Polydeoxyribonucleotide Regulation of Inflammation

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Significance: Dysregulated inflammation is a critical factor in the development of a wide range of diseases. Controlling inflammatory responses can be challenging because available treatment options are often limited. This review discusses the ability of polydeoxyribonucleotides (PDRN) to modulate inflammation and the evidence that supports it.

Recent Advances: PDRN is known in the clinical field for its regenerative properties. This action is partially related to the stimulation of the purinergic system through adenosine A2A receptors (A2ARs). Recently, the topical anti-inflammatory effects of PDRN have aroused much interest, with a growing body of research supporting its use for the management of several inflammatory states.

Critical Issues: Impaired tissue repair and several metabolic disorders are associated with chronic inflammation. Despite the growing clinical concern, an optimal and sustainable therapy for different inflammatory conditions has not yet been established, and current treatments are often limited by their short-term efficacies and side effects.

Future Directions: The present review provides an updated summary of the ability of PDRN to control inflammation as observed in several *in vitro* studies, simplified models of the main biological mechanisms mediating its anti-inflammatory effect, and confirmed *in vivo* and clinical models. It analyzes the therapeutic potential of PDRN in terms of its mechanism of action through A2AR activation, its efficacy and its complications compared with those of current anti-inflammatory drugs.

Keywords: adenosine A2A receptors, inflammation, inflammatory diseases, PDRN, polydeoxyribonucleotides

SCOPE AND SIGNIFICANCE

THE PRESENT REVIEW focuses on the effect of polydeoxyribonucleotides on the management of inflammation. In this study, we provide an overview of the effects of PDRN tested in several experimental studies. In all the reported cases, PDRN successfully reduced inflammation.

TRANSLATIONAL RELEVANCE

Treatments commonly used to control inflammation are often ineffective in several clinical settings because of

their short-term efficacies or side effects that affect major organ systems. PDRN acts by engaging adenosine A2 receptors (A2ARs), key players in promoting wound healing and neovascularization. Thus, understanding the molecular interactions between PDRN-induced A2AR activation and inflammation could provide effective tools to improve healing in several diseases.

CLINICAL RELEVANCE

Although inflammation plays a central role in tissue repair, uncon-

trolled inflammation underlies several physiological and pathological conditions, for example, aging and metabolic disorders. Current anti-inflammatory drugs, although effective, often exhibit severe limitations under specific conditions. In this regard, PDRN has been suggested to have intriguing therapeutic potential.

BACKGROUND

Inflammation is a physiological process designed to maintain tissue homeostasis in response to a wide range of stimuli, for example, infection, injury, and tissue stress.¹ Inflammatory responses are orchestrated by different immune cell lineages, as well as stromal and endothelial cells, fibroblasts, and smooth muscle cells, which secrete numerous inflammatory mediators.²

Overall, inflammation may be defined as an effector arm of the immune system,³ although it may be harmful to the organism when prolonged. Chronic inflammation is closely linked to metabolic disorders, such as type 2 diabetes, insulin resistance, and obesity,⁴ and it is also a risk factor for cardiovascular diseases.⁵

Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids currently represent the gold standard for various diseases. However, long-term NSAID therapy correlates with adverse gastrointestinal, renal, and cardiovascular effects,⁶ and similarly, long-term treatment with glucocorticoids poses serious health challenges.⁷

Purinergic receptor signaling

Adenosine is an endogenous purine nucleoside that modulates key physiological processes, including inflammation.⁸ Its effects are mediated by the P1 receptor family, which are G-protein-coupled receptors. A1 and A3 receptors inhibit adenylate cyclase, and conversely, A2A and A2B receptors stimulate cAMP production.⁹ This receptor system acts as a quick sensor of tissue injury and, at the same time, as a first-aid mechanism for tissues and organs.

A2ARs are expressed in several human tissues,⁸ but their high expression in immune cells suggests a role in many inflammatory processes.¹⁰

Polydeoxyribonucleotide

Polydeoxyribonucleotide is a DNA-derived drug consisting of a mixture of double-stranded deoxyribonucleotides with a chain length of 80–2,200 base pairs.

PDRN is commonly extracted and purified from the gonads of salmon trout (*Oncorhynchus mykiss*), which provide high-quality DNA without immunological side effects.

Because of its chemical structure, the compound circulates freely in the plasma, is distributed in tissues according to their blood supply, and is degraded by unspecific plasma or membrane-bound DNA nucleases, which make its active oligo- and mononucleotides available for biological activity.¹¹

PDRN may be considered a prodrug that provides active deoxyribonucleotides for purinergic receptor binding. More specifically, released adenosine acts on adenosine A2A receptors, as suggested by Thellung *et al.*¹² through the use of 3,7-dimethyl-1-propargylxanthine, a selective A2AR antagonist that abrogates the effects of PDRN (Fig. 1).

Moreover, PDRN-derived nucleotides can also act through a “salvage pathway,” an efficient energy-saving metabolic pathway for nucleic acid synthesis. Bases and nucleotides from PDRN, once incorporated into the DNA of damaged or hypoxic cells, promote DNA formation and reactivate cell proliferation and growth¹³ (Fig. 1).

PDRN can therefore act both on adenosine receptors but can also promote cell proliferation by providing new “building blocks” for cells through salvage pathway and it is precisely because of this dual action that the use of PDRN may be preferable over simple A2AR agonists. Thus, this evidence has prompted researchers to hypothesize that PDRN could be a therapeutic tool to control inflammation through A2AR stimulation.

METHODS

For the present review, the MEDLINE database (through PubMed), Google Scholar, and the Cochrane Central Register of Controlled Trials were searched from their earliest available dates to October 2019. The search terms, including subheadings and keywords, were polydeoxyribonucleotide, inflammation, adenosine A2A receptors, and tissue repair. Reference lists of the included studies and relevant reviews were also searched. No language restriction or filters were applied. Two authors (M.T.C. and G.C.) screened the titles and abstracts of the retrieved studies for inclusion.

