

RESEARCH HIGHLIGHT

Pioglitazone improves pelvic ganglion neuronal survival

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Cavernosal nerve injury is a common complication after radical prostatectomy and causes erectile dysfunction (ED). Our recent publication established that pioglitazone (PGZ) improves cavernosal nerve function after crush injury in the rat model by both neural protection and neuroregeneration. This result is clinically significant for the many men who undergo treatment for localized prostate cancer. A better understanding of the effects of PGZ on pelvic ganglion neurons after cavernosal nerve injury is warranted. In this Research Highlight, we discuss the implications of our investigation from a molecular and clinical perspective.

Keywords: Pioglitazone; Erectile Dysfunction; Cavernosal nerve injury

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The cavernosal nerves are mixed autonomic nerves that project from the major pelvic ganglion (MPG) to innervate the lower urinary tract and reproductive organs. Stimulation of these nerve fibers induces an erection through smooth muscle relaxation in the corpus cavernosum^[1]. Consequently, injury to the cavernosal nerves causes erectile dysfunction (ED). Pelvic surgery, including radical prostatectomy (RP), can damage these nerves through several mechanisms, including mechanical traction, transection, electrocautery, ischemia, and inflammation. This insult induces neuropraxia and axonal degeneration, which leads to ED^[2]. The success of post-operative reinnervation of the end organ after RP depends on the ability of injured neurons to survive and change to a regenerative phenotype. Unfortunately, current pharmacologic options for patients with post-RP ED have been met with limited success^[3]. Therefore, identifying protective agents that promote neural growth and regeneration are vitally important to improve post-operative erectile function in men undergoing pelvic surgery.

The rat model for bilateral cavernosal nerve crush injury (BCNI) has been used to simulate cavernosal nerve injury during pelvic surgery. Quinlan *et al.* originally demonstrated the technique for the identification and manipulation of the cavernosal nerve in the rat model^[4]. Burnett *et al.* applied this technique to evaluate erectile hemodynamics with the measurement of intracavernosal pressure (ICP) following nerve injury^[5]. His study demonstrated that ICP decreased following nerve crush injury. Mills *et al.* evaluated ICP as a percent of the mean arterial pressure (MAP) to account for the effect of systemic blood flow on erectile function^[6]. As a result of these technical advances, we utilize the ratio of ICP to MAP to assess the efficacy of erectile response following cavernosal nerve injury.

Aliperti *et al.* investigated the effect of pioglitazone (PGZ) treatment on rat erectile function after BCNI using the aforementioned techniques^[6]. Thiazolidinediones (TZD), including PGZ, are peroxisome proliferator-activated receptor

γ (PPAR γ) ligands used for treating type 2 diabetes. There is evidence suggesting that TZDs are vasculoprotective by poorly understood mechanisms [7, 8]. PGZ was selected because of its vasculoprotective and neuroprotective effects in models of cerebrovascular accidents [9]. PGZ is indicated in reducing atherosclerotic plaque *in vivo*. In this study, animals were divided into sham, nerve crush, and nerve crush with PGZ treatment (with two groups of varying PGZ dosages). Nerve crushed animals treated with PGZ experienced dose-dependent improvements in the ICP/MAP ratio. The high-dose PGZ group demonstrated a significant increase in the ICP compared to the nerve crush group and exhibited similar responses as in the sham group. Immunofluorescence, quantitative polymerase chain reaction and Western blotting of cavernosal tissue demonstrated that animals treated with high-dose PGZ had increased synthesis of endothelial nitric oxide and neuronal nitric oxide (nNOS) [6]. The low-dose PGZ group showed a smaller increase in ICP compared to the high-dose PGZ group.

Katz *et al.* was the first to document that PGZ promoted neuroprotection and neuroregeneration of the cavernosal nerves [10]. The study was composed of four groups, a sham group, a nerve crush group, and two groups treated with PGZ after nerve crush. Of the two treatment groups, one group received both pre- (4 days) and postsurgical (14 days) treatment with pioglitazone (BCNI + pre + post). The other group received only postsurgical treatment (BCNI + post). Length of pretreatment was based on the use of 2–5 days of preventive therapy in studies of pioglitazone in neuroprotection [11, 12].

