Photobiomodulation in acute traumatic brain injury

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Photobiomodulation in acute traumatic brain injury: a systematic review and meta-analysis

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Abstract

Photobiomodulation (PBM) is a therapeutic modality which has gained increasing interest in neuroscience applications, including acute traumatic brain injury (TBI). Its proposed mechanisms for therapeutic effect when delivered to the injured brain include anti-apoptotic and anti-inflammatory effects. This systematic review summarises the available evidence for the value of PBM in improving outcomes in acute TBI and presents a meta-analysis of the pre-clinical evidence for neurological severity score (NSS) and lesion size in animal models of TBI. A systematic review of the literature was performed, with searches and data extraction performed independently in duplicate by two authors. Eighteen published articles were identified for inclusion: seventeen pre-clinical studies of *in vivo* animal models; and one clinical study in human patients. The available human study supports safety and feasibility of PBM in acute moderate TBI. For pre-clinical studies, meta-analysis for NSS and lesion size were found to favour intervention versus control. Sub-group analysis based on PBM parameter variables for these outcomes was performed. Favourable parameters were identified as: wavelengths in the region of 665 nm and 810 nm; time to first administration of PBM ≤ 4 hours; total number of daily treatments ≤3. No differences were identified between pulsed and continuous wave modes or energy delivery. Mechanistic sub-studies within included *in vivo* studies are presented and were found to support hypotheses of anti-apoptotic, anti-inflammatory and pro-proliferative effects, and a modulation of cellular metabolism. This systematic review provides substantial meta-analysis evidence of the benefits of PBM on functional and histological outcomes of TBI in *in vivo* mammalian models. Consideration of study design and PBM parameters should be closely considered for future human clinical studies.

Introduction

Traumatic brain injury (TBI) is a global health problem and a significant cause of mortality and life-long disability. Worldwide, annual incidence of TBI is estimated at over 50 million[1,2], with annual costs to the global economy around US$400 billion[1]. No effective disease-modifying therapy has been identified to control the variety of post-injury responses and prevent secondary injury to brain tissue, and cell damage/loss[3]. These pathophysiological mechanisms include the well-established factors such as raised intracranial pressure (ICP) and cerebral hypoxia, and current therapeutic paradigms are centred on monitoring and maintaining intracranial homeostasis, principally with respect to pressure and oxygenation[4]. However, other pathways of injury are gaining increasing recognition: mitochondrial dysfunction[5], neuroinflammation[6], excitotoxicity[7] and oxidative stress[8]. Pharmacological therapeutics typically target discrete pathways or mechanisms, but have thus far failed to demonstrate clear benefit in the context of this multi-faceted pathology[9]. Trialling of multiple novel therapies in a clinical setting is challenging without stepwise integration of single novel therapies into standard of care, which individually may not surpass the threshold for detectable therapeutic benefit. Single therapies which are able to target multiple mechanisms within the pathophysiological pathway therefore hold considerable therapeutic potential.

Photobiomodulation (PBM) is the application of red/near-infrared (R/NIR) light (600-1100 nm) to biological tissue for the purpose of therapeutic advantage, restoration of function or enhancement of physiology[10]. The concept of the biological effect of photons from across the electromagnetic (EM) spectrum is well established. Photons with low wavelength and high energy (gamma rays) are administered as radiotherapy,

and photons in the ultraviolet part (UVB) of the EM spectrum are integral in the biological synthesis of cholecalciferol. The use of 5-aminolavulanic acid, preferentially taken up by high-grade glioma tissue, and converted to protoporphyrin IX which fluoresces under violet-blue (405 nm) light, has become central in oncological neurosurgery to maximise resection margins[11]. PBM is similarly based on a photobiological interaction of longer, lower energy photons.

Though the mechanism of PBM is not fully established[12,13], cytochrome c oxidase (CCO), the terminal component of the electron transport chain, is thought to be the principle chromophore of absorption of R/NIR light[14]. Absorption of R/NIR photons by CCO is thought to lead to downstream modulation of reactive oxygen species production *via* effects on mitochondrial membrane potential (MMP), an increase in nitric oxide dissociation, and raised ATP production[15]. PBM is additionally proposed to potentiate light-sensitive ion channels[16], resulting in increased intracellular calcium concentrations, in turn affecting levels of cyclic adenosine monophosphate (cAMP) and modulation of downstream transcription factors. Together, these molecular interactions are proposed to lead to the various reported effects (anti-apoptotic, anti-inflammatory, pro-proliferative[12–15]).

The application of R/NIR light, due to its high scatter and absorbance in biological tissue resulting in attenuated tissue irradiance at depth, lends itself more readily to topical application for externally accessible tissues. The scientific and clinical interest in such contexts has a broad evidence base, particularly in: dermatological pathology[17], burns[18], wound healing[15], cancer-related lymphedema[19] and oral inflammatory conditions[20]. Following these successes, attention to the targeting of R/NIR light at deeper biological tissues has since increased in recent years[21–24], including the central nervous system[25–29].

The application of PBM in the field of TBI has gathered interest and scientific evidence in the last decade. The application of PBM as a therapeutic intervention for the injured brain has taken two principle forms: (1) in the acute setting post-injury, intended as a neuroprotective/neurorestorative therapy; and (2) in the rehabilitative setting in the chronic phase post-injury, for the purpose of improving symptomatology or neurocognitive/neuropsychological function. Pre-clinical studies on the efficacy of PBM in acute TBI have generated positive results, utilising moderate or severe models of TBI in rodent species[30], with promising results in an early clinical study[31]. The methodology, outcome measures and hypothesised mechanisms in chronic contexts differ greatly from acute applications, and as such, the chronic/rehabilitative phase of TBI recovery is beyond the scope of this review.

The efficacy of PBM in ischaemic stroke had too been considered promising[32]. Such promise culminated in a phase III randomised controlled trial - the NEST-3 study[33] which did not demonstrate efficacy and was terminated early. However, the study design has stimulated criticism[34], the themes of which represent ongoing scientific “unknowns” in PBM research. The optimal variables of dosing are yet to be established, including irradiance (both surface and after tissue penetration), time of initiation post-injury, optimal duration of therapeutic course, and optimal brain regions for irradiance. Formally establishing the evidence base in TBI is essential for informing future directions in both pre-clinical, translational and clinical research, to minimise the risk of future clinical PBM studies in TBI using suboptimal parameters.

Aims

This systematic review and meta-analysis seeks to comprehensively identify and summarise the scientific literature on the use of PBM in acute TBI. The variables of PBM implemented in identified studies will be analysed with respect to the observed outcomes.

Methods

A systematic review of the literature was performed on 4th October 2021 following the methodology of the Cochrane Handbook for Systematic Reviewers and presented in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[35].