RESULTS AND DISCUSSION

PDRN and tissue regeneration

Healthy tissue repair proceeds through overlapping cellular processes, including inflammation, cell recruitment, proliferation, matrix deposition, and tissue remodeling. The failure of even one of these mechanisms results in chronic (nonhealing) wounds.

Impaired repair activity is associated with major injuries and conditions, such as aging, cancer, diabetes, infection, and vascular diseases.¹⁴ Currently,

Figure 1. Diagram representing the general mechanism of action of PDRN. PDRN acts on two possible pathways. The former is called the salvage pathway and relies on providing cells with nucleotides that they can reuse to synthesize new nucleic acids. The latter requires membrane-bound adenosine receptors (A2ARs) and controls several aspects of tissue homeostasis. A2AR, adenosine A2A receptor; PDRN, polydeoxyribonucleotide.

the challenge lies in developing a compound capable of modulating the different phases of healing.

Satisfactory results have been obtained by using PDRN to promote the healing of tissues, including skin,^{13,15,16} cartilage,^{17,18} bone,^{19,20} vascular endothelium,²¹ and corneal epithelium.²² Not surprisingly, however, most *in vitro* studies on tissue regeneration have been conducted on fibroblasts,²³ the major players in skin wound healing.²⁴ PDRN promotes cell viability and migration without any cytotoxic effect, even in neonatal fibroblast cells.²⁵

PDRN and the control of the inflammatory response

PDRN reduced the expression of proinflammatory cytokines in several *in vitro* (Table 1), as confirmed also *in vivo* (Table 2) and clinical models (Table 3).

The role of adenosine as a feedback regulator of inflammation has long been established. Adenosine limits the extent of inflammation by reducing leukocyte recruitment, as well as the expression of adhesive molecules and the generation of toxic

Table 1. The table summarizes the *in vitro* studies investigating the effects of PDRN on wound healing

Cell Model	PDRN Dose	PDRN vs. CTR Outcome	References
Macrophage cell line RAW 264.7	ZA + LPS +1, 10 or 100 $\mu\text{g/mL}$ for 24 h	$\uparrow$ cell viability and vascularization; anti-inflammatory effects.	41
Macrophage cell line RAW 264.7	LPS +100 $\mu\text{g/mL}$ for 1, 2 and 3 h	$\uparrow$ vascularization; anti-inflammatory effects.	43
Human chondrosarcoma cell line SW1353	IL-1 β + 100 $\mu\text{g/mL}$ for 24 h	Anti-inflammatory effects.	45
Human chondrocytic cell line SW1353	IL-1 β + 100 $\mu\text{g/mL}$ for 24 h	Proangiogenic effects; >wound closure.	58
Human chondrocytes from RA cartilage specimens	IL-1 β + 25, 50 or 100 $\mu\text{g/mL}$ for 24 h	Anti-inflammatory effects.	18
Wound biopsies from diabetic foot ulcers	Mixture of 10 mg l ⁻¹ PDRN + L-carnitine, calcium ions and proteolytic agents for >6 months	$\uparrow$ cell viability; $\uparrow$ epidermal and connective wound - healing markers.	65
Human embryonic fibroblasts (H2EL)	—	$\uparrow$ cell viability; Proangiogenic effects.	21
Vascular endothelial cells (VE)	—	—	—
Fibroblast neonatal cell (HDF-n)	12.5, 25, 50, 100, or 200 $\mu\text{g/mL}$ for 24, 48 and 72 h	no cytotoxicity; $\uparrow$ cell proliferation; $\uparrow$ cell migration.	25

Upward arrows indicate an increase in the endpoint.

PDRN, polydeoxyribonucleotide; CTR, control; ZA, zoledronic acid; LPS, lipopolysaccharide; IL-1 β , interleukin-1 β ; HA, hyaluronic acid; OA, osteoarthritis; RA, rheumatoid arthritis.

Table 2. The table summarizes the *in vivo* preclinical studies investigating the effects of PDRN on wound healing

