR SURFACE EPITHELIUM.

Although the elements of the OGRS are present in ocular surface epithelium, the question arises as to their functionality. Corneal explants in organ culture have

demonstrated the effect of media supplementation with OGF or NTX on the outgrowth of corneal epithelium from the explants and onto the surface of the tissue culture dish[38]. Control outgrowths of epithelium in such cultures grew in concentric circles of decreasing differentiation. In contrast, the outgrowths of epithelial cultures exposed to supplemental OGF not only grew more slowly compared to control cultures, but the concentric waves of outgrowth were poorly organized. Conversely, corneal epithelium in cultures supplemented with NTX grew more rapidly than controls, and in an organized pattern in contrast to OGF supplemented cultures.

5.2 EFFECTS OF THE OPIOID GROWTH REGULATORY SYSTEM ON DNA SYNTHESIS AND CELL DIVISION.

From a cytologic perspective, what is the effect of OGF and NTX on epithelium in such culture? Supplementation of the culture medium with OGF suppresses DNA synthesis and, therefore, cell division of cultured corneal epithelium. Conversely, adding NTX to the medium increases DNA synthesis and cell division resulting in the enhanced outgrowth of epithelium[38]. These effects reflect the antagonistic effects of OGF and NTX when functioning within the OGRS. Specifically, because OGF is neither maximally nor minimally produced in homeostatic epithelium, there is a balance within the OGRS that can be manipulated to either upregulate or downregulate DNA synthesis and cell division.

5.3 EFFECTS OF THE OPIOID GROWTH REGULATORY SYSTEM ON NORMAL CORNEAL EPITHELIAL WOUND HEALING.

An *in vivo* demonstration of these antagonistic effects of manipulation of the OGRS can be found in the healing of standardized wounds in the rat corneal epithelium in which the circumference of the wound is outlined using a trephine and the encircled epithelium removed. In this wound model, administering either intraperitoneal or topical NTX increases the speed with which the corneal epithelium heals compared to controls in rats or rabbits[39,40]. Although these results are highly suggestive it was necessary to confirm that they were mediated specifically by blocking the interaction of OGF with the OGFr.

5.4 CONFIRMATION OF OPIOID GROWTH FACTOR RECEPTOR DEPENDENCE AND SPECIFICITY.

Using the Helios gene gun system, one can achieve particle mediated gene transfer. This technique enabled the delivery of sense and antisense complimentary OGFr-specific DNA into corneal epithelial cells thereby causing the cells either to upregulate or downregulate the production of OGFr[41,42]. If one injects sense DNA into the cell on the vector particle, there's an increase in the production of the receptor. Conversely, antisense DNA given to the cell suppresses the production of the OGFr. Thus, using the gene gun it is possible to control the amount of the OGFr produced in the cell. What is the biologic corollary? If one gives sense, DNA thereby causing overexpression of the OGFR one finds that the standardized corneal epithelial wounds heal more slowly than controls in response to this treatment. On the other hand, if antisense DNA is injected into the cells so that there is suppression of the production of the receptor, there is downregulation of the OGRS and an increased rate of corneal epithelial wound healing[41,42].

5.5 NALTREXONE SAFETY.

The safety of treatment with NTX will be discussed from several perspectives in this

review. The initial evaluation was a seven-day study of systemic NTX treatment in rats. DNA production and mitoses were evaluated in the basal layer of the peripheral corneal epithelium in these animals (all percentage changes cited in this review were significant at least at the $P \leq 0.05$ level). NTX treated animals synthesized increased DNA 69% to 85% more than control animals. Similarly, the epithelial thickness increased by 8% to 38%. This increase reflected increased packing density; however, there was no toxicity or proliferative pathology seen in the epithelium. Rather, treatment appeared to accelerate normal homeostatic processes. There was negligible apoptosis or necrosis.

6. Opioid Growth Regulatory System and normal human corneal epithelium.

The effect of manipulation of the OGRS on the healing rates of standardized corneal epithelial wounds was tested on organ cultured human ocular anterior segments that were not suitable for transplantation[43]. When the culture medium was supplemented with NTX, the corneal epithelium healed completely significantly more rapidly than controls. On the other hand, when OGF was added to the culture medium, the rate of corneal epithelium healing decreased significantly. Importantly, NTX increased the labeling index of the epithelial cells by 152%, and increased the rate of cell division. Conversely, OGF depressed the labeling index by 75% and 82% in the peripheral cornea and limbus respectively demonstrating its effect in downregulating cell division. This experiment nicely illustrates the "one bite (or one treatment) makes you taller and the other makes you smaller" antagonistic effect that OGF and NTX exhibit when competing within the OGRS for access to the OGFr in healing human corneal epithelium.