Systematic searches were performed independently in duplicate. The following databases were searched, from their respective inception to October 4th 2021: Medline, Embase, Cochrane Central, Scopus, Google Scholar and Web of Science. Reference lists of pertinent articles on the topic were hand-searched for suitable articles. Reference lists of identified articles for inclusion were also hand-searched for suitable articles. An example search strategy for Medline is given below:

Search:

1) Exp Low-Level Light Therapy
2) Photobiomodulation.mp
3) Exp Phototherapy/
4) Phototherapy.mp
5) Low level laser therapy.mp
6) 1 or 2 or 3 or 4 or 5
7) Exp Brain Injuries, Traumatic/
8) Exp Craniocerebral Trauma/
9) Head injury.mp
10) 7 or 8 or 9
11) 6 and 10

Study Selection

Studies were independently screened for inclusion by two reviewers (A.R.S., and Z.A.). Initial screening based on title and abstract were performed, followed by full text screening. Eligibility for study inclusion was defined as: use of PBM therapy (red/near-infrared) in acute traumatic brain injury. Any methodology was accepted, and studies involving *in vitro/in vivo* animal or human subjects were included. Studies which only reported computational modelling methods were excluded. Articles were excluded where blue or white light therapy were used, and where PBM was used in chronic or rehabilitative phase of injury. Conference abstracts were excluded to avoid duplicate reporting where insufficient detail was available to in abstracts to identify such cases; where full manuscripts were available for conference proceedings, these were screened against included manuscripts to exclude duplicate reporting.

Data Extraction

Data were extracted from included reports using a piloted form. Data extracted from pre-clinical studies included; species and injury model used, number of replicates, PBM therapy variables (wavelength, irradiance, timing, doses, light source, delivery site), outcomes, and findings of any mechanistic sub-studies. Data were extracted for meta-analysis for neurological severity score and lesion size indices, and other functional and histological outcomes were extracted for narrative presentation. Where data were not directly reported as values, values were extrapolated from published figures. From clinical studies, patient characteristics, PBM therapy variables, any comparator used, outcome measures and outcomes were recorded.

Synthesis of Results

This systematic review presents a combination of meta-analysis and narrative presentation of available data. Specific outcome measures were deemed suitable for meta-analysis where ≥ *n* = 3 studies had presented data (either directly or suitable for accurate extrapolation) in this regard. Remaining outcomes reported in included articles will be presented in tabular and narrative form without statistical analysis.

Risk of bias

Risk of Bias in Individual Studies was assessed using SYRCLE's tool for assessing risk of bias[36], and overall risk of bias was determined as low, moderate or high for each included article. For clinical trials, Cochrane Risk of Bias tool[37] with overall risk of bias determined via design quality assessment as good, fair or poor for each included article.

Statistical analysis

Data for meta-analysis were extracted as mean, standard deviation (SD) and n number. Where standard error of the mean (SEM) were presented, SD was calculated using SEM and n number. Review Manager 5.4.1 (Cochrane Collaboration, 2022) was used for meta-analysis. Effect size was calculated with a 95% confidence interval, using a standardised mean difference model due to heterogenous data reporting. A fixed effects model was used, and where heterogeneity was calculated by Chi^2 test as ≥50%, a random effects model was used.

Results

The search strategy identified 1784 records, with 1459 remaining after removal of duplicates, leaving 93 full-text articles which were assessed for eligibility against the inclusion/exclusion criteria after abstract screening. Eighteen studies met the criteria for inclusion, with 17 pre-clinical studies being conducted in animal models[38,39,48–54,40–47], and one clinical study using human participants[31]. The full flow chart for search results is shown in the PRISMA diagram in Figure 1.

[Insert Figure 1]

Human studies

We identified a single study of PBM in acute TBI in human participants. Figuero Longo et al. (2020)[31] randomised 68 patients with moderate TBI to receive either sham therapy or transcranial PBM within 72 hours of injury. The PBM therapy application used an 810 nm LED array within a custom helmet, with reported average irradiance to the scalp of 36 mW/cm^2 for 20 min applications. The authors calculated that their system provides a surface energy of 1.3 J/cm^2 per session based on cadaveric modelling. Patients received 3 x 20 min sessions, at least 12 hours apart. Twenty-eight patients received at least one PBM session, and were evaluated for diffusion MRI outcomes and Rivermead post-concussion questionnaire (RPQ) in comparison to control group. There were significant changes in radial diffusivity, mean diffusivity and fractional anisotropy at the late subacute stage between PBM and sham groups, though not for axial diffusivity or other time points. RPQ scores were somewhat improved in PBM group versus sham, though not of statistical significance, though it is noteworthy that the study is not appropriately powered to identify significance for functional outcomes.

Animal studies

We identified 17 studies of PBM in animal models of TBI[38,39,48–54,40–47] (Table 1). All studies used an *in vivo* model of TBI, with one study using neuronal cell cultures as an additional model[39]. Four studies used rats[40,41,45,53], one study used a transgenic (X-1 KO) mouse[54] and 13 used wild-type mice[38,39,52,42–44,46,48–51]. Seven studies used controlled cortical impact (CCI) models[38,42,45,49–52], six used weight drop (WD) [43,44,46–48] (one in a repeated TBI model[53]), two used controlled scalp impact[39,54], one open lateral fluid percussion (LFP)[41], and one blast-induced neurotrauma (BINT)[40].

The available reported PBM variables for therapeutic regimes are given in Table 1, with all used permutations of a variable given (where a study has used multiple therapeutic regimes). Notably, 15 studies used a wavelength in the 800-810 nm region, either alone or in comparison with another wavelength, with two studies not using a wavelength in this region[41,45] (using 670 or 830 nm). Full width half of maximum values were not reported. Two studies used a light-emitting diode (LED)[41,45], 15 employing a laser source. Two studies delivered PBM directly to the cortical surface of the lesion site[42,46] with 15 studies applying the source to skull or scalp. Four studies used a pulsed wave (PW) source[38,39,44,54], alone or on combination with continuous wave (CW), one used nano-pulsed laser therapy (NPLT)[40], and 12 used CW alone. One study used PBM in combination with metabolic substrates[39], 16 using PBM alone. All studies commenced PBM within 8 hours of injury. Ten studies used PBM protocols with a single dose of PBM [38–41,43,44,46–48,54], with seven using alternative and additional course durations up to 15 days[53].

Meta-analysis

Two outcomes were sufficiently reported in the included studies to permit robust meta-analysis: neurological severity score (NSS) and lesion size. NSS was predominantly

reported as baseline NSS (± SD/SEM) and end-point NSS (± SD/SEM). Insufficient detail was available in any included study, or in the wider literature, to calculate or infer the ΔSD, and so the end-point NSS was used in a standardised mean difference model.

Neurological Severity Score

Meta-analysis was performed for the nine studies reporting NSS values in PBM and control groups. Given the frequent use of sub-study groups based on varying therapeutic protocols, a total of 28 therapeutic protocols from across nine studies are included in the analysis (Figure 2). Overall, effect size is -1.55 [-2.10, -1.01] in favour of PBM versus control ($p < 0.00001$, I^2 82%).

[Insert Figure 2]

Given the heterogeneity of therapeutic regimes employed, both inter- and intra- article, sub-group analysis was performed based on distinct variables: CW vs PW, wavelength of PBM, timing of first dose post-injury, total number of doses and incident energy per dose (calculated for cortical surface energy) (Table 3). Both continuous and pulsed wave PBM have statistically significant effects on NSS. Ando et al., 2011 reported statistically significant advantage of PW at 10 Hz over CW, but not of PW at 100 Hz. Wavelengths in the region of 665/810 nm are associated with positive effects, but not 730/980 nm. A first dose after 4 hours post-injury or a course of treatment with >3 doses were not effective, and all energy values were associated with reduced NSS. Dosing regimes for sub-studies and therapeutic protocols included in meta-analysis are described in Table 2.

