Local insulin for wound healing:

a Systematic Review and Bayesian Network Meta-analysis

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Author's contributions: JRG and MMJ conceptualized and designed the study. AB developed and conducted the search strategy. JRG, KRB, and MMJ performed the text screening. KRB, SCW, and GKB performed the risk of bias analysis. JRG, KRB, SKM, and MMJ performed the data abstraction. JRG performed the statistical analysis of the data. GKB and MMJ provided supervision of the project. All authors contributed to manuscript writing and editing.

ABSTRACT

Background: Small clinical trials have demonstrated that local insulin promotes wound healing through pro-angiogenic effects, immunoregulation, cellular recruitment and differentiation. However, because of the trials' small sample sizes and safety concerns over the use of insulin for non-FDA approved uses, its widespread acceptance is limited. The aim of this systematic review and network meta-analysis (NMA) was to assess the safety and relative effectiveness of the use of local insulin application in wounds using a Bayesian approach.

Methods: Medline, CENTRAL, EMBASE, Scopus, LILACS, ClinicalTrials.gov, and grey literature sources were searched for human studies assesing the local use of insulin vs. any comparator for wound healing since inception to October 2020. Data on safety, wound characteristics, treatment characteristics, and outcomes was extracted. An NMA was conducted on safety, clinical and histological outcomes. The study protocol is registered in PROSPERO (CRD42020169119).

Results: A total of 949 reports were found, of which 23 were included in the NMA, totaling 1,240 patients. The studies evaluated six different therapies, and most comparisons were against placebo. Meta-analyses showed a standardized-mean difference of -1.8 mg/dL blood glucose change with insulin treatment and a lack of reported adverse events. Statistically significant clinical outcomes identified include reduction in wound size, increased healing rate, lower PUSH scores, fewer days to attain complete closure, and increased odds for complete wound closure with insulin use. Likewise, significantly increased neo-angiogenesis and granulation tissue were also found.

Conclusion: Local insulin is a highly effective, low-cost, and safe alternative for treating wounds in diabetic and non-diabetic patients.

Keywords: Insulin, wounds, network meta-analysis, wound healing, neo-angiogenesis

1. INTRODUCTION

Wounds are a significant health issue, affecting over 8.2 million individuals in North America alone [1]. Acute wounds account for an excess of 17.2 million hospital visits per annum [2], and it is estimated that over 6.5 million people live with a chronic wound [3]. A recent study calculated annual expenses for treating wounds in USD 35.8 billion, with most costs linked to the outpatient setting, particularly among post-surgical patients and the diabetic [4]. For these reasons, research on reliable and safe strategies to promote wound repair is a pressing need.

Insulin is a hormone that has been demonstrated to enhance the wound healing potential both *in vitro* and *in vivo* [5]. Insulin use is associated with immunoregulation and reduction of inflammation [6,7], keratinocyte and fibroblast migration and differentiation [8,9], as well as angiogenic effects [10,11]. Additionally, it has demonstrated systemic effects by shifting a wound's catabolic environment into anabolism, thereby promoting its closure [12]. Individual randomized clinical trials (RCTs) have found consistent effects in diabetic [13,14] and non-diabetic subjects [15,16] for promoting wound closure and a lack of significant adverse events. Nonetheless, because insulin use has the potential drawback of causing hypoglycemia and hypokalemia, these trials have included a limited number of participants. Therefore, the absence of solid conclusions, particularly about its safety, has prevented the widespread acceptance of insulin as a therapy to promote wound healing.

A previous meta-analysis of 8 studies failed to identify significant effects for wound healing, except for decreasing the wound area [17]. However, the study's search strategy was not exhaustive, and its methodology presents several limitations, mainly including only studies where insulin was compared to placebo and thus failing to include any studies where more than two treatments were compared. Because several new clinical trials exploring the local use of

insulin to promote wound healing have been published since the meta-analysis done by Sridharan and Sivaramakrishnan [17], and to date, no study has analyzed the pooled-aggregates of insulin safety, the aim of this systematic review and network meta-analysis of RCTs was to assess the safety and relative effectiveness of the use of local insulin application in wounds using a Bayesian approach.