7. Opioid Growth Regulatory System and diabetes.

As noted previously, between 17.5% and 65.3 % of confirmed diabetic individuals will experience complications secondary to ocular surface disease[12,17]. Having demonstrated the impact of the OGRS on the healing normal ocular surface epithelium, our hypothesis was that increased OGF in diabetes leads to these and other ocular surface complications. Specifically, we anticipated that increased OGF contributes to decreased tear production, delayed corneal epithelial wound healing, and decreased corneal sensitivity in diabetes. Finally, we proposed that blocking the interaction between OGF and OGFr utilizing NTX would reverse each of these major ocular surface complications by downregulating the OGRS. The remainder of this section of this review provides evidence for the role of the OGRS in diabetic ocular surface complications.

7.1 ELEVATED OPIOID GROWTH FACTOR IN DIABETES.

OGF and OGFR have been demonstrated to be elevated in the corneal epithelium of diabetic rats[44]. Elevated levels of OGF have been found in the plasma of diabetic patients[45-47]. Similarly, genetically obese diabetic db/db mice, which are an animal model of Type 2 diabetes, also exhibit elevated plasma OGF levels[48]. Nevertheless, association does not necessarily indicate causation. What is the evidence for the specific impact of the OGRS on ocular surface complication in diabetes?

7.2 OPIOID GROWTH REGULATORY SYSTEM AND EPITHELIAL WOUND HEALING IN DIABETIC ANIMALS.

Standardized corneal epithelial abrasions heal more slowly in diabetic rats than in control animals[44]. Conversely, treatment with NTX accelerated wound healing. It also is significant to note that in the preceding study, DNA synthesis in the diabetic rats was decreased by 90% compared to nondiabetic controls. In contrast, NTX treatment restored the epithelial to normal levels and the labeling index increased by up to eight-fold in diabetic animals compared to diabetic control rats.

7.3 EFFECT OF GLUCOSE CONTROL AND NALTREXONE TREATMENT ON CORNEAL EPITHELIAL WOUND HEALING.

What is the impact of insulin treatment and blood glucose control on wound healing compared to treatment with NTX?

Intensive insulin therapy leading to normal glycemia in rats with diabetes prevents the delay in wound healing of ocular surface epithelium that is observed in poorly controlled diabetic rats[49]. Even topical insulin helped to normalize corneal epithelial healing in untreated diabetic rats that had standardized corneal epithelial wounds. In these animals the epithelial wounds healed 35% more slowly than normal control animals compared to topical insulin-treated diabetic corneal wounds, which healed 19% to 60% faster than control diabetic animals[50]. Importantly, topical insulin treatment had no effect on corneal thickness, intraocular pressure, apoptosis, or serum glucose levels in this study. In contrast to NTX, topical insulin had no effect on the healing of corneal epithelial wounds in normal animals.

Finally, it should be noted that NTX and insulin were independently effective in accelerating corneal epithelial wound healing in type 1 diabetic rats[51]. However, combined insulin and NTX were no more effective than either one when used independently. It's possible, therefore, that insulin and NTX have similar mechanisms of action, or that each can maximize wound healing, leaving no opportunity for a further increase by the complimentary modality. It also should be noted that insulin had no effect on wound healing in normal animals and NTX had no impact on blood glucose levels in diabetic animals. Finally, the relationship between insulin and the OGRS may provide a fertile avenue for research in preventing diabetes itself[52].

7.3.1 Safety of topically applied insulin and naltrexone.

A study to determine the safety of insulin and NTX during the healing of these animals was performed. We found that there were no differences from normal rats or insulin treated diabetic controls with regard to intraocular pressure, corneal thickness, endothelial cell number, or epithelial apoptosis necrosis or organization in rates treated with over a 10,000-fold dosage range.