Lesion Size

Ten studies reported outcomes of lesion size at study endpoint, encompassing 21 therapeutic protocols. Overall effect size was -1.55 in favour of PBM, [-2.19, -0.95] (p <0.00001, I^2 = 75%). Standardised mean difference was used due to heterogeneity in reported effect and measurement techniques. Forest plot for lesion size across included studies is illustrated in Figure 3. Dosing regimes for sub-studies and therapeutic protocols included in meta-analysis are described in Table 2.

[Insert Figure 3]

Alternative functional outcomes

Morris Water Maze (MWM) was an outcome measure used in n = 5 studies[39,40,42,51,52]. However, there was significant heterogeneity between training schedules, timing of testing post-injury (varying from 7 to 55 days post-injury), the use of hidden platform, visible platform and probe trials. As such, these were not deemed suitable for meta-analysis and the findings of individual studies for MWM will be described in the narrative below. Similarly, other functional outcomes were used by studies with varying frequency: forced swim test (n = 1)[38], tail suspension test (n = 1)[38], wire grip and motion test (n = 2) [49,51], rotarod score (n = 1)[41], bilateral asymmetry test (n = 1)[41], beam balance scores (n = 1)[40], nose poke tasks (n = 1)[45], open field, elevated plus maze, Y maze and fear conditioning (all n = 1)[53], and discussed below.

Khuman et al., (2012) [42] performed MWM trials, with inclusion of probe trials and analysis of progressive improvement. Statistically significant improvements in outcomes were mixed, though 60 J/cm^2 delivered either transcranially or directly for a single administration demonstrated a statistically significant improvement versus controls in all measured MWM domains. All PBM groups versus controls exhibited

greater progressive improvement ($p < 0.0001$). Administered directly, 60, 120 and 210 J/cm^2 but not 30 or 105 J/cm^2 resulted in MWM hidden platform latency improvements, and 60 and 120 J/cm^2 elicited probe trial improvements. Single dose transcranial administration of 60 J/cm^2 resulted in improvements in all MWM domains if given 1 hour after injury, but not after 4 hours or daily for 7 days. Esenaliev et al., (2018) used transcranial nano-pulsed laser therapy (NPLT) in a blast induced neurotrauma (BINT) model. NPLT was demonstrated to improve MWM ($p < 0.05$) at 7 days post-injury, though this effect was not observed on subsequent days' MWM testing.

Xuan et al., (2016)[52] also used the MWM test at 22 and 49 days post injury. Groups with TBI receiving 3x daily PBM treatments at day 22 demonstrated significant improvements in latency scores for both platform and probe trials, compared to TBI controls ($n = 6$, $p < 0.05$ and 0.01 respectively). The effect was not significant at day 49 though the effect was still observed, and the group receiving 14 days of treatment had results similar to TBI controls at both timepoints post-injury.

Giacci et al., (2014) found no significant differences between control, 670nm and 830nm treatments at 7 days post-injury in rotarod score or in the bilateral asymmetry test contralateral vs ipsilateral latency. Xuan et al., (2013) [49] used a wire grip and motion test and showed no statistically significant improvement in vestibular function in any treatment group. Xuan et al. (2014) [51] utilised the wire grip and motion test (WGMT) at 7, 14, 21 and 28 days post-TBI using single or triple doses of PBM (810 nm, 25 mW/cm^2, 12 min transcranial). The authors demonstrated statistically significant improvements in WGMT performance in both single ($p < 0.05$) and triple ($p < 0.001$) doses at 28 days post-injury, with significant improvements recognised also at 14 and 21 days in PBM versus control. Khuman et al. (2012) [42], also found no improvement on motor recovery in a stationary wire grip test. Esenaliev et al., (2018)

[40] used transcranial nano-pulsed laser therapy (NPLT) in a blast induced neurotrauma (BINT) model. NPLT was demonstrated to improve beam-balance test scores on day 1 post-injury ($p < 0.01$), beam walk test scores on day 1 and 2 post-injury ($p < 0.001$ and < 0.05 respectively), not observed at later time points. Quirk et al., (2012) [45], (670 nm, 50mW/cm^2, 5 min exposure time, daily exposure for 72h) found some improvements in movement domains (nose poke tasks) though not all, and their functional testing was limited to this assessment.

Ando et al., (2011) [38] found statistically significant improvements in the forced swim test (for depression) in the group receiving PW 10Hz ($p < 0.05$) at 28 days post-injury, and significant improvements in the tail suspension test for anxiety and depression in both PW 10 Hz and PW 100 Hz at 28 days post-injury. CW groups did not demonstrate statistically significant improvements in these domains. Yang et al (2020) [53] performed more extensive functional assessments in their repeated TBI model. Open field (mobility), elevated plus maze (anxiety), Y maze (spatial memory) and fear conditioning testing all demonstrated statistically significant improvements attributable to PBM administration after repeated closed TBI.

Histological outcomes

Wu et al., (2012)[9] marked fractional defects on morphometric brain sections with H&E staining, and identified that 665 and 810 nm LLLT resulted in statistically significant reductions in numbers of mean fractional defect areas ($p < 0.05$ and < 0.01 respectively, $n = 10$ per group).

Mechanistic outcomes

Metabolic activity

Ando et al., (2011) [38] performed ATP fluorometric assay for specimens immediately after laser exposure versus control, finding no significant differences between control and intervention arms. However, a trend suggested a mild increase of cortical ATP content with 10Hz PW treatment, correlating with this as the treatment condition with greatest effect on improving NSS and lesion size. Zhang et al., (2014)[54], though principally using W-1 KO mice, performed ATP assay on WT mice with and without LLLT after mTBI, demonstrating that at 24h after injury there was a significant increase in cortical ATP levels with LLLT ($p < 0.001$, $n = 6$ per group). Dong et al., 2015[39], included LLLT therapy to cultured SH-SY5Y neuronal cells in varying conditions. They report that hypoxia (induced by $CoCl_2$) related cell death was significantly reduced by exposure to 3-10 J/cm^2 LLLT at 2h post-exposure to hypoxia, with increasing survival with increasing energy or exposure periods (up to 10J/cm^2 x3). The effect was augmented with the addition of mitochondrial substrates to the media (glucose/lactate/pyruvate). They further demonstrated that this increased survival with LLLT exposure correlated with suppression of lactate level at 0-18hr, increase in ATP level, reduction in ROS level and increase in mitochondrial membrane potential. In an *in vivo* model of hypoxia (using cortical topical oxyrase) they reported complete prevention of hypoxia related hippocampal tissue loss due to Oxyrase in the presence of LLLT.

