Pharmacological PTEN inhibition: potential clinical applications and effects in tissue regeneration

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Although the human body can heal, it takes time, and slow healing and chronic wounds often occur. Thus, identifying novel therapies to aid regeneration is needed. Here, we conducted a systematic review following the Preferred Reporting Items for Systematic Reviews guidelines and assessed preclinical studies on phosphatase and tensin homolog (PTEN) inhibitors and their effects on tissue repair and regeneration. In conditions associated with neurodegeneration, tissue injury and ischemia, the PTEN-regulated PI3K/AKT signaling pathway is activated. The use of PTEN inhibitors resulted in better tissue response by reducing the healing time and lesion sizes or inducing neuronal regeneration. Notably, all studies included in this systematic review indicated that pharmacological inhibition of PTEN enhanced the repair process of the eye, lung, muscle and nervous system.

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The physiological process of tissue repair following an injury is essential for restoring the integrity and function of the tissues, organs and body systems. Disruption of tissue integrity results in the activation of molecular and cellular mechanisms that are common to different tissues and mostly encompass overlapping phases of inflammatory response, cell migration and proliferation, angiogenesis and tissue remodeling [1]. It is accepted that developmental genes that are pressed into work earlier in life also participates in the response to an injury [2–4]. In this sense, phosphatase and tensin homolog (PTEN), a tumor suppressor gene, is transiently downregulated so that tissue repair might be enhanced.

PTEN is a known negative regulator of the PI3K/AKT/mTOR signaling pathway, which, by its turn, regulates cell proliferation [5]. Studies on the PTEN molecular function revealed that it is essential for embryogenesis and organ development, and that disrupted PTEN resulted in decreased apoptosis and enlarged organs in mouse models [6]. PTEN pharmacological inhibition leads to PI3K/AKT/mTOR upregulation, hence improving cell proliferation and migration, which are crucial factors to the tissue repair [7]. Therefore, a transient downregulation of PTEN has been regarded as a promising strategy to aid tissue repair [3,4]. In fact, conditions such as neurodegeneration, ischemia, tissue injuries and insulin-resistant metabolic disorders benefit from increased signaling through the PI3K/AKT/mTOR pathway, which makes inhibition of PTEN a potential pharmacological approach [8,9].

Bisperoxovanadium (bpV) compounds, such as bpV(phen) (bisperoxovanadium 1,10-phenantroline), bpV(pic) (bisperoxovanadium 5-hydroxypyridine) and bpV(HOPic) (bisperoxovanadium 5-hydroxypyridine-2-carboxylic acid), have been proposed as reversible and relatively specific PTEN inhibitors [10–13], which points to their potential and beneficial effects on the tissue repair process.

Considering such information, our systematic review (SR) aims to investigate the potential evidence for the effects PTEN inhibitors exert on tissue regeneration and injury repair. The motivational question to our review is: does pharmacological inhibition of PTEN enhance the healing after an injury?

Methods

Study design & review question

This SR was reported in accordance with the Preferred Reporting Items for Systematic Reviews Checklist [14]. This review aimed to systematically search and report the currently available data on the biological effects of PTEN pharmacological inhibition on tissue repair or regeneration.

Eligibility criteria

Inclusion criteria

Articles that evaluated the efficacy of PTEN pharmacological inhibition on the tissue repair using *in vitro* and *in vivo* studies were selected for our SR.

Exclusion criteria

Studies were excluded for the following reasons: studies with PTEN inhibitors that analyze effects not associated with wound healing, tissue repair or regeneration; studies that do not use PTEN inhibitors; studies that use methods for PTEN inhibition other than pharmacological or that assess RNA-based agents; studies that administer the PTEN inhibitor before injury (injury prevention or protection); studies written in languages other than English. Reviews, letters, personal opinions, book chapters, conference abstracts or nonpeer review literature were also excluded.

Information sources & search strategy

The studies considered for inclusion in the SR were identified using individual search strategies on the following electronic databases: Cochrane, Embase, PubMed, Scopus and Web of Science. The gray literature search was performed using Google Scholar. The search included all articles published up to 8 February 2018, with no initial time restriction. Duplicated entries were removed by reference manager software (EndNote®, Thomson Reuters, NY, USA). The reference lists of articles were screened to identify any additional studies. The search strategies for PubMed were: (#1 bpV[phen] or bpVphen or bpV-phen or 'bpV phen' or bpV[hopic] or bpVhopic or bpVhopic or 'bpV hopic' or bpV[pic] or bpVpic or bpV-pic or 'bpV pic' or bpV[bipv] or bpVbipv or bpV-bipv or 'bpV bipv' or vo-ohpic or voohpic or vo[ohpic] or vo-oh[pic] or 'vo-oh pic' or 'vo oh pic' or sf1670 or sf-1670 or 'sf 1670' or vanadium or bisperoxovanadium or bisperoxovanadate; #2 'pten inhibitor' or 'pten inhibitors' or 'pten antagonist' or 'pten antagonists'; #3 (#1 or #2); #4 wound or cicatrization or healing or regeneration or cicatrize or heal or regenerate or migration or proliferation or migrate or proliferate or induction or induct or recovery or recover or acceleration or accelerate or decrease or repair; #5 (#3 and #4). All search strategies used on the databases can be found in Supplementary Table 1.

Study selection

The study selection was completed in two phases. In Phase I, three authors (AEM Marques, GA Borges & LP Webber) independently reviewed the titles and abstracts, and excluded the studies that were clearly not related to the topic. In Phase II, two authors (AEM Marques & GA Borges) independently read the full text of all selected articles and excluded studies according to the selection criteria (Supplementary Table 2). Disagreements between the evaluators were solved by consensus; otherwise, another reviewer (CH Squarize) was involved in making a final decision.

Data collection process & analysis

One author (GA Borges) collected key information from the articles. A second reviewer (LP Webber) cross-checked the information and accuracy. Any disagreements were resolved by discussion and consensus among the authors (GA Borges, LP Webber & CH Squarize). The following information was collected: year of publication, author(s), country, experimental model (e.g., details of the *in vivo* and *in vitro* experiments), treatment regimen and drug concentrations, experiment design and techniques and reported results. Due to the observational nature and reported outcomes, only the qualitative analysis was performed. Experimental (e.g., variability among organs and

tissue types, characteristics and outcomes) and methodological (e.g., variability in study design and risk of bias) heterogeneity were considered.

