The effects of topical liposomal resveratrol on incisional and excisional wound healing process

Abstract

Background and Design: The objective of this study was to investigate the wound healing activity of different concentrations of liposomal trans-resveratrol formulations on incisional and excisional wounds in rats.

Materials and Methods: The wound healing effect was tested by an excisional and incisional wound model. Wound closure was measured for 12 days. On the 12th day of the study, maximal load, maximum stress, stress, and % of elongation values were evaluated in the incisional wound. In addition, angiogenesis, granulation tissue thickness, epidermal and dermal regeneration values, and macroscopic photographic analyses were evaluated in the excisional wound.

Results: When the wound tissue surface healing rates were evaluated, similar effects were observed at the end of the 10th and 12th days between the 5% Res group and the commercial product containing 1% Centella asiatica extract used as the reference molecule. Histological evaluation showed that 1% Res and 5% Res groups induced significant wound healing activity compared to the control group. Furthermore, 1% Res and 5% Res groups increased wound healing rates by promoting granulation tissue, epidermal, and dermal regeneration as well as angiogenesis.

Conclusion: Liposomal formulations containing 1% and 5% resveratrol were found to have positive effects on the healing process, both on incisional and excisional wound tissues.

Keywords: Resveratrol, liposome, incisional wound, excisional wound, axial tensile-elongation tests

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Sonuç: %1 ve %5 resveratrol içeren lipozomal formülsyonların, hem eksizyonal hem de insizyonal yara dokularında ileyleme süreci üzerinde olumlu etkileri olduğu bulunmuştur.

Anahtar Kelimeler: Resveratrol, lipozom, insizyonal yara, eksizyonal yara, eksenel çekme-uzama testleri

Introduction

When the skin tissue is damaged, the body responds quickly with functional regeneration, which is called wound healing. The wound healing process has 5 important steps, homeostasis and inflammation, granulation tissue formation, neovascularization, epithelialization, and remodeling. Numerous molecules that act on this process at different stages have been used in research to accelerate or regulate wound healing. Resveratrol is one of these molecules. Resveratrol (3,5,40-trihydroxy-trans-stilbene) is abundant in Polygonum cuspidatum roots, grapes, peanuts, plums, strawberries and red wine. Previous studies have shown that resveratrol has anti-inflammatory, immunomodulatory, cardioprotective, antioxidant, neuroprotective, hepatoprotective and antioxidant properties. In rodent models, resveratrol has been shown to inhibit lipopolysaccharide-induced acute lung inflammation by inhibiting TLR4/NF-kBp65/MAPKs signaling cascade and NLRP3 inflammasome activation. Reactive oxygen species (ROS) are natural byproducts of cellular metabolism and cause cell death at extreme levels. SIRT1 reduces ROS levels and enhances cell activity. Resveratrol increases SIRT1 in human pulmonary alveolar epithelial cells, reduces ROS production, protects cell membrane potential, and prevents apoptosis in alveolar epithelial cells, thereby reducing hyperoxia-associated lung damage. The antioxidant properties of resveratrol are not limited to the lungs alone. It has been shown in experimental studies that rats are protected against ischemia-reperfusion injury in the heart. In a number of studies resveratrol has been shown to be effective at different doses for skin cancer, breast cancer, prostate cancer, lung cancer, colon cancer and liver cancer.

Yaman et al. investigated the incisional wound healing process in rats given oral resveratrol in their research in 2013 and concluded that oral resveratrol was effective. Another study by Tang et al. showed that resveratrol inhibits the formation of pathological scar tissue and inhibits fibroblast proliferation by decreasing mTOR and 70S6K expression. Increasing the absorption of a molecule into the target tissue by adding it into liposomes is often preferred recently. One of the most important advantages of using liposomes is that liposomes can be prepared in desired sizes and thus pass through regions such as the blood-brain barrier where the molecules they carry do not normally pass through. For example, the study of Ethemoglu et al. investigated the effect of trans-resveratrol loaded in liposomes on penicillin-induced epileptic activity model. When trans-resveratrol is used orally, its effectiveness is limited due to low water solubility, stability, and bioavailability. Nanocapsulation has proven that molecules are effective in increasing water solubility, chemical stability, and bioavailability of active compounds. The use of liposomes as a drug carrier allows the drug to reach adequate penetration and concentration in the target tissue and reduce systemic side effects to a minimum. Liposomes are potentially beneficial vehicles for the topical delivery of active pharmaceutical ingredients. There is no significant changing on skin structure in previous study of empty liposomes. Three dimensional images of volunteers skin was evaluated 2 weeks before-after study. According to results active ingredient (sodium hyaluronate) containing liposomes have significant effect on wrinkles and integration of skin structure. However, empty liposomes have no important differentiation effect of skin structure. In another research, wound epithelialization was measured by computerized planimetry as percentage original wound area, it was significantly increased wound epithelialization rats receiving active ingredient insulin-like growth factor-I (IGF-I) containing liposomes when compared to sham, or IGF-I (p<0.05). Sham formulations have negligible effect on wound epithelialization.