7.4 SUMMARY OF NALTREXONE EFFECT ON THE HEALING OF DIABETIC CORNEAL EPITHELIAL WOUNDS.

Given the findings regarding the effect of topical NTX on corneal epithelial wound healing in models of type 1 and type 2 diabetes, we concluded that NTX has an effect on the first major reflection of ocular surface disease and diabetes, which is abnormal corneal wound healing. Moreover, it does so in a non-toxic manner[53].

8. Effect of naltrexone blockade of the Opioid Growth Factor receptor in the Opioid Growth Regulatory System on three other major ocular surface complications of diabetes: poor epithelial adhesions to the underlying basement membrane, decreased corneal sensitivity (corneal neuropathy), and dry eye.

8.1. DIABETIC EPITHELIUM TO BASEMENT MEMBRANE CELLULAR ADHESION.

An important problem in diabetes is that these individuals have very poor adhesion of the epithelium to the underlying basement membrane. The effect of NTX treatment on this problem was investigated. Topical NTX treatment for seven days following abrasion in normal rats was investigated in insulin treated and control rats[54]. There were no differences in adhesion structures or basal and super basal layers of the corneal epithelium in all NTX treated groups, whether normal, diabetic or insulin treated diabetic animals and were similar to normal vehicle treated control animals. This study suggests that humans treated with topical NTX may be able to reform normal adhesion complexes of their diabetic epithelium and, in part at least, reverse the recurrent erosions that can be seen in diabetic humans.

8.2 DIABETIC CORNEAL NEUROPATHY.

Diabetic corneal neuropathy correlates with peripheral neuropathy, particularly when the corneal neuropathy is documented with clinical confocal microscopy[55-60]. Corneal aesthesiometry also can be helpful in the clinical assessment of corneal neuropathy[59,61-63]. Corneal neuropathy accompanies delayed corneal epithelial wound healing[64]. Corneal nerve damage can be induced by obesity related to type 2 diabetes[65]. Moreover, diabetic corneal neuropathy is reversible as evidenced by the fact that corneal nerve regeneration has been demonstrated after simultaneous kidney and pancreas transplantation and other therapy[66,67].

When rats that had eight weeks of diabetes were evaluated relative to corneal sensitivity, there was a 2-fold reduction in corneal sensitivity in these animals[53]. However, when these animals were treated with NTX, there was a twofold increase in cornea sensitivity and, essentially, a restoration of normal corneal sensitivity in these NTX treated, diabetic animals. It should be noted that when NTX therapy was discontinued, corneal sensitivity regressed to being 1.9-fold less than normal animals and comparable to control diabetic animals, thus, the period of normalcy can only be attributed to NTX therapy. NTX also restored normal corneal sensitivity in the mouse model of type 2 diabetes[68].

8.2.1 Summary of naltrexone effects on corneal neuropathy.

Topical NTX treatment restores corneal sensitivity to normal levels in both type 1 and type 2 diabetic animals. This finding is consistent with other studies cited above, which suggest that corneal neuropathy is reversible.

8.3 DIABETIC DRY EYE.

8.3.1 Normal tear production and naltrexone effect.

The third major reflection of diabetic ocular surface disease is dry eye. Before discussing dry eye *per se* it should be noted that there is

a cyclic tear production in normal rats. When these animals are at their nadir of tear production, NTX treatment restores tear production to normal levels[69]. There is no effect on the contralateral fellow eye and the production of tears returns to the normal cyclic change if NTX is discontinued. Neither one drop of NTX nor vehicle had any impact on tear production if the level of the tear production was already at the normal level. It appears that NTX only raises tear production level up to the normal rate and does not surpass it in normal animals. Conversely, one drop of OGF significantly reduced tear production in rats that were at their normal level. It is also important to note that opioid blockade with NTX did not raise production to a supernormal level, so it appears that the opioid growth regulatory system also has an effect on tear production in normal rats but cannot raise tear production above normal levels.

8.3.2 Diabetic dry eye and naltrexone effect.

Animals that have had diabetes for eight weeks exhibited tear production that was reduced by 32% to 53% compared to normal animals or those treated with NTX.[70] NTX treatment restored tear production to normal levels. If that treatment was discontinued, tear production dropped back to the abnormal level, thereby indicating that it was the NTX that actually had the effect of restoring tear production to normals. Tear production also was restored to normal in the model of type 2 diabetes, which is the *db/db* mouse[68].