2. METHODS

The Preferred Reporting Items for Systematic Review and Meta-Analysis for Network Meta-Analyses (PRISMA-NMA) statement [18] was followed as a reference protocol standard. A PRISMA-NMA Protocol checklist is included as Supplemental material.

2.1 Search Strategy and Selection Criteria

The following databases were searched for relevant studies in October 2020: Medline (via Ovid 1946 to October 15, 2020; via PubMed, 2020/10/03 to 2020/12/31); The Cochrane CENTRAL Register of Controlled Trials (via Wiley, from Inception to October 2020); Embase (via Ovid 1947 to October 2020), Scopus (via Elsevier); Latin American and Caribbean Health Sciences Literature (LILACS) (via Global Index Medicus). Additionally, all database searches were rerun on April 2021.

The search strategies designed by an experienced librarian (AB) used subject headings and text words to identify RCTs evaluating the outcomes of local insulin therapy for wound healing. The Medline strategy was applied to all databases, with modifications to search terms as necessary.

No language limits were applied. Search strategies were peer-reviewed by a second librarian. All search strategies can be consulted in the Supplementary material.

In addition, a clinical trials registry (ClinicalTrials.gov) and grey literature sources (Google Scholar, Proquest Dissertations & Theses Global) were searched in December 2020 and April 2021, respectively). Finally, a citation search was performed in Scopus and Web of Science to identify records from the reference lists and citing papers of included studies (December 2020). No changes to the original search stategies were required. For all database records, deduplication was performed by the librarian with the bilbiographic management software EndNote [19]. Deduplication settings were modified for each of the twelve rounds of iterative duplicate identification, followed by a manual verification for duplicates. Screening was performed with the online software Rayyan QCRI [20].

2.2 Eligibility Criteria

Included studies were based on the following eligibility criteria:

Population: Studies of children and adults with acute or chronic wounds were included. We applied no age or sex restrictions.

Interventions and Comparator: Studies comparing the local (i.e. locally injected or topical) use of any insulin formulation for wound healing and any comparator, including placebo, were included.

Outcomes: The primary outcomes are 1) safety outcomes, including change in glucose levels and presence of adverse events, 2) clinical effectiveness outcomes, including change in wound size,

the healing rate of wounds, the proportion of fully closed wounds, change in clinical scores of wound healing (i.e. PUSH score), and required time for the complete healing of the wound, and 3) histological effectiveness outcomes, including change in blood vessel density and area of granulation tissue.

2.3 Study Design

RCTs and non-randomized controlled trials (quasi-experimental) of any length duration were included in the study.

2.4 Screening and Extraction Process

Titles and abstracts were screened independently in duplicate. Any citation identified by either of the screeners as potentially relevant was advanced to full-text review. Two independent investigators (JRG and KRB) then screened full texts for inclusion; any discrepancies were resolved by discussion between the two screeners and, when necessary, with a senior member of the review team (GKB or MMJ). All data abstraction was performed in pairs (2 of JRG, KRB, SKM or MMJ).

The wound type was categorized as either acute (i.e. burns, post-surgical wounds) or chronic (i.e. diabetic foot ulcers, pressure ulcers) [21]. Whether the study's population consisted of diabetic or non-diabetic patients was also categorized. The type of insulin used was categorized as short-acting or intermediate/long-acting [22]. For each outcome, data was extracted from the baseline and from the last reported time point when available. For effectiveness outcomes, the relevant data

included the mean change from baseline and a measure of variance. Whenever possible, additional outcomes were calculated based on the data provided (i.e. healing rate in mm^2/day from change in wound size). If results were available only in figures with exact values not given, we used the DigitizeIt (CERN - European Organization for Nuclear Research, 2015) software to estimate the values. Two reviewers (JRG and MMJ) derived estimates independently, and we used the mean of their estimates. A summary of the included papers and the data extracted from them is available in Table 1.