Disease Model	Animal Model	PDRN Therapy	PDRN vs. CTR Outcome	References
Collagen II-induced arthritis (CIA)	DBA/1 mice (6 w)	8 mg/kg i.p. for 3 w	Clinical improvement of CIA and anti-inflammatory effects.	18
Experimental periodontitis (EPD)	Sprague-Dawley rats (12 w)	2 × 0.75% PDRN gel for 1 w	Anti-inflammatory and antiapoptotic effects.	46
LPS-induced lung injury	Sprague-Dawley rats (9 w)	8 mg/kg i.p. for 24 and 72 h	↑ A2ARs expression; anti-inflammatory and anti-apoptotic effects.	66
Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ)	New Zealand White rats	2, 4, and 8 mg/kg local injection, 2 times a day for 20 d	↓ severity of osteonecrosis; restoration of osteoclast activity; anti-inflammatory effects; ↑ vessel density.	44
Wound model	Diabetic mice (8 w)	Intradermal injection for 12 d	Faster re-epithelialization of collagen fibers formation; ↓ wound depth; ↑ vascularization.	25
Chondrocutaneous Composite Grafts	New Zealand White rabbits.	0.75 mg/kg intradermal injection on 1, 3, 6, 9, and 12 d after surgery	↑ graft viability; ↑ vascularization.	61
Random Pattern Skin Flaps	Sprague-Dawley rats (7 w)	8 mg/kg i.p. for 6 d	Survival of random pattern skin flaps; ↑ granulation tissue formation; ↑ vascularization.	16
Ischemic skin flap	Sprague-Dawley rats	8 mg/kg i.p. for 3, 5 and 10 d	↑ revascularization; complete re-epithelialization and well-formed granulation tissue.	53
Experimental thermal injury	C57BL/6 mice	8 mg/kg i.p. for 2 w	Anti-inflammatory and antiapoptotic effects.	54
Incisional wound healing	Sprague-Dawley rats (10 w)	8 mg/kg i.p. for 3 or 7 d	Scar prevention; proper collagen deposition; anti-inflammatory effects.	52
Incisional wound healing	C57BL/KsJ-m1/1Leptdb mice (14 w)	8 mg/kg i.p. for 12 d	Epidermal regeneration; proangiogenic effects.	60
Laser-induced skin wound	Sprague-Dawley rats (8 w)	8 mg/kg i.p. for 1 w	Faster epidermal regeneration and thick granulation tissue formation; proangiogenic effects.	59
Excisional wound healing	Sprague-Dawley rats	Topical administration for 4 w	↓ wound size ↓ cell apoptosis.	68
Excisional wound healing	Hairless mice (8 w)	8 mg/kg i.p. for 1 w	Best wound closure with earlier collagen deposition and increased re-epithelialization in classic PDRN-treated group.	64
Experimental colitis	Sprague-Dawley rats (14 w)	8 mg/kg i.p. for 1 w	Normal colon appearance and epithelial regeneration; ↓ lipid peroxidation and neutrophil infiltration; anti-inflammatory and antiapoptotic effects.	47
Chronic soft tissue ulcer	C57-BL/KsJ-m+/+Lept Diabetic mouse	—	↑ re-epithelialization and well-formed granulation tissue; ↑ wound healing; ↑ revascularization; ↓ infection.	21
Acetic acid-induced gastric ulcers (GU)	Mongolian gerbils (10/12 w)	4-8-16 mg/kg i.p. for 2 w	↓ ulcer size; ↑ VEGF; anti-inflammatory and antiapoptotic effects.	50
Indomethacin-induced gastric ulcers (GU)	Sprague-Dawley rats (10 w)	8 mg/kg i.p. for 2 w	↓ ulcer size; ↑ VEGF; ↑ A2ARs, cAMP, PKA; anti-inflammatory effects.	49
Spinal cord injury (SCI)	C57BL6/J mice	8 mg/kg/i.p. for 10 d	Improved locomotor performance; < neural damage; anti-inflammatory effects; anti-apoptotic effects; Wnt/β-catenin pathway stimulation.	51
Peripheral nerves damage	C57B mice	5 mg/kg/i.p. for 1 w	Nerve regeneration.	67

Upward arrows indicate an increase in the endpoint; downward arrows indicate a decrease.

w, week; d, day; i.p., intraperitoneally; A2AR, adenosine A2A receptor; VEGF, vascular endothelial growth factor; cAMP, cyclic adenosine-3',5'-monophosphate; PKA, protein kinase A.

oxygen metabolites in neutrophils through A2AR activation.²⁶ The inhibition of early and late inflammatory reactions by selective A2AR agonists has been confirmed in *in vivo* models of respiratory system diseases,²⁷ for some of which corticosteroid therapy has not yet been sufficiently substantiated.²⁸

Encouraging results have also been obtained in animal models of bowel diseases^{29,30}; and in *in vivo*

and clinical studies in several autoimmune diseases,³¹ for example, rheumatic diseases^{32,33}; for example, adenosine was shown to reduce inflammatory cell infiltrates and enhance T_{reg} activity in animal models of obesity by reducing adipose tissue inflammation and improving glucose homeostasis.³⁴ Furthermore, A2AR activation in bone marrow-derived cells exerts a protective effect against

Table 3. The table summarizes the clinical studies investigating the effects of PDRN on wound healing