Neuroinflammation

Khuman et al., (2012) [42] performed quantitative analysis of microglial activation. 60 J/cm^2 resulted in a significant reduction in activated microglia compared to control treatment ($p = 0.03$, $n = 4$/group). Khuman et al., (2012) [42] also found no significant

difference between treatment and control for: magnitude of brain oedema at 24h, brain tissue loss or protein nitrosylation. Yang et al. [53] also found that PBM lowered MAP2 dispersion, increased APP and MBP and reduced cleaved Tau, with the authors concluding that PBM attenuates axonal injury after repeated TBI. Yang et al., (2020) [53] found that PBM was associated with attenuated astrogliosis at the lesion site, and attenuated the increases of IBA1 and IL-1B associated with pro-inflammatory shifts post-injury. PBM associated elevation of IL-10, in combination with reduction of IL-1B was attributed by the authors to a shift from “pro-inflammatory” to “anti-inflammatory” phenotypes after exposure to PBM. NPLT-treated rats (Esenaliev et al., (2018)[40]) demonstrated lower CD68+ microglial cells (marker of microglial activation). Xuan et al., (2016) [52] stained for glial fibrillary acidic protein in multiple brain areas in their study using 3/14 day PBM courses post-injury. Whilst the 14 day course did not correlate with functional improvements (MWM, NSS), the 3 day course did improve these outcomes. Both 3/14 day courses were associated with reduced GFAP staining in all tested brain areas at 56 days post-injury, to a similar extent.

Apoptotic markers

Quirk et al., (2012)[45], performed Western blotting on tissue extracts from four brain regions in TBI and sham +/- PBM administration. Glutathione levels, vastly increased in injury site versus same brain area in sham, were not significantly changed in the lesion site with the application of PBM. However, they identified a statistically significant reduction in Bax levels (pro-apoptotic protein) after application of PBM compared with controls. Levels of Bcl-2 (anti-apoptotic marker) were also significantly increased after PBM compared with no therapy. B-actin levels were not significantly altered. Esenaliev et al., (2018) [40] in their transcranial NPLT BINT model found that at PID3 in injured cortical neurons (FJC^{neg}) from rats exposed to NPLT had lower

expression of BAX, CASPASE-3 and STAT3, with higher levels of BNF expression. This effect was not observed on PID7. Similarly, NPLT-treated rats showed lower immunofluorescence with caspase-3 staining on PID3, with no observed immunofluorescence at PID7. Similarly, Yang et al. [53] also found a significant and marked reduction in cytosolic caspase-3 and caspase-9 associated with PBM administration after repeated closed head injury. Xuan et al., (2014) [51] found significant reductions in caspase-3 expression at day 4 post-injury in both single ($p < 0.05$) and triple ($p < 0.01$) treatment regimes versus control.

Neuronal damage and proliferation

Yang et al, (2020) [53] in PBM treated rats after repeated closed head injury, found that synaptic degradation was prevented by PBM based on expression of synaptophysin and spinophilin in hippocampal CA1 region. NPLT-treated rats (Esenaliev et al., 2018[40]) demonstrated higher cell proliferation in the subgranular zone (BrdU immunohistochemistry). Xuan et al., (2013) [49] demonstrated statistically significant reduction in degenerating neurons in 3 day treatment regime specimens through Fluoro-Jade C staining compared with sham treatment ($n = 8$, $p < 0.05$). Xuan et al., (2013) [49] also showed statistically significant increases in BrdU staining (proliferating cells) with 3 days' treatment vs sham ($n = 8$, $p < 0.001$). Xuan et al., (2014) [51] found significant increases in BrdU/DAPI ratio in hippocampal DG at day 7 post-injury in both single ($p < 0.01$) and triple ($p < 0.001$) treatment regimes versus control, and at day 28 post-injury (single ($p < 0.05$) and triple ($p < 0.01$)) . Similar results were also shown for neurogenic SVZ and in perilesional tissue all at 7 and 28 days post-TBI. Xuan et al., (2014) [51] found significant increases in PBM treated specimens versus control in microtubule-associated migration protein double-cortin (DCX) suggestive of presence of maturing neurons. DCX/DAPI ratio in DG and perilesional tissue was increased in

treated subjects at 7 and 28 days, and in SVZ at 7 but not 28 days. Xuan et al., (2014) [51] also performed staining for neuron-specific class III β-tubulin (TUJ/1) (expressed in differentiating neural progenitor cells) at 7 and 28 days post-injury in PBM and control specimens, finding an association between increased TUJ1/DAPI ratio and PBM treatment at both timepoints in both DG and SVZ. Xuan et al (2015)[50]demonstrated a transient upregulation in brain derived neurotrophic factor (BDNF) at day 7 post-injury in DG and SVZ, not observed in the perilesional region. The lesion and SVZ at 28 days post-injury showed upregulation of Synapsin-1, suggestive of synaptogenesis.

Cerebral blood flow

Shemesh et al. (2022) [46] used a terminal anaesthesia model, with TBI and subsequent craniotomy for dual-wavelength speckle contrast imaging , with 20 minutes of baseline data, delivery of PBM directly, and a further 20 minutes of data taken. Cerebral blood flow, saturation and overall oxygen consumption increased in animals receiving PBM therapy versus control.

Experimental factors

Transmittance

Ando et al., (2011) [38] performed profiling of the laser power transmission to brain tissue for each of their treatment protocols, measuring transmission through scalp alone, and skull and scalp. Using this data, they calculated an approximation of irradiance to cortical surface based on the dimensions of overlying scalp/skull in their model. Oron et al., (2007) [43] similarly used cadaveric mouse tissue to simulate their protocol with a power meter positioned within the cranium to directly measure transmitted irradiance. Shemesh et al. (2022)[46] also directly measured power outputs to calculate incident dose at the “sample plane” (cortical surface in their direct delivery model with a 200 mW output source at 20 cm from cortex).

Temperature

Khuman et al, (2012) [42], recorded temperature changes in their model of direct application of PBM to the cortical surface: PBM treatment "increased brain temperature by 0.2 ± 0.1°C (30 J/cm^2), 2.5 ± 0.4°C (60 J/cm^2), and 4.1 ± 0.3°C (120 J/cm^2), over the 2-min application period. In the transcranial protocol, LLLT (60 J/cm^2) increased brain temperature by 1.8 ± 0.1°C". The authors reported that brain temperature returned to baseline within 3–5 min.

Risk of Bias Assessment

Risk of bias assessment for the included human clinical study is presented in Table 4, with overall study quality determined as "Fair". Overall risk of bias for animal studies was high across most studies, as illustrated in Figure 4.

[Insert Figure 4]

Discussion

This systematic review presents a comprehensive summary of the available literature on the use of PBM therapy in the context of acute TBI. The meta-analysis, though limited in its scope due to literature heterogeneity, combined with supplementary narrative summary, presents clear and robust data of the therapeutic benefit of PBM in pre-clinical models on functional, histological and radiological TBI outcomes. The presented data on specific mediators amalgamates a broad variety of mechanistic insights into the action of PBM in TBI. Subgroup analysis based on variables of PBM parameters also offers an important quantitative summary toward establishing the translational potential for PBM.