In order to better define the mechanism of PGZ, we analyzed several molecular markers reflective of neuronal health, including neuronal nitric oxide synthase (nNOS), neurturin (NTN), glial cell line-derived neurotrophic factor family receptor alpha-2 (GFR α 2), and β -III tubulin. We studied the MPG and cavernous nerve tissue to evaluate the molecular and biochemical effect of PGZ treatment in promoting erectile function. We again used immunofluorescence, quantitative polymerase chain reaction, and Western blotting to evaluate nerve regrowth and regeneration.

nNOS catalyzes the production of nitric oxide (NO) and is present in neuronal tissue [13]. The cavernosal nerves promote penile erection via a nitric oxide (NO)-mediated mechanism. NO acts on the end organ, the corpus cavernosum, to relax smooth muscle and induce vasodilation. In this experiment, nNOS expression was significantly increased in the pelvic ganglia of rats treated with PGZ after cavernosal nerve crush compared to the ganglia of rats that received crush injury with no treatment. In fact, the level of nNOS expression in

the treatment group was similar to sham level. In addition, rats that were treated with PGZ both before and after the nerve crush injury showed greater protein nNOS expression than those that only received post-injury PGZ therapy. The trend of increased nNOS gene expression in the pelvic ganglia of both treatment groups confirms PGZ's neuroprotective effects. The surviving nerves had an enhanced ability to compensate for the injury by upregulating nNOS synthesis.

NTN is a member of the glial cell line-derived neurotrophic factor family. It promotes the survival and regeneration of pelvic parasympathetic nerves through activation of the glial cell line-derived neurotrophic factor family receptor, specifically GFR α 2. *In vitro* studies reveal that NTN and GFR α 2 promote survival and neuronal growth factors in the MPG [14]. Administration of pre and post-injury treatment with PGZ reversed the effect of BCNI, causing a significant elevation in GFR α 2-labeled cells and an elevation in NTN-labeled cells compared with the crush-only and sham groups. PGZ treatment upregulated the expression of both NTN and GFR α 2 genes in the pelvic ganglion at the mRNA level as well. This demonstrates that PGZ can increase the number of neurons projecting from the MPG toward the penis after cavernosal nerve damage.

β -III tubulin, a major structural protein within the microtubules, is a necessary component for axonal growth. Following sciatic nerve injury, adult rats were found to upregulate β -III tubulin in the dorsal root ganglion [15]. In this study, we did not find a significant increase immunohistochemically in β -III tubulin-stained cells or levels of β -III tubulin protein in the pelvic ganglion after BCNI or PGZ treatment. However, qRT-PCR revealed significant upregulation of β -III tubulin mRNA following PGZ treatment. This finding indicates that PGZ may impact β -III tubulin protein expression with a longer treatment time.

The findings above are consistent with earlier findings regarding the impact of PGZ on pelvic neural tissue after crush injury, showing evidence that suggests cavernosal nerve and MPG recovery [6]. PGZ has also shown efficacy in treating ED in patients responding poorly to sildenafil [16]. The proposed mechanism involves decreased inflammation enhanced neuronal survival and regeneration [17]. The molecular pathway for this neuroprotective pathway is undefined, though one explanation involves activation of the peroxisome proliferator-activated receptor- γ (PPAR γ). In a model of optic nerve injury, PGZ inhibited apoptosis of retinal ganglion cells through the PPAR γ mechanism [11]. Cao *et al* postulated that PPAR- γ promotes Schwann cell differentiation, which augments peripheral nerve survival and regeneration [12]. Alternatively, Rahimian *et al* theorized that

the neuroprotective effects of PGZ after sciatic nerve ischemia and reperfusion injury are secondary to its antioxidant properties^[18]. It is also possible that PGZ improves cavernosal nerve function via upregulation of insulin growth factor 1 (IGF-1) receptor. IGF-1 is a growth factor found in the circulation and is also produced locally in multiple tissues, including nerves and the vasculature. IGF-1 acts via the AKT-signaling pathway, a stimulator of cell growth and proliferation^[19]. In human aortic cells, PGZ has been demonstrated to upregulate IGF-1 independently of PPAR-gamma^[20]. IGF-1 has been demonstrated to stimulate nerve regeneration and survival in models of sciatic nerve injury and CNS neuronal injury after hypoxemic injury^[21]. Therefore, it is possible that an IGF-1 dependent mechanism may play a role in the neuroprotective and regenerative properties of PGZ.