Risk of bias & quality assessment

In vivo studies were assessed according to an adaptation of the ARRIVE guidelines proposed by Suarez-Lopez Del Amo *et al.* [15] (Supplementary Table 3). Two authors (GA Borges & LP Webber) independently assessed each *in vivo* study, and any disagreements were resolved by discussions involving a third author (CH Squarize). Low risk of bias was estimated when data were deemed clearly sufficient or key procedures on animal handling and experimenting were described. A high risk of bias was estimated when the information was insufficient or key procedures were not described. An unclear or possible risk of bias was estimated when the information was not fully described. The summary of the risk of bias across the included studies is represented according to the Cochrane guidelines. The lack of available standard quality assessment tools for *in vitro* studies prevented the estimation of the risk of bias for the included *in vitro* studies.

Summary measures

The primary outcome for this SR was the capacity of PTEN inhibitors to enhance healing, increase migration or proliferation and block senescence or apoptosis. The protein and gene expression profile after treatment with PTEN inhibitors was considered as secondary outcome. Any outcome measurements was regarded in this review (i.e., categorical and continuous variables).

Results

Study selection

A total of 3723 abstracts were identified across the five electronic databases. After duplicate articles were removed, 3370 citations remained. Following the inclusion and exclusion criteria, a comprehensive evaluation of the abstracts was performed on Phase I of the study selection. A total of 3288 articles were excluded, resulting in 86 articles to be fully assessed in Phase II. After a comprehensive analysis, 69 studies were excluded (Supplementary Table 2) and 17 articles [16–32] were included in the qualitative synthesis. The process of study selection is depicted in Figure 1.

Study characteristics

The selected studies were performed in USA (n = 9), China (n = 4), France (n = 1), Italy (n = 1), Japan (n = 1) and the UK (n = 1); and published between 2007 and 2018. The descriptive characteristics of each article were summarized in Tables 1–3. The studies were categorized into three groups, considering the type of cells, tissue and organ, which were: eye/lung, muscle and nervous system (Tables 1–3 & Figure 2A & B).

In the studies categorized in group 1 (eye/lung; n = 5), *in vitro* assays were mostly conducted (Figure 2A). They evaluated the repair process via cell monolayer scratch assay, time-lapse tracking and migration chambers. Protein expression changes were assessed (through western blot or immunofluorescence) in all group 1 studies (Table 1). Either bpV(pic) or bpV(phen) was used in the studies, except for one study that used both compounds (Figure 2B).

The studies gathered in group 2 (muscle; n = 3) presented *in vitro* and *in vivo* results (Figure 2A). These studies investigated myotube formation, muscle regeneration or repair (through *in vitro* scratch models or histological assessment of *in vivo* models), and protein and gene expression after PTEN inhibition (Table 2). One study used bpV(HOpic) and two other studies used a bpV compound that was not specified by the authors (bpV*, Figure 2B).

Most of the included studies were categorized in group 3 (nervous system, n = 9), all reporting *in vivo* results (Figure 2A). These studies assessed neurological scores and functional recovery after treatment, lesion volume (morphometry and histometric analysis), and changes in the protein or gene expression (Table 3). BpV(pic), bpV(phen) and SF1670 (i.e., N-[9,10-dihydro-9,10-dioxo-2-phenanthrenyl]-2,2-dimethyl-propanamide) were the PTEN inhibitors used. One study did not specify which bpV compound was administered (bpV*, Figure 2B).

During the selection of the studies, we also identified *in vitro* and *in vivo* studies that reported the effects of PTEN suppression on tissue regeneration through RNAi approaches. These studies used mainly miRNA, siRNA or other RNAi methods to suppress the expression of PTEN on tissues or cells of the nervous system, muscle, skin, trophoblast cells, bone marrow, and cornea (Supplementary Table 4). Although promising, the current status of PTEN inhibition using RNA-based approaches to treat injuries has not been modified, tested or available in a pharmaceutical grade.

Figure 1. Flow diagram of the literature search and selection criteria adapted from Preferred Reporting Items for Systematic Reviews. From 3370 articles (after duplicates were removed), 86 were screened in Phase I of study selection (after assessment of titles and abstracts) and 17 were included in Phase II (after reading of complete article). PTEN: Phosphatase and tensin homolog.

Table 1. Summary of descriptive characteristics of included studies – Group 1 – lung and eye (n = 5).