Establishing an optimum modality

The subgroup analysis presented in this review identified that the available evidence supports both continuous wave (CW) and pulsed wave (PW) as therapeutic modes of dose administration. The greater effect size of PW is suggestive that there may be an advantage to this approach, though this analysis is not able to draw conclusions on the relative merits of PW in comparison to CW. Both approaches generated an effect size favouring intervention with statistical significance. It is noteworthy however that Ando et al., (2011)[38] compared CW with PW (10 Hz) and PW (100 Hz) and found PW 10 Hz the most effective, with a statistically significant further improvement of outcomes compared with CW. Oron et al., (2012) too found that PW at 100 Hz was associated with improved NSS recovery at 56 days post-injury compared with CW. The application and mechanistic effect of pulsed wave light delivery is not fully understood and warrants further well controlled studies.

Given the presence of one paper in the literature describing the technique, insufficient evidence on the application of NPLT in TBI is available to draw conclusions as to its efficacy, particular in comparison with CW/PW.

The subgroup analysis also identifies a clear dichotomy amongst the used wavelengths: wavelengths in the region of 665 and 810 nm corelated with therapeutic effect, and wavelengths in the region of 730 and 980 nm did not. This apparent failure of 730/980 nm wavelengths to elicit detectable therapeutic benefit in *in vivo* models of TBI has two potential explanations: (1) 730/980 nm do not elicit the same phototherapeutic and biochemical effects at a cellular level in TBI; (2) penetrance of 730/980 nm photons to the target tissue is impaired in comparison with other wavelengths. These potential causes of therapeutic failure are not able to be informed by the current available evidence identified here. Longer wavelengths penetrate further, however 980 nm light has increased levels of absorption by water and oxygenated haemoglobin than the shorter wavelengths[55], which may in part explain this phenomenon. A further consideration is that no included study reported full width at half maximum value; a measure of the bandwidth of a light source at 50% capacity (though spectral transmittance was reported by Giacci et al., 2014[41] for their spinal cord injury model). As such, it is not possible to evaluate whether sources outside the emission maxima of 660 and 810 nm may have included these peaks.

Establishing an optimum dose

In the subgroup analysis on overall dose, all three subgroups of dose demonstrated efficacy with statistical significance. The optimal dose is a subject of much debate in PBM research which has not been resolved with wide-ranging and thorough reviews from across the large body of PBM literature in multiple tissue types[56]. The well-established “biphasic dose response” of PBM creates potential problems of both

under- and overdosing of target tissue[57,58]. The Hamblin laboratory have argued that greater mitochondrial concentration (as in cerebral tissue) is more likely to respond favourably to relatively low levels of fluence, and have a greater propensity for failure due to overdose[56]. However, the doses delivered at any depth within cerebral tissue due to impedance from scalp, skull, periosteum, dura, cerebrospinal fluid (CSF), blood (and brain itself) is greatly attenuated even in rodent tissue: this issue is amplified in larger mammals such as humans[59]. Delivery varies further on factors such as skull thickness, differing between species, patients and anatomical locations. In the absence of subject-specific complex computational modelling, even establishing “the dose” reaching the target tissue is difficult, more so if attempting to resolve this to discrete anatomical locations within the organ. Study therefore on “the optimum dose” is an even greater challenge.

Establishing an optimum dose regime

Subgroup analysis has also demonstrated clearly that two key factors in the PBM dosing regime should be considered. Firstly, the time to initial dose is of great importance: the therapeutic effect appears to be lost or greatly diminished when first dose administration is given more than around six hours post-injury in rodent models. Furthermore, the beneficial effects of PBM appear to be lost where repeated administrations are given on a daily basis over a number of days, though the optimum duration of a treatment course cannot be established from the current literature. Xuan et al (2013)[49] examined this directly, and showed that in their model the efficacy increased with 3x vs 1x doses, but any derived benefit was lost if the course continued to 14x doses. Whilst direct comparative data is limited to this study, Yang et al (2020) found considerable therapeutic benefits, particularly in functional outcomes, with doses given over a 15 day course.

Establishing an optimum delivery technique

The specifics of delivery varied greatly between studies using a transcranial delivery method. PBM parameters (irradiance, fluence and exposure time), positioning factors (direct to skull, direct to scalp, or a specified distance from the scalp) and specimen factors (underlying durotomy/craniotomy/closed surgical wound) all have considerable effects on the dose delivery to the target tissue.

The included results are not able to inform conclusions regarding the potential benefits of varying specifics of a transcranial application method, nor inform conclusions on the benefits of either direct or transcranial application of PBM. Only one study used both direct and transcranial approaches (Khuman et al., (2012)[42]) and they were not directly compared. Dosing at 60 J/cm^2 60-80 min post-injury were concluded by the authors as the main factors in eliciting functional improvements in MWM testing, with this dose performing best in either direct or transcranial administration. Administered directly, 60/120/210 J/cm^2 but not 30/105 J/cm^2 resulted in MWM hidden platform latency improvements, and 60/120 J/cm^2 elicited probe trial improvements. Across all MWM outcomes, 60 J/cm^2 given at 60-80 min post-injury, whether delivered directly or transcranially were deemed to elicit similar modest improvements (though direct statistical comparison was not presented). The remaining outcomes were not significantly improved by any PBM regime tested in this study.

Whilst this review has principally considered the incident energy exposure to injured target tissue (i.e. delivery to neural/glial cells), there is growing interest in the systemic effects of PBM. Given the low penetrance of R-NIR light to the brain from external application, it has been hypothesised that transcranial PBM has contributory effects from photon absorption in superficial tissues (scalp with its rich vascularity, skull with calvarial bone marrow). PBM directed to the tibia in a mouse model of Alzheimer's

disease has been shown to activate mesenchymal stem cells (MSCs)[60]. Calvarial bone marrow niches have interfaces with CSF, which is a possible conduit for activated MSCs from skull in transcranial PBM[61].

Further to this, PBM application to anatomically distant sites has been observed to result in therapeutic effects in neurological disorders. Termed the ”abscopal effect”, therapeutic action distant to irradiated tissue has been recognised in radiotherapy since 1953[62,63], and more recently considered in PBM[64]. Recent research in Parkinson’s disease has identified therapeutic benefit for improvement of clinical signs in a short series of patients receiving PBM to the abdomen and neck[65], with similar benefits to those observed with transcranial delivery[29]. In animal models however, direct stimulation appears to carry greater benefit[66,67]. The mechanism for this is not known, but has been proposed as activation of remote immune and stem cells which become systemically active after PBM[66]. It is noteworthy here that the deep brain target in Parkinson’s disease (substantia nigra) receives significantly attenuated doses in humans, even with transcranial application.

Establishing the translational effect in humans

This review has focused on the acute phase of injury. The applications of PBM in patients with TBI in clinical studies is however much more broad when considering chronic or rehabilitative contexts and has been well summarised elsewhere[30,47]. This systematic review identified one study reporting the application of PBM in acute TBI in human subjects. Whilst the study was not sufficiently powered to detect any functional benefit, this study provides some evidence of a physiological effect of transcranial PBM through radiological outcomes. Whether this will translate to clinically relevant effects should be the subject of further study, but Figuero Longo and colleagues[68] were able to demonstrate a favourable safety profile to support further study. Similarly

to the presented pre-clinical literature, the included clinical study raises issues with regard to measurement and reporting of delivered dose. Data on fluence at a cortical level was not presented. Furthermore, the total number of doses received by enrolled patients varied and was not transparently reported nor considered in the data analysis. For this reason, the overall quality of the study was deemed “fair”, and consideration for dose, dose regime and dose delivery should be a key consideration in the planning of further clinical trials of PBM in TBI.