To our knowledge, this is the first study to demonstrate the neuroprotective effects of pioglitazone on the pelvic ganglion in a model of post-prostatectomy ED. Furthermore, it is the first report to compare the effects of preventive and reactive PGZ therapies for nerve injury. The results indicate that PGZ provides a protective effect on pelvic ganglion neurons after cavernosal nerve injury. This effect was most apparent with combined pre- and postoperative therapy. Preventive therapy with pioglitazone increased the number of nitrergic penis-projecting neurons in the pelvic ganglion compared with the crush group and sham groups at 2 weeks post BCNI, as evidenced by increased immunoreactivity (IR) for nNOS and GFR α 2. This may have been a result of enhanced survival or increased regeneration of pelvic ganglion neurons after cavernosal nerve injury.

A trend of increased nNOS gene expression in the pelvic ganglia of both treatment groups also points to pioglitazone's neuroprotective effects. Changes in the expression of the neurotrophin, NTN, and its receptor, GFR α 2, also indicate the neuroprotective effect of pioglitazone. The increased levels of NTN and GFR α 2 suggest that PGZ enhances neurotrophic support of both the pelvic ganglia and axons innervating the penis after cavernosal nerve injury. PGZ also significantly upregulated β -III tubulin gene expression, which provides a favorable environment for the regrowth of injured axons.

A potential criticism of this study is the clinical applicability of this model. The 6.5-mg/kg dose in the rat translates to 455 mg daily in a 70-kg adult male, which is higher than the Food and Drug Administration (FDA)-approved dose of 15-45 mg daily. This causes concern given the association between PGZ and the exacerbation of congestive heart failure. The FDA has issued a black-box warning related to PGZ and congestive heart

failure^[22]. Nonetheless, this study suggests future directions for further investigation of the utility of this class of TZD in the prevention of post-prostatectomy ED caused by cavernosal nerve injury. Our group is currently investigating the specific mechanism through which PGZ acts on the cavernosal nerve following a crush injury. Further understanding of this mechanism may allow development of certain pharmacologic agents that can provide the benefits of PGZ without the drawbacks.

Conflicting interests

The authors have declared that no conflict of interests exist.

Author contributions

Study conceived by EK. Administrative support provided by WJGH. Data collected and assembled by EK, DH. Data analyzed by EK, DH. Manuscript drafted by DH, EK. Manuscript edited by DH, KJD, EK, WJGH. Project supervised by WJGH.

Abbreviations

TZD: Thiazolidinedione; PGZ: Pioglitazone; ED: erectile dysfunction; RP: radical prostatectomy; BCNI: bilateral cavernosal nerve crush injury; ICP: intracavernosal pressure; MAP: mean arterial pressure; nNOS: neuronal nitric oxide; NTN: Neurturin; GFR α 2: glial cell line-derived neurotrophic factor family receptor alpha-2; NO: nitric oxide; PPAR γ : proliferator-activated receptor- γ ; IGF-1: Insulin-like growth factor-1; IGF-1R: Insulin Growth Factor 1 Receptor; MPG: Major Pelvic Ganglion.

References

1. Dean RC, Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. *Urol Clin North Am* 2005;32:379-395.
2. McCullough AR. Sexual dysfunction after radical prostatectomy. *Rev Urol* 2005;7 Suppl 2:S3-s10.
3. Segal R, Burnett AL. Erectile preservation following radical prostatectomy. *Ther Adv Urol* 2011;3:35-46.
4. Quinlan DM, Nelson RJ, Partin AW, Mostwin JL, Walsh PC. The rat as a model for the study of penile erection. *J Urol* 1989;141:656-661.
5. Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide: a physiologic mediator of penile erection. *Science* 1992;257:401-403.
6. Aliperti LA, Lasker GF, Hagan SS, Hellstrom JA, Gokce A, Trost LW, et al. Efficacy of pioglitazone on erectile function recovery in a rat model of cavernous nerve injury. *Urology* 2014;84:1122-1127.

