Inflammatory Reaction and Blood Flow in Experimental Wounds Inoculated with *Staphylococcus aureus*

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**Key Words.** Inflammation · *Staphylococcus aureus* · Granulation tissue · Wound healing · Wound infection

Abstract. Wound healing and granulation tissue formation can be accelerated by inoculation with live pathogenic microorganisms. For further elucidation of this phenomenon the present work was undertaken to study the effects of *Staphylococcus aureus* microorganisms on the inflammatory reaction and blood flow in developing granulation tissue in rats. Cylindrical hollow sponge implants were used as an inductive matrix for the growth of granulation tissue. In control animals 1 ml of wound fluid was withdrawn from the central dead space of the implant immediately after implantation and replaced with 1 ml of physiological saline. In experimental animals the implants were injected with live staphylococci, $10^2$ or $10^5$ microorganisms/ml. Wound fluid was analyzed 3, 7, 10 and 14 days after implantation, whereas measurements of local blood flow and albumin extravasation in the granulation tissue were made after 7 days. Implants inoculated with $10^3$ organisms developed infection with pus formation while implants contaminated with $10^2$ organisms showed no infection. In wound fluid specimens collected from the infected implants correlation between the number of polymorphonuclear leukocytes and prostaglandin E$_2$ concentration was statistically significant. The most prominent finding in contaminated but uninfected implants was an enhanced local blood flow. This may explain some of the mechanisms leading to *S. aureus* induced acceleration of wound healing.

Introduction

The inflammatory phase in wound healing is considered to be a preparatory process for the formation of new tissue [26]. Extinction of the inflammatory process with steroids or elimination of key cells in the inflammatory response counteracts the normal wound healing [19]. On the other hand, if the inflammatory response is too excessive, the repair process is prolonged [3, 7]. Obviously, there must be an optimal inflammatory re-
in order to achieve rapid wound healing. The content of the term 'optimal' is not exactly known. It has been suggested that controlled stimulation of the inflammatory response actually might result in facilitated repair, indicating that under normal circumstances injured tissues exist in conditions that are suboptimal for wound healing.

Several reports indicate that wound healing can be accelerated to a certain extent by inoculation with live pathogenic microorganisms [2, 4, 10, 17, 18, 21]. In a previous study [9], inoculation of experimental wounds, i.e. subcutaneously implanted cellulose sponges with \(10^2\) Staphylococcus aureus organisms enhanced the tissue ingrowth and formation of collagen when studied 14 days after implantation. However, larger numbers \((10^3-10^5)\) of inoculated staphylococci induced an overt purulent infection and there was no stimulation of the repair process. The result of that study seemed to corroborate the hypothesis that a controlled enhancement of the inflammatory response by a certain number of staphylococci, not large enough to overcome the defense mechanisms of the body, facilitated wound healing.

Of course, it is not possible to apply these findings directly to clinical situations where stimulated wound healing would be desirable. However, a thorough characterization of the inflammatory process and wound environment under the experimental conditions used might give valuable information for further aims to facilitate wound healing through controlled stimulation of the inflammatory response. The present study was designed to investigate the wound inflammatory response and blood flow in sponge implants of rats inoculated with either a high dose of staphylococci causing an overt wound infection or a low dose of staphylococci causing no infection but instead facilitating wound healing [9].

Material and Methods

A standardized experimental wound model described by Niinikoski et al. [16] was used and is described in detail elsewhere [9].

Eighteen rats were studied in three groups of 6 animals. In the control group the implants were treated immediately after implantation by withdrawing 1 ml of wound fluid from the central dead space and replacing it with 1 ml of physiological saline. In the experimental groups the aspirated wound fluid was substituted with a corresponding volume of saline containing live staphylococci (S. aureus: strain 25923, American Type Culture Collection, Rockville, Md., USA), \(10^3\) or \(10^5\) microorganisms/ml. All aspirations and injections were carried out under strictly aseptic conditions and great care was taken to avoid contamination of implants with airborne oxygen.