8.3.3 Long term safety of naltrexone and dry eye.

A 30-day safety study of topical NTX in type 1 diabetic dry eye was conducted. It demonstrated that twice daily NTX restored tear production to normal levels in diabetic rats and restored corneal sensitivity to normal[71]. Importantly, there were no histologic abnormalities noted in the epithelium.

8.3.4 Sex differences in diabetic ocular surface complications.

There are sex differences in the magnitude of type 1 diabetic ocular surface complications[72]. Type 1 diabetic female rats have an increased magnitude and earlier onset of dry eye and decreased sensitivity complications compared to males[73]. Moreover, there's a strong negative correlation with serum estrogen and OGF levels. Serum OGF levels are higher in female than in male diabetic rats. Nevertheless, NTX delays the onset of ocular surface complications regardless of sex.

Unlike epithelial wound healing, tear production and corneal sensitivity are decreased in type 1 female rats[74]. Even in the presence of insulin, these findings are quite different from those found in diabetic corneal epithelial wound healing. Additionally, serum and tissue OGF levels and receptor levels are increased in these female diabetic animals.

9. Naltrexone treatment prevents diabetic ocular surface complications.

So far, this review has focused on the treatment of diabetic ocular surface complications; however, in some cases, diabetic complications in both sexes can be prevented by NTX treatment. Specifically, if NTX treatment was begun at the time that type 1 diabetes was induced by streptozotocin treatment, epithelial wound healing remained normal as did tear production, and corneal sensitivity. In effect, the development of these complications was

prevented[75]. Moreover, serum OGF levels were normalized with systemic NTX and tissue levels were normalized with topical NTX.

10. Human safety studies.

Having performed appropriate animal studies, a 24 hour safety study in humans was initiated and completed by Dr. David Liang.[76] The research was funded by Telemedicine and Advanced Technology Research Center (TATRC-United States Army). At one week following 24-hour administration of NTX, no adverse effects were found.

A subsequent 30-day phase two clinical trial of NTX for diabetic dry eye was funded by the Life Sciences Greenhouse of Central Pennsylvania and the Ben Franklin Technology partners of Central and Northern Pennsylvania and was conducted by a Contract Research Organization (CRO). It was a single center randomized double masked placebo controlled clinical study of NTX in diabetic individuals with dry eye. There were 30 treated and 30 control patients. The CRO concluded that the results support and extend the safety of the NTX to include subjects with diabetes and those with dry eye disease and treatment programs to include twice daily dosing for 30 days. Moreover, although designed primarily as a safety study and underpowered to determine efficacy, both active versus vehicle and active versus baseline assessments demonstrated positive trends for NTX and the relief of dry eye disease.

11. Summary of impact of naltrexone treatment on ocular surface complications in diabetes.

The previous sections of this review have documented the ability of treatment with NTX to reverse the OGRS-mediated diabetic ocular surface complications of decreased tear production, reduced cell division leading to delayed corneal epithelial wound healing and decreased corneal sensitivity. Figure 3. summarizes the impact of NTX treatment in reversing the ocular surface complications of diabetes.

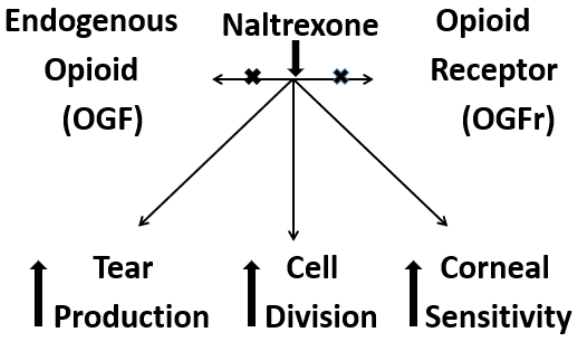

Figure 3. Illustrates that blockade of the interaction of OGF with its specific receptor, OGFr, reverses these adverse diabetic surface complications mediated by the OGRS.

12. Wider implications for the Opioid Growth Regulatory System in diabetes.

The studies outlined above have demonstrated that NTX is safe and effective in treating diabetic ocular surface complications, specifically delayed epithelial wound healing, abnormal corneal sensitivity, and dry eye. Nevertheless, it is reasonable to ask if there are broader implications for the OGRS in the pathobiology of diabetic complications? Is this just a local phenomenon? The answer is no.