2.5 Risk of Bias

The risk of bias (RoB) was assessed using the R.o.B. 2.0 tool [23] by two independent investigators (KRB and SCW) and visualized with the Robvis tool [24]. Discrepancies were resolved by discussion with a senior member of the team (GKB). None of the investigators assessing RoB are listed as co-authors of included studies.

2.6 Study Protocol

The research plan was developed by experts in wound care, patients with chronic wounds, methodologists, and an information specialist. We registered a protocol in PROSPERO (CRD42020169119), and it was published in full before data collection.

2.7 Statistical Analysis

A network meta-analysis was performed for each outcome using a random-effects model within a Bayesian framework using the *gemtc* package in R, version 4.0.1 (R Foundation for Statistical Computing, 2020). For continuous outcomes, the generated model corresponds to a generalized linear model with an identity link and for binary outcomes, to a generalized linear model with a logit link. Random effects on the treatment parameters were included in the models, which allows each study to have a different but related treatment effect. Because of heterogeneity values over 90% reported in the previous meta-analysis by Sridharan and Sivaramakrishnan [17], non-informative prior distributions for effectiveness model parameters were used. Convergence of chains was assessed using the Gelman-Rubin statistic and by visual inspection of trace plots. Consistency was assessed by comparing the direct and indirect evidence by using a node-splitting approach. Finally, network plots for each analysis were generated. Subgroup analyses were then done separating trials in diabetic vs. non-diabetic patients, acute vs. chronic wounds, and short-acting vs. intermediate/long-acting insulin formulations. Summary results are presented as the summary of the mean difference (SMD) or odds ratio (OR) with their 95% credible intervals (95% CrIs).

3. RESULTS

3.1 Included Studies

Our searches yielded 974 reports. After the removal of duplicates, 682 records were screened, and from them, 36 full-text articles were assessed for eligibility. Ultimately, 25 studies were included in the systematic review and 23 in the meta-analysis, which corresponds to 1,240 patients (Figure 1). The included studies evaluated six different therapies, and most comparisons

were against placebo. The mean sample size per group was 21 (range, 5 to 55). Fourteen (61%) trials were done on diabetic patients, 19 (82%) were done on chronic wounds, and the mean number of days of insulin application was 14 (range 1 to 84). The mean insulin dose used was 10 U per 10 cm^2 of wound area (range 0.1 to 30). Two manuscripts contained multiple trials [25,26], which were analyzed separately, and one study was reported exclusively in a trial registry, for which we obtained unpublished data in personal communication [27]. In almost all the cases, the last reported time point corresponded to the number of days of insulin administration (median of 14 days, range 6 to 84 days) (Table 1).

RoB analysis showed 5 (22%) trials with a low risk of bias, 11 (48%) with some concerns, and 8 (30%) with a high risk of bias (Supplementary material). The primary sources of identified bias were lack of randomization or blinding of studies and the absence of trial registration. However, no significant differences in interest outcomes were found when comparing studies at low/medium vs. high risk of bias.

Each included study contained data on at least one outcome of interest (safety or effectiveness). No included study had data on all outcomes. Treatment network comparisons include data ranging from 4 to 15 trials. Treatment network graphs for all outcomes are in Figure 2.

3.2 Safety of Local Insulin Therapy

Data from 9 trials (8 two-arm and 1 multi-arm, total n = 539) comparing insulin vs. placebo was extracted [14,16,27–33]. As shown in Figure 3, except for high insulin doses, the mean change in blood glucose was not different from placebo. Insulin treatment induced an SMD of -1.8 (95%CrI -8.2 to 3.2) mg/dL blood glucose change. No significant differences were found when

comparing non-diabetic vs. diabetic subjects (SMD 0.68, 95%CrI -11.2 to 10.49), or short vs. intermediate/long-acting insulin types (SMD 1.34, 95%CrI -10.3 to 12.0).