Establishing a mechanism

Through presentation of molecular sub-studies from the included literature, this review has illustrated the broad range of potential therapeutic mechanisms of PBM in TBI. A full discussion of the complex and not fully elucidated mechanism of PBM is beyond the scope of this review, however there are key themes observed from the included articles. In summary, PBM therapy in acute TBI appears to correlate with: (1) increases in ATP levels[38,39,54]; (2) reduction in reactive oxygen species[39]; (3) increase in mitochondrial membrane potential[39]; (4) reduction in microglial activation/astrogliosis[40,42,53]; (5) reduction of pro-inflammatory mediators[52]; (6) reduction in apoptotic markers[40,45,51,53]; (7) decreased neuronal degeneration[49,53]; (8) increased cell proliferation and maturation[49,51]; and (9) increased cerebral blood flow[46]. Given the heterogeneity of PBM parameters and injury models, it cannot be concluded whether such mechanisms are specific to a particular PBM parameter/injury interaction or representative of a broad mechanism of effect which is common to PBM modalities in acute TBI. However, the presence of beneficial effects on multiple discrete TBI pathophysiological processes is encouraging for its prospective translational benefit.

This systematic review has identified multiple studies reporting reduction in neural degeneration in TBI after PBM, reducing hippocampal (CA1) synaptic degradation[53] and degenerating neurons[49]. There is also significant evidence to support neurogenic and synaptogenic activity stimulated by PBM. Included articles have identified increased subgranular cell proliferation[40]; increased hippocampal, SVZ and perilesional cell proliferation[49,51]; and neuronal maturation and differentiation of neuronal progenitor cells[51]. Upregulation of BDNF post-PBM has been correlated with increased expression of Synapsin-1, with the authors concluding that early BDNF upregulation may mediate synaptogenesis in later (28 day post-injury) recovery phase[50]. In the wider literature, BDNF upregulation has been observed in PBM treated animal models of Alzheimer's disease, and proposed as a neuroprotective mechanism for PBM[69]. Similarly, increased neurogenesis[70,71] and synaptogenesis[70] has been observed in occlusive and pro-thrombotic stroke models in the rat with PBM treatment. Upregulation of BDNF expression with PBM has also been demonstrated in an organotypic hippocampal slice model[72] and a methanol-induced toxicity occipital cortex damage model[73].

Whilst the accumulated evidence presented here offers insights into potential downstream pathways mediated therapeutic effects in acute TBI, understanding of an overall mechanism of action for R/NIR light on injured brain tissue remains incomplete. Integral to photobiological interaction, photons must be absorbed by a biological molecule to elicit an effect, *i.e.* a chromophore. CCO has been understood as a primary chromophore of PBM[70,74,75], with photon energy resulting in the dissociation of nitric oxide (NO)[76], increasing MMP and ATP production, and modulating ROS production[73]. Whilst there is a clear role of the mitochondria in mediating the biological effects of PBM, the specific role of CCO is not fully confirmed.. A novel mechanism

disparate to CCO has recently emerged for 980 nm light: activating heat or light sensitive calcium ion channels[77]. It remains possible, or even likely, that there are further chromophores which contribute to the wide-ranging biological effects of PBM, with discrete unknown downstream mechanisms, with specific chromophores related to wavelength of PBM.

Risk of bias

The risk of bias across the included animal studies was found to be generally high. In most cases, reporting of factors as per SYRCLE guidelines were not adhered to. All studies described baseline characteristics and had clear primary outcomes, however randomization and blinding were overlooked by the majority of studies. To ensure replicable and robust data to inform the ongoing direction of PBM research in TBI, adherence to such guidelines is strongly encouraged for future studies.

Controls

Included studies in this review have used control procedures which include the equivalent restraint required to facilitate PBM, but without its application. However, it is arguable that this is an inadequate control: application of a broadband light source at equivalent irradiance would be a more robust approach. An ideal source would elicit similar transient temperature changes to a PBM source, in order to control also for this secondary effect of PBM therapy and would robustly identify that wavelength-specific doses of incident light are the beneficial factor. A typical broadband source would deliver a fraction of its energy in the form of wavelengths of therapeutic interest. Such wavelengths could also be filtered. If exploring such options as controls, spectral irradiance should be comprehensively measured and presented in the study reporting.

PBM parameter reporting

The detail with which PBM parameters were employed and reported in each study and sub-study varied greatly. Average irradiance and exposure time were reported across studies, but reporting of results of cadaveric modelling with direct measurement of transmitted PBM was infrequent. No study presented data from computational modelling of dose delivery or distribution. This enhances difficulties in the between-study comparison of efficacy: variability lies not only in PBM parameters but also in the transmission of those parameters to the target tissue. This is a salient and common shortcoming of much of the PBM literature[78]. The accurate reporting of radiometry in future studies should be prioritised.

Limitations

There are a number of limitations for this study. Due to heterogeneity in the included studies' selection of outcome measure, the meta-analysis is not comprehensive in scope and is able to represent only a subsection of the literature. Furthermore, due to common outcome measure preference within laboratories, high availability of NSS data or lesion size data is inherently biased toward a selective pool of publications. A narrative review on PBM in TBI and stroke[32] from the Hamblin laboratory presents an excellent summary of the available data, with outputs categorised by research group. Subgroup analyses were performed here to maximise the value of meta-analysis by providing some insight into the efficacy of varying PBM parameters. This however is not entirely robust given the inclusion of only a single study in some sub-groups, and conclusions should be guarded with that consideration. Similarly, the statistical component of the meta-analysis cannot account for the duplicate appearance of control groups as though they were independent, where a single control group is common across multiple sub-studies where the data is taken from a single paper. As

described above, PBM parameter reporting varied in its quality, and in some instances the dose for PBM parameters was calculated based on available beam transmittance data, and may not be entirely representative of the "true" figure which was not directly measured in the reported data.

This review does not comprise the available evidence for applications of PBM in chronic TBI, though a review summarising this data is available in the literature[30]. The study of chronic TBI is predominantly in case reports[79–82] or short case series[83–85] from human applications, in contrast to the evidence in acute TBI which is predominantly in animal models. This is likely attributable to the availability of replicable acute TBI models in animals (in contrast to chronic TBI models), and the practical logistics of patient recruitment in chronic (versus acute) TBI contexts. An ongoing randomised controlled trial for chronic TBI patients is likely to provide more robust evidence on the efficacy[86]. In contrast, applications of PBM in patients with stroke have predominantly been in the acute setting (NEST studies[33,87,88]) and there is little quality evidence for applications in the chronic rehabilitative phase post-ischaemic stroke[89]. Whilst some comparisons between pathologies (TBI, stroke) and time-points (acute, chronic) can be considered, the mechanisms, pathophysiology, interventional goals and outcome measures are discrete and specific, supporting the focussed nature of this systematic review and meta-analysis.