<i>Clinical Study</i>	<i>Study Design</i>	<i>Experimental Group</i>	<i>Intervention</i>	<i>PDRN Therapy</i>	<i>Follow Up</i>	<i>PDRN vs. CTR Outcome</i>	<i>References</i>
Genital lichen sclerosis	Case-control	28 M 45 y	0.05% CP (<i>n</i> =14); PDRN (5.625 mg/3 mL) +0.05% CP (<i>n</i> =14).	Intradermal PDRN infiltration for 8 sessions + daily topical CP.	6 months	Anti-inflammatory effects; long- lasting improvement in symptoms; no side effects.	70
Genital lichen sclerosis	Case series	21 M 56.95 y	1 or 2 vials PDRN (5.625 mg/3 mL)	Weekly locoregional intradermal or submucosal PDRN injections for 2 cycles of 10 sessions.	12–24 months	Improvement in irritative symptoms and in the trophism and the elasticity of the skin; no side effects.	71
Knee osteoarthritis	Double-blind RCT	30 M/F 65.08 y	PDRN (5.625 mg/3 mL) + HA (<i>n</i> =15) HA (<i>n</i> =14)	3 Intra-articular PDRN injections at weekly intervals.	6 months	↓ pain; ↑ physical function; ↑ functional activity; no side effects.	74
Radiation-induced oral mucositis	Case series	3 M=1; F=2 67 y	PDRN	PDRN spray 2 times/day or 3–6 times/day a month after RT ended.	Weekly until a month after RT ended	↓ pain; ↓ erythema and desquamation; no side effects.	72
Radiation-induced cystitis (CRC)	Case series	8 M=4; F=4 69.7 y	10 mL PDRN 50%	Biweekly intravesical PDRN instillation for 2 months.	4 months	↓ CRC symptoms	73
Pes anserine bursitis (PA)	Case report	1 F 50 y	PDRN (5.625 mg/3 mL)	Ultrasound-guided PDRN injection	>8 months	↓ pain; full range of motion; no side effects.	75
Hemiplegic shoulder pain in subacute stroke patients	Case-control	20 M/F 66.25 y	Triamcinolone (<i>n</i> =10) PDRN (5.625 mg/3 mL) (<i>n</i> =10)	2 intra-articular injections at weekly intervals.	>1 month	Improvement in symptoms; no side effects.	79
Lateral epicondylitis (LE)	Case series	2 M 62 y	PDRN (5.625 mg/3 mL)	Ultrasound-guided PDRN injections	2 months	↓ pain; resolution of hypervascularity; no side effects.	80
Tibial tendon dysfunction	Case report	1 F 67 y	PDRN (5.625 mg/3 mL)	5 PDRN prolotherapy injections at weekly intervals.	>12 months	Improvement in the arch of the foot; ↓ pain; no side effects.	81
Rotator Cuff Tendinopathy	Case series	32 M/F 52.5 y	PDRN (5.625 mg/3 mL)	5 ultrasound-guided PDRN injections at weekly intervals.	3 months	↓ pain; ↓ disability; no side effects.	82
Chronic Supraspinatus Tendinopathy	Case-control	106 M/F 52.5 y	Conservative treatment (<i>n</i> =51) PDRN (5.625 mg/3 mL) (<i>n</i> =55)	3 ultrasound-guided PDRN injections at weekly intervals.	3–6 months	↓ pain; no side effects.	83
Partial-thickness tear of supraspinatus tendon	Case series	17 M/F 57.9 y	PDRN (5.625 mg/3 mL)	3 ultrasound-guided PDRN injections every 2 weeks.	3 months	↓ pain; improvement in forward flexion and internal rotation in shoulder; no side effects.	84
Tendinopathy	Case series	138 M/F 45 y	PDRN (3 mL)	3–5 weekly subcutaneous peritendon PDRN infiltrations + Daily intramuscular administration for a total of 15–20 vials.	—	↓ pain; resumption of functional conditions; no side effects.	85
Chronic plantar fasciitis	Double-blind RCT	40 M/F 53 y	Placebo (<i>n</i> =20) PDRN (2.812 mg/1.5 mL) (<i>n</i> =20)	Weekly PDRN injection into the tender region of the heel, medial to the insertion of the plantar fascia for three weeks.	3 months	↓ pain; improvement in symptoms; no side effects.	86
Ischiofemoral impingement syndrome	Case series	2 M 23.5 y	PDRN (5.625 mg/3 mL)	Ultrasound-guided PDRN prolotherapy at 1/2 weeks intervals for 5 sessions	>6 months	↓ pain.	87
Radiating leg pain	Case report	1 M 51 y	PDRN (5.625 mg/3 mL)	4 Injections around the piriformis muscle at biweekly intervals.	Every 2 months for 2 months	Long-term relief.	88

(continued)

Table 3. (Continued)

Clinical Study	Study Design	Experimental Group	Intervention	PDRN Therapy	Follow Up	PDRN vs. CTR Outcome	References
Complex regional pain syndrome (CRPS) type 2	Case report	1 F 32 y	PDRN (5.625 mg/3 mL)	Ultrasound-guided PDRN prolotherapy.	1 month	Improvement in allodynia and hyperalgesia.	89
Postherpetic brachial plexopathy	Case report	1 F 73 y	PDRN (5.625 mg/3 mL)	4 ultrasound-guided PDRN injections at weekly intervals.	>6 months	↓ motor weakness; ↓ neuropathic pain.	92
Cervical nerve root injury	Case report	1 F 54 y	PDRN (5.625 mg/3 mL)	2 × ultrasound-guided cervical nerve root block (NRB) using PDRN at weekly intervals.	2 months	↓ motor weakness; ↓ neuropathic pain.	93
Lumbosacral radiculopathy	Case report	1 M with 2DM 44 y	PDRN (5.625 mg/3 mL)	4 epidural PDRN injections at weekly intervals.	>6 months	↓ pain; no side effects.	96
Carpal tunnel syndrome	Case report	1 F with 2DM 41 y	PDRN (5.625 mg/3 mL)	2 ultrasound-guided PDRN injections at weekly intervals.	6 months	Improvement in symptoms; no side effects.	95

Upward arrows indicate an increase in the endpoint; downward arrows indicate a decrease.

M, male; F, female; y, years (mean age); CP, clobetasol propionate; HA, hyaluronic acid; RCT, randomized controlled trial; RT, radiotherapy; 2DM, type 2 diabetes mellitus.

ischemia/reperfusion injury in several organs^{35–39} and sepsis.⁴⁰

Han *et al.*⁴¹ administered PDRN to Raw 264.7 macrophages after stimulating them with zole-dronic acid, a nitrogen-containing bisphosphonate used to inhibit severe bone resorption, and lipopolysaccharide (LPS) in an *in vitro* bisphosphonate-related osteonecrosis of the jaw (BRONJ) model.⁴² Cell viability, as assessed by the MTT assay, was enhanced and NO production was decreased in the experimental group. The dose-dependent anti-inflammatory effect of PDRN was revealed by the suppression of LPS-induced iNOS, IL-1 β , IL-6, TGF- α , as assessed by western blotting.

In agreement with Castellini *et al.*⁴³ who treated LPS-primed macrophages with PDRN, these results have been recently confirmed in a BRONJ rat model, which showed that PDRN reduced the severity of osteonecrosis by decreasing the inflammatory infiltrate, restoring osteoclast activity and promoting blood vessel formation.⁴⁴

Baek *et al.*⁴⁵ investigated the possible use of DNA polymeric molecules as a new alternative treatment for osteoarthritis, a condition characterized by the destruction of cartilage through inflammation. Transcriptome analysis and validation revealed that human chondrosarcoma cells stimulated with IL-1 β expressed lower levels of the IL-6, IL-1 β , IL-8, and chemokine (C-C motif) ligand three genes after treatment with PDRN compared with routine hyaluronic acid (HA) treatment. PDRN reduced the expression of proinflammatory high-mobility group box chromosomal protein 1 (HMGB1), TNF α , and IL-6, and increased the levels of anti-inflammatory IL-10 in IL-1 β -stimulated human chondrocytes from rheumatoid arthritis patients.¹⁸

The same study confirmed the anti-inflammatory effect of PDRN using a rodent model of collagen II-induced arthritis (CIA); the intraperitoneal (i.p.) administration of PDRN attenuated the severity of arthritis, and reduced the circulating levels of late proinflammatory cytokines and their expression in cartilage. Likewise, PDRN also exerted a positive effect on periodontal inflammation⁴⁶ in an experimental periodontitis rat model.