A total of 18 (76%) of the included studies mentioned recording of adverse events [14,16,26–29,31–42]; however, "no adverse events were recorded" was reported as the norm. The absence of nausea, light-headedness, or drowsiness was reported in most trials. The absence of pain or infection of the wound site after injection of the insulin was reported in 4 trials [27,31,35,39]. The only reported adverse event was the presence of white crystal deposit in the wounds after administration of insulin, compatible with zinc deposits [43]. No trial reported hypoglycemic events. Given the absence of reported adverse events, robust and interpretable relative safety estimates were impossible to estimate.

3.3 Clinical Effectiveness Outcomes

Change in wound size as percentage of the wound was extracted from 11 trials (10 two-arm and 1 multi-arm, total n = 738) [27,29,32,34,36,37,40–42,44,45]. Insulin treatment reduced the wound size by SMD 27.0% (95%CrI 19.0 to 36.2). Medium and high insulin doses and no treatment were not different from placebo. A reduction in wound size of SMD 2.5 cm^2 (95%CrI 1.1 to 4.3) compared with placebo was found from data of 9 2-arm studies (total n = 558) [27,29,36,37,40–42,44,45]. The healing rate of wounds was extracted from 15 trials (14 2-arm and 1 3-arm, total n = 784) [16,26–28,34,36,37,40–46]. Compared with placebo, insulin treatment increased the healing rate by SMD 23.0 mm/day (95%CrI 16 to 32). Zinc treatment was not different from placebo. Two two-arm studies (total n = 110) reported data on changes in PUSH score for pressure ulcers [33,37]. Compared with placebo, insulin treatment led to a reduction of 2.7 (95% CrI 0.5 to 5) points in

the score. The number of days required for complete closure of the wound was also reduced with insulin therapy compared with placebo. Data from 11 trials (8 2-arm, 2 3-arm, and 1 multi-arm, total n = 437) [14,16,25,26,32,34,36,39,47] showed a reduction of 10 days (95%CrI 3.5 to 17). Finally, the OR of achieving complete closure of the wound was significantly improved with insulin therapy compared with placebo. Data from 3 trials (2 2-arm and 1 3-arm) [36,39,47] showed an OR of 20.0 (95%CrI 2.7 to 330). No significant differences vs. placebo were found for medium doses of insulin (Figure 4).

Except for the healing rate and time to achieve complete closure of the wound, subgroup analyses did not show statistically significant differences for the quantitative outcomes in non-diabetic vs. diabetic subjects and between acute vs. chronic wounds. In non-diabetic patients, insulin use showed a higher rate of healing (SMD 9.44 mm/day, 95%CrI 4.5 to 14.5) compared to diabetic patients. Likewise, non-diabetic patients required an SMD 2.8 (95%CrI 0.7 to 6.6) fewer days to achieve complete closure of the wound than those with diabetes. The use of insulin in acute wounds led to an increased reduction of SMD 14 (95%CRI 1.4 to 25) days to achieve complete closure of the wound, compared to chronic wounds. Finally, compared to shorter-acting formulations, intermediate and long-acting insulin led to smaller wound size, both in percentage (SMD -10.9%, 95%CrI -15.2 to –6.7) and area measurements (SMD -2.1 cm^2, 95%CrI -3.8 to -0.5), as well as fewer days required to achieve complete closure rate (SMD -1.5 days, 95%CrI –2.7 to -0.3).

3.4 Histological Effectiveness Outcomes

Change in blood vessel density was extracted from 4 two-arm trials (total n = 84) [27,31,35,39]. Compared with placebo, insulin treatment increased blood vessel density by SMD 30 /mm^2 (95%CrI 1 to 68). Likewise, change in granulation tissue area was increased with insulin treatment. Data from 5 2-arm trials (total n = 190) [14,27,31,35,41] showed an increase of 25% (95%CrI 8.8 to 39.8) (Figure 5). No differences were observed between non-diabetic vs. diabetic subjects. However, compared to chronic wounds, acute wounds showed an increase in granulation tissue of 8.9% (95%CrI 0.4 to 18.4). Finally, compared to shorter-acting formulations, intermediate and long-acting insulin formulations also increased granulation tissue area by SMD 7.7% (95%CrI 0.4 to 13.9).

