THE EFFICACY OF RECOMBINANT HUMAN ACTIVATED PROTEIN C (rhAPC) vs ANTITHROMBIN III (AT III) vs HEPARIN, IN THE HEALING PROCESS OF PARTIAL-THICKNESS BURNS: A COMPARATIVE STUDY

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SUMMARY. This is an experimental study regarding the positive effect of recombinant human activated protein C (rhAPC) in the healing process of partial-thickness burns, in comparison to antithrombin III and heparin. On a porcine model we induced superficial partial-thickness and deep partial-thickness burns and performed intravenous administration of the elements of study during the first 48 h. The progress of the condition of the injured tissues was evaluated by histopathological examination at specific time intervals. The results showed an improved healing response of the specimens treated with rhAPC compared to those treated with antithrombin III, heparin, and placebo.

Keywords: burns, healing, recombinant human activated protein C (rhAPC)

Introduction

Skin vitality is dependent on good microcirculation. The stasis observed in the microcirculation, which eventually may lead to necrosis and prolong the time of healing, is a crucial point in thermal injury. Studies of microcirculatory behaviour after thermal injury have revealed that the participation of superoxides and thrombi contributes to this phenomenon.

We attempted a study in a porcine model in which a thermal injury was induced. Our purpose was to evaluate medical therapies. Although many drugs have been proposed for the topical treatment of burned surfaces, the majority are antibacterial and moisturizing agents. Only a few drugs have been used intravenously to speed up the healing process and preserve microcirculation, heparin and antithrombin III being among the commonest.

We therefore observed the effects of the intravenous administration of heparin and antithrombin III in the healing process of superficial partial-thickness and deep partial-thickness burns and compared their action to that of recombinant human activated protein C (rhAPC), a new powerful antithrombotic, anti-inflammatory and profibrinolytic substance that is associated with a substantial reduction in mortality for patients with severe sepsis. A placebo group was also used.

rhAPC and burns

There are no previous data about the efficacy of rhAPC for burn patients, and burn injuries represent a potentially septic situation.

Our goal was to preserve the microcirculation and thus interrupt the “spontaneous conversion” of deep partial-thickness burns to full-thickness burns, to promote tissue repair, and to decrease inflammation.

Aim

Our aim was to conduct an experimental comparative study on the healing effect of rhAPC versus heparin ver-
sus antithrombin III in the treatment of partial-thickness burns.

Heparin and antithrombin III are among the commonest drugs used intravenously to speed up the healing process in burn injuries and preserve the microcirculation.3,4

Materials and method

We used 24 pigs, aged 5-6 weeks, weighing 10-14 kg each. On the back of each animal we created superficial and deep partial-thickness burns under general anaesthesia. For the creation of the burn wounds a preheated (221 °C, 430 °F) soldering iron with a modified tip was used to create squares of burn injury measuring 2 x 3 cm. The temperature of the tip was measured using a K-type thermocouple contact thermometer. The depth of the burn wounds was calculated by histopathological examination. Five intermittent vertical applications of the soldering iron, using its own weight (100 g) for 5 sec, created a superficial partial-thickness burn (SPTB) (average depth, 0.73 mm). Ten vertical applications were made for 10 sec creating a deep partial-thickness burn (DPTB) (average depth, 0.95 mm) (Fig. 1).

The total time duration for the creation of all burn injuries in each pig was 6 min.

After the creation of the burn wounds, each element of study was infused into each specimen via a jugular catheter. The pigs were divided into four groups: group A (placebo group) received NaCl 0.9% for 48 h at a rate of 4 cc/kg/h; group B was given a bolus of heparin (50 IU/kg for 5 min); group C had continuous infusion of rhAPC for 48 h at a rate of 24 μg/kg/h; and group D received AT III in titrated doses, maintaining plasma levels of 120-200% for 48 h (Table I).

All the animals were treated topically with povidone iodine every 8 h and received 1.2 g cefuroxime daily.