Pallio *et al.*⁴⁷ investigated the effects of PDRN in two rat models of colitis characterized by extensive tissue damage due to severe inflammation. The authors observed a significant reduction in the extent and severity of mucosal alterations, epithelial regeneration, and reduced infiltration of inflammatory cells. Moreover, PDRN reduced the circulating levels of IL-1 β and TNF- α .

Thus, PDRN could be a promising therapeutic approach for inflammatory bowel diseases because of its increased safety compared with that of the common NSAIDs⁴⁸ as recently demonstrated by Ko *et al.*,⁴⁹ using a rat model of gastric ulcer induced by the oral administration of indomethacin, a commonly used NSAID. PDRN reduced gastric inflammation, decreased the expression of the proinflammatory cytokines, TNF- α , IL-6, and IL-1 β , and promoted gastric mucosal regeneration to a greater extent than proton pump inhibitors.

These results are consistent with the findings of Jeon *et al.*,⁵⁰ who investigated the effects of PDRN treatment on acetic acid-induced gastric ulcers in gerbils.

Recently, Irrera *et al.*⁵¹ tested the effects of PDRN on neural damage. In an experimental model of spinal cord injury (SCI), the i.p. administration of PDRN 1 h after SCI reduced the marked increase

in $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and neutrophil infiltration, as assessed by MPO activity and histological results.

Most interestingly, it has been suggested that the effects of PDRN on wound healing are mediated by its anti-inflammatory and collagen synthesis properties. Jeong *et al.*⁵² not only observed significantly reduced inflammatory cell infiltration by histology after the administration of PDRN on both days 7 and 14 postsurgery, but also that PDRN treatment significantly reduced the expression of HMGB-1, and the addition of exogenous HMGB-1 abolished the effects of PDRN on scar reduction and collagen deposition.

Polito *et al.*⁵³ investigated the effects of i.p. injections of PDRN on skin flaps using a similar rodent model. Western blot assays showed that PDRN reduced HIF-1 α expression and upregulated iNOS and nitrites in the early stages of healing.

Bitto *et al.*⁵⁴ also suggested that PDRN promoted physiologic wound repair by investigating its effects on experimental second-degree burns in mice after 7 and 14 days. Consistent with previous reports in different experimental models, PDRN has been shown to decrease the levels of the proinflammatory cytokine $\text{TNF-}\alpha$ and increased VEGF, eNOS, iNOS, and NO_2/NO_3 levels, as determined by western blotting.

PDRN and the resolution of inflammation

Blood vessel restoration is still an unresolved issue in regenerative medicine. Angiogenesis and vasculogenesis represent key processes in tissue repair because vessels support cells in injured tissues by providing nutrients and oxygen.

Adenosine promotes tissue vascularization mainly by enhancing vascular endothelial growth factor (VEGF) secretion. VEGF, a key mediator in blood vessel growth, plays a critical role in the resolution of inflammation and is therefore thought to be instrumental to subsequent wound healing.²⁴ A bulk of data has shown that adenosine exerts an anti-inflammatory effect by suppressing the classical macrophage activation pathway, predominantly through A2AR stimulation.

Furthermore, recent studies have underlined how the resolution of inflammation relies not only on the suppression of proinflammatory mechanisms but also on a complex array of active anti-inflammatory signals that modulate and resolve inflammation.⁵⁵ The existing evidence supports the hypothesis that PDRN also controls these biological mechanisms. Macrophages represent the major VEGF producers in resolving inflammatory states,²⁶ and A2AR activation on TLR-activated macrophages induces a switch from a proinflammatory phenotype to an anti-inflammatory phenotype

and an increase in VEGF and anti-inflammatory IL-10 expression.

Moreover, the A2AR-mediated shift from a proinflammatory profile to an anti-inflammatory profile has also been detected in mature dendritic cells.⁵⁶

Interestingly, PDRN promotes angiogenesis, unlike other DNA-derived drugs, such as defibrotide, which shows an inhibitory effect.¹¹ An increase in VEGF expression was observed in several *in vitro* models, including LPS-activated macrophages,^{41,43} which are considered a major source of VEGF in wound healing⁵⁷ (Fig. 2).

PDRN increased PDGF, ANG-2, and VEGF and increased cell migration (as assessed by the scratch assay) in a human $\text{IL-1}\beta$ -stimulated chondrocytic cell line, a common model of osteoarthritis.⁵⁸ Chen *et al.*²¹ treated human embryonic fibroblasts and vascular endothelial cells with short poly-N-acetyl glucosamine nanofibers and polydeoxyribonucleotide (sNAG+PDRN) or PDRN alone.

The authors observed an increase in cell proliferation by the CCK8 assay after PDRN treatment and an increase in cytokines and VEGF. Again, the administration of PDRN markedly increased CD31 staining and microvessel density. Furthermore, PDRN encapsulation in sNAG also showed a positive effect even in *in vivo* model, aiding the healing of chronic ulcers in diabetic rats.