Centella asiatica extract (CAE) is a molecule that facilitates the repair process of the wound tissue and has been shown to exhibit antimicrobial activity. For this reason, it has been preferred as a reference molecule in many studies including our study.

In many previous studies, the tensile strength was measured to determine the amount of improvement in the skin tissue in incisional wound healing. In addition to the parameters examined in previous studies, we aimed to obtain more data by measuring the parameters of maximum load, stress, strain, and elongation on the healing of incisional wound tissue in our study. Maximum load expresses the maximum strength after the maximum tensile force applied to the skin sample. The pulling force applied to the skin tissue prolongs the skin tissue sample. When the maximum load value is reached, the tissue starts tearing. Stress (Mpa) is defined as the amount of force acting on the unit area. It is caused by applying a force perpendicular to the surface area to extend the skin sample in the direction of the pulling force and is also called tensile stress. Strain (mm/mm) refers to the extent to which the skin sample under load changes its shape compared to the condition before the load is applied. This definition is a concept that allows the mathematical expression of the deformation of materials with elastic properties. Elongation refers to the % change of the difference between the length of the skin tissue before starting the tensile test and the length of the skin immediately preceding the break.

This study aimed to investigate the effect of resveratrol loaded in liposomes on incisional and excisional wound tissue topically.

Materials and Methods

Animals

Male Wistar-Albino rats weighing 250-300 gr were used in the study. The rats were housed in regular cages with food and water ad libitum, at room temperature (24°C) with artificial light from 7.00 am to 7.00 pm. Before performing in vivo experiments, ethical clearance approval...
was obtained from the Local Ethical Committee (Istanbul Medipol University, Turkey, approval number: 44, date: 07.08.2018). Informed consent is not necessary as it is an animal experiment. The experimental animals were divided into 4 groups of 8 animals per group, control, commercial product containing 1% CAE, group of containing 1% resveratrol (1% Res) and group of containing 5% resveratrol (5% Res).

Preparation of liposome formulations containing trans-resveratrol
The chemicals used to prepare the liposome formulations were supplied from the relevant companies [hydrogenated phosphatidylcholine, LIPOID GmbH, Germany, Dicetyl phosphate, Sigma Aldrich, United States of America (USA), Cholesterol, Fluka Research Chemicals, USA]. Liposomal dispersion formulation was prepared by thin film technique. Briefly, liposome was prepared by dissolving the 100 μM of phospholipids and lipophilic active substance (trans-resveratrol, Interpharma, Chezc Republic) in 30 mL chloroform-methanol mixture in a round-bottom flask. The organic solvents were removed using a rotary evaporator under reduced nitrogen pressure to form a thin film over the wall of round-bottom flask. The dried film was then hydrated over a water bath with 10 mM Tris Buffer pH 5.5 above phase transition temperature. After multilamellar vesicle preparation, sonication process and incorporation technique were applied, respectively. Final trans-resveratrol concentrations were 1% (w/v) and 5% (w/v), respectively (Table 1). Then liposomes were sonicated during 5 minutes x 3 times to provide content uniformity. All dispersions were kept at 4 °C until usage under nitrogen.

Once the liposomes were prepared, their sizes, zeta potentials and Poly Disperity Index (PDI) values were measured (Table 2). According to the results obtained, the mean particle size distribution of empty liposome increases. When we evaluated liposomes from the point of view of PDI, resveratrol-loaded liposomes had a larger particle size than empty liposomes, and PDI values were between 0.1 and 0.5, which is the ideal value. According to all these data, liposomes containing 5% Res may be preferred because they have a larger diameter than liposomes containing 1% Res, 5 times more active ingredient and higher Zeta potentials.