Table I - The 24 pigs were divided into four groups. Each of the elements of study was treated intravenously starting after completion of burn injuries

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo - NaCl 0.9%</th>
<th>Group B - Heparin</th>
<th>Group C - Activated protein C</th>
<th>Group D - AT III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 cc/kg/h for 48 h</td>
<td>50 IU/kg over 5 min</td>
<td>24 μg/kg/h for 48 h</td>
<td>Maintain plasma levels of 120-200%</td>
</tr>
</tbody>
</table>

Cannulation of the jugular vein

After cannulation of the jugular vein, a subcutaneous canal was created in the neck area, and the catheter surfaced from the dorsum of the cervical area. From there it was connected to a micrometric pump through a Foley elastic catheter. In this way the animal was free to move but unable to chew or tear the catheter apart, thus facilitating the continuous infusion for 48 h (Figs. 2-4).
Assessment

The assessment was performed with skin biopsies obtained from the burned areas on day 0 (baseline-normal skin) and on days 1, 3, 7, 13 and 20 post-burn. Histological evaluation of the healing process was performed on the zones of erythema, stasis, and necrosis.

The following features were studied:

a) The depth of the zone of cellular necrosis resulting from direct thermal injury.

b) The depth of zone of cellular injury resulting from the thermal damage also from the biochemical and biophysiological effects of the cell destruction.

c) The overall depth of the zone of cellular necrosis along with the zone of cellular injury.

Comparison between the depths of the zones was the determining factor for the assessment of the speed of healing (which presented with various degrees of re-epithelialization).

The statistical analysis was performed with a value of \( p < 0.05 \) considered statistically significant. The comparison of results from the four groups was performed using the two-way mixed ANOVA test and pairwise analysis was performed with the Krug-Willis test for the examination of the differences between the four therapies, as a percentage change from baseline to each time of measurement of the depth of the total burn injury.

Photo-documentation was performed throughout the experiment showing the histology of the skin before and immediately after the burn wound (Figs. 5-7).

Results

Clinically, there was no macroscopic difference between the four groups on day 3 post-burn either in superficial partial-thickness burns (SPTB) or in deep partial-thickness burns (DPTB).

However, by day 13 post-burn, all eschars had detached from the SPTB wounds and almost all had detached in the DPTB wounds in the rhAPC group (Fig. 8).
In the heparin group most of the eschars detached in the SPTB whereas there was only partial detachment in the DPTB on day 13 post-burn (Fig. 9).

On day 13 post-burn the eschars in the placebo group were only partially detached in some of the SPTB and still firmly adhered in the DPTB (Fig. 10).

It is interesting to note that in the AT III group most of the necrotic SPTB eschars were also detached as well as some of the eschars in the DPTB on day 13 post-burn (Fig. 11).

**Histological assessment**

The histological examination shows that the statistical difference between the total burn injury depths with regard to the four groups was already manifested from day 3, with rhAPC demonstrating less depth of total injury in both superficial and deep partial-thickness burns (Tables II, III).

In the superficial partial-thickness burns, after day 13, the results of the rhAPC group were comparable to those of the AT III and placebo groups. However, by day 20, there was a statistically significant difference in healing between rhAPC and all other groups. The rhAPC group showed a 75% decrease in wound depth in comparison to a 17% decrease in the heparin group, a 52% decrease in the AT III group and a 53% increase in wound depth in the placebo group (Table II, Chart 1).
In deep partial-thickness burns, after day 13, the results of the rhAPC group were comparable to those of the AT III group. However, by day 20, there was a statistically significant difference in healing between rhAPC and all other groups. The rhAPC group showed an almost 80% decrease in wound depth in comparison to 28.6% decrease in the heparin group, a 59.4% decrease in the AT III group, and a 59.9% increase in wound depth for the placebo group. It is obvious that the rhAPC group demonstrates a much better healing curve (Table III, Chart 2).

**Discussion**

Burns injuries are among the worst traumas known. The larger the burn injury, the more severe are the consequences and the higher the chances are of an adverse outcome or even death. As the temperature increases, protein coagulation takes place, and this causes destruction of the protein architecture. New aberrant bonds are formed, creating macromolecules not similar to the original structures. The cell necrosis is complete, usually beginning at the skin surface, where the heat energy was absorbed most directly, extending downwards. This zone is called the zone of coagulation. The zone of stasis lies deeper and peripheral to the zone of coagulation. In this zone the damage is less and most cells are initially viable. However, the blood flow becomes progressively impaired and finally stops. This development of ischaemia results in necrosis of the already affected cells. Peripheral to this zone is the zone of hyperaemia, which is characterized by minimal cellular injury and prominent vasodilatation with increased blood flow, due to vasoactive mediators that were produced as part of the inflammatory response. Complete cellular recovery usually happens from this zone up only when capillaries eventually grow back upwards. Healing occurs rapidly and completely through migration to the surface of epithelial stem cells which survive in deeper portions of the hair follicles as well as the sweat and sebaceous glands. Relatively little scarring occurs in a superficial injury, due to the limited inflammatory phase, which is cut short by wound closure (re-epithelialization) occurring within two weeks.

