European Journal of Pharmaceutical Sciences 104 (2017) 230–239

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Review

The efficacy of probiotics as pharmacological treatment of cutaneous wounds: Meta-analysis of animal studies

Christos G. Tsiouris[a], Martha Kelesi[a], Georgios Vasilopoulos[a], Ioannis Kalemikerakis[a], Effie G. Papageorgiou[b,*]

[a] Department of Nursing, Faculty of Health and Caring Professions, Technological Educational Institution of Athens, Greece
[b] Department of Medical Laboratories, Faculty of Health and Caring Professions, Technological Educational Institution of Athens, Greece

ARTICLE INFO

Keywords:
Probiotics
Wound healing
Cutaneous wound
Meta-analysis
Lactobacillus
Kefir

ABSTRACT

The aim of the current meta-analysis of animal studies was to evaluate the efficacy of probiotics as pharmacological treatment of cutaneous wounds. A systematic electronic literature search was conducted and in total six animal studies which undertake twelve experiments met our inclusion criteria. We used the percentage (%) of wound area at the end of the first week after initial wounding to evaluate the efficacy of the probiotic treatment. The heterogeneity was estimated as statistically significant ($p < 0.0001$) and therefore the meta-analysis was performed with the random-effect model. Based on the estimated Hedges' g (Hedges, 1982), the administration of probiotics was associated with acceleration of the wound contraction ($g = -2.55$; 95%CI = $-3.59, -1.50$; $p < 0.0001$). The meta-regression analysis showed that the moderator sterile kefir extract has the greater effect on the overall estimated efficacy of probiotic treatment ($g = -5.6983$; $p = 0.0442$) with bacteria probiotic therapies (70% kefir gel, *L. brevis*, *L. fermentum*, *L. plantarum*, *L. reuteri*) following ($g = -2.3814$; $p = 0.0003$). For bacteria dose moderator, the results showed that increase in bacterial dose corresponds to increase of the estimated overall effect size ($g = -10.2056$; $p = 0.0053$). The linear regression test of funnel plot asymmetry showed absence of publication bias. In conclusion, the results indicate that probiotics administration is an effective pharmacological treatment of cutaneous wounds. However, due to the heterogeneity among studies, further research is required.

1. Introduction

The majority of epithelial linings of our body, such as the skin and mucosa, are colonized by a great number of microorganisms that constitute the so-called normal microflora. These microorganisms outnumber 10 times the human body cells. Normal microflora is constituted mainly by commensal bacteria. These bacteria cooperatively interact with their host and they are crucial for its health (Tlaskalová-Hogenová et al., 2011; Patel and DuPont, 2015; Cogen et al., 2008).

Wound healing is a natural biological process that can be affected by many moderating factors. Some of them can lead to improper or impaired wound healing and others can improve wound healing and resolve impaired wounds (Guo and DiPietro, 2010). One of the major factors affecting the healing process is the interaction of the wound with the microbial microflora (Bowler et al., 2001).

Microbial colonization occurs in all wounds, chronic or acute. Understanding the correlation between different microbial communities and wound healing capability is an intense area of research (Scales and Huffnagle, 2013). Recent studies, suggest that changes in local cutaneous microflora, as well as alterations in the gastrointestinal tract microflora, can affect positively or negatively the healing process through various ways, especially through the production of antimicrobial molecules and regulation of immune and inflammatory response (Peral et al., 2009; Rahimzadeh et al., 2014; Poutahidis et al., 2013).

Probiotics are live bacteria or yeasts which exert health-promoting effects to the host (Schrezenmeir and de Vrese, 2001). Preclinical and clinical studies emphasize their efficacy in preventing various infectious, immune-mediated and inflammatory diseases (Wong et al., 2013). Probiotics have the ability to balance the gut microflora and improve the gastrointestinal barrier. In addition, they contribute to the reduction of low-density lipoprotein (LDL) levels and total cholesterol levels, and also suppress inflammation and modulate local and systemic immune functions (Wong et al., 2013; Hakansson and Molin, 2011; Wolvers et al., 2010; Jones et al., 2012a).

According to the evidence so far, probiotics can be useful in the prevention and treatment of difficult healing wounds by regulating the

* Corresponding author at: Technological Educational Institution of Athens, Department of Medical Laboratories, Ag Spyridonos, Egaleo, Athens 12243, Greece.
E-mail address: efipapag@teiath.gr (E.G. Papageorgiou).

http://dx.doi.org/10.1016/j.ejps.2017.04.002
Received 6 October 2016; Received in revised form 1 April 2017; Accepted 2 April 2017
Available online 07 April 2017
0928-0987/

interactions between the host and microbes (Wong et al., 2013). More specifically, studies in laboratory animals showed that certain probiotic bacteria can positively affect the wound healing process by topical administration (e.g. *L. brevis*, *L. plantarum* and L. *fermentum*) or *per os* (*L. reuteri*) (Scales and Huffnagle, 2013; Peral et al., 2009; Poutahidis et al., 2013; Zahedi et al., 2011a; Jones et al., 2012b).