Study (year), country	Experimental model	Treatment (drug; conc.)	Methodology/assessments	Result summary	Ref.
Lai <i>et al.</i> (2007), USA	IVT: hUAECs, BEAS2B, DU147, LNCaP and PC3-PTEN-null	bpV(phen); 0.1–50 μ M bpV(pic); 0.1–10 μ M	Viability and cytotoxicity assays; glutathione assay – to assess the specificity of investigated compounds to PTEN; scratch assay; migration assay; mechanical wound model; western blot	<ul style="list-style-type: none"> PTEN present in hUAECs, BEAS2B and DU147 cells and lack endogenous PTEN in LNCaP and PC3 cells bpV(phen) resulted in a dose-dependent $\uparrow$p-AKT (in hUAECs, BEAS2B and DU147 cells) and $\uparrow$p-GSK3 (in BEAS2B cells) No toxicity to BEAS2B cells exposed to up to 5 μM for bpV(phen) or bpV(pic) in 24 h or to bpV(pic) in 48 h No toxicity to hUAECs within the 1–2 μM range for both compounds in up to 96 h bpV(phen) (1 μM) and bpV(pic) (0.5 μM) $\uparrow$ wound closure of BEAS2B and hUAECs cells bpV(phen) (1 μM) $\uparrow$ migration of BEAS2B cells bpV(phen) (1–2 μM) re-established differentiated hUAECs cells monolayer TEER in $\downarrow$ time, with $\uparrow$ recovery rate 	[20]
Kakazu <i>et al.</i> (2008), USA	IVT: HCE and wounded corneas (rabbit) in tissue culture	bpV(phen); 1–10 μ M	Wound closure evaluation (alizarin red dye and stereomicroscopy); western blot; immunofluorescence	<ul style="list-style-type: none"> bpV(phen) (6 μM) $\uparrow$ cornea repair ($\downarrow$ wound area), and $\uparrow$p-cMET, $\uparrow$PI3K (p-p85), $\uparrow$p-AKT-1 and $\downarrow$PTP1B activity in HCE cells bpV(phen) (10 μM) $\uparrow$p-p70S6K (p-S6) and $\uparrow$p-ERK1/2 	[19]
Cao <i>et al.</i> (2011), USA	IVT: HCE and -wounded cornea (enucleated eye; adult rats) in tissue culture	bpV(pic); 0.1–10 μ M	Single-cell migration assay; scratch and hole wound healing assay; cornea wound re-epithelialization monitoring; immunofluorescence; western blot	<ul style="list-style-type: none"> bpV(pic) (0.1–10 μM) $\uparrow$ single-cell migration and repair. bpV(pic) (1–10 μM) $\downarrow$ in time of wound healing (from 30 to 20 h) in cornea. bpV(pic) (1 μM) $\uparrow$p-AKT 	[16]
Mihai <i>et al.</i> (2012), USA	IVT: hUAECs and BEAS2B	bpV(phen); 0.1–2 μ M	Mechanical wound model – evaluation of TEER; proliferation (EdU assay) western blot; migration assay; atomic force microscopy; fluorescence microscopy (cytoskeleton analysis)	<ul style="list-style-type: none"> bpV(phen) (1–2 μM) $\uparrow$ recovery of differentiated hUAECs cells monolayer integrity ($\uparrow$ TEER in $\downarrow$ time and $\uparrow$ recovery rate) bpV(phen) (1–2 μM) $\downarrow$ proliferation in the wound region and in the monolayer model in BEAS2B cells bpV(phen) (1–2 μM) $\uparrow$p27 in BEAS2B cells bpV(phen) (1 μM) $\uparrow$ migration in BEAS2B cells (as early as 2 h after treatment) bpV(phen) (1 μM) $\downarrow$ cellular stiffness in BEAS2B cells No different actin distribution or quantification in BEAS2B cells bpV(phen) (0.1–2 μM) did not alter p-FAK, but $\uparrow$p-ERK1/2 	[24]
Mao and Sun (2015), China	IVV: Retinal detachment (adult Sprague–Dawley rats)	bpV(pic); 40 ng/kg, 400 ng/kg, or 4 μ g/kg (SR)	ONL evaluation; TUNEL assay; immunohistochemistry; western blot; ELISA	<ul style="list-style-type: none"> Untreated RD group: photoreceptors disorderly and loosely arranged, inner segments partly missing and outer segments mostly detached bpV-treated group: photoreceptors were closely arranged and outer segments partly (40 ng/kg) or mostly (400 ng/kg and 4 μg/kg) preserved bpV(pic) (4 μg/kg) prevented reduction of ONL thickness ratio and $\uparrow$ number of cells in ONL on days 3 and 7 bpV(pic) (4 μg/kg) $\downarrow$ TUNEL-positive cells in days 1–3 bpV(pic) (4 μg/kg) $\uparrow$PIP3 levels, $\uparrow$p-AKT, $\uparrow$p-PDK, $\uparrow$Bcl-2, $\downarrow$cytosolic cytochrome-c and $\downarrow$cleaved-caspase-3, $\uparrow$p-AKT in the ONL and $\uparrow$Bcl-2 immunoreactivity in the inner nuclear layer, ganglion cell layer and ONL 	[21]

$\downarrow$: Decrease(d)/downregulation; $\uparrow$: Increase(d)/upregulation; BEAS2B: Transformed human lung epithelial cell line; C2C12: Mouse myoblast cell line; DRGN: Dorsal root ganglion neuron; DU147: PTEN functional prostate cancer cell line; HCE: Human corneal epithelial cell; hUAEC: Primary human upper airway epithelial cell; IBZ: Ischemic boundary zone; IP: Intraperitoneal injection; IVT: *In vitro*; IVV: *In vivo*; LNCaP: PTEN-null prostate cancer cell line; MCAO: Middle cerebral artery occlusion; min: Minute; MSC: Muscle stem cell; OLP: Oligodendrocyte progenitor; ONL: Outer nuclear layer; p: phosphorylated; PCN: Primary cortical neuron; qPCR: Real time polymerase chain reaction; RD: Retinal detachment; SR: Subretinal injection; TEER: Trans-epithelial electrical resistance.

Table 2. Summary of descriptive characteristics of included studies Group 2 – muscle (n=3).

Author (year) country	Experimental model	Treatment (drug; conc.)	Methodology/assessments	Result summary	Ref.
Castaldi et al. (2007), Italy	IVT: C2C12; mouse primary satellite cells and neonatal cardiac myocytes IVV: GFP-C2C12 (injected into femoral artery of α -SG null mice – muscular dystrophy model)	IVT: bpV [†] ; 10 μ M IVV: bpV [†] ; 10 μ M (cells were treated prior to be injected into the mice model)	IVT: immunoprecipitation and western blot; RT-PCR; cell cytometry IVV: RT-PCR; immunofluorescence and histochemistry	<ul style="list-style-type: none"> • IVT: bpV[†] (10 μM) $\downarrow$ myotube formation (48–72 h); $\downarrow$ MyoD, $\downarrow$ Myf5 and $\downarrow$ Myog gene expression (indicating suppressed myogenic differentiation); $\downarrow$ G₁ and $\uparrow$ in S cell-division cycle phases (12–48 h); $\uparrow$ cyclin D1 (<i>Ccnd1</i>), $\uparrow$ Pna, $\downarrow$ Pax7 and $\uparrow$ Mdr1 gene expression; $\uparrow$ Sca-1, $\uparrow$ c-kit, $\uparrow$ CD45, $\uparrow$ CD34, $\uparrow$ CD11b, $\uparrow$ Mac-3, $\uparrow$ Gr-1, $\uparrow$ Ter119 $\uparrow$ p-c-Jun (AP1), $\uparrow$ p-IkBα; $\uparrow$ p65 (NF-κB) nuclear translocation; activated AP1 and NF-κB transcriptional activity. • IVV: Very few untreated GFP-positive cells observed, restricted within damaged areas of the skeletal muscle tissue; Untreated C2C12 cells did not give rise to α-SG positive fibers • bpV[†] (10 μM)-treated GFP-positive cells spread throughout the muscle, mostly in the interstitial muscle (day 5); many fibers expressed α-SG (by day 21) 	[30]
Dimchev et al. (2013), UK	IVT: C2C12	bpV(HOPic); 1 or 10 μ M	Creatine kinase assay; microscopical analysis; scratch assay; migration assay (tracking of leading cells from the wound edges); western blot; qPCR	<ul style="list-style-type: none"> • bpV(HOPic) (10 μM) $\downarrow$ myotube formation (72 h), $\downarrow$ creatine kinase enzymatic activity (72 h), $\downarrow$ MyoD and $\downarrow$ Myog gene expression (24 h), indicating suppressed myogenic differentiation • bpV(HOPic) (1 μM) $\uparrow$ the number of cells infiltrating the wound (38%); $\uparrow$ the velocity and migration distance from the edges (41%); $\uparrow$ p-AKT and $\uparrow$ p-ERK1/2 	[18]
Smeriglio et al. (2016), France	IVT: C2C12; MSCs; myofibers (mice extensor digitorum longus muscle) IVV: GFP-H1-C2 or MSCs (transplanted into nu/nu male mice with focal cryoinjury in tibialis anterior muscle)	IVT: bpV [†] ; 10–30 μ M IVV: bpV [†] ; 10–30 μ M (cells were treated prior to be injected into the mice model)	IVT: Cell morphology assessment; western blot; RT-qPCR; chromatin immunoprecipitation assay; immunofluorescence IVV: histological assessment and immunofluorescence; qPCR	<ul style="list-style-type: none"> • IVT: $\uparrow$ elongation and fusion (myotubes formation) in untreated cells • bpV[†] (30 μM) kept cells mononucleated and $\downarrow$ fusion; • bpV[†] (30 μM) $\downarrow$ Pax7, $\downarrow$ Pax7, $\downarrow$ Myog, $\uparrow$ Sca-1 and $\uparrow$ Pax1 (promotes early muscle stem cell gene expression profile) • bpV[†] (30 μM) $\downarrow$ H4-Ac, $\downarrow$ H3K4-3Me $\downarrow$ H3K27Me3 in C2C12 and MSC; Chromatin regions upstream Pax7 and Sca-1 modified toward an active conformation • Myofiber-associated Pax7⁺ cells expressed Pax7 and coexpressed MyoD, however bpV[†] (10–20 μM) $\downarrow$ the average number of MyoD⁺ nuclei/fiber; the number of Pax7⁺ and Pax1⁺ nuclei/fiber remained unchanged • bpV[†] (10–20 μM) $\downarrow$ MSC proliferation rate • IVV: Muscles transplanted with bpV[†]-treated cells underwent a well-organized regeneration with centrally nucleated myofiber in contrast to control (moderate muscle regeneration with disorganized myofibers) • bpV[†] (30 μM) $\uparrow$ engraftment of transplanted cells that robustly fused with regenerating myofiber • bpV[†] (30 μM) $\uparrow$ Pax7, $\downarrow$ MyoD expression (by day 30) • bpV[†] (30 μM) $\uparrow$ number of βGAL⁺ SCs in regenerating fibers 	[26]