Ko *et al.*⁴⁹ demonstrated that PDRN increased PKA, CREB, A2AR, and VEGF levels in a rat model of indomethacin-induced gastric ulcers suggesting that PDRN inhibits the production of proinflammatory cytokines by promoting phosphorylation of CREB through the cAMP-PKA pathway and induced VEGF expression by A2AR stimulation. Consistently, Jeon *et al.*⁵⁰ showed overexpression of VEGF in PDRN-treated animals in a gastric ulcer model.

Figure 2. Two main macrophage phenotypes are known to act on inflammation and healing. M1 macrophages are proinflammatory cells that secrete a vast array of cytokines that participate in inflammation. M2 macrophages, conversely, actively aid the resolution of inflammation and promote wound healing. PDRN has been shown to increase the expression of IL-10 and VEGF, which are known products of M2 cells. It can therefore be hypothesized that PDRN might act by promoting the shift of macrophages from the M1 to the M2 phenotype. VEGF, vascular endothelial growth factor.

I.p. injections of PDRN for 7 days reduced skin erythema, increased epithelial confluence, decreased crusting, and generally improved the appearance of laser-induced skin wounds in a rat model comparable to fractional laser resurfacing; moreover, wound healing was faster in the PDRN-treated group. Histology indicated that PDRN induced rapid epidermal regeneration and thick granulation tissue formation and significantly enhanced microvessel density, as further confirmed by the increase in the amount of VEGF, assessed by ELISA, and the greater number of PECAM-1/CD31-positive microvessels.⁵⁹

Galeano *et al.*⁶⁰ also used incisional skin wounds on the backs of diabetic mice to test the effect of i.p. injections of PDRN for 12 days. The administration of PDRN in diabetic mice significantly increased *VEGF* mRNA expression to physiological levels 3 and 6 days after injury. Western blot analysis of tissues from PDRN-treated animals revealed a greater increase in Angiopoietin-1 and a restored expression pattern of VEGF and Transglutaminase-II (TG-II), a key matrix protein during healing. The regenerative effects of PDRN were recently confirmed in an *in vivo* study. Histological analysis showed that PDRN promoted wound healing and faster re-epithelialization and angiogenesis through increased expression of VEGF and CD31 in diabetic mice.²⁵

Chung *et al.*¹⁶ elevated and repositioned caudally pedicled random pattern skin flaps on the dorsal skin of rats. Animals received daily i.p. injections of PDRN. Histology showed that the average necrotic area of the flap was significantly smaller and that the granulation tissue thickness score was significantly higher in the PDRN group on day 7 after surgery. The immunohistochemical analysis showed more VEGF-positive cells and a greater PECAM-1/CD31-positive microvascular density in the PDRN group.

Polito *et al.*⁵³ investigated the effects of i.p. injections of PDRN on skin flaps using a similar rodent model. Laser Doppler perfusion imaging indicated that PDRN improved the blood perfusion of ischemic flaps, promoting complete recovery and graft healing. RT-PCR and western blot analysis indicated that PDRN enhanced VEGF expression, as confirmed by immunostaining for CD31. Similarly, PDRN restored perfusion by increasing neovascularization, as assessed by histological and optical methods, in auricular chondrocutaneous composite grafts in rabbits.⁶¹

PDRN and matrix degradation

Deficiencies in proangiogenic factors, such as VEGF, and the degradation of matrix components resulting from the faulty regulation of proteases

and their inhibitors are critical in wound pathogenesis,⁶² as suggested by the increased expression of matrix metalloproteinases (MMPs) in nonhealing human skin wound fluid.⁶³ The proteolytic imbalance has been attributed, in part, to uncontrolled inflammation.

Baek *et al.*, however, showed that PDRN decreased MMP13 and increased aggrecan expression. Moreover, PDRN promoted cell spreading and migration capabilities *in vitro*, as determined by a scratch assay. This could indicate, within the limits of this model, an increase in epithelial repair.⁵⁸ Similarly, Bitto *et al.*¹⁸ investigated the histological features of a rodent model of CIA and observed an increase in preserved cartilage by histology after treatment with PDRN.

Daily i.p. injections of PDRN of different molecular weights, that is, low-molecular-weight PDRN (<50 kDa), mid-molecular-weight PDRN (50–1,500 kDa), and high-molecular-weight PDRN (>1,500 kDa) were administered to hairless mice with excisional skin wounds for a week. Immunohistochemistry revealed that the molecular weight of PDRN did not affect wound closure but did affect the quality of wound regeneration.⁶⁴ Among the examined sizes, mid-molecular-weight PDRN promoted earlier collagen deposition as well as better wound closure and increased re-epithelialization.

Gennero *et al.*⁶⁵ demonstrated that a specific PDRN-based formulation was also effective in improving the *ex vivo* maintenance of tissue biopsies for more than 1 year, increasing epithelial and fibroblast markers, for example, Collagen I and Collagen IV, in cell extracts.

Jeong *et al.*⁵² investigated scar formation in rats after the injection of PDRN. For this purpose, dorsal skin excision was performed in 30 rats, which then received vehicle or 8 mg/kg PDRN i.p. for 3 or 7 days. On day 7, Hematoxylin and Eosin and Masson's Trichrome staining demonstrated complete wound re-epithelialization and the formation of sound granulation tissue; on day 14, more collagen was deposited within significantly narrower scars in the presence of PDRN, and western blotting revealed increased levels of type I and type III collagen. Moreover, faster collagen fiber deposition was recently observed in diabetic mice after PDRN treatment.²⁵

PDRN and antiapoptotic effects

In a periodontitis rat model, PDRN reversed the increase in the levels of proapoptotic BAX observed after the onset of periodontitis and decreased BCL-2 protein expression.⁴⁶ In a rat model of LPS-induced lung injury, PDRN decreased the activation of

caspases 3, 8, and 9, as determined by immunohistochemistry, suppressed the expression of the proapoptotic protein BAX and proinflammatory mediators, and enhanced the expression of the antiapoptotic protein BCL-2 and A2AR, as determined by western blotting.⁶⁶

Pallio *et al.* confirmed that PDRN reduced BAX expression and restored BCL-2 expression in two rat models of colitis characterized by extensive tissue damage due to severe inflammation.⁴⁷ Jeon *et al.*⁵⁰ induced gastric ulcers in gerbils and observed a decrease in the BAX/BCL-2 ratio in PDRN-treated animals. In agreement with these findings, a TUNEL assay showed that PDRN suppressed DNA fragmentation, and immunohistochemistry demonstrated that PDRN suppressed caspase-3 expression.