Future directions

This study, in accordance with previous work[56], has identified parameter heterogeneity and incomplete reporting as factors which impede accurate conclusions to be taken from PBM literature reviews. Accurate and comprehensively reported radiometry should be prioritised in future work, in order for clearly established dose *delivery* to target tissue to be compared between studies[78]. Given the lack of a well-established

therapeutic dose window in TBI, accurate representation of dose delivery for comparison with outcome measures is key to advancing this knowledge.

Transcranial approaches to PBM have a clear benefit in animal models, as illustrated here, but PBM delivery via this route is inherently impeded by the thickness of scalp and skull when translating this approach to humans. Thorough clarification of the effective dose window will be invaluable, alongside computational simulation, in determining whether this approach is the optimal route for clinical practice, or whether more elaborate or novel approaches may improve delivery and functional outcomes[90–95].

Conclusion

This review has provided clear evidence of the beneficial effects of PBM in acute TBI. Whilst specific parameters for optimum effect cannot be determined, the literature supports: wavelength selection in the regions of 665 or 810 nm; minimising time to first treatment; and limited total applications of daily PBM. Included studies comprising mechanistic considerations support the hypotheses that PBM reduced cellular apoptosis, reduced microglial activation and neuroinflammation, attenuated neuronal degeneration, promoted neurogenesis/synaptogenesis and modulated metabolism. Precise radiometry reporting in the literature for the purposes of comparability between studies is encouraged for future work. Whilst clinical data in acute TBI is limited to a single study, a considered approach toward study protocols should be taken in order to ensure that further clinical study is utilising optimal parameters and is conducted to a high standard.

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Authorship confirmation statement

All authors have read and approved the full version of this article. AS was responsible for conceptualizatation and manuscript preparation. AS and ZA performed the searches, risk of bias assessments, extracted the data from the included articles and performed the analysis. All authors contributed to the development of the ideas/writing/final review of the submitted article.

Conflict of interest statement

The research group has submitted a patent pending application relating to the invasive delivery of PBM (UK Patent Application No 2006201.4).

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Table 1. Summary table of included studies. BINT = blast-induced neurotrauma; CCI = controlled cortical impact; CW = continuous wave; LFP = lateral fluid percussion; OD = once a day; BD = twice a day; PW = pulsed wave; WD = weight drop. * denotes where a protocol has been used but irradiance parameters have not been presented.

	Species	*TBI Model*	*Severity*	*Wavelength (nm)*	*Site of administration*	*Mode*	*Laser/LED*	*Average irradiance (mW/cm2)*	*Cortical irradiance (mW/cm2)*	*Exposure time (min)*	*Energy (J/cm2) per dose*	*Cortical surface energy (J/cm2) per dose*	*First dose post-injury (hours)*	*Total doses (regime)*
Ando et al., 2011[38]	Mouse	CCI	Severe	810	Scalp (over craniotomy)	CW, 10 Hz, 100 Hz	Laser	50	3-7.5	12	36	*	4	1
Dong et al., 2015[39]	Mouse	Scalp impact	Moderate	810	Scalp	10 Hz	Laser	150	8.9	4	36	1.8 to 2.5	4	1
Esenaliev et al., 2018[40]	Rat	Closed BINT	Mild to moderate	808	Scalp	NPLT (20Hz)	Laser	*	*	5	*	300	1	1
Giacci et al., 2014[41]	Rat	Open LFP	Mild	670, 830	Scalp (over craniotomy)	CW	LED	*	*	30	28.4 / 22.5	*	0	7 (OD)
Khuman et al., 2012[42]	Mouse	CCI	Not stated	800	Cortex or scalp over craniotomy	CW	Laser	250, 500, 1000	*, 250, 500, 1000	2, 7	30, 60, 105, 120, 210	*, 30, 60, 105, 120, 210	1 or 4	1
Oron et al., 2007[43]	Mouse	Skull WD	Mild	808	Skull	CW	Laser	*	10 or 20	2	*	1.2-2.4 J/cm2	4	1
Oron et al., 2012[44]	Mouse	Skull WD	Mild to moderate	808	Skull	CW, 100 Hz, 600 Hz	Laser	21	10	2	2.52	1.2	4, 6 or 8	1
Quirk et al., 2012[45]	Rat	CCI	Moderate	670	Scalp (over craniotomy)	CW	LED	50	*	5	15	*	Not stated	6 or 20 (BD)
Shemesh et al., 2022[46]	Mouse	Scalp WD	Severe	810	Cortex	CW	Laser	50	50	15	45	45	0.16	1
Wu et al., 2010[47]	Mouse	Skull WD	Moderate to severe	660, 810, 980	Scalp	CW	Laser	150	*	4	36	*	4	1
Wu et al., 2012[48]	Mouse	Skull WD	Moderate to severe	665, 730, 810, 980	Scalp	CW	Laser	150	*	4	36	*	4	1
Xuan et al., 2013[49]	Mouse	CCI	Severe	810	Scalp (over craniotomy)	CW	Laser	25	*	12	18	*	4	1/3/14 (OD)
Xuan et al., 2014[51]	Mouse	CCI	Moderate to severe	810	Scalp (over craniotomy)	CW	Laser	25	*	12	18	*	4	1/3 (OD)
Xuan et al., 2015[50]	Mouse	CCI	Severe	810	Scalp (over craniotomy)	CW	Laser	50	*	12	36	*	4	1/3 (OD)
Xuan et al., 2016[52]	Mouse	CCI	Severe	810	Scalp (over craniotomy)	CW	Laser	25	*	12	18	*	4	3/14 (OD)
Yang et al., 2020[53]	Rat	Skull WD (repeated)	Mild	808	Scalp	CW	Laser	350	25	2	*	*	2	15 (OD)
Zhang et al., 2014[54]	Mouse (X-1 KO)	Scalp impact	Mild	810	Scalp	10 Hz	Laser	150	Not stated	4	36	1.8-2.5	4	1

Table 2. Dosing regimes of studies and sub-studies included in meta-analyses. Scalp* denotes scalp route of administration with underlying craniotomy. # = data not available, cortical irradiance is calculated from available data, or extrapolated from available data using transmittance penetration data (Ando et al., 2011 where λ = 800-810 nm). * data not available and incalculable. CW = continuous wave; PW = pulsed wave; NSS = neurological severity score.