[†]bpV compound was not specified by the authors.

$\downarrow$: Decrease(d)/downregulation; $\uparrow$: Increase(d)/upregulation; BEAS2B: Transformed human lung epithelial cell line; C2C12: Mouse myoblast cell line; DRGN: Dorsal root ganglion neuron; DU147: PTEN functional prostate cancer cell line; h: hour; HCE: Human corneal epithelial cell; hJAE: Primary human upper airway epithelial cell; IBZ: Ischemic boundary zone; LFB: Luxol fast blue; LNCaP: PTEN-null prostate cancer cell line; IP: Intraperitoneal injection; IVT: *In vitro*; IVV: *In vivo*; MCAO: Middle cerebral artery occlusion; min: minute; MSC: Muscle stem cell; OLP: Oligodendrocyte progenitor; ONL: Outer nuclear layer; p: phosphorylated; PC3: PTEN-null prostate cancer cell line; PCN: Primary cortical neuron; qPCR: Real time polymerase chain reaction; RD: Retinal detachment; RT-PCR: Reverse transcription polymerase chain reaction; SAH: Subarachnoid hemorrhage; SCI: Spinal cord injury; SR: Subretinal injection; TEER: Trans-epithelial electrical resistance.

Table 3. Summary of descriptive characteristics of included studies – Group 3 – nervous system (n = 9).

Study (year) country	Experimental model	Treatment (drug; conc.)	Methodology/assessments	Result summary	Ref.
Yang <i>et al.</i> (2007), USA	IVT: injury with 6-OHDA (adult female Sprague–Dawley rats)	bpV(phen); 3 or 10 μM (0.5 $\mu\text{l/h}$) continuous flow	Histological assessment (labeling with fluorogold neuronal tracer); immunofluorescence; western blot	<ul style="list-style-type: none"> bpV(phen) (3–10 μM) $\uparrow$ presence of neurons, $\uparrow$ the number and length of dendritic processes and $\uparrow$ tyrosine phosphorylation in the <i>substantia nigra</i> bpV(phen) (3–10 μM) $\uparrow$ dopaminergic innervation in the <i>neostriatum</i> 	[32]
Nakashima <i>et al.</i> , (2008), USA	IVT: SCI at T9 vertebra level (adult female Sprague–Dawley rats)	bpV(phen); 30–300 μM (0.5 $\mu\text{l/h}$) continuous flow	Functional tests; histological assessment (CTB labeling); immunofluorescence	<ul style="list-style-type: none"> bpV(phen) (30 and 100 μM) $\downarrow$ loss of sensory axons and white matter; the area of spared dorsal column white matter at the injury epicenter was greatest at 100 μM bpV(phen) (100 μM) improved hindlimb grid-walking performance when compared with control bpV(phen) (30 and 100 μM) $\uparrow$ CTB-labeled innervation of gracile nucleus and myelinated sensory axons; $\downarrow$ Iba-1 and $\uparrow$ RECA-1 	[31]
Walker <i>et al.</i> , 2012, (USA)	IVT: unilateral cervical SCI (adult female Sprague–Dawley rats)	bpV(pic); 400 $\mu\text{g/kg}$ – 2 $\times$ /day (IP)	Histological assessment (cresyl violet acetate staining); immunofluorescence; behavioral and functional tests; western blot	<ul style="list-style-type: none"> bpV(pic) (400 $\mu\text{g/kg}$) $\downarrow$ lesion, $\downarrow$ cavity volume, $\uparrow$ number of motoneurons in the surrounding injury epicenter and $\uparrow$ RECA-1^{+} gray matter area (indicative of vascularization) bpV(pic) (400 $\mu\text{g/kg}$) recovered the skilled forelimb function and coordination bpV(pic) (400 $\mu\text{g/kg}$) did not significantly alter PTEN, but $\uparrow$p-AKT and $\downarrow$LC3II/LC3I ratio ($\downarrow$ autophagic activity upregulation) 	[27]
Mao <i>et al.</i> , (2013), China	IVT: PCN (from mouse embryos) IVT: Transient MCAO stroke model (adult male CD-1 mice)	IVT: bpV(phen); 0.1 μM IVV: bpV(phen); 200 $\mu\text{g/kg/day}$ (IP)	IVT: measurement of neurite outgrowth (PCN submitted to emulated ischemic injury) IVV: neurological score and functional tests; Infarct volume assessment (TTC staining); Bielschowsky silver staining (a marker for axons, to assess axonal regrowth and density); immunohistochemistry; western blot	<ul style="list-style-type: none"> IVT: bpV(phen) (0.1 μM) $\uparrow$ neurite outgrowth after oxygen-glucose deprivation. IVV: bpV(phen) (200 $\mu\text{g/kg}$) improved neurological scores (days 11–14) and asymmetrical motor deficits (day 13) bpV(phen) (200 $\mu\text{g/kg}$) did not alter infarct volume (day 4) While Bielschowsky silver staining and MBP immunoreactivity were decreased in the striatal IBZ (day 14) after treatment with saline, bpV(phen) (200 $\mu\text{g/kg}$) restored Bielschowsky silver staining and MBP levels comparable to sham control bpV(phen) (200 $\mu\text{g/kg}$) resulted in significant $\uparrow$p-AKT $\uparrow$p-S6 in striatal IBZ, as well as $\downarrow$PTEN expression 	[22]