**Table II** - Healing effect in superficial partial-thickness burns indicated by histological evaluation of wound depth (WD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3 post-burn</th>
<th>Day 7 post-burn</th>
<th>Day 13 post-burn</th>
<th>Day 16 post-burn</th>
<th>Day 20 post-burn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>45% increase in WD</td>
<td>15% decrease in WD</td>
<td>40% decrease in WD</td>
<td>24% increase in WD</td>
<td>53% increase in WD</td>
</tr>
<tr>
<td>Heparin</td>
<td>5% decrease in WD</td>
<td>25% increase in WD</td>
<td>18% increase in WD</td>
<td>6% decrease in WD</td>
<td>17% decrease in WD</td>
</tr>
<tr>
<td>rhAPC</td>
<td>25% decrease in WD</td>
<td>40% decrease in WD</td>
<td>53% decrease in WD</td>
<td>61% decrease in WD</td>
<td>75% decrease in WD</td>
</tr>
<tr>
<td>AT III</td>
<td>16% decrease in WD</td>
<td>37% decrease in WD</td>
<td>44% decrease in WD</td>
<td>49% decrease in WD</td>
<td>52% decrease in WD</td>
</tr>
</tbody>
</table>

**Table III** - Healing effect in deep partial-thickness burns indicated by histological evaluation in wound depth (WD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3 post-burn</th>
<th>Day 7 post-burn</th>
<th>Day 13 post-burn</th>
<th>Day 16 post-burn</th>
<th>Day 20 post-burn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>37.5% increase in WD</td>
<td>7.6% decrease in WD</td>
<td>19.2% decrease in WD</td>
<td>26.9% increase in WD</td>
<td>59.9% increase in WD</td>
</tr>
<tr>
<td>Heparin</td>
<td>16.4% increase in WD</td>
<td>34.3% increase in WD</td>
<td>11.8% increase in WD</td>
<td>7.4% increase in WD</td>
<td>28.6% decrease in WD</td>
</tr>
<tr>
<td>rhAPC</td>
<td>26.1% decrease in WD</td>
<td>21.6% decrease in WD</td>
<td>43.3% decrease in WD</td>
<td>61.8% decrease in WD</td>
<td>79.7% decrease in WD</td>
</tr>
<tr>
<td>AT III</td>
<td>5.7% decrease in WD</td>
<td>25.8% decrease in WD</td>
<td>50% decrease in WD</td>
<td>55% decrease in WD</td>
<td>59.4% decrease in WD</td>
</tr>
</tbody>
</table>

**Chart 1** - Healing effect of the substances studied in superficial partial-thickness burns by assessment of depth of total burn injury in time.

**Chart 2** - Healing effect of the study substances in deep partial-thickness burns by assessment of depth in total burn injury in time.
In deep partial-thickness wounds most of the dermis is destroyed and a few epithelial cells remain only in the deepest parts of the hair follicles and of the sweat and sebaceous glands. As the epithelial cells have to migrate from the depths, and as a result of the loss of stem cells, re-epithelialization is greatly retarded in these wounds. The control of invasive burn wound infection through the use of effective topical chemotherapy, prompt surgical excision, and timely closure of the burn wound has resulted in unsurpassed survival rates. Even so, infection remains the most common cause of death in these severely injured patients. A burn patient suffering from decreased blood supply becomes ischaemic, hypoxic, and highly oedematous. The wound healing response can be divided into three distinct, but overlapping phases: 1) homeostasis and inflammation, 2) dermal and epidermal proliferation, and 3) maturation and remodelling. The first response after disruption of tissue integrity is to control the damage produced to the vascular system. The initial response to deep burns involves a transient 5- to 10-min period of intense vasoconstriction that aids in homeostasis. This is followed by active vasodilatation that usually becomes most pronounced approximately 20 min after the injury and is accompanied by an increased capillary permeability. Histamine is believed to be a key chemical mediator responsible for the vasodilatation and the danger in vascular permeability. Shortly after burning, platelet adhesion occurs at the site of the burn. One of the major problems we face in the evaluation and management of burn victims is the gradual increase in tissue damage as far as depth and total body area of the affected skin is concerned. The direct skin damage due to the cause of burn (Zone I) often represents only 30% of the final damage to the tissues of the area. This area is surrounded by a second zone where due to the release of toxic agents (hyper oxides, free radicals, lysosomes) from the destroyed cells, venous stasis is observed which leads to the formation of thrombus in the microvessels and finally cellular death which result in an increase of tissue damage of more than 30%. A number of agents and substances have been used in an effort to preserve the blood flow in microcirculation mainly by preventing thrombosis. These substances belong to the anticoagulants and blood flow facilitators (such as heparin, urokinase, prostaglandin E1, magnesium sulphate, IIb/IIIa inhibitors of platelets) and others.