	f	λ (nm)	Average irradiance (mW/cm^2)	Average Irradiance (cortex) (mW/cm^2)	Exposure time (minutes)	Administration	First dose post-injury (hours)	Doses	Brief results of intervention versus control (p value where available)
Oron et al., 2007[43]	CW	808	10 or 20	0.899	2	Skull	4	1	Improved NSS at 28 days ($p < 0.05$)
Wu et al., 2010 (a)[47]	CW	665	150	*	4	Scalp*	4	1	Improved NSS at all time points to 28 days ($p < 0.05$) and lesion size ($p < 0.05$)
Wu et al., 2010 (b)[47]	CW	810	150	9.39	4	Scalp*	4	1	Improved NSS at all time points to 28 days ($p < 0.05$) and lesion size ($p < 0.01$)
Wu et al., 2010 (c)[47]	CW	980	150	9.39	4	Scalp*	4	1	No effect on NSS at 28 days
Ando et al., 2011 (a)	CW	810	50	5.25	12	Scalp*	4	1	Moderately effective for improvement of NSS at 28 days
Ando et al., 2011 (b)	10 Hz	810	50	5.25	12	Scalp*	4	1	Most effective for improvement of NSS at 28 days ($p < 0.001$) ,and brain lesion volume ($p < 0.01$)
Ando et al., 2011 (c)	100 Hz	810	50	5.25	12	Scalp*	4	1	Moderately effective for improvement of NSS at 28 days
Khuman et al., 2012 (a)[42]	CW	800	500	0.5	2	Cortex	1	1	No effect on lesion size at 14 days ($p = 0.12$)
Oron et al., 2012 (a)[44]	CW	808	21	10	2	Skull	6	1	Effective for improvement of NSS at 28 and 56 days ($p < 0.05$)
Oron et al., 2012 (b)[44]	CW	808	21	10	2	Skull	8	1	No effect on NSS at 56 days
Oron et al., 2012 (c)[44]	CW	808	21	10	2	Skull	4	1	Effective for improvement of NSS at 56 days ($p < 0.001$) ,and brain lesion volume ($p < 0.01$)
Oron et al., 2012 (d)[44]	100 Hz	808	21	10	2	Skull	4	1	Effective for improvement of NSS at 56 days ($p < 0.001$) ,and brain lesion volume ($p < 0.01$)
Oron et al., 2012 (e)[44]	600 Hz	808	21	10	2	Skull	4	1	Effective for improvement of NSS at 56 days ($p < 0.001$)
Wu et al., 2012 (a)[9]	CW	665	150	*9.39	4	Scalp	4	1	Effective for improvement of NSS at 28 days ($p < 0.05$)
Wu et al., 2012 (b)[9]	CW	730	150	*9.39	4	Scalp	4	1	No effect on NSS at 28 days
Wu et al., 2012 (c)[9]	CW	810	150	9.39	4	Scalp	4	1	Effective for improvement of NSS at 28 days ($p < 0.001$)
Wu et al., 2012 (d)[9]	CW	980	150	9.39	4	Scalp	4	1	No effect on NSS at 28 days
Xuan et al., 2013 (a)[49]	CW	810	25	2.63	12	Scalp*	4	1	No effect on NSS or lesion size at 28 days
Xuan et al., 2013 (b)[49]	CW	810	25	2.63	12	Scalp*	4	3	Effective for improvement of NSS at 28 days ($p < 0.001$) and lesion size ($p < 0.01$)
Xuan et al., 2013 (c)[49]	CW	810	25	2.63	12	Scalp*	4	14	Effective for improvement lesion size at 28 days ($p < 0.01$)
Giacci et al., 2014 (a)[41]	CW	670	0.02	*<1	30	Scalp*	0	7	No effect on lesion size at 7 days
Giacci et al., 2014 (b)[41]	CW	830	0.01	*<1	30	Scalp*	0	7	No effect on lesion size at 7 days
Xuan et al., 2015 (a)[50]	CW	810	50	3.13	12	Scalp*	4	1	Effective for improvement of NSS at 28 days ($p < 0.05$)
Xuan et al., 2015 (b)[50]	CW	810	50	3.13	12	Scalp*	4	3	Effective for improvement of NSS at 28 days ($p < 0.001$)
Zhang et al., 2014[54]	10Hz	810	150	8.85	4	Scalp	4	1	NSS at day 7 significantly reduced ($p < 0.001$)
Xuan et al., 2016 (a)[52]	CW	810	25	2.63	12	Scalp*	4	3	Improved NSS versus control and versus 14 day treatment duration from week 2 to week 8 ($p < 0.001$) post-injury
Xuan et al., 2016 (b)[52]	CW	810	25	2.63	12	Scalp*	4	14	Improved NSS versus control at week 7 ($p < 0.01$) and week 8 ($p < 0.001$) post-injury
Yang et al., 2020[53]	CW	808	350	25	2	Scalp	2	15	Improved NSS throughout protocol ($p < 0.05$)

Table 3. Subgroup analyses for endpoint neurological severity score. Subgroups stratified from those in Figure 2 and Table 1. NSS at 28 days with exception of Yang et al., 2020, Zhang et al., 2014[54] (both 14 days), Oron et al (c-e) (27 days)[44]. CW = continuous wave; PW = pulsed wave, * = heterogeneity not applicable due to small sample size. Energy per dose at cortical surface is calculated where not reported by respective study as described in Table 2 caption.

Variable (n interventional animals)	Effect size [95% CI]	p-value	I^2 for heterogeneity (p value)
Mode			
CW (220)	-1.31[-1.88, -0.73]	**< 0.00001**	82% (<0.00001)
PW (42)	-2.94 [-4.48, -1.39]	**0.0002**	79% (0.0006)
Wavelength			
660-670 nm (18)	-1.19 [-1.91, -0.47]	**0.001**	0% (0.98)
730 nm (10)	-0.11 [-0.99, 0.77]	0.81	*
800-815 nm (216)	-1.93 [-2.59, -1.26]	**< 0.00001**	83% (<0.00001)
980 nm (18)	0.06 [-0.59, 0.72]	0.85	0% (0.86)
First dose timing post-injury			
≤ 4 hours (249)	-1.60 [-2.18, -1.02]	**< 0.00001**	83% (<0.00001)
> 4 hours (13)	-1.20 [-3.22, 0.81]	0.24	79% (0.03)
Number of treatments			
≤3 (217)	-1.81 [-2.36, -1.27]	**< 0.00001**	77% (<0.00001)
>3 (45)	0.31 [-1.18, 1.80]	0.69	88% (0.0003)
Energy per dose at cortical surface			
$0 < E \leq 1$ (J/cm^2) (16)	-2.75 [-3.95, -1.55]	**< 0.00001**	*
$1 < E \leq 2$ (J/cm^2) (101)	-2.77 [-4.33, -1.21]	**0.0005**	90% (<0.00001)
$2 < E \leq 3$ (J/cm^2) (95)	-1.12 [-1.68, -0.55]	**0.0001**	67% (0.001)
$E > 3$ (J/cm^2) (50)	-1.36 [-1.90, -0.83]	**< 0.00001**	26% (< 0.00001)

Table 4. Risk of bias assessment for human clinical studies, based on Cochrane RoB tool[37].

	Randomization	Deviations from intended interventions	Missing outcome data	Measurement of outcome	Selective reporting	Overall
Figueiro Longo et al., 2020[31]	Low	High	Low	Some concerns	Low	Fair

Figure 1. PRISMA flow chart of systematic review process

Figure 2. Forest plot of end neurological severity score (standardised mean difference). NSS at 28 days with exception of Yang et al., 2020, Zhang et al 2014[54] (both 14 days), Oron et al (c-e) [44] (27 days). Control total value includes duplicates from varying test conditions compared with single control group.

Figure 3. Forest plot of lesion size (histologically or radiologically determined). Values at 28 days post-injury with exception of Oron et al., 2012 (56 days) [44], Khuman et al., 2012[42] (14 days) and Giacci et al., 2014 (7 days). Control total value includes duplicates from varying test conditions compared with single control group.

Figure 4. Risk of bias analysis for the included studies using the SYRCLE tool.