[†]bpV compound was not specified by the authors.

$\downarrow$: Decreased/d/downregulation; $\uparrow$: Increased/d/upregulation; BEAS2B: Transformed human lung epithelial cell line; C2C12: Mouse myoblast cell line; CTB: Cholera toxin subunit B; DRGN: Dorsal root ganglion neuron; DU147: PTEN functional prostate cancer cell line; FAK: Focal adhesion kinase; h: hour; HCE: Human corneal epithelial cell; hUAEC: Primary human upper airway epithelial cell; IBZ: Ischemic boundary zone; Ikb α : Inhibitor of κB ; LFB: Luxol fast blue; LNCaP: PTEN-null prostate cancer cell line; IP: Intraperitoneal injection; IVT: *In vitro*; IVV: *In vivo*; MBP: Myelin basic protein; MCAO: Middle cerebral artery occlusion; min: minute; MSC: Muscle stem cell; OLP: Oligodendrocyte progenitor; ONL: Outer nuclear layer; p: phosphorylated; PCN: Primary cortical neuron; RD: Retinal detachment; RT-PCR: Reverse transcription polymerase chain reaction; SAH: Subarachnoid hemorrhage; SCI: Spinal cord injury; SR: Subretinal injection; TEER: Trans-epithelial electrical resistance.

Table 3. Summary of descriptive characteristics of included studies – Group 3 – nervous system (n = 9) (cont.).

Study (year) country	Experimental model	Treatment (drug; conc.)	Methodology/assessments	Result summary	Ref.
Walker and Xu (2014), USA	IVV: unilateral cervical SCI (adult female Sprague-Dawley rats)	bpV(pic); 400 µg/kg – 2×/day (IP)	Histological assessment (cresyl violet acetate and LFB stainings for calculation of spared myelinated tissue); immunofluorescence; assessment of penumbral motor neuron atrophy	<ul style="list-style-type: none"> • bpV(pic) (400 µg/kg) ↑ white matter and ↑ LFB⁺ myelin around injury epicenter • bpV(pic) (400 µg/kg) ↑ the number of CC1⁺ oligodendrocytes • bpV(pic) (400 µg/kg) ↑ the soma area of ventral horn motor neurons (and exhibited similar size and morphological appearance to the normal tissue (sham-operated group)) 	[28]
Chen <i>et al.</i> (2015), China	IVV: intravascular perforation – SAH model (male Sprague-Dawley rats)	bpV(pic); 200 µg/kg – every 6 h (IP)	Neurological score; SAH score; brain water content; Evans blue dye extravasation; TUNEL assay; Nissl staining; western blot	<ul style="list-style-type: none"> • No difference was reported regarding the SAH grading scores of vehicle-treated and bpV-treated groups • bpV(pic) (200 µg/kg) improved neurological scores; ↓ neuronal degeneration, ↓ TUNEL-positive neurons in the hippocampal CA1 region • bpV(pic) (200 µg/kg) significantly ↓ water content in brain (except for brain stem) and ↓ Evans blue extravasation in the right and left/ipsilateral hemispheres • bpV(pic) (200 µg/kg) significantly ↓PTEN, ↑p-PTEN, ↓GluR1, ↑GluR2 and ↑GluR3 	[17]
Mao <i>et al.</i> (2015), China	IVV: transient MCAO (adult male Sprague-Dawley rats)	bpV [†] ; 200 µg/kg/day (IP)	Neurological score; infarct volume assessment; qPCR; ELISA; western blot.	<ul style="list-style-type: none"> • bpV[†] (200 µg/kg) improved neurological score and ↓ infarct volume • bpV[†] (200 µg/kg) ↑IL-10 and ↓ TNF-α concentrations in the IBZ; ↓PTEN (mRNA and protein levels), ↑PI3K, ↑AKT and ↑p-GSK3β in the striatal IBZ 	[23]
Walker <i>et al.</i> (2015), USA	IVV: unilateral cervical SCI (adult female Sprague-Dawley rats)	bpV(pic); 400 µg/kg – 2×/day (IP)	Behavioral and functional tests; histological assessment (cresyl violet acetate staining); immunofluorescence	<ul style="list-style-type: none"> • bpV(pic) (400 µg/kg) improved recovery of sensorimotor function, ↓ lesion area, ↓cavity area, ↑ spared tissue area, promoted ventral horn neuron sparing and ↑ SMI-31⁺ and RECA-1⁺ area as a fraction of Schwann cells-graft area (indicative of axonal growth into the graft as an index of lesion size) 	[29]
Pham and Tu (2018), Japan	IVV: Sciatic nerve axotomy (in a Type-2 diabetic C57BL/6J mice)	SF1670; 200 nM (0.11 µl/h) continuous flow	Functional and morphological nerve regeneration tests (paw withdrawal thresholds measured with calibrated von Frey filaments and stereomicroscopy); sciatic nerve transection-regeneration model (fluoro-ruby staining)	<ul style="list-style-type: none"> • SF1670 (200 nM) ↑ functional recovery in both diabetic and non-diabetic mice (responded to von Frey filaments at 3 weeks, compared with no response in vehicle-treated groups) • SF1670 ↑ the number of fluoro-ruby-labeled neurons after axotomy (indicating that it may promote the axonal transport in the axon and nerve regeneration after nerve transection) 	[25]

[†]bpV compound was not specified by the authors.