The effects of PDRN on apoptosis do not appear to be limited to the gastrointestinal tract, as Irrera showed that PDRN preserved neuronal structure, prevented cell death, restored BAX and BCL-2 expression, and modulated Wnt signaling, a key pathway in normal organ development and tissue homeostasis, using an animal model of neural damage.⁵¹ This confirmed a previous study by Park *et al.* that demonstrated that PDRN facilitated nerve regeneration by promoting VEGF expression after sciatic nerve transection in a mouse model.⁶⁷

Yun *et al.*⁶⁸ demonstrated that PDRN, alone or in combination with HA, decreased wound size and cell apoptosis by a TUNEL assay.

PDRN and the regulation of inflammation: clinical endpoints

Skin and mucosal diseases. PDRN was successfully used in patients with lichen sclerosus, an autoimmune inflammatory dermatosis. Compared with corticosteroid monotherapy, eight sessions of subdermal PDRN infiltration combined with daily topical corticosteroid administration induced long-lasting improvements in inflammation, atrophy, hypertrophy, erosion, leukoplakia, and pigmentation.^{69,70}

The effectiveness of PDRN in the management of genital lichen sclerosus was evaluated in a subsequent pilot study.⁷¹ Twenty-one patients, including diabetic and hypertensive subjects, were treated with weekly intradermal or submucosal PDRN injections for 2 cycles of 10 sessions. PDRN improved irritative symptoms, trophism, and the elasticity of the skin and again showed excellent tolerability.

Podlesko *et al.*⁷² investigated the use of an oral spray containing PDRN for the treatment of oral mucositis, the most common adverse effect of radiotherapy (RT) and/or chemotherapy, in a small ($n=3$) set of patients. PDRN was administered to

mucositis-affected areas during RT 2–6 times/day, and all patients received PDRN for at least a month after RT ended. Patients reported decreased pain and oral mucositis after 2–3 days of treatment.

PDRN also showed positive effects on RT-induced cystitis.⁷³ PDRN was intravesically instilled bi-weekly for 2 months in eight patients (CRC) previously subjected to RT for pelvic cancer with chronic radiation cystitis unresponsive to conventional medical treatment. Four months after the last infusion, hematuria, which is characteristic of hemorrhagic cystitis, and CRC symptoms significantly improved.

Orthopedic pathologies. In a double-blind RCT, compared with HA alone, the anti-inflammatory effect of PDRN was proven to enhance the efficacy of HA in the management of knee osteoarthritis by improving functional activity and physical function and reducing pain at 6 months of follow-up.⁷⁴ A case report described the use of PDRN for a common soft tissue pain disease, anserine bursitis, as an alternative to standard treatments. Ultrasound-guided PA bursa injection of PDRN significantly reduced pain at the 2-week follow-up visit, and complete functional recovery was achieved at the 8-month follow-up examination.⁷⁵

Numerous studies have confirmed the efficacy of PDRN in reducing pain and symptoms of inflammation in tendinopathy, a musculoskeletal disorder for which an optimal treatment has not yet been established.⁷⁶ Glucocorticoids represent the most common treatment choice for this pathology.

However, *in vitro* and *in vivo* evidence has suggested that glucocorticoids decrease the proliferation of tendon cells and extracellular matrix (ECM) synthesis and, generally negatively affect the mechanical properties of tendons.⁷⁷ Conversely, PDRN enhanced growth factor secretion, collagen synthesis, and restored tensile strength.⁷⁸ Moreover, PDRN had a similar anti-inflammatory effect in subacute stroke patients with hemiplegic shoulder pain, suggesting that it could be a valid alternative for this musculoskeletal complication due to its increased safety.⁷⁹

PDRN could also be a valid alternative to corticosteroids for lateral epicondylitis (LE) treatment.⁸⁰ After undergoing ultrasound-guided PDRN injection, two patients reported improvements in LE symptoms and complete resolution of hypervascularity without complications. PDRN also yielded promising results in a case report on posterior tibial tendon dysfunction as the result of ankle syndesmotom surgery. The patient, who experienced side effects from long-term NSAID ad-

ministration, reported good improvements in pain without complications after PDRN prolotherapy.⁸¹

The use of PDRN as an injected proliferant in prolotherapy, which is commonly used for chronic musculoskeletal diseases, could also be a viable therapeutic option for rotator cuff tendinopathy (RCT), a condition characterized by shoulder pain and disability. Weekly PDRN treatment improved pain and function and reduced disability for at least 3 months after therapy in a cohort of 32 patients with RCT.⁸²

These results were consistent with a case/control study with a 6-month follow-up to evaluate the efficacy of PDRN injections compared with conservative treatments for RCT.⁸³ Between-group comparisons revealed differences in outcome measurements at month 3. Specifically, the experimental group ($n=55$), which received weekly PDRN injections, demonstrated a greater reduction in pain and disability.

Similarly, a recent pilot study in 17 patients with RCT reported decreased shoulder pain and disabilities of the arm, hand, and shoulder, and improvements in forward flexion and internal rotation.⁸⁴ This is in agreement with a trial by Gervaso P.⁸⁵ showing that painful symptoms disappeared or were significantly reduced in a large ($n=138$) cohort of patients with chronic tendinopathy treated weekly with PDRN by subcutaneous peritendon infiltration and daily by intramuscular administration.

