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QUERCETIN ON HEALING OF INFECTED CUTANEOUS WOUNDS OF DEXAMETHASONE-TREATED RATS

Biological Science

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ABSTRACT

PURPOSE: To evaluate the actions of quercetin on healing of infected cutaneous wounds of Dexamethasone-treated rats.

MATERIAL AND METHODS: Open cutaneous excision wound was created on the back of the 40 Wistar rats that received dexamethasone for 10 consecutive days. These animals were divided into four groups: Dexamethasone (D), Dexamethasone-Infection (DI), Dexamethasone-Quercetin (DQ), and Dexamethasone-Infection-Quercetin (DIQ). Linear measurements of wound edges were obtained. Skin and subcutaneous tissue were removed for pathological anatomic analysis of the wound. This study has been approved by Universidade do Vale do Sapucaí Ethics Committee on Animal Usage.

RESULTS: Wound areas (mm) before and after healing were, respectively, in D (626.4±34.9 *vs* 338.5±57.8 p>0,05); DI (512.7±31.1 *vs* 384.2±39.0; p=0.01); DQ (567.5±30.0 *vs* 334.9±52.5 p>0,05); DIQ (522.1±56,1 *vs* 306.2±73.1 p>0,05). before and after healing were, respectively, in D (626.4±34.9 *vs* 338.5±57.8 p>0,05); DI (512.7±31.1 *vs* 384.2±39.0; p=0.01); DQ (567.5±30.0 *vs* 334.9±52.5 p>0,05); DIQ (522.1±56,1 *vs* 306.2±73.1 p>0,05). After wound healing Colony-forming Units, were 88 x 10^6/ml in DI and 50 x 10^6/ml in DIQ, p = 0,02.

CONCLUSION: Quercetin accelerated healing of infected cutaneous wounds of dexamethasone-treated rats.

KEYWORDS

Wound Healing. Dexamethasone. Quercetin. Infection. Rats

INTRODUCTION

Quercetin is a natural flavonoid found abundantly in almost all edible vegetables and fruits. Clinical and experimental studies have shown that quercetin has great therapeutic potential in the prevention and treatment of different diseases, including cardiovascular, neurodegenerative, câncer, diabetes, obesity, infeccion and inflammation[1-6].

Quercetin has long been recognized for its antibacterial activity[7]. It was shown to inhibit both Gram-positive and Gram-negative bacteria such as S. aureus, Streptococcus, Pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus spp., Shigella spp. and E. coli[8,9].

Studies have showed that quercetin could inhibited proinflammatory cytokine production and subsequent inflammation. The quercetin component could depress an initial reaction leading to excessive inflammation by quenching reactive oxygen species (ROS), generated by inflammatory cells[10,11].

Dexamethasone is a drug of the glucocorticoids group. It markedly affects most aspects of wound healing. When it is administered early after injury delay the appearance of inflammatory cells and fibroblasts, the deposition of ground substance and collagen, regenerating capillaries, contraction, and epithelial migration[12]. Glucocorticoids exert many complex quantitative and qualitative immunosuppressive effects that induce cellular immunodeficiency and consequently might increase host susceptibility to various viral, bacterial, fungal, and parasitic infections[13].

In this study, we evaluated the actions of quercetin on healing of infected cutaneous wounds of Dexamethasone-treated rats.

MATERIAL AND METHODS

This experimental study was conducted from January of 2018 to February of 2019. All procedures with animals have been approved by Universidade do Vale do Sapucaí (UNIVAS) Ethics Committee on Animal Usage, by 210/14 protocol.

Forty male *Wistar* rats, which were 3 month-old, were utilized; they were provided by UNIVAS *vivarium*. Animals had free access to water and to rat's food (Nuvilab®) and remained on isolated cages during ten consecutive days, under a temperature range from 21º to 25°C, alternating light/dark cycles.

All animals were anesthetized with intraperitoneal injection of Ketamine (60mg/Kg) plus Xylazine hydrochloride (8 mg / kg). The backs of the rats were shaved and cleaned with polyvidone–iodine. An open cutaneous excision wound of 20 mm in length and 20 mm in width was made on the back of each rat near the area of the shoulder blades, where rats could not reach their own wounds. Wound margins were measured in length and width with a digital caliper.

The animals were randomized into four groups: Dexamethasone (D; n = 10), Dexamethasone-Infection (DI; n = 10), Dexamethasone-Quercetin (DQ; n = 10), and Dexamethasone-Infection-Quercetin (DIQ; n = 10). All animals received intraperitoneal dexamethasone injection (Decadron®) 1mg/Kg/day and DQ and DIQ groups received intraperitoneal Quercetin injection 50mg/Kg/day, both drugs for ten consecutive days.

Wounds of DI and DIQ groups received suspensions containing *Staphyloccus aureus*. These bacteria were isolated from lower limb wounds of female patients and cultured on blood agar. From the stock cultures, the Staphylococci were transferred to the Thioglycollate medium (Himedia®), incubated at 37°C for 24 hours and then spread on a mannitol salt agar medium and then again incubated at 37ºC for 24 hours. The concentration for the inoculation of *Staphyloccus. aureus* was 1x10^6 CFU (colony- forming units)/ml and it was obtained using a sterile swab, which was touched on the surface of 4 to 5 great colonies isolated in the mannitol salt agar plate and emulsified in 3 ml of water for the inoculum (autoclaved deionized water). The final turbidity was equivalent to a 0.5 McFarland turbidity standard. The turbidity was confirmed by the use of the MicroScan® turbidity meter with an interval of 0.08 ± 0.02 (Procedure manual for Gram-Positive Dehydrated Panels-MicroSan®-SIEMENS Panels). A ml of suspension containing approximately 1 x 10^6 colony-forming units

(CFU) of *Staphyloccus. aureus* was applied to the wound surface, immediately after wounding while the animals were anesthetized. Swabs were taken from the wound on day 0, 3 and 10. The collected swabs were immediately sent to the laboratory for testing. In the quantitative count study, 2 ml of normal saline was added to each of the samples. The sample was vortexed thoroughly and a 10-fold serial dilution was performed. Eight hundred microliters of each sample dilution were spread onto Tryptic Soy Agar (TSA). Two replicates were carried out for each dilution, and the agar plates were incubated at 37°C for 24 hours. The colonies were counted, and results were tabulated.

The animals were monitored immediately postoperatively for spontaneous breathing efforts and movement. After surgery, each animal was housed in an individual cage in a room and fed with standard rat diet and water, post-operative subcutaneous injection of morphine 0,02 mg/kg was given.

After ten days all animals were anesthetized again and the wound tissue was excised and fixed in 10% neutral buffered formalin. It was dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. On the glass slides, five-micron-thick sections of the epidermis, dermis, and subcutaneous panniculus carnosus muscle have been mounted. After dewaxing the sample, it was rehydrated to distilled water and stained with hematoxylin and eosin and Masson's Trichrome. All subsequent analyses were performed by an experienced pathologist without knowledge of the previous treatments. Based on the degree of re-epithelization, presence of fibroblasts and fibrocytes, vascular proliferation, and collagen organization, a four-tiered grading system was adopted to evaluate the historical differences of different samples. The evaluations were carried out quantitatively: 0 = absent; 1 < 25%; 2 = 25%-75% and 3 > 75% .

Statistical data analysis was performed by BioEstat *software,* version 5.3. We used D'Agostino test of normality. Numeric variables with normal distribution were compared using Analysis of Variance and Tukey tests. Nonparametric data were compared by Kruskal-Wallis test. It has been adopted $p<0.05$ for rejecting the null hypothesis.\

RESULTS

Table 1- Wound areas in initial and final of the experiment, in Dexamethasone (D), Dexamethasone-infection (DI), Dexamethasone-Quercetin (DQ), and Dexamethasone-infection-Quercetin (DIQ) groups.

Wound Areas (mm)		D (n=10)	DI (n=10)	DQ (n=10)	DIQ (n=10)
Experiment	Initial	626.4±34.9	512.7±31.1	567.5±30.0	522.1±56.1
	Final	338.4±57.8	384.2±39.0*	334.9±52.5	306.2±73.1

* p = 0,01

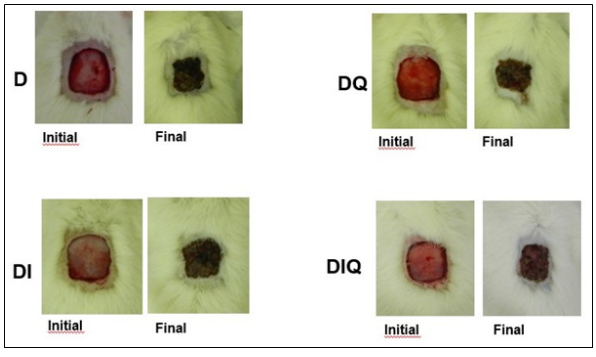

Figure 1- Macroscopic images of wound healing, in initial and final of the experiment, in Dexamethasone (D, n=10), Dexamethasone-Infection (DI, n=10), Dexamethasone-Quercetin (DQ, n=10), and Dexamethasone-Infection-Quercetin (DIQ, n=10) groups.

Table 2- Histological parameters of wound healing in Dexamethasone (D), Dexamethasone-infection (DI), Dexamethasone-Quercetin (DQ), and Dexamethasone-infection-Quercetin (DIQ) groups.

Histological Parameters	D (n=10)	DI (n=10)	DQ (n=10)	DIQ (n=10)	p
Neutrophilis	1	2	1	1	0,001
Fibroblasts	2	2	2	2	0,5
Collagen	1	1	1	1	0,4
Neovascularization	2	2	2	2	0,3
Reepithelialization	1	1	1	1	0,3

Scores: 0 = absent 1 < 25% 2 = 25%-75% 3 > 75%

Table 3- Colony-Forming Units (CFU/ml) in initial and final of the experiment, in Dexamethasone-infection (DI) and Dexamethasone-infection-Quercetin (DIQ) groups.

Colony-Forming Units (CFU/ml)		DI (n=10)	DIQ (n=10)	p
Experiment	Initial	40 x 106	40 x 106	0,9
	Final	88 x 106	40 x 106	0,02

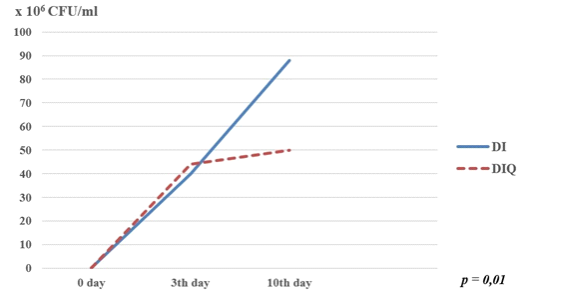

Figure 2- Colony-forming Units in 0, 3th and 10th days of the experiment in Dexamethasone-Infection and Dexamethasone-Infection-Quercetin (DIQ, n=10) groups.

DISCUSSION

This experimental model showed that Quercetin improved healing of the wound infected by *Staphyloccus aureus*. This finding is according to the literature[14,15]. Anti-inflammatory properties of Quercetin has been studied both *in vitro* and *in vivo***.** A study carried out *in vitro* research and showed that Quercetin was capable of modifying the metabolism of platelet arachidonic acid. It also reported that this flavonoid blocked both the cyclooxygenase and lipooxygenase pathways at relatively high concentrations, while at lower concentrations the lipooxygenase pathway was the primary target of inhibitory activity. In contrast, the inhibition of cyclooxygenase and the consequent increase in intracellular cyclic AMP appeared to be the major mechanism involved in the antiaggregating effect of Quercetin. Its antiagregating activity was relatively low but they were able to inhibit the first wave of cyclic AMP-induced aggregation and increased the cyclic AMP response to PGI. This flavonoid may have significant *in vivo* effects on homeostasis of the immune system and on the behaviour of secondary cell systems involved in the inflammatory response. However, they concluded that more work is required to strengthen this hypothesis. Flavonoids were demonstrated as possessing *in vivo* anti-inflammatory properties and had good anti-inflammatory activity without the ulcerogenic side-effects of other anti-inflammatory drugs[16,17].

The wound repair process involves steps that include inflammation around the site of injury, angiogenesis and the development of granulation tissue, repair of the connective tissue and epithelium, and ultimately remodeling that leads to a healed wound. The progression from an injured site to a healed wound is potentially slowed or arrested by a number of different events and conditions. One event that impedes wound healing is colonization of the wound bed by microorganisms[18]. In addition to the production of a variety of toxins and proteases, the presence of microorganisms in a wound bed may also lead to a prolonged inflammatory response. The host inflammatory response is remarkably effective at eliminating the invading microbial population, but that same process, over time, may also damage the surrounding tissues. This study describes the morphohistological features of healing of the wound, in rats, and the changes associated with infection by *Staphyloccus aureus* obtained by inoculation of the bacteria. The infected wounds an acute inflammatory reaction. Polymorphs, mainly neutrophils, were much more in evidence. The use of Quercetin was important in reducing the wound's microbial load. Once a wound becomes infected, healing is delayed. Increased bacteria on the surface and in wounded tissue increases the metabolic requirements of the wound and of the host's response to that heavy bacterial load. Bacteria produce endotoxins, exotoxins, proteases, and local tissue injury. The presence of a bacterial burden in a wound stimulates a proinflammatory environment. The presence of bacteria

induces also migration of monocytes, macrophages, and leukocytes, all of which initially act in an appropriate fashion but later produce a response that is exaggerated and deleterious. This is evidenced by the fact that wounds associated with a heavy bacterial burden often show healing failure[19-22].

CONCLUSION

Quercetin accelerated healing of infected cutaneous wounds of dexamethasone-treated rats.

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