Comparison of effects of unfractionated heparin and low molecular weight heparin on skin wound healing of rats

Compara¸c˜ao dos efeitos da heparina n˜ao fracionada e heparina de baixo peso molecular na cicatrizac¸˜ao de feridas na pele de ratos

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ABSTRACT

PURPOSE: To compare the effects of unfractionated heparin (UH) and a low molecular weight heparin (LMWH) on skin wound healing of rats.

METHODS: Forty eight male Sprague-Dawley rats underwent 8mm full thickness dorsal skin wounds and were randomly assigned to three equal groups. In experimental group A, heparin sodium was injected at a concentration of 1000U/kg. In experimental group B, enoxaparin was injected at a concentration of 1mg/kg. Physiologic saline (0.5ml) was administered to the control group. Injections were made subcutaneously, once daily, for seven days. At 7th and 10th days tissue samples were taken from all rats. Histologic examination of these tissues was made under light microscope and scored.

RESULTS: Histological examination showed a significant difference between the 7th and 10th day groups in wound healing. It was observed that wound healing of LMWH injected group is better. This difference is statistically significant at 10th day.

CONCLUSIONS: Daily administration of single doses of unfractionated heparin and a low molecular weight heparin improves wound healing positively. Low molecular weight heparin induces wound healing more than unfractionated heparin.

Key words: Heparin, Low-Molecular-Weight. Wound Healing. Skin. Rats.

RESUMO

OBJETIVO: Comparar os efeitos da heparina n˜ao fracionada (HNF) e da heparina de baixo peso molecular (HBPM) na cicatrizac¸˜ao de feridas cutˆaneas de ratos.

M´E TODOS: Quarenta e oito ratos machos Sprague-Dawley foram submetidos `a ferida na pele dorsal com espessura total de 8mm e foram distribu`idos aleat´oriamente em tr´es grupos iguais. No grupo experimental A, a heparina s´odica foi injetada a uma concentra¸c˜ao de 1000U/kg. No grupo experimental B, a enoxaparina foi injetada a uma concentra¸c˜ao de 1mg/kg. Solu¸c˜ao salina fisiologica (0,5ml) foi administrada para o grupo controle. As inje¸c˜oes foram feitas por via subcutˆanea, uma vez por dia, durante sete dias. Nos dias 7º e 10º amostras de tecido foram obtidas de todos os ratos. O exame histol´ogico destes tecidos foi realizado em microsc´opio de luz.

RESULTADOS: O exame histol´ogico mostrou uma diferen¸c˜a signiﬁcativa entre os grupos no 7º e 10º dias na cicatrizac¸˜ao das feridas. Observou-se que a cicatrizac¸˜ao de feridas do grupo com heparina de baixo peso molecular foi melhor. Esta diferen¸c˜a foi estatisticamente signiﬁcativa no 10º dia.

CONCLUSÕES: A administra¸c˜ao di´aria de doses ´unicas de heparina n˜ao fracionada e de heparina de baixo peso molecular melhora a cicatrizac¸˜ao de feridas. A heparina de baixo peso molecular induz melhor a cicatrizac¸˜ao de feridas do que a heparina n˜ao fracionada.

Introduction

The wound healing process is defined as a series of events that starts with inflammation and is followed by cell immigration, angiogenesis, provisional matrix synthesis, accumulation of collagen, and re-epithelialization. This process is very complex and it consists of a complex interaction among inflammatory cells, biochemical mediators, extracellular molecules, and the microenvironment. Collagen is the most essential basal, skeletal protein employed in healing processes. The increase in collagen content during wound repair may be attributed to an increase of collagen synthesis and/or proliferation of fibroblasts. Many factors can interfere with one or more phases of healing process, thus causing impaired wound healing. These factors include oxygenation, infection, age, and sex hormones, stress, diabetes, obesity, medications, alcoholism, smoking, and nutrition may be considered.

Unfractionated heparin (UH) and low molecular weight heparin (LMWH) are routinely used in the preoperative period. Heparin rapidly inhibits the anticoagulant activity mediated by anti-thrombin III (AT III). Anti-thrombin slowly interacts with thrombin without heparin. Although heparin-AT III complex inactivates thrombin, LMWH and anti-thrombin complex is more specific for Factor Xa. In recent years because of short half-life of heparin causes bleeding LMWH has preferred and it was reported that for the patients treated with LMWH propagation of thrombus, risk of thromboembolism and especially major bleeding incidence are significantly lower than heparin. Besides length of stay in hospital is shorter and hospital charges are more lower for the patients treated with LMWH. These features make LMWH reliable for DVT prophylaxis and treatment.

How does using heparin derivatives on wound healing after surgery is still a controversial subject. While protecting the patient from thromboembolism, safety of operative field shouldn’t be compromised. For this reason it is very important to know if UH and LMWH which are commonly used in clinic is effective or not. Purpose of this study is to compare the effects of UH and LMWH on wound healing histologically in rats underwent full thickness wound.