Topical application of specific probiotic species leads to strengthening of immune system response, reduction of inflammation and acceleration of wound healing process (Rahimzadeh et al., 2014; Zahedi et al., 2011a; Nasrabadi and Ebrahimi, 2011; Atalan et al., 2003). More specifically, probiotics bacteria produce exopolysaccharides that have immunostimulatory activity and are able to activate macrophages and lymphocytes (Zahedi et al., 2011a; Foligné et al., 2010; Nayak et al., 2010). The lactic acid bacteria that are used as probiotics, as *L. plantarum,* produce*,* apart from exopolysaccharides, also lactic acid, as the major metabolic end-product of carbohydrate fermentation. Lactic acid has antibacterial properties and inhibits the proliferation of pathogenic microorganisms and therefore lactic acid bacteria or probiotic mixtures in which they found, like Kefir, have been tested for their wound healing properties (Nasrabadi and Ebrahimi, 2011a; Nasrabadi and Ebrahimi, 2011b; Sonomoto, and Yokota, A. 2011; Atalan et al., 2003; Rahimzadeh et al., 2015; Huseini et al., 2012; Farnworth, 2006; Farnworth, 1999). Furthermore, a wound healing, nitric oxide gas (gNO)-producing, probiotic patch using lactic acid bacteria in an adhesive gas permeable membrane has been tested for treating ischemic and infected full-thickness dermal wounds in rabbit models and showed increased wound closure (Jones et al., 2012b).

Dietary intake of lactic acid bacteria has been shown to down-regulate host inflammatory responses, confer more rapid progression of inflammatory events during wound healing and compresses the classical wound repair cascade. In addition, ingestion of lactic acid bacteria leads to rapid collagen deposition, which is very important for proper wound repair (Poutahidis et al., 2013).

Impaired wound healing, as in the case of chronic, ischemic or infected wounds, is a major challenge for both health professionals and patients. The use of common antimicrobial agents is becoming constantly more and more ineffective in the treatment of common pathogens infections, as also it contributes to the emergence, dissemination, and evolution of antibiotic resistance (Gorwitz, 2008; Anstead et al., 2007; Nordmann et al., 2007; Linares, 2001; Davies and Davies, 2010). Therefore alternative pharmacological therapies which do not rely on the use of common antimicrobial agents are becoming more and more needed in the wound management (Jones et al., 2012b). Based on the above-mentioned therapeutic effects of probiotics in wound healing process, by either topical application or *per os* administration, their potential use in the treatment of wounds and ulcers should be taken into account.

Here, we report on a meta-analysis of data from controlled *in vivo* studies testing the efficacy of probiotics as a pharmacological treatment of cutaneous wounds in animal models. We further assessed whether probiotic species, gas NO, route of administration, wound infection, ischemia, treatment day, the frequency of administration, initial wound area and microbial dose per wound, affect the efficacy of probiotic therapy. Also, we examine the heterogeneity of published studies that were included in this meta-analysis and assess the presence of publication bias.

2. Methods

2.1. Search strategy

Using prespecified inclusion and exclusion criteria (see below), we identified all publications reporting experiments in laboratory animals that compare the use of probiotics with a control in cutaneous wounds, by searching (from inception to July 2016) two electronic databases (MEDLINE and EMBASE), with search results limited to those indexed as describing animal experiments.

The structured search strategy used the following format of search terms: (probiotic OR commensal microbiota OR microbiome OR symbiotic OR microbial symbionts OR *lactobacillus* OR *Bifidobacterium* OR *lactobacilli* OR *Saccharomyces* OR Bacteriotherapy OR kefir OR kefir products) AND (wound healing OR wound OR cutaneous wound OR wounds OR burn). No language restriction was imposed. In addition, the reference lists of identified studies were manually checked to identify other potentially eligible trials. This process was performed iteratively until no additional articles could be identified.

2.2. Inclusion and exclusion criteria

We included experiments where functional outcome in a group of animals exposed to cutaneous wound and treated topically or *per os* with probiotics was compared with functional outcome in a control group of animals. We excluded individual comparisons that did not report (or where we could not calculate) the number of animals, the mean outcome, or its standard deviation in each group. Also, we excluded duplicate studies and experiments that have repeated data or did not report outcomes associated with the wound surface and the wound contraction.

2.3. Data extraction and outcome measures

Two authors independently extracted the following data from each experiment: first author, year of publication, animal characteristics, number of animals, probiotic group, route of administration, number of wounds in both treated and control groups, coexisting factors such as infection and ischemia that possibly affect the wound healing process, mean outcome, standard deviation in each group, frequency of treatment administration (No. of Adm./treatment days), initial wound area (day 0, WA_0), wound area on the seventh day after induction of wounds (WA_7), microbial dose per wound and the depth of wounds. If the WA_7 was not given by the study, we extracted the wound area on the sixth day after wounding (WA_6) and examined the treatment day as a moderator variable. We convert wound area measures (WA_7 or WA_6) to a percentage (%) of wound area (WA_7% or WA_6%) considering the initial wound area (day 0, WA_0) as 100%.

It is important to note that we extracted wound area at the end of the first week (7th or 6th day) after initial wounding, because at this time the maximum response of cell proliferation and matrix deposition is occurs, while the inflammation phase is nearing its end (Enoch and Price, 2004). Therefore, based on the importance of cellular events of wound healing that are occur within this one week period (Enoch and Price, 2004; Yussof et al., 2012), we used the percentage (%) of wound area at the end of this first week to evaluate the efficacy of probiotics as pharmacological treatment of cutaneous wounds.

Where a publication reported more than one experiment, or where an experiment reported more than one individual comparison, we considered these separately and extracted data for each, correcting the weighting of these studies in the meta-analysis to reflect the number of experimental groups served by each control group.

2.4. Quality assessment

Study quality of individual studies was assessed according to published criteria (Horn et al., 2001; Antonic et al., 2013; Macleod et al., 2004).

These criteria were:

(i) publication in a peer-reviewed journal
(ii) statements describing control of temperature
(iii) randomization to treatment group
(iv) allocation concealment
(v) blinded assessment of outcome