Table 1. Liposome formulation and composition

<table>
<thead>
<tr>
<th>Code</th>
<th>Composition</th>
<th>pH</th>
<th>Molar ratio</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Res</td>
<td>HPC:DCP:CHOL+resveratrol (1% w/v)</td>
<td>5.5</td>
<td>10:1:4</td>
<td>Milky dispersion</td>
</tr>
<tr>
<td>5% Res</td>
<td>HPC:DCP:CHOL+resveratrol (5% w/v)</td>
<td>5.5</td>
<td>10:1:4</td>
<td>Milky dispersion</td>
</tr>
</tbody>
</table>

Table 2. Characterization result of liposome formulations

<table>
<thead>
<tr>
<th>Code</th>
<th>Composition</th>
<th>Size (nm)</th>
<th>Zeta Potential (mV)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Res Free</td>
<td>HPC:DCP:CHOL (resveratrol free)</td>
<td>134.20±4.38</td>
<td>-22.4±0.12</td>
<td>0.112±0.002</td>
</tr>
<tr>
<td>1% Res</td>
<td>HPC:DCP:CHOL+resveratrol (1% w/v)</td>
<td>168.80±8.20</td>
<td>-26.8±0.26</td>
<td>0.146±0.001</td>
</tr>
<tr>
<td>5% Res</td>
<td>HPC:DCP:CHOL+resveratrol (5% w/v)</td>
<td>192.20±8.44</td>
<td>-27.2±0.82</td>
<td>0.175±0.004</td>
</tr>
</tbody>
</table>

Anesthesia and wound creation protocols
Rats were anesthetized with a combination of 60 mg/kg ketamine and 6 mg/kg xylazine intraperitoneally. The back area was shaved and the area was cleaned with 70% alcohol. The modified wound model was used to create the wound model considering the information in the literature. Two excisional wound tissues were formed using a 5 mm punch biopsy instrument on the left side of the midline in the shaved back region, one cm apart from each other and 1.5 cm away from the midline. Except for the control group, Linear full-thickness incisional wound was formed in a length of 2 cm using a No. 11 surgical scalpel to remain 1.5 cm to the right of the midline in the dorsal region. Incisional wound edges were sutured using 3/0 silk thread. In the control group, only the excisional wound model was formed as described above. Drug formulations and CAE were administered topically to the relevant groups at 10.00 am every day for 11 days. On day 12 of the study, the rats were sacrificed under anesthesia, tissue samples were taken for histological evaluation and wound tension measurement.

Macroscopic evaluation
From the day when the excisional wound tissue was formed, the every other day photographs were taken and the surface areas were measured with the ImageJ program. Afterwards, wound healing rates were calculated by the formula given in the literature.

Histological evaluation
On day 12, the animals were sacrificed by decapitation and the skin of the back including the wound area was removed. Full-thickness biopsy samples extended from the outside margin to the center of the treated area. All tissue specimens were fixed in 10% neutral formalin for at least 25 h at room temperature. After fixation, wound samples were dehydrated in graded ethanol series, cleared in xylene and embedded in paraffin. 5 μm thick sections (Thermo Microm HM 340E,
Waltham, US) were mounted on glass slides, dewaxed, rehydrated with distilled water and stained with hematoxylin-eosin according to routine procedures for light microscopy. The images were taken under a Nikon Eclipse Ni (Nikon Instruments Europe BV, Amsterdam, Holland) research microscope. Measurement was performed by two independent researchers blind to the drug administration groups. Wound healing for each group was evaluated via use of the scoring system previously described by Galeano et al.39 for epidermal and dermal regeneration as: score 1 for poor epidermal organization in ≥60% of the tissue, score 2 for incomplete epidermal organization in ≥40% of the tissue, score 3 for moderate epithelial proliferation in ≥60% of the tissue and score 4 for complete epidermal remodeling in ≥80% of the tissue. For thickness of the granulation tissue, scoring was: 1 for thin granulation layer, 2 for moderate granulation layer, 3 for thick granulation layer, 4 for very thick granulation layer. For angiogenesis, only mature vessels were counted and identified by the presence of erythrocytes in the lumen. To distinguish well formed capillaries from poorly formed ones, we evaluated the presence or absence of edema, congestion, hemorrhage, thrombosis and intravascular or intervascular fibrin formation. Score 1 describes altered angiogenesis (one to two vessels/site) characterized by high degree of edema, hemorrhage, occasional congestion and thrombosis, score 2 describes few newly formed capillary vessels (3-4/site), moderate edema and hemorrhage, occasional congestion, intravascular fibrin deposition and absence of thrombosis, score 3 describes newly formed capillary vessels (5-6/site) and score 4 describes newly formed and normal appearing capillary vessels (>7/site).39,41.