In another placebo-controlled study, PDRN proved more effective for the treatment of chronic plantar fasciitis. PDRN was injected weekly into the tender region of the heel medial to the insertion of the plantar fascia for 3 weeks and there were noticeable improvements in symptoms.⁸⁶

Diseases of the peripheral nerve system. Kim *et al.*⁸⁷ reported the use of PDRN in patients with ischiofemoral impingement syndrome in whom surgery was not recommended. Young patients who received ultrasound-guided prolotherapy with PDRN reported excellent pain relief with a long-term effect for >6 months.

PDRN provided satisfactory results for nerve injury treatment. Recently, PDRN was used in a case of radiating leg pain due to a ganglion cyst that was compressing the sciatic nerve and was surgically unresectable.⁸⁸ After ineffective treatment with corticosteroids, the patient was subjected to three PDRN injections at intervals of 2 weeks and exhibited greater improvements in symptoms than with conservative treatment. Improvements in allodynia and hyperalgesia have been achieved after

PDRN prolotherapy in acute stage complex regional pain syndrome type 2, for which no specific treatment has yet been detected.⁸⁹

Moreover, even when nerve injury was associated with bone fracture, PDRN, which is known to promote osteoblast proliferation,⁹⁰ replaced corticosteroids and NSAIDs, the application of which was limited because of their inhibitory effects on bone healing.⁹¹ Recently, PDRN was shown to be a viable alternative to corticosteroids for the management of postherpetic brachial plexopathy.⁹² Ultrasound-guided PDRN in a patient with cervical nerve root injury, a complication of traumatic cervical spine fracture, improved motor weakness and sensory symptoms after the second injection.⁹³

The safety profile of PDRN has prompted its use in patients with metabolic syndromes, such as diabetes, a clinical condition for which corticosteroids have glucose-related adverse effects.⁹⁴ Reports on the success of PDRN injections for the management of carpal tunnel syndrome⁹⁵ and lumbosacral radiculopathy⁹⁶ in patients with diabetes mellitus have been recently published.

SUMMARY

Dysregulated inflammation impairs wound healing, and chronic inflammation is associated with a wide range of pathologies. The treatment of inflammation therefore has critical significance in the management of several diseases. Most current anti-

Figure 3. Summary of the action of PDRN through the activation of A2ARs; receptor stimulation increases the levels of cAMP, thus orchestrating diverse molecular mechanisms ranging from the control of matrix deposition to cell survival, neovascularization, and the control of inflammation. cAMP, cyclic adenosine-3,5'-monophosphate.

inflammatory drugs have demonstrated strong therapeutic potential. However, their use has been proved to be limited by their short-term efficacies and side effects.

In the last few decades, PDRN has generated much interest due to its regenerative properties. PDRN is a prodrug that generates active deoxyribonucleotides, nucleosides, and bases, which activate A2ARs (Fig. 3). PDRN has been shown to promote cell proliferation, proper ECM deposition, and angiogenesis and blunts inflammation in several preclinical and clinical studies. Promising results for skin regeneration as well as musculoskeletal tissues have also been obtained.²³ The regenerative effects of PDRN may be at least in part linked to its anti-inflammatory action. PDRN prevents excessive scar formation and enhances physiological tissue repair by suppressing HMGB1.⁵²

Therefore, since the anti-inflammatory effects of PDRN seem to play a crucial role, clarifying the molecular mechanisms underlying its action could help to better define its therapeutic potential and even broaden its clinical field applications.

The present review analyzed different recent experiments on the effects of topical PDRN on the control of inflammation. *In vitro* studies have consistently shown that PDRN modulates inflammatory signals, such as TNF- α , NO, and IL-6. Unsurprisingly, most *in vivo* studies, however, were conducted on tissue wound models, as this is the primary area of application of PDRN. Although PDRN exhibited a clear anti-inflammatory activity in these studies, no pure inflammation model has yet been published.

More specific studies are therefore needed to assess the *in vivo* anti-inflammatory activity of PDRN using adequate established animal models.⁹⁷

Conversely, the anti-inflammatory activity of PDRN has been confirmed by several diverse clinical studies on inflammation, ranging from oral mucositis to tendinopathy. The main limits of these studies, however, are their limited sample sizes and study

TAKE-HOME MESSAGES

- A growing body of research has demonstrated that PDRN, through A2AR activation, is able to reduce local inflammatory responses.
- PDRN has been shown to modulate inflammation in several conditions of impaired wound healing for which effective therapeutic strategies do not yet exist.
- Its long-term efficacy and absence of side effects suggest that PDRN is a promising therapeutic strategy for inflammatory states.

designs. Bigger RCTs with robust endpoints are needed to obtain more solid evidence of the anti-inflammatory activity of this compound. Such studies, however, appear justified based on the promising evidence gathered so far in the available literature.

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Abbreviations and Acronyms

A2AR = adenosine A2A receptor
 BRONJ = bisphosphonate-related osteonecrosis of the jaw
 cAMP = cyclic adenosine-3,5'-monophosphate
 CIA = collagen II-induced arthritis
 ECM = extracellular matrix
 HA = hyaluronic acid
 i.p. = intraperitoneal
 IL-1 β = interleukin-1 β
 LE = lateral epicondylitis
 LPS = lipopolysaccharide
 MMP = matrix metalloproteinase
 NSAID = nonsteroidal anti-inflammatory drugs
 OA = osteoarthritis
 PDRN = polydeoxyribonucleotide
 RA = rheumatoid arthritis
 RCT = rotator cuff tendinopathy
 RT = radiotherapy
 SCI = spinal cord injury
 VEGF = vascular endothelial growth factor
 ZA = zoledronic acid