7. Granberry MC, Fonseca VA. Cardiovascular risk factors associated with insulin resistance: effects of oral antidiabetic agents. *Am J Cardiovasc Drugs* 2005;5:201-209.
8. Giannini S, Serio M, Galli A. Pleiotropic effects of thiazolidinediones: taking a look beyond antidiabetic activity. *J Endocrinol Invest* 2004;27:982-991.
9. Kovanecz I, Ferrini MG, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone prevents corporal veno-occlusive dysfunction in a rat model of type 2 diabetes mellitus. *BJU Int* 2006;98:116-124.
10. Katz EG, Moustafa AA, Heidenberg D, Haney N, Peak T, Lasker GF, et al. Pioglitazone Enhances Survival and Regeneration of Pelvic Ganglion Neurons After Cavernosal Nerve Injury. *Urology* 2016;89:76-82.
11. Zhu J, Zhang J, Ji M, Gu H, Xu Y, Chen C, et al. The role of peroxisome proliferator-activated receptor and effects of its agonist, pioglitazone, on a rat model of optic nerve crush: PPARgamma in retinal neuroprotection. *PLoS One* 2013;8:e68935. Epub 2013/07/23.
12. Cao Y, Wang Q, Zhou Z, Wang Y, Liu Y, Ji Y, et al. Changes of peroxisome proliferator-activated receptor-gamma on crushed rat sciatic nerves and differentiated primary Schwann cells. *J Mol Neurosci* 2012;47:380-388.
13. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* 2012;33:829-37, 37a-37d.
14. Laurikainen A, Hiltunen JO, Thomas-Crusells J, Vanhatalo S, Arumae U, Airaksinen MS, et al. Neurturin is a neurotrophic factor for penile parasympathetic neurons in adult rat. *J Neurobiol* 2000;43:198-205.
15. Moskowitz PF, Oblinger MM. Sensory neurons selectively upregulate synthesis and transport of the beta III-tubulin protein during axonal regeneration. *J Neurosci* 1995;15:1545-1555.
16. Gholamine B, Shafiei M, Motevallian M, Mahmoudian M. Effects of pioglitazone on erectile dysfunction in sildenafil poor-responders: a randomized, controlled study. *J Pharm Pharm Sci* 2008;11:22-31.
17. Eto M, Sumi H, Fujimura H, Yoshikawa H, Sakoda S. Pioglitazone promotes peripheral nerve remyelination after crush injury through CD36 upregulation. *J Peripher Nerv Syst* 2008;13:242-248.
18. Rahimian R, Fakhfour G, Rasouli MR, Nouri M, Nezami BG, Paydar MJ, et al. Effect of pioglitazone on sciatic nerve ischemia/reperfusion injury in rats. *Pediatr Neurosurg* 2009;45:126-131.
19. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Kline WO, et al. The IGF-1/PI3K/Akt Pathway Prevents Expression of Muscle Atrophy-Induced Ubiquitin Ligases by Inhibiting FOXO Transcription Factors. *Molecular Cell* 2004;14:395-403.
20. Higashi Y, Holder K, Delafontaine P. Thiazolidinediones up-regulate insulin-like growth factor-I receptor via a peroxisome proliferator-activated receptor gamma-independent pathway. *J Biol Chem* 2010;285:36361-36368.
21. Sjoberg J, Kanje M. Insulin-like growth factor (IGF-1) as a stimulator of regeneration in the freeze-injured rat sciatic nerve. *Brain Res* 1989;485:102-8. E
22. Marks DH. Drug utilization, safety and clinical use of Actos and Avandia. *Int J Risk Saf Med* 2013;25:39-51.