**Wound tension measurements in incisional wound tissue (axial tensile-elongation experiments)**

Axial tensile-elongation experiments were carried out on a TA Instruments QA-800 mechanical analyzer at Yeditepe University Engineering Faculty. In order to standardize the data obtained from the skin samples taken after the incisional wound model, the cross-sectional areas of skin tissues to be subjected to tensile-elongation tests were measured separately. During the measurement, the skin tissues were attached to the fixed ends of the mechanical analyzer. All pull tests were initiated by resetting the force sensor. At certain intervals, a draw of 100 microns was applied. At the same time, the device continued to record by continuously measuring the stress-strain responses with applied force. Maximum load, maximum stress, strain and percent elongation values calculated in skin tissue.

**Statistical Analysis**

For statistical data comparisons, a standard software package (SPSS 20 for Windows, SPSS Inc., Chicago, IL, USA) was used. Differences between groups were analyzed by one-way ANOVA, followed by least significant differences tests. All values were given as mean ± S.E.M. P values <0.05 were considered significant.

**Results**

There was no significant difference between the groups when compared with the control group at 2nd and 8th days in the evaluation of the surface of the excisional wound. However, there was a significant difference on the 4th day, between CAE and the control group (p=0.048), on the 6th day, between the control group and 1% Res (p=0.005) and 5% Res (p=0.004) groups, on the 10th day, between the control group and CAE (p=0.01), 1% Res (p=0.026) and 5% Res (p=0.000) groups, on the 12th day between the control group and CAE (p=0.037) and 5% Res (p=0.003) groups (Figure 1).

- In the histological scoring of the healing of the excisional wound tissue, epidermal and dermal regeneration, granulation tissue formation and angiogenesis were better in the 1% (p=0.697) and 5% resveratrol (p=0.245) groups than in the control group, but not statistically significant (Figure 2).

- When the maximum load, maximum stress, strain and % elongation values of the incisional wound were evaluated, there was a significant decrease in all groups compared to the control group. On the other hand, no significant difference was found between the other groups compared with the control group. In particular, the group containing 5% Res compared to CAE, the preferred reference molecule for wound healing, was almost identical (Figure 3). There was no statistically significant difference between the other groups except for the control group.

**Discussion**

The wound healing process includes stages such as inflammation, cellular proliferation and remodeling, and cell-cell and cell-matrix interactions during these stages. Numerous studies have shown that essential oils of various medicinal plants increase the wound healing with active ingredients from aromatic plants and fruits. Resveratrol is a molecule with anti-inflammatory, immunomodulator, cardioprotective, antioxidative, neuroprotective, hepatoprotective, antioxidant and...
The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

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Günal et al.

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Günal et al.

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Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.

The effects of topical liposomal resveratrol on wound healing process: A systematic review

Günal et al.
tissue. Therefore, when we compare control group values with our other study groups, it is expected that the data will be higher in the control group. No statistically significant difference was found between the groups except the control group. Maximum load, maximum stress, strain and % elongation values that tissues were able to achieve were almost similar to those used as the reference molecule (Figure 3). In the case of axial retraction in a developing skin tissue, the closer the recovery of the damaged tissue to the strong skin tissue, the better the healing is. Proliferation of fibroblasts during wound healing is one of the factors affecting wound tensile. Histological evaluation parameters show us that the values obtained as a result of incisional stretching experiments are valuable.

Study Limitation
Although our study investigated the effects of liposomal formulations at different concentrations of resveratrol using incisional and excisional wound healing modalities, such as cytokines, growth factors, matrix metalloproteinases, known to be effective in the wound healing process, have not been evaluated by methods such as ELISA and RT-PSR.

Conclusion
Liposomal formulations containing 1% and 5% resveratrol at wound healing were found to have positive effects on healing process, both in excisional and incisional wound tissues. The study is a preliminary study and verification of the obtained data with molecular methods will enable to obtain data that will shed light on new treatment modalities.

Ethics
Ethics Committee Approval: Before performing in vivo experiments, ethical clearance approval was obtained from the Local Ethical Committee of Istanbul Medipol University. (approval number: 44, date: 07.08.2018).

Informed Consent: Informed consent is not necessary as it is an animal experiment.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References
27. Aslan I: Studies on liposome, gel and lipogelosome formulations containing sodium hyaluronate. Faculty of Pharmacy, Institute of Health Sciences, Cosmetology: Yeditepe University; 2010.