12.1. THE SCOPE OF ANOTHER SIGNIFICANT DIABETIC COMPLICATION.

The diabetic foot is one of the most expensive complications in diabetes[77]. In the United States alone, 1.6 million individuals have diabetic foot ulcers at any one time[78]. During their lifetime, a diabetic individual has a

greater than 30% risk of developing a foot ulcer and 40% of these ulcers recur during the year after the initial episode[79]. Fortunately, most patients present for evaluation with earlier stage foot ulcers[80,81]. Nevertheless, 50% to 60% of these ulcers become infected with 20% of the moderate to severe ulcers leading to lower extremity amputation[78]. In 2014, the U.S cost of diabetic foot management between 2007 and 2011 was estimated to be $9-13 billion in addition to the costs associated with diabetes management *per se*[82].

The treatment for early-stage foot ulcers includes debridement, off-loading, and routine wound care. Additionally, other treatments that have been tried include a fibrin-leukocyte patch, placenta-derived products, sucrose octasulfate dressing, hyperbaric oxygen, and negative pressure wound therapy[78]. Nevertheless, there is a need for improved treatment methods for all stages of these ulcers[83].

12.2 OUR HYPOTHESIS REGARDING THE ROLE OF THE OPIOID GROWTH REGULATORY SYSTEM IN THE PATHOGENESIS OF DELAYED AND INCOMPLETE DIABETIC WOUND HEALING.

Wound healing of the diabetic foot is complex and it is characterized by delayed and incomplete wound healing, in part, reflecting compounding accompanying pathophysiologic processes such as peripheral artery disease and neuropathy[84].

Our hypothesis was that type 1 and type 2 diabetes were accompanied by dysfunction of the OGRS and associated upregulation of the negative growth factor OGF leading to delayed and incomplete wound healing thereby disrupting the homeostatic processes found in normal wound healing. Specifically, as will be demonstrated, we found depressed cell migration and division, decreased angiogenesis, reduced fibroblast proliferation, depressed collagen production and maturation leading to incomplete wound healing and long-term poor wound strength in the healing of experimentally induced diabetic cutaneous wounds on the dorsum of rats. We hypothesized further that adding topical NTX to these healing wounds would prevent the binding of OGF to OGFr and, therefore, result in normalized wound healing. In support of this hypothesis, as we will demonstrate, we found that NTX treatment resulted in reversal of depressed cell migration and division, restoration of angiogenesis, and increased fibroblast proliferation leading to increased collagen production and maturation. These salutary effects, in turn, resulted in more complete wound healing and a normal wound strength when measured 60 days after injury.

12.3 DIABETIC WOUND HEALING IN RESPONSE TO NALTREXONE TREATMENT.

Overall, treatment with NTX speeds full-thickness cutaneous wound closure in type 1 diabetic rats[85]. These treated animals had a wound that was 13% to 57% smaller than diabetic controls. Moreover, wound contraction is a very important part of the wound healing process in rats. Wound contraction was 50% less in diabetic animals but was restored to normal in NTX treated animals[85]. There were no differences in the histology of NTX treated wounds compared to normal control animals.

What are the specific NTX effects leading to these results? There is increased DNA synthesis

in type one diabetic rat wounds treated with NTX. Specifically, when NTX was mixed into sterile lubricant and applied to the wounds of these diabetic animals, the labeling indexes increased by 103% to 147%[85]. Therefore, increased DNA synthesis and increased cell division occur in these wounds in response to NTX treatment. NTX also stimulated the expression of factors key to angiogenesis[86]. Moreover, using sirius red staining, it was demonstrated that NTX treatment stimulated wound remodeling in diabetic rats, which is another key component of normal wound healing[87]. Finally increased collagen production and maturation were demonstrated in NTX treated healing diabetic wounds[87].

Having demonstrated that individual aspects of wound healing were normalized by NTX treatment, we sought to determine the completeness of the healing process in diabetic and NTX treated animals. Using a standardized instrument that measures the tensile strength of tissue, we determined the tensile strength of apparently healed wounds at 60 days after the initial wounding[87]. Diabetic wounds had significantly less strength than did wounds that had been treated with NTX. Conversely, NTX treated wounds had a tensile strength that was essentially the same as that of normal tissue. Therefore, NTX treatment fully restored wound strength in these animals thereby demonstrating the net effect of each of the ways that it had facilitated the normal wound healing process in diabetic animals.