↓: Decrease(d)/downregulation; ↑: Increase(d)/upregulation; BEAS2B: Transformed human lung epithelial cell line; C2C12: Mouse myoblast cell line; CTB: Cholera toxin subunit B; DRGN: Dorsal root ganglion neuron; DU147: PTEN functional prostate cancer cell line; FAK: Focal adhesion kinase; h: hour; HCE: Human corneal epithelial cell; HUAE: Primary human upper airway epithelial cell; IBZ: Ischemic boundary zone; IκBα: Inhibitor of κB α; LFB: Luxol fast blue; LNCaP: PTEN-null prostate cancer cell line; IP: Intraperitoneal injection; IVT: *In vitro*; MBP: Myelin basic protein; MCAO: Middle cerebral artery occlusion; min: minute; MSC: Muscle stem cell; OLP: Oligodendrocyte progenitor; ONL: Outer nuclear layer; p: phosphorylated; PCN: Primary cortical neuron; RD: Retinal detachment; RT-PCR: Reverse transcription polymerase chain reaction; SAH: Subarachnoid hemorrhage; SCI: Spinal cord injury; SR: Subretinal injection; TEER: Trans-epithelial electrical resistance.

Figure 2. Distribution of the use of phosphatase and tensin homolog inhibitors in *in vitro* and *in vivo* studies and targeted tissues, organs and system. (A) Number of studies in each group distributed according to study design. Note that four out of five articles in group 1 (eye and lung) were *in vitro* studies; in contrast to group 3 (nervous system), in which 100% of the studies were *in vivo* ($n = 9$ out of 9). Likewise, studies on group 2 (muscle, $n = 3$) presented *in vitro* results alone ($n = 1$) or alongside with *in vivo* results ($n = 2$). (B) Number of studies in each group distributed according to PTEN inhibitors. BpV(pic) and bpV(phen) were the most used bpV compounds (see groups 1 and 3). BpV(HOpic) and SF1670 were only used in one study each. Note that a bpV compound (bpV*), generally described as bpV by the authors, was used in three studies (see groups 2 and 3). (C) Studies distributed according to study design and PTEN inhibitors. Most *in vivo* studies were conducted with bpV(pic) ($n = 5$), while bpV(phen) was mostly used *in vitro* ($n = 4$). bpV: Bisperoxovanadium; bpV(phen): Bisperoxovanadium 1,10-phenantroline; bpV(pic): Bisperoxovanadium 5-hydroxypyridine; bvp(HOpic): Bisperoxovanadium 5-hydroxypyridine-2-carboxylic acid; PTEN: Phosphatase and tensin homolog.

Figure 3. The concentrations of Bisperoxovanadium compounds used *in vitro* were lower than 10 μM and resulted in PTEN inhibition and AKT activation. (A) The graph shows the concentrations of the PTEN inhibitor reported in the publications with *in vitro* experiments, distributed according to tissue groups (lung/eye, muscle and nervous system). Concentrations administered in the studies remained within the 0.1–10 μM range, as emphasized by the dotted rectangle; (B) PTEN inhibitors induced an increase in p-PTEN and p-AKT. Note that concentrations equal to or lower than 2 μM resulted in increased AKT phosphorylation or PTEN inactivation (phosphorylation). P-AKT: phosphorylated-AKT; PTEN: Phosphatase and tensin homolog; p-PTEN: Phosphorylated-PTEN.

The *in vivo* studies assessed repair of injuries on the cervical spinal cord [27–29,31], brain (subarachnoid hemorrhage induced by intravascular perforation, transient middle cerebral artery occlusion model for ischemia and dopaminergic neurons in the *substantia nigra*) [17,22,23,32], sciatic nerve [25], retina (retinal detachment model) [21] and muscle (cryoinjury and α -sarcoglycan null muscle dystrophy model) [26,30] (Tables 1–3). Overall, the *in vitro* studies were conducted on C2C12 mouse muscle cells [18,26,30], BEAS2B human lung epithelial cells [20,24], primary mouse myocytes/myoblasts [26,30], primary human upper airway epithelial cells [20,24], human corneal epithelial cells [16,19] and enucleated corneas from rabbits [19] or rats [16] (Tables 1–3). BpV(pic) was the most administered compound *in vivo*, while bpV(phen) was the most used *in vitro* (Figure 2C).

Results of individual studies & groups

Group 1

Lai *et al.* [20], Kakazu *et al.* [19], Cao *et al.* [16] and Mihai *et al.* [24], reported that the *in vitro* repair process was accelerated after administration of bpV(pic) at doses varying from 0.1 to 2 μM , or bpV(phen) at 1–10 μM (Figure 3A). Increased cell migration was reported with bpV(phen) or bpV(pic) at 1 μM [4,16,24]; and neither bpV(phen) nor bpV(pic) were cytotoxic to lung epithelial cells in concentrations ranging from 0.5 to 5 μM [20]. Additionally, bpV(phen) at 1 and 2 μM significantly reduced cell proliferation, which suggested that the enhanced wound closure observed was not a consequence of increased proliferation [24]. Mao and Sun [21] studied the effects of

bpV(pic) in a retinal detachment animal model and reported positive outcomes, considering the better arrangement of photoreceptors and increased thickness and number of cells in the outer nuclear layer.

Molecular changes were reported among the studies from group 1. The most common molecular change was the increase in p-AKT (Figure 3b) [16,19–21], as well as increased p-P70S6K [19], PI3K (phosphorylation of subunit p85) [19], PIP3 levels [21], p-ERK1/2 [19,24], p-cMet [19], p27 [24], p-PDK [21], p-GSK3 [20], p-Bad [21] and Bcl2 [21]. A decrease in PTP1B activity was noticed [19], as well as decreased cytosolic cytochrome-c and cleaved caspase-3 [21].

Group 2

The studies on group 2 addressed muscular regeneration and indicated that treatment of muscle cells with bpV compounds decreased myotubes formation [18,26,30], enhanced *in vivo* muscle regeneration [26,30] and increased *in vitro* cell migration [18]. While Dimchev *et al.* [18] studied bpV(HOpic) in concentrations of 1 and 10 μ M, Castaldi *et al.* [30] and Smeriglio [26] used doses ranging from 10 to 30 μ M, although they did not specify the bpV compound used.

Aligned with the results from group 1, an increase in p-AKT was observed (Figure 3) [18]. Additionally, other molecules that are important to muscle biology were assessed. PTEN inhibition resulted in decreased expression of *Myod* and *Myog* [18,26,30], which led the authors to suggest a potential reduction on myogenic cell differentiation. An increased expression of the progenitor markers Sca-1 and Pw1 and the *Pw1* gene was observed [26]. The expression of *Pax7* [26,30] and *Myf5* [30] was decreased, and an increased expression of *Ccnd1*, *Pcna* and *Mdr1* genes [30], as well as Sca-1, c-kit, CD34, CD11b, Mac-3, Gr-1, Ter119, p-ERK1/2, p-cJun, p-IkBa and p65 proteins were reported [30].