4. DISCUSSION

Wound healing is achieved through the interaction of multiple neuro-humoral mechanisms that govern the interplay between the skin and its precursor cells with the immune and vascular systems [48,49]. Thus, it is a complex series of reactions and interactions among cells and mediators, some of which can be supplemented exogenously [50,51]. Our meta-analysis results show that insulin is a potent anabolic hormone that promotes wound regeneration of diabetic and non-diabetic individuals by promoting neo-angiogenesis and increased production of granulation tissue, which in turn accelerates the rate of wound healing and reduces the number of days required to achieve complete healing (Figure 6). Moreover, the safe use of local insulin to promote wound healing is hinted by our results, as no significant blood glucose changes were identified in the meta-analysis of the included studies, even for non-diabetic subjects. However, caution interpreting the results must be taken due to the scarcity of other adverse events reporting. Mention of a severe hypoglycaemic event is only reported as a case report by Coid [52];

however, the patient in question, a 59-year-old woman with severe multiple sclerosis and a sacral ulcer, received a single dose of over 80 UI of regular insulin.

Human insulin is synthesized as a single-chain precursor that suffers several post-translational modifications that include cleavage and folding to finally be stored as an A-chain comprised of 21 amino acid residues linked to a 30 residue B-chain by two disulphide bonds. Once assembled, mature insulin is naturally crystallized and stored in β-cells through its interaction with zinc complexes [53]. Mimicking this, the pharmacological-grade insulin is produced by recombinant technologies and crystallized at acidic pH through the addition of zinc salts. The degree of crystallization and assembly of insulin monomers into more complex oligomers is then considered the rate-limiting step for the bioavailability of the insulin formulations, therefore prolonging its half-lives [54]. While studies on diabetic patients have demonstrated that longer-acting insulin forms exhibit lower risk for hypoglycaemic events compared to basal insulin analogs [55], in our meta-analysis, we did not find significant differences in the drop of blood glucose in studies using short vs. medium or long-acting analogs. However, the latter formulations' use led to improved clinical and histological outcomes, namely reduction in wound area, fewer days to heal, and increased granulation tissue. We believe these differences may be explained by the increased bioavailability of the drug in the wound bed, which caused sustained activation of the insulin receptor in the target cells. Taken together, these results favour the use of the longer types of insulin formulations for future studies and in clinical practice.

In contrast to the results reported by Sridharan and Sivaramakrishnan [17] that showed a lack of effect of insulin to increase the wound healing rate, the number of blood vessels or the area of granulation tissue, all of our outcome measurements showed significant differences favouring the use of insulin compared to placebo. We believe these differences are due to the higher number of

articles included in our study (22 vs. 7), the inclusion of studies that tested more than two treatment arms, the calculation of novel outcome measures from the reported data, and the use of Bayesian statistics rather than probabilistic ones. Our study's limitations include a wide heterogeneity in the design of the included studies, particularly those regarding the dose of insulin, how it was administered, and the number of treatment days. However, we believe this heterogeneity suggests an even stronger effect of insulin to promote wound healing than observed. Even doses as low as 0.1 UI and short duration treatments led to increased wound healing rates. For the widespread use of insulin to promote wound healing, we recommend using the median dose reported in the studies, which is 10 UI per 10 cm^2 of wound area. Higher doses, particularly those used by Wang et al. [39] and Zeng et al. [32], were associated with worse healing outcomes. The authors of these papers attribute this paradoxical effect to immune dysregulation of the wound micro-environment; however, further studies are needed to sustain this claim. Due to significant heterogeneity in the study design, it was impossible to determine the best duration of the treatment. However, based on our clinical experience and research [27,31,35], a minimum of 10 to 15 days of treatment are recommended. Interestingly, our results suggest that insulin administration either as a topical spray/soaked gauzes or injected into the wound bed produces similar outcomes. Therefore, we recommend its topical use whenever possible to avoid pain and infection issues with parenteral doses. A summary of the recommendations and a treatment algorithm based on them is presented in Figure 7.