**Burns and heparin**

In recent years a multitude of studies by many authors have researched the action of heparin in burns. Heparin is an endogenous glycosaminoglycan. It has been used parenterally, topically, by inhalation, in pellets, and in bio-engineered membrane. Heparin relieved pain, inhibited clotting and inflammation, restored blood flow, and enhanced long-term healing. It preserved lung and improved its function, preserved intestinal integrity, and reduced bacterial translocation. Collagen restoration was enhanced and healed skin was smoother.4-6,16-25

Heparin appears to be the most popular substance used in the research for the treatment of burns.

**Burns and antithrombin III**

AT III is an endogenous glucoprotein synthesized in the liver. It revokes the action of various clotting factors including factors Xa, XIa kalikrein and thrombin. Its antithrombotic action is increased 1000-fold in the presence of heparin. AT III’s anti-inflammatory properties are exerted by the decrease of the production of cytokines (IL-6), of lysosomal proteases and binding molecules and to its recently established action as a serine protease inhibitor. These anticoagulant and anti-inflammatory characteristics make it one of the most common substances used in research on burn treatment.5,7,26-31

**Burns and activated protein C**

Protein C is a serine protease and naturally occurring anticoagulant that plays a role in the regulation of haemostasis through its ability to block the generation of thrombin production by inactivating Factors Va and VIIIa in the coagulation cascade. Human protein C is produced in vivo primarily in the liver. In conjunction with other proteins, aPC functions as perhaps the most important downregulator of blood coagulation, resulting in protection against thrombosis. In addition to its anti-coagulation functions, activated protein C has anti-inflammatory effects and also exerts profibrinolytic properties that facilitate clot lysis. Also, activated protein C effectively prevents endotoxin-induced pulmonary vascular injury. The connection between inflammation and the activated protein C anticoagulant pathway is most elegantly discussed in an article by Charles T. Esmon.19 Last but not least, recombinant human activated protein C is used in the treatment of severe sepsis.7

The protein C enzyme system represents a major physiological mechanism of anti-coagulation, anti-inflammation, and fibrinolysis. Even though activated protein C is a very powerful anticoagulant, and anti-inflammatory drug, its use was never reported in the early stages of burn injury (first 7 post-burn days) either in clinical or in experimental studies – up to now. The IV administration of activated protein C during the first 48 postburn hours can exert a beneficial effect directly in zone II (or Zone of Stasis) of the burn injury, by preventing coagulation and inflammation in this area thus effectively stopping the progress of the burn injury, resulting in overall smaller volume of damaged tissue to be repaired.9,30,47
Conclusion

Recombinant human activated protein C (rhAPC) demonstrates a substantially better healing response than heparin and AT III in both superficial and deep partial-thickness burns, as indicated by the decrease in the depth of the burn injury.

Compared to Heparin and AT III, rhAPC offered a statistically significant improvement in the healing of both superficial and deep partial-thickness burns. On day 20 post-burn there was a 75% healing effect of rhAPC in superficial partial-thickness burns, compared to 17% for heparin and 52% for ATIII. For the same time period, regarding deep partial-thickness burns, rhAPC demonstrated an impressive 79.7% healing effect compared to heparin (28.6%) and ATIII (59.4%).

Future clinical and experimental studies should focus on further evaluating the therapeutic utilization of rhAPC for severely burned patients.


Mots-clés: brûlures, guérison, protéine recombinante humaine activée C (PrhAC)

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