Similarly, NTX treatment restores DNA synthesis in models of type 2 diabetes with a similar result to that seen in the models of type 1 diabetes. Specifically, NTX treated *db/db* diabetic mice had a BRDU labeling index (LI) indicating DNA production that was 52% compared to untreated diabetic animals that had a LI of only approximately 19%[88]. Further histologic examination of the NTX treated tissue in normal mice demonstrated 1.7-fold thicker hyperplastic epithelium than untreated normal control mice. Similarly, the epithelium of wounds of diabetic mice treated with NTX were 1.5-fold thicker than in control diabetic mice. No other abnormalities were noted.

12.4 SUMMARY OF IMPACT OF NALTREXONE TREATMENT ON CUTANEOUS WOUND HEALING COMPLICATIONS IN DIABETES.

The previous sections demonstrated multiple ways in which diabetes inhibits complete cutaneous wound healing resulting in reduced tensile strength when apparently healed wounds are tested even 60 days following initial wounding. Figure 4 summarizes the impact of blockade of the OGRS utilizing NTX in reversing the main manifestations on incomplete cutaneous wound healing thereby restoring normal tensile strength to healed wounds.

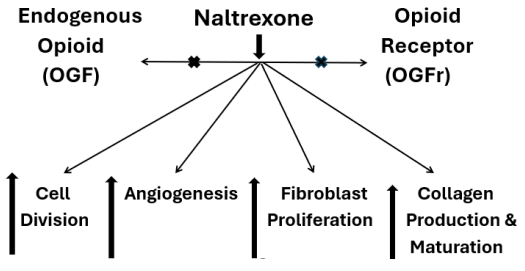

Figure 4. Illustrates NTX reversal of the major manifestation of incomplete diabetic wound healing thereby restoring normal tensile strength to the healed wound.

12.5 PRECLINICAL ANIMAL TRIAL.

In anticipation of future clinical trials of NTX in the treatment of diabetic cutaneous wounds, the U.S. Food and Drug Administration (FDA)

mandated a safety study involving the 30-day application of gel containing 1%, 2% and 10 % NTX to a standard area of the uninjured skin on the dorsum of normal Göttingen mini-pigs[89]. A contract research organization (CRO) was retained to perform the study, which included histopathologic examination of the treated tissue. Other studies performed were blood analyses including serum NTX levels, gross organ morphology, and examination of clinical signs of overall health such as food consumption. The study was funded by a grant from the QED program in Philadelphia, PA and the PA Department of Health.

At the conclusion of the study, the CRO performed necropsy studies on all animals and found no statistically significant changes in organ weights, body weights, and other physiological parameters. Moreover, there were no biologically significant differences between the treated and untreated sections when the tissues were examined histologically. There were no findings contraindicating moving to human clinical trial of NTX for the treatment of diabetic cutaneous wounds.

13. Conclusions.

Increased levels of OGF have been found in the serum of diabetic humans and in animal models of diabetes. We have demonstrated that the OGRS was disordered in animal models of type 1 and type 2 diabetes. Relative to the diabetic eye, this dysregulation resulted in the ocular surface abnormalities of dry eye, delayed healing of corneal epithelial wounds and decreased corneal sensitivity. We have demonstrated, further, that all of these abnormalities can be reversed by administering the OGF antagonist, NTX. Similarly, we have demonstrated abnormalities in the cutaneous wound healing of animal models of type 1 and type 2 diabetes including delayed wound closure, decreased angiogenesis, reduced fibroblast proliferation, and decreased collagen production and maturation, all of which resulted in poor tensile strength of apparently healed wounds. NTX treatment reversed each of these components of abnormal diabetic wound healing. Finally, the findings regarding the effects of NTX on ocular surface and cutaneous wound healing complications in diabetes, and the associated safety studies will need to be confirmed by definitive clinical trials if the proprietary NTX formulation is to move from bench to bedside.

14. A final question.

Given the positive impact that has been demonstrated for NTX treatment on the diverse diabetic complications of ocular surface disease and cutaneous wound healing, will it prove useful for the treatment or even prevention of other diabetic complications?

Conflict of Interest Statement:

JWS, ISZ, and PJM are inventors of IP held by Penn State University Research Foundation (PSRF) related to composition and use of formulations for treatment of dry eye and wound healing. The authors receive no monetary or other compensation.

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Author contribution

JSW: Conceptualization, writing - original draft and editing, final revision

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