Original Research Article

Healing effects of Quercetin on full thickness epidermal thermal injury in Wistar rats

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A b s t r a c t

There are many difficulties in treatment and management of a thermal injury, especially after topical application of the therapeutic agents. Quercetin is a well known agent, which exhibit antioxidant, anti-inflammatory and angiogenic functions.
This study was carried out to investigate the effect of quercetin on thermal injury healing in a rat model.
Ninety female Wistar rats were used. Animals were inflicted with a reproducible full-thickness burn and randomized into three groups to receive no treatment (control group, CG), local application of a quercetin solution (quercetin group, QG) as well as application of the glyceryl trioctanoate, the solvent used to prepare the solutions (solvent group, SG). The size and healing progress of each wound was recorded and evaluated by means of clinical evaluation, planimetry and histological examination on days 0, 3, 6, 12, 21, and 31.
Even though a significantly accelerated wound healing and faster re-epithelialization was recorded in QG compared to other groups, quercetin application failed to lead to a rapid healing of full-thickness burns.
The use of quercetin could to be an alternative treatment of burn wounds but further research is needed to evaluate the effective doses for speeding up healing time.
Keywords: thermal injury, wound healing, quercetin

Introduction

When skin is exposed to a burn injury, the damage includes cell death and tissue necrosis, oxidative stress with formation of free radicals, inflammation, infection, formation of edema, loss of body fluids [1] and in general, many difficulties in treatment and management of a burn.
Depth of burns defines the healing of the burn wound. The healing process involves the inflammatory response of the body (vascular and cellular response) [2,3] and, in partial thickness burns, re-epithelialization in the form of keratinocyte migration from viable skin appendages in dermis. Finally, angiogenesis and fibrogenesis assist in dermal reconstitution [4].
Several agents have been used for local treatment of burns wounds. Each agent usually targets one or more complications that reveal after a burn injury, or they enhance the process of the burn wound healing.
Quercetin is one of the most widely distributed flavonoids present in fruits and vegetables. Quercetin presents a sum of biological activities including antioxidant effects [5] by scavenging free radicals [6] and anti-inflammatory [7,8] by inhibiting the 5-lipoxygenase pathway of arachidonic acid [9].
Although the *in vitro* anti-infallmatory and antioxidant effects of various flavonoids have been extensively studied, data from *in vivo* studies focusing on the use of flavonoids in the treatment process of burn wound injuries are scarce [10,11].
In our study we evaluated the topical application of quercetin on full thickness epidermal burn wound in Wistar rats. The rate of wound closure was measured for a specific time of the post-burn period as well as macroscopic and histological evaluation was performed to determine the efficacy of quercetin.

Materials and methods

Chemicals and Reagents

Quercetin dihydrate minimum 98% HPLC, (Sigma-Q0125) was dissolved in glyceryl trioctanoate (T9126, Sigma-Aldrich, St. Louis, MO, USA) giving a final concentration of 0.16g/ml. The solutions were kept at 4 C throughout experimentation.

Animal care

Female Wistar rats, 5-month-old, weighing 195-240 g, were used in this study. The animals were caged at controlled room temperature (20±2 C) under 12h/12h of light/darkness conditions and were fed *ad libitum* with rat chow and water. The animals were handled with human care in accordance with the National Institutes of Health guidelines and the European Union directive for the care and the use of laboratory animals (Greek presidential decree No. 160 1991 implementation of the EEC Directive 86/609/EEC) and according to the permission number 20EEP02.

Infliction full thickness epidermal burn wound

A reproducible full-thickness burn was inflicted on the back of each animal with the use of a rectangular steely stamp, 1 cm thick, measuring 4 cm2 of surface area as described previously [12].