Group 3

A total of nine studies composed group 3 [17,22,23,25,27–29,31,32], which concerned the effects of pharmacological inhibition of PTEN on organs, tissues and cells of the nervous system. Seven studies indicated that the treatment improved neurological score, induced behavior and functional recovery [17,22,23,25,27,29,31], and reduced lesion volume [22,23,27–29,31,32]. An increased number of neurons surrounding the injury epicenter was also reported [27,28,31,32]. Mao *et al.* [22] noticed a significant increase in neurite outgrowth on primary cortical neurons treated with bpV(phen); and Walker *et al.* [29] reported that bpV(pic) induced significant axonal growth. Pham and Tu [25] noted that SF1670 promoted axonal transport in a nerve transection-regeneration model. Mao *et al.* [22] reported a reduction in the number of TUNEL-positive neurons and in neuronal degeneration after treatment with bpV(pic), as well as a reduction in water content on the brain and in disruptions of the blood–brain barrier. Except for the three studies that administered SF1670 in a continuous flow [25] or infused bpV(phen) [31,32], all the other *in vivo* studies used either bpV(pic) or bpV(phen) via intraperitoneal (IP) injections at doses of 200 or 400 μ g/kg for 1–14 days (Figure 4A).

The *in vivo* studies showed either a decrease in PTEN expression [17,22,23] or PTEN inactivation (phosphorylation) (Figure 4B) [17]. Additionally, markers of PTEN inactivation were observed, such as increased PI3K [23], p-AKT [22,27] and p-S6 [22]. The studies addressed whether important pathways related to nervous system repair were affected, reporting an increase in p-GSK3 β [23], IL-10 [23], MBP [22], RECA-1 [27,29,31], SMI-31 [29], CC1 [28] and GluR2/GluR3 [17]. Additionally, decreases in the TNF α [23], GluR1 [17] and Iba-1 [31] expression, as well as in the LC3II/LC3I ratio [27] were reported.

Synthesis of results

All studies included in this SR indicated that the inhibition of PTEN enhanced the repair process, either by reducing the recovery time and lesion size or volume, increasing cell proliferation and migration or inducing neuronal regeneration in injury models. Injuries in the spinal cord [27–29,31], eye [16,19,21], muscle [18,26,30] and lungs [20,24] were frequently studied. Majority of the studies focused on the tissue regeneration induced by PTEN inhibitors on the nervous system. These studies were the most prominent in altering the PI3K–AKT–mTOR signaling pathway *in vivo*. Walker *et al.* (2012) [27] and Mao *et al.* (2013) [22] observed an increase in p-AKT levels after PTEN inhibition by bpV(pic) and bpV(phen), respectively. Mao *et al.* [23] showed that a bpV compound decreased PTEN protein and mRNA levels, which led to increased PI3K and AKT.

Notably, the *in vitro* studies included in this SR reported a positive cell response to PTEN inhibitors at the concentration range of 0.1–10 μ M (Figure 3A) [16,18–20,22,24,30]. The exception was one study that used

Figure 4. Results of *in vivo* studies. (A) Administration of PTEN inhibitors and doses that were applied in *in vivo* experiments, distributed according to tissue groups. bpV(pic) and bpV(phen) were administered as intraperitoneal injections at concentrations of 200 or 400 μg/kg in group 3 (nervous system). bpV(pic) was administered as subretinal injections in doses varying from 0.04 to 4 μg/kg (group 2 – eye and lung). SF1670 and bpV(phen) were administered as continuous infusion at concentrations varying from 0.2 to 300 μM (0.11–0.5 μl/h) in group 3 studies. (B) Studies that observed a difference in the protein expression pattern after treatment with PTEN inhibitors. bpV(pic) and bpV(phen) were able to decrease PTEN expression or inactivate it (phosphorylation) and increase p-AKT. bpV: Bisperoxovanadium; bpV(phen): Bisperoxovanadium 1,10-phenantroline; bpV(pic): Bisperoxovanadium 5-hydroxypyridine; PTEN: Phosphatase and tensin homolog.

a nonidentified bpV compound at 20–30 μM [26]. Interestingly, the bpV compounds were able to increase phosphorylation of PTEN and AKT even in concentrations equal to or lower than 2 μM (Figure 3B).

The *in vivo* administration of bpV(pic) and bpV(phen) was mostly IP at doses varying from 200 to 400 μg/kg. In the cases of subretinal injections, bpV(pic) was administered at doses varying from 40 ng/kg to 4 μg/kg. There was a large variability in the doses and drugs used for infusion. SF1670 was infused continuously at 200 nM (0.11 μl/h flow) [25]; and infusions of bpV(phen) ranged from 3 to 300 μM (0.5 μl/h flow; Figure 4A) [31]. As seen in Figure 4B, bpV(pic) and bpV(phen) were found to decrease PTEN expression and induce phosphorylation of PTEN and AKT in the *in vivo* studies.

Quality assessment & risk of bias

The quality assessment of the *in vivo* studies identified a moderate-to-low risk of bias. Most studies satisfactorily characterized ethical statements, statistical methods and experimental outcomes. A moderate risk of bias was identified in items concerning experimental design (e.g., randomization, blinding and definition of experimental

and control groups), housing and husbandry (conditions and welfare-related assessment and interventions) and allocation of animals to experimental groups (e.g., randomization). Additional criteria of the risk of bias analyses, such as information on the sample size and characteristics of the experimental animal (e.g., species, strain, sex, developmental stage, weight and source), were sufficiently described in 50–60% of the studies. The overall risk of bias assessment is summarized in the Supplementary Figure 1.

Discussion

This SR assessed the effects of pharmacological PTEN inhibition, specifically on the tissue repair process and the biological events it comprises. The 17 included studies reported that PTEN inhibitors induce repair and promote tissue regeneration by increasing cell proliferation, cell migration and/or favorable gene/protein expression profile.

PTEN inhibition as a therapeutic strategy has been broadly studied in the past 15 years, since some of these inhibitors were first reported. In this SR, four PTEN inhibitors were investigated in the included studies, and three of them were vanadium compounds. Vanadium compounds have been applied in medicine and clinical research as therapy for several conditions, such as diabetes, tropical infectious diseases, gastrointestinal infections and even AIDS [33].