Methods

The study protocol was reviewed and approved by the Research Review Committee and the Animal Care Committee at the Gulhane Military Medical Academy (Ankara, Turkey). Three-month-old male Sprague-Dawley rats (n:48) weighting between 275 to 325g were used. Forty eight rats were randomly assigned to three equal groups including experimental group A, experimental group B and control group. Each group consisted of 16 rats and all were given standard care and dietary regimen during the experimental period.

Anesthesia and surgical procedure

Rats were injected 4mg/kg body weight xylazine HCl (alfazine) for premedication and 60mg/kg body weight ketamine (alfamine) for general anesthesia. The skin of the rats was shaved in the dorsa-lateral region and full thickness skin wounds were established by 8mm diameter of punches. In experimental Group A, heparin sodium was injected subcutaneously at a concentration of 1000U/kg. In experimental Group B, enoxaparin was injected subcutaneously at a concentration of 1mg/kg. Physiologic saline (0.5ml) was administered to the control group. Subcutaneously once daily injections were begun twelve hours before skin excision, six hours after surgery and continued postoperative period for seven days. After induction of high dose general anesthesia the rats, experimental and control were sacrificed on days 7th and 10th and tissue samples were taken.

Histological analysis

The wound area was dissected and cut into 1.5 x 1.5 cm full thickness strip including intact tissue. In order to examine fibroblastic proliferation, epithelialization of healing construction and phases of collagen histologically, tissue samples were taken from the wounded area at 7th and 10th days. They were fixed into 10% formaldehyde for two days then these blocks were embedded in paraffin and 4-5µm sections were cute from paraffin blocks by using microtome (Microm HM355s, Japan) for microscopic examination. Then paraffin blocks were stained by hematoxylin-eosin and scored. For each sample, 10 different fields at a magnification of ×20 were examined and histopathological findings were categorized for the presence of epidermal regeneration, collagen bundles, fibroblastic proliferation and vascularity (Table 1). While the samples exactly show the features of the phase that they are in were scored as 1, samples that are between two phases was scored as 0.25 point.
TABLE 1 - Microscopic criteria for evaluation and scoring of wound healing.

<table>
<thead>
<tr>
<th>Criterion/ Scores</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td>1. Epidermal regeneration</td>
<td>Absence of epithelium</td>
<td>Single-layer epithelium with partial closure</td>
<td>Multilayer epithelium with complete closure</td>
</tr>
<tr>
<td>2. Collagen fibers</td>
<td>Very thin, sparse and irregular</td>
<td>Thin, partly sparse and irregular</td>
<td>Dense and regular</td>
</tr>
<tr>
<td>3. Numbers of fibroblasts</td>
<td>A few</td>
<td>Moderate</td>
<td>A lot</td>
</tr>
<tr>
<td>4. Vascularity</td>
<td>Absence</td>
<td>Occasional presence and light scattering</td>
<td>Confluence of cells</td>
</tr>
</tbody>
</table>

Statistical analysis

Data were analyzed using SPSS for Windows v.15.0. Kruskal-Wallis was used to compare means of continuous variables for different groups. The groups which were determined to be different were compared in multiple pairs by Mann-Whitney-U test with Bonferoni correction significance was set at p<0.05.

Results

Histologic analysis of wound healing sites at 7th day

Control group: It was considered that organization of collagen fibrils in dermis layer were sparse and irregular (Figure 1a).

Group A: Dermis was not well organized and characterized by sparse collagen fibrils that were partly organized (Figure 1b).

Group B: Epidermal organization was close to normal and neovascularization areas were determined beneath the epidermis layer. Collagen fibrils in dermis layer were more regular than control group (Figure 1c).

Histologic analysis of wound healing sites at 10th day

Control group: It was considered that collagen fibrils in dermis layer were sparse and irregular. Dermal organization was better than control group at 7th day. Hemorrhagic areas were determined beneath the epidermis layer (Figure 2a).

Group A: Collagen fibrils were sparse and irregular in some places but hair follicles were much more than other groups (Figure 2b).

Group B: Collagen fibrils were more regular than...
group A. Mononuclear cell infiltration was observed in between epidermis and dermis layers (Figure 2c).

![Figure 2](image)

**Figure 2** - Histological sections of wound healing at 10th day. **a**. In the control group, collagen fibrils are appeared sparse and irregular. **b**. In the group A, collagen fibrils are appeared partially sparse and irregular in some places. **c**. In the group B, mononuclear cells infiltration is presented between epidermis and dermis layers. Epidermis (ep), H&E (20x).

After evaluation of the groups among themselves according to the histological scoring, experimental Group B had the best healing at 7th and 10th days (7th day: Mean±SD 1.4±0.13 10th day: Mean±SD 1.75±0.19) and difference between groups is statistically significant (p<0.000). When we evaluate the groups one by one in experimental group healing was the same and in other groups healing is better at 10th day but the difference is not statistically significant (Table 2).