Study protocol

The animals were randomly divided into three groups (30 rats each), according to the substance applied to the burn wound: Control group (CG): no dermal application; Solvent group (SG): solvent application (glyceryl trioctanoate); Quercetin group (QG): application of quercetin solution.
The volume of the quercetin solution applied daily on the burn wound, as well as that of the solvent, was set at 0.30ml/4 cm2 of the burn surface area.
Burn wounds size was measured on days (post burn day, PBD) 0, 6, 12, 21, and 31 with the use of a high precision planimeter (HAFF planimeter No 313 – Gebruder HAFF GMBH, Germany) after tracing their borders on a plastic film [12] and photographed with a digital camera (Olympus FE-190, 6.0 Megapixel, 3x Optical Zoom Japan). Total body surface area (TBSA) of the animals was measured as previously described [13].

Macroscopic evaluation

Macroscopic evaluation was performed on days 0, 3, 6, 12, 21, 31 for the following parameters: a. Presence or absence of necrotic eschar (skin sloughing); b. Haemorrhage; and c. Purulent discharge, as an indication of infection. The animals were also monitored for 15 minutes after the treatment application.

Histology

A triangular-shaped specimen centred over the burn wound, (including the whole thickness of the wound, as well as the panniculus carnosus, and extending to the surrounding healthy tissue) was harvested from the sacrificed animals (six animals from each group) on post burn days 3, 6, 12, 21, and 31. The specimens were fixed in 4% neutral buffered formalin, paraffin embedded, cut in 5 m thick sections, and stained with haematoxylin-eosin [12].

Statistical analysis

Data are expressed as mean ±S.D. The statistical significance between data means was determined by Student's t-test and two-way analysis of variance (ANOVA) was used for statistical evaluation of differences between groups (SPSS version 16.0, Statistical Package for the social Sciences software, SPSS, Chicago, USA). P-values $p < 0.05$ were considered as significant.

Results

Clinical - macroscopic findings

No animal deaths were recorded through out the experimental protocol, no severe complications were recorded, and all macroscopic findings are presented in Figure 1.
The main findings of macroscopic evaluation can be summarized into the following: a. On days 0, 3, and 6, macroscopic findings were almost identical in all groups; b. In the course of time, faster fall of burn eschar was noticed in SG and QG as compared to CG and c. In the course of time, there was a higher infection rate of burn wounds in SG compared to QG and CG.
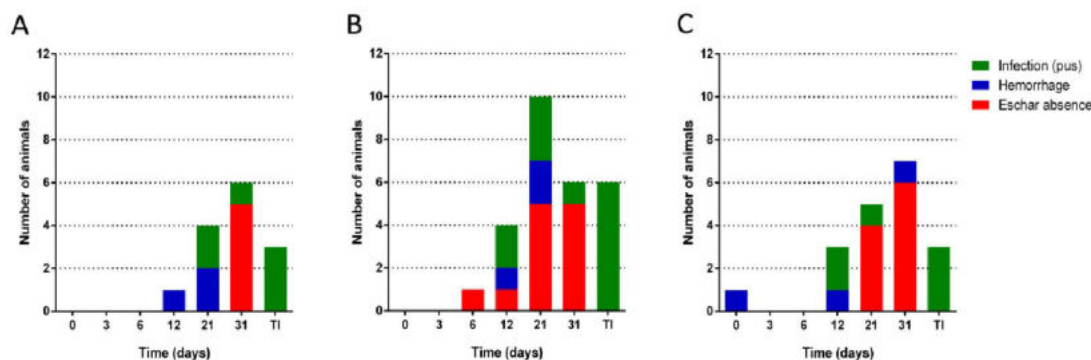

Figure 1. The main findings of macroscopic evaluation. (A), Control group; (B), Solvent group; (C), quercetin group; TI, Total infections

Planimetric evaluation

The burn wound area (4 cm^2) inflicted with a rectangular steely stamp represented in average 1.1% of TBSA of the experimental animal rats used in this study. The results of planimetric evaluation of burn wound area, expressed as (%) of the CG on days 0, 3, 6, 12, 21, and 31 after induction of burn wounds are shown in Figure 2.
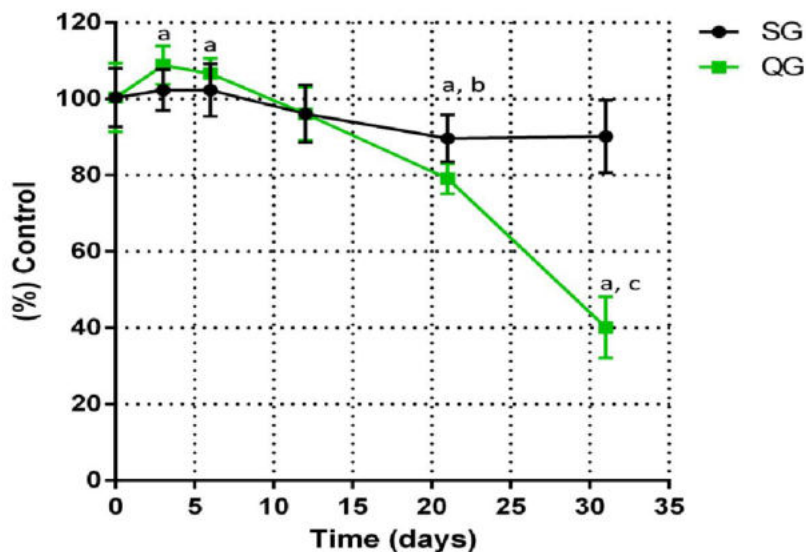
On PBD3 and PBD6, QG presented a significant increase of burn surface area ($p<0.05$). On PBD12 burn surface area of QG and SG was the same as the CG. From that time point and on the burn surface area of the QG steadily decreased and was calculated at 80% of the CG at PBD21 (90% of the CG for the SG) and 40% of the CG at PBD31 (90% of the CG for the SG). Furthermore, on PBD31 the burn wound was almost completely re-epithelialized in QG.

Figure 2. Burn wound size in rats measured by planimetry and expressed as (%) of the control burn wound size on post-burn days 0, 3, 6, 12, 21 and 31. Data are presented as mean±STDEV. [a] Significant different between CG and QG ($p<0.05$); [b] Significant different between CG and SG ($p<0.05$); [c] Significant different between SG and QG ($p<0.05$)

Histological evaluation

Sections with characteristics of acute inflammation were seen for the CG and SG on PBD3 and PBD6 (Stage 1). Interestingly, in PBD3, the sections of QG the polymorphonuclear cells were significantly reduced in number, compared to control (Stage 1). In PBD6, despite the absence of well-developed granulation tissue in QG, abundant edema was accompanied by the presence of more fibroblasts than in CG and SG, and few neo-capillaries (Stage 2).

In PBD12 a clearly developed capillary network and several fibroblasts as well as appearance of mature granulation tissue were established in the QG (early Stage 3). These changes were accompanied with the absence of inflammatory cells in PBD21 (late Stage 3). The presence of immature granulation tissue was noted in the sections of CG and SG (Stage 2) but by PBD21 mature granulation tissue with flattened, fibroblasts and dense mature capillary network was noted (Stage 3) (**Figure 3**).