Wound fluid was analyzed 3, 7, 10, and 14 days after implantation, whereafter the rats were sacrificed. Polymorphonuclear leukocytes (PMNs) were counted in Bürker's chamber after staining with gentian violet. The concentration of prostaglandin \(E_2\) \((\text{PGE}_2)\) was determined by a radioimmunoassay (NEN Chemicals GmbH, Dreieich, FRG).

The blood flow measurements were made 7 days after implantation using a radioactive microsphere technique described by Lundberg and Smedegård [12]. In brief, the rats of the three groups, each containing 5–9 animals, were tracheostomized and catheters were inserted into both femoral arteries and the left ventricle of the heart. To measure albumin extravasation in granulation tissue \(5 \mu\text{Ci}\) of \(^{125}\text{I}\) labelled human serum albumin \((^{125}\text{I}\text{HSA}; 50 \mu\text{Ci}/\text{ml}; Amersham International Ltd., Apersham, UK) were injected intracardially. Thereafter, to measure blood flow, two separate sets of 15-μm microspheres labelled with \(^{17}\text{Co}\) or \(^{65}\text{Zn}\) (3M Co., Minneapolis, MN, USA) were administered into the left ventricle, one 20 min and the other 40 min after the \(^{125}\text{I-HSA}\) injection, the two isotopes being varied randomly. The arterial reference sample was drawn from the right femoral artery. Blood flow and cardiac output were calculated by means of a computer program [20].
and expressed as an average of the two determinations.

Results are expressed as means ± standard error. The significance of differences in means between the control and experimental groups was tested by a repeated measured two-way analysis of variance, where the grouping factor used was the treatment group and the within factor the day of measurement or the site of the skin sample. The results of wound fluid PGE\textsubscript{2} and PMNs were analyzed after In transformations, because of the high variation in the data. The pairwise comparisons between daily mean values were made using Student's t test with the Bonferroni correction after discovering by the two-way analysis of variance that there was an overall variation between these mean values. The dependence between In(PGE\textsubscript{2}) and In(PMN) in the group inoculated with 10\textsuperscript{5} organisms was tested using one-way analysis of covariance with repeated measures. Statistical processing was carried out using a BMDP computer program library [5].

Results

Implants inoculated with 10\textsuperscript{5} organisms invariably developed infection with pus formation while implants inoculated with 10\textsuperscript{2} organisms developed no macroscopical infection. Infection seemed to increase the total number of wound fluid PMNs over the control level from day 7 onwards (fig. 1). However, due to high variation of the data no statistically significant differences were detected between the groups. In implants inoculated with 10\textsuperscript{2} bacteria the amounts of wound fluid PMNs were near to the control range.

The response of wound fluid PGE\textsubscript{2} concentration to the increasing size of the bacterial inoculum was almost analogous to that of wound fluid PMNs (fig. 2). In infected implants the correlation between the amounts of PMNs and the concentration of PGE\textsubscript{2} after In transformations was statistically significant (p < 0.01). In implants inoculated with 10\textsuperscript{2} organisms the In(PGE\textsubscript{2}) rose significantly between days 3 and 7 (p < 0.05) and decreased between days 7 and 10 (p < 0.05). In implants inoculated with 10\textsuperscript{5} organisms the In(PGE\textsubscript{2}) was significantly above the control level on day 14 (p < 0.01).

In implants inoculated with 10\textsuperscript{2} or 10\textsuperscript{5} microorganisms the mean blood flow was 57 and 26\% higher, respectively, than that in control implants (table I). However, only the former difference was statistically significant (p < 0.01). No essential differences between the groups were detected in the blood flow of
the skin overlaying the implant or more caudal skin. Furthermore, no significant differences were observed in the extravasation of $^{125}$I-HSA in the granulation tissue or skin samples between the groups.

**Discussion**

According to earlier studies, wound healing can be accelerated to a certain extent by inoculation of live staphylococci. In 1921, Carrel [4] observed that healing was faster in the open wounds of dogs treated with various concentrations of *S. aureus* microorganisms than in control wounds protected from the environment by a dressing. Botsford [2] treated skin wounds in guinea pigs with a culture of *Staphylococcus albus* and found that a mild infection exerted a favorable effect on the rate of gain in the wound tensile strength. In the work of Levