Comparative Study of Wound-Healing Activity of Dihydroquercetin Pseudopolymorphic Modifications

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Wound-healing activity of the crystalline form of dihydroquercetin and its microtubular pseudopolymorphic modification obtained by crystal engineering was compared using the rat model of IIIA degree burn. The rate of wound healing in the group treated with microtubular pseudopolymorphic modification of dihydroquercetin was 4.8±0.1%, which was higher by 11.6% than in the group treated with crystalline form (4.3±0.1%). Bioavailability analysis on MDCK cell culture showed that the apparent permeability coefficient of microtubular pseudopolymorphic modification was higher than that of crystalline form by 31.1% (19.4±0.2×10⁻⁴ and 14.8±0.3×10⁻⁴ cm/sec, respectively). It was proven that the use of crystal engineering improved the biopharmaceutical parameters of dihydroquercetin and increased its pharmacological efficiency.

Key Words: dihydroquercetin; burns; polymorphism; biopharmacy; regenerative medicine

Dihydroquercetin (DHQ), or taxifolin, is characterized by a wide range of pharmacological activity. In Russia, antioxidant drug Diquertin is produced on the basis of this flavonoid [2]. Much attention is now attracted to DHQ due to unique combination of pharmacological effects and its high safety profile [10,12]. Recent studies are focused on the search of new biological targets using in silico approach [5,8]; pharmacological properties of this substance [1], mechanisms of antioxidant activity [9], and its metabolism and pharmacokinetics are extensively studied [14]. The development of new DHQ forms characterized by higher water solubility and better bioavailability is a priority research area [13]. Crystal engineering is a new way for the modification of the biopharmaceutical parameters of flavonoids, including DHQ [4].

DHQ is produced in the form of a pharmaceutical substance (FS-000388-270812) that has a crystalline structure (DHQ-C) according to the data of X-ray powder diffraction analysis. Using crystal engineering approach, a new microtubular pseudopolymorphic modification (DHQ-T) was obtained [6].

The aim of this study was to study and compare the wound healing activity of polymorphic modification of DHQ (microtubular and crystalline) using the rat model of IIIA degree burn.

MATERIALS AND METHODS

The study was carried out on 3-month-old male Sprague-Dawley rats (n=48; mean weight 270 g) provided by Institute of Medical Plant Development (Beijing). The maintenance, feeding, and sacrifice were conducted with strict adherence to GOST 33215-2014 (Guidelines for the Maintenance and Care of Laboratory Animals. Rules for the Equipment of Premises and Organization of Procedures) and GOST 33216-2014 (Guidelines for the Maintenance and Care of Laboratory Animals. Rules for the Maintenance and Care of Laboratory Rodents and Rabbits).
sign of this experiment was approved by the Ethics Committee of the I. M. Sechenov First Moscow State Medical University.

The IIIA burn was modeled by a widely used method [7,11]: the rats were deeply anesthetized with chloral hydrate and a metal plate (m=200 g, d=8 cm²) heated to 105±5°C was applied to the shaved skin of the back for 20 sec.

In 24 h, the animals were divided up into 4 groups (12 rats per group). Group 1 served as a negative control (application of 1 ml water). Group 2 rats were treated by applying 1 ml suspension of DHQ-C (Lavitol, Ametis) with the concentration of 50 mg/ml on the wound. Group 3 animals were treated with DHQ-T suspension [6]. In group 4 (positive control), the wounds were treated with 1 ml sea-buckthorn oil (Wulumuqi Huajinshan Shipin Co. Ltd.). All preparations were applied daily.

On days 1, 3, 5, 8, 11, 14, 17, 20, and 24, the body weight and temperature were measured. Wound healing was controlled by the Popova’s contact method and non-contact method of digital photography with a square standard using GNU Image Manipulation Software 2.10.18 (GNOME Foundation) [37]. The wound healing rate (W) was calculated by the formula:

\[ W = \frac{S_0 - S_n}{S_0} \times 100\% , \]

where \( S_0 \) and \( S_n \) are the mean square of the wound before and on day \( n \) of treatment. The mean rate of wound healing was determined from the plot using the data of all measuring days.

The \textit{ex vivo} experiments were carried out on passage 25-28 MDCK cell cultures obtained from the Cell Culture Center of Chinese Academy of Medical Sciences (Beijing). The cells were grown in DMEM (Corning) supplemented with 10% fetal calf serum (Gibco) and penicillin and streptomycin (Gibco) (Corning) supplemented with 10% fetal calf serum (Gibco) (Corning) supplemented with 10% fetal calf serum (Gibco). The cells were grown in DMEM (Corning) supplemented with 10% fetal calf serum (Gibco) and penicillin and streptomycin (Gibco) (Corning) supplemented with 10% fetal calf serum (Gibco). The cells were grown in DMEM (Corning) supplemented with 10% fetal calf serum (Gibco) and penicillin and streptomycin (Gibco) (Corning) supplemented with 10% fetal calf serum (Gibco). The cells were grown in DMEM (Corning) supplemented with 10% fetal calf serum (Gibco). The cells were grown in DMEM (Corning) supplemented with 10% fetal calf serum (Gibco) and penicillin and streptomycin (Gibco) (Corning) supplemented with 10% fetal calf serum (Gibco). The cells were grown in DMEM (Corning) supplemented with 10% fetal calf serum (Gibco) and penicillin and streptomycin (Gibco) (Corning) supplemented with 10% fetal calf serum (Gibco).

The apparent permeability (\( P_{app} \)) was calculated by the formula:

\[ P_{app} = \frac{(dC/dT) \times V}{A \times C} \]

where \( dC/dT \) is the change of DHQ concentration in the receiver chamber over time, \( V=1.5 \text{ ml}, A=4.7 \text{ cm}^2 \), \( C \) is initial DHQ concentration in the culture medium (mg/ml).

The data were analyzed using LibreOffice Calc 6.2 (The Document Foundation), including potting and calculation of the mean values, confidence interval, and correlation coefficient.

RESULTS

At the beginning of the study the mean body weight of rats in groups 1, 2, 3, and 4 were 279.4±11.2, 276.1±7.1, 268.2±10.4, and 277.1±7.8 g, respectively; the body temperature was about 38.0±0.9°C, \textit{i.e.} the groups were homogenous and congruerrous. After burn modeling, a decrease in body weight (by ~5.4%) was observed in all groups presumably due to distress.
Later, this parameter returned to initial values. No significant differences in body temperature were observed.

The area of burns and the wound healing rates are presented in Table 1. According to the results of the contact method, the mean wound healing rate in groups 1, 2, 3, and 4 were 4.0±0.1, 4.3±0.1, 4.6±0.1, and 4.2±0.1%, respectively. These results were consistent with the results of the non-contact method (4.1±0.1, 4.3±0.1, 4.8±0.1, and 4.4±0.1% in groups 1, 2, 3, and 4, respectively). In group 3, the wound completely healed by day 22, in group 2 and 4, healing was completed by day 24. In group 1, most wounds did not heal by the end of the experiment.

| Parameter | Group 1 | Group 2 | Group 3 | Group 4 |
|-----------|---------|---------|---------|---------|
| Wound area, cm² |          |         |         |         |
| day 1     | 7.8±1.0 | 8.7±0.5 | 9.0±0.5 | 8.4±0.7 |
| day 8     | 6.3±0.6 | 5.7±0.3 | 5.1±0.5 | 5.7±0.2 |
| day 14    | 3.2±0.5 | 2.9±0.5 | 2.5±0.5 | 3.0±0.6 |
| day 24    | 0.6±0.8 | 0.6±0.7 | 0.2±0.2 | 0.6±0.6 |
| Wound healing, % |        |         |         |         |
| day 8     | 19.2    | 34.5    | 43.3    | 36.0    |
| day 14    | 59.0    | 66.7    | 72.2    | 66.3    |
| day 24    | 92.3    | 93.1    | 97.8    | 93.3    |
| Wound area, cm² |          |         |         |         |
| day 8     | 33.4    | 34.4    | 21.0    | 15.3    |
| day 14    | 64.1    | 70.0    | 70.2    | 64.1    |
| day 24    | 92.3    | 92.0    | 93.4    | 92.7    |

Based on the results of in vivo experiment, we hypothesized that the differences in pharmacological effects of the polymorphic modifications of DHQ are determined by their biopharmaceutical characteristics. To test this hypothesis, ex vivo analysis of permeability was performed.

Evaluation of the toxicity of both polymorphic modifications revealed a logarithmic dependence between the concentrations of DHQ samples and cytotoxicity (Fig. 1). The nonlinear dose dependence can be explained by low water solubility of DHQ and formation of suspensions at higher concentrations. Hence, the negative effect on MDCK cells was caused by physical impact of solid DHQ particles,

![Fig. 1. The dependence on of MDCK cell viability on DHQ concentration.](image1)

![Fig. 2. Time course of DHQ concentration during permeability assay.](image2)
rather than DHQ toxicity. The limit of cell viability for both samples was ~75%, so both DHQ forms demonstrated high safety profile. Permeability analysis (Fig. 2) revealed no significant differences between the concentration curves of the studied DHQ forms. The Papp of DHQ-T was significantly higher than that of DHQ-C: $19.4\pm0.2\times10^{-4}$ and $14.8\pm0.3\times10^{-4}$ cm/sec, respectively. These findings support our assumption that the differences in wound healing efficacy of polymorphic modifications of DHQ are determined by better permeability of DHQ-T.

Thus, DHQ-T demonstrates the same safety profile as DHQ-C and better wound healing activity. The increase of pharmacological efficacy of DHQ-T correlates with the better biopharmaceutical characteristics.

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