Finally, in PBD31 a squamous epithelial cell layer consisted of only a few layers of immature keratinocytes was seen in CG and SG whilst in QG keratinocytes formed a mature epidermis. Underneath, a dense fibrous connective tissue was noted (Stage 5).
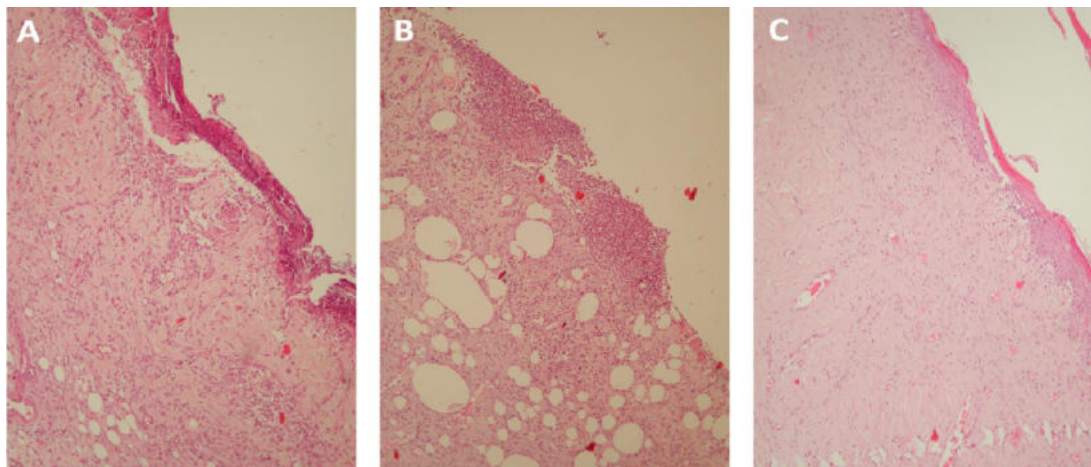
Figure 3. Histological evaluation of the healing process. Representative sections were taken from animals of the control group CG (A), SG (B) and QG (C) on PBD21. In the sections of CG and SG, there is a central area of ulceration. This central area is absent in the QG. In the latter the granulation tissue is more mature and extensive and re-epithelization appears. (Haematoxylin-eosin x 200)

Discussion

According to the findings of this study quercetin has the potential for use as a therapeutic agent for burn wound healing.

On PBD21, a reduction of the burn wound in 4 of the 6 animals of QG was recorded and in this particular group, only one animal had visual inflammation with pus with no other parameter mentioned such as hemorrhage, or accumulation of serum fluid under the burn eschar, (compared to SG and CG). Evidence of the planimetric measurements showed 21% reduction of the burned area on QG, on PBD21, compared to PBD0, which is the best healing effect in all subgroups. Finally, the histological evaluation suggests that in QG, inflammatory cells were no longer observed, and the granulation tissue was mature.

It has been suggested that reactive oxygen species (ROS) produced by macrophages and neutrophils due to the burn injury lead to oxidative injury affecting not only the burned skin but also all the surrounding [14,15]. Quercetin inhibits free radical process in cells, thus protects cutaneous tissue-type cell populations, fibroblasts, keratinocytes and endothelial cells from oxidative stress [16]. Moreover, to the ability of quercetin to scavenge free radicals was partly attributed the better dermal wound healing in rats treated with incorporated collagen matrices rather than normal collagen matrix [17].

Thermal injury leads to tissue damage and activates inflammatory responses [18]. Quercetin inhibits lipoxygenase activity in mouse epidermal cells [19] and mouse skin [20] and prevents collagen breakdown (by inhibiting matrix-metalloproteinase 1) and thus prove beneficial to treat inflamed skin as well as photoaged skin [21]. It can also block histamine release and expression of

proinfallamtory cytokines in mast cells, thus preventing acute skin inflammation [22].

Chronic inflammation could also lead to fibrosis. An experiment in mice showed that oral supplementation with quercetin inhibits radiation-induced skin fibrosis that can lead to several pathologies of the skin, for example keloid and hypertrophic scars [23]. Previous *in vitro* experiments showed that quercetin can also inhibits keloid fibroblasts and hypertrophic scar-derived fibroblasts proliferation that commonly occurs after injuries (e.g. burns) [24].

Conclusions

In summary, quercetin presented strong thermal injury healing effect in Wistar rats according to planimetric, macroscopic and histological evaluation. Although quercetin failed to produce a short-term healing of the full-thickness burns in order to prevent surgery, the results are promising. Optimization of quercetin dosage as well as the form of application is needed in order to speed up healing time and maximise potential antioxidant and anti-inflammatory effects of quercetin.

Author's contribution

EG was responsible for the conduction of the experiments. YS and IV assisted her during her experiments (infliction of the burn wounds, anaesthesia, planimetric measurements, and macroscopic observation). AB and DP performed the histological evaluation of the samples. EG, YS and VR were responsible for the manuscript preparation. SK and AE were in charge of this experimental procedure (design of the study) and assisted with the interpretation of the results.

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