The only active comparator that we identified through our search strategy was zinc chloride [25,43]. Beyond its use as a stabilizer of the insulin monomers, zinc salts have also demonstrated to help modulte wound healing and are a required co-factor for a number of metaloproteinases [56]. Zinc deficiency has been found to be associated with delayed wound healing [57,58], increased

inflammation [59], and impaired debris and bacterial wound clearance [60]. In contrast, topical zinc application has been shown to promote wound granulation and epithelization [61–63]. The results of our NMA do not show significant differences for zinc vs. insulin or zinc vs. placebo to improve healing outcomes. Nonetheless, because only two trials used zinc as an active comparator, the effects of zinc to promote healing were most likely underpowered and as such, are yet to be determined. Despite our extensive search strategy, we did not identify any trials comparing insulin to growth factors or bioactive peptides (i.e. β-TGF, VEGF, etc.). This represents an interesting gap in the literature that future studies need to address. If found to be equivalent to growth factor supplementation, topical insulin may have the potential to offer a low-cost alternative for tissue engineering. Finally, another potential limitation to our results is the heterogeneity in how wound size measurements were collected over the different studies. Transparent films (n = 8, 36%), rulers (n = 8, 36%), and planimetry photograph (n = 6, 28%) were used in the studies included in our analysis. Because all of them are manual methods, bias and variability in measuring the outcomes cannot be ruled out. For future studies and clinical practice, we recommend using automated digital methods that use fiducial markers to increase the consistency of results [64,65]. Nonetheless, the study's identified limitations were controlled through sub-group analyses, finding no differences between the trials according to the risk of bias score. Therefore, despite these concerns, our summary of state of the art on the use of insulin to promote wound healing should be useful to stakeholders, including patients, clinicians, guideline developers, and health technology assessors.

In conclusion, in this meta-analysis of 23 studies and 1,180 patients, the local use of insulin to promote wound healing is a safe and effective alternative to promote wound healing. Namely, an increase in the neo-angiogenesis and granulation of the wound bed, which in turn leads to

increased healing rates, reduction in the wound size, fewer days required to heal, increased odds of attaining complete closure rate and lower PUSH scores. While the absence of adverse events cannot be entirely ruled out, our result strongly suggests that if present, they are mild. Furthermore, we demonstrate that the local use of insulin does not cause significant blood glucose changes. For these reasons, we believe insulin is a highly effective, low-cost, and safe alternative for treating wounds in diabetic and non-diabetic patients. However, in our opinion, its use should be focused on those wounds at high-risk of non-healing and for those in which other mainstream strategies have already failed.

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Figure 1. Study selection process

A total of 23 articles were selected based in the inclusion and exclusion criteria. Two of them included multiple trials, which were analyzed by separate. Thus, a total of 26 reports are included in the meta-analysis.

Figure 2. Network graphs of studies included in the clinical and histological effectiveness meta-analysis

The width of each line connecting two treatment nodes is proportional to the number of head-to-head trials for that comparison.

Figure 3. Network graph and forest plot of change in blood glucose concentration

The width of each line connecting two treatment nodes in the network graph is proportional to the number of head-to-head trials for that comparison. In the forest plot, each treatment is compared with placebo. Except for high insulin dose, no significant differences in blood glucose level before and after treatment administration were found. 95% CrI: 95% credibility interval.

Figure 4. Forest plots of clinical effectiveness outcomes

In all cases, each treatment is compared with placebo. Except for complete wound closure, which is represented as the odds ratio (OR) of achieving this outcome, data is presented as change in the standardized mean difference (SMD) scale. For change in wound size, days to heal, and change in PUSH score, negative values represent improvement over placebo. For healing rate, positive values indicate improvement over placebo. 95% CrI: 95% credibility interval.

Figure 5. Forest plots of histological effectiveness outcomes

In all cases, each treatment is compared with placebo. Data is presented as change in the standardized mean difference (SMD) scale. Positive values indicate improvement over placebo. 95% CrI: 95% credibility interval.