Therapeutic oligonucleotides have been recognized as highly precise, being able to target disease-relevant proteins or genes that are unreachable by small molecules and proteins [34]. Since 2016, five oligonucleotides (i.e., defibrotide, eteplirsen, nusinersen, inotersen and patisiran) have been approved as treatments for a variety of disorders. Such favorable circumstance prompts the continuing development of therapeutic oligonucleotides into a next-level major class of drugs [35]. The first siRNA-based drug, Patisiran, was approved as a treatment in humans in 2018 [35]. Christie *et al.* (2010) [36] demonstrated that, both *in vivo* and *in vitro*, PTEN siRNA and the PTEN inhibitor bpV(pic) could significantly improve the axon regeneration after peripheral nerve injury. Also, Lai *et al.* (2007) [20] reported that the rate of wound closure was significantly accelerated in lung epithelial cells after the inhibition of PTEN through both siRNA and the inhibitors bpV(pic) and bpV(phen). Studies that reported the effects of RNA-based PTEN suppression on tissue regeneration were summarized in Supplementary Table 4. Based on the fact that there is no commercially available RNA-based PTEN suppressor, this SR included only studies that assessed pharmacological PTEN inhibiting agents.

In high concentrations, bpV compounds may induce PTP inhibition and considered that PTEN shares with the PTPs the same active site motif, such compounds were also hypothesized to inhibit PTEN. Schmid *et al.* [12] observed that bpV compounds with polar *N,O* ligands, such as bpV(pic) and bpV(HOpic), favor binding PTEN, while bpV compounds with the neutral *N,N* ligands, such as bpV(phen), are more indiscriminate, targeting both PTEN and the PTPs (PTP-1B and PTP- β). All in all, those authors indicated that bpV compounds are specific to inhibiting PTEN in concentrations 10- to 100-fold lower than the ones that inhibit PTPs. With bpV(pic) and bpV(HOpic), particularly, PTEN was inhibited in concentrations lower than 10 and 5 μM , respectively [12]. Notably, as identified by this SR (Tables 1–3), bpV(pic) and bpV(HOpic) also showed a positive influence on the injury repair process in concentrations within the 0.1–1 μM range.

The prospect of administrating vanadium compounds as therapeutic agents is still, in part, delayed by the possibility of systemic safety risks and toxicity related to the accumulation in high phosphate tissues [37]. However, the topical and local applications may be considered in light of the potential positive effects on the regeneration process (topical use: WEBBER ET AL., SUBMITTED). Furthermore, the vanadium element as a constituent of the Ti-6Al-4V alloy is widely employed in manufacturing implants and prosthetic appliances, due to its enhancing effects on new bone formation and osteointegration [38]. Vanadium has also enhanced endochondral ossification when combined to a fibrous composite scaffold aimed to aid the treatment of bone defects [39]. Additionally, vanadium appears to be needed by humans in minimal amounts and can be found in food such as shellfishes, herbs and mushrooms [40].

The articles included in this SR have not evaluated or addressed oncogenicity. Even so, studies recommend the transient inhibition of PTEN or the use of PTEN inhibitors for a short window of time [7,20,21,24,], which would potentially reduce any possible oncogenicity.

Our search strategy, and additional search strategies we conducted on the databases, did not result in clinical trials that tested PTEN inhibitors on human patients. A SR of randomized clinical trials (Phase I) by Smith *et al.* [41] concluded that short-term vanadium-based therapy was tolerated in diabetic patients, even though efficacy was low and variability between patients high. It is important to consider that the systemic use of vanadium compounds

may result in toxicity. Yet, advances in drug delivery may help overcome the potential secondary effects or toxicity of vanadium compounds [42].

The majority of the studies included in this review regarded the effects of systemic application of bpV compounds on nervous system healing (group 3), indicating that they were actively beneficial to tissue regeneration. Additional studies even reported that such molecules instigate biological processes such as myelination [43] and neurite outgrowth [44], both of importance to functional recovery after injury. A relatively smaller amount of studies investigated the effects of bpV compounds on muscle (group 2), lung and eye (group 1) regeneration, and a lack of studies on skin and cutaneous lesions was observed. Nevertheless, it is known that the DNA-based suppression of PTEN is beneficial to the cutaneous repair process [4,45]; therefore, the topical use of PTEN inhibitors in the skin and mucosa might be advantageous, considered their potential as wound healing enhancer and the possible decreased toxicity for their topical application and are underway (SQUARIZE CH, PERSONAL COMMUNICATION).

The activated form of PI3K leads to phosphorylation of PIP2, converting it to PIP3. PTEN inhibits the PI3K/AKT/mTOR signaling pathway through dephosphorylation of PIP3 [46]. As observed in this review, AKT phosphorylation is a recurrent biomarker for the action of PTEN inhibitors, considered that p-AKT was reported increased. It is an expected outcome, since inhibition of PTEN results in an upregulation of the PI3K/AKT/mTOR pathway, meaning an increased AKT phosphorylation. Studies on nonpharmacological inhibition of PTEN demonstrated similar results [4,7,45], which emphasizes the proregeneration effects of this approach.

Conclusion

This SR indicated that the pharmacological inhibition of PTEN enhanced the repair process of the eye, lung, muscle and nervous system. It also increased AKT activation (p-AKT), which may serve as a potential molecular biomarker for drug delivery and efficacy. Additional studies are necessary to test the efficacy of these inhibitors in other systems and to advance their potential clinical use further.

Future perspective

Further studies on the topical application of PTEN inhibitors will hopefully reveal their potential as a therapeutic approach to skin and mucosa injuries and as an epithelial regeneration enhancer. Investigating the effects of pharmacological inhibition of PTEN may have on other tissues, organs and systems is also expected, considered the positive results experienced so far and here described.

Summary points

- This systematic review investigated the effects of phosphatase and tensin homolog (PTEN) inhibitors on tissue regeneration.
- The systematic review process yielded 17 studies that met the selection criteria and were included in this study.
- The data collected in this study indicated that PTEN inhibitors (bpV compounds and SF1670) improves the repair process and regeneration of eyes, lungs, muscle and nervous system.
- The most used PTEN inhibitor *in vivo* was bisperoxovanadium 5-hydroxypyridine (bpV[pic]).
- The concentrations of PTEN inhibitors varied from 0.1 to 10 μ M in most studies, and doses varied according to the administration route *in vivo*.
- PTEN inhibitors increase p-AKT, which might serve as a marker for successful drug delivery and efficacy.

Author contributions

CH Squarize and RM Castilho designed and supervised the research; AEM Marques, GA Borges and LP Webber performed the search and analyzed data; and GA Borges, ENS Guerra and CH Squarize wrote the paper.

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Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/rme-2019-0065

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