<table>
<thead>
<tr>
<th>Days/ Groups</th>
<th>Control (n=16)</th>
<th>Group A (n=16)</th>
<th>Group B (n=16)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th day</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.44±0.11</td>
<td>1.0±0.19</td>
<td>1.4±0.13</td>
<td>0.000*</td>
</tr>
<tr>
<td>10th day</td>
<td>1.0±0.22</td>
<td>1.0±0.19</td>
<td>1.75±0.19</td>
<td>0.000*</td>
</tr>
<tr>
<td>P*</td>
<td>0.17</td>
<td>0.90</td>
<td>0.90</td>
<td></td>
</tr>
</tbody>
</table>

Evaluation of the groups in pairs at 7th day there is no significant difference between experimental groups but comparison of the experimental groups and control group in pairs a significant difference was found (p<0.000) and also at 10th day a significant difference was found between experimental groups and between experimental Group B and control group (p<0.003) (Table 3).

<table>
<thead>
<tr>
<th>Groups/ Days</th>
<th>7th day</th>
<th>10th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A –Control</td>
<td>0.000*</td>
<td>0.15</td>
</tr>
<tr>
<td>Group B –Control</td>
<td>0.000*</td>
<td>0.003*</td>
</tr>
<tr>
<td>Group A-Group B</td>
<td>0.06</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

**Discussion**

The normal healing includes hemostatic/inflammatory phases, maturation phase and remodeling phase. An effect of heparin on wound healing is still controversial subject. Studies examined the wound healing claimed that UH and LMWH shows anti-proliferative effects on fibroblast, endothelial cells, osteoblasts and angiogenesis. Heparin administration may...
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be caused hemorrhagic complications and increase the sero-hemorrhagic drainage after surgery. Prolonged sero-hemorrhagic drainage may have changed the content of wound fluid and so it influences the thrombin formation. A decrease in thrombin and fibrin clot formation causes negative effect in the early phases of healing. In our study we did not encountered macroscopically hematoma or bleeding is during heparin administration. Heparin might be expected to impair the early phases of healing process but in this study histologically we found the numbers of fibroblasts are more and collagen fibrils are more regular in experimental groups at 7th and 10th day then control group.

The influence of heparin administration on wound healing in rats has been investigated by Cen et al. They received subcutaneously heparin on experimental burned rats and this caused a significant shortening of healing time. Our results on LMWH are in line with this observation. Similar effect has been observed by Kweon et al. who administered heparin/chitosan complex topically. After two weeks of treatment the dorsal full thickness skin excision was nearly completely healed when it was compared with the respective controls. Li et al. have shown that the promotion of LMWH on the gastric ulcer healing was independent from the anticoagulant effect. In the present study we analyzed the effects of UH and a LMWH on wound healing process at 7th and 10th days. Gunerhan et al. examined the effects of UH and LMWH on wound healing and indicated a positive effect between experimental groups at 7th day but the difference was not significant. In our study when we compared the effects of UH and LMWH on wound healing, similarly the difference was not significant between experimental groups at 7th day but wound healing was faster for experimental group that heparin used at 10th day (p<0.003).

Oliveira et al. histopathologically examined the abdominal wall healing in rats treated with enoxaparin for one week and sacrificed at the end of the 3rd, 7th and 14th days after laparotomy surgery was performed. The result of histopathological examinations the organization of collagen was better in control groups than experimental groups enoxaparin used at 7th day but there was no difference between groups at 14th day. This shows that heparin has no effect on final maturation of wound healing at 14th day. In our study statistically significant difference was observed between groups at 7th and 10th day in terms of wound healing. This result shows that heparin has effect on wound healing until the 10th day.

One of the most important problems regarding studies with heparin related to dose and duration of application. In most of the studies concerning wound healing treatment dosage of DVT was preferred for heparin. For example Oliveira et al. used enoxaparin (1 mg/kg/day) on abdominal wound healing for seven days. Arikan et al. had a similar study. They applied 1mg/kg enoxaparin per day for seven days on abdominal wound healing. Also Kus et al. performed a similar study for the same period and dose of application. Another dose problem of heparin studies on wound healing are direct comparison between UH and LMWH doses is not possible due to the differences in units. During our study we have not found a literature related with optimal dose for wound healing. Therefore, adhering to existing literatures, we determined application dose of enoxaparin as 1mg/kg and heparin dose as 1000 U/kg. In addition, adhering to duration of heparin existing prophylaxis we made application to rats during seven days.

Conclusions

The low molecular weight heparin preparations have more attractive pharmacokinetic profile than UH preparations and this feature makes them more popular in the last decades. Histologically both of the preparations increased the rate of re-epithelialization, therefore, unfractionated heparin and low molecular weight heparin play a positive role in tissue remodeling but when effects of these two preparations compared with each other low molecular weight heparin has more positive effects on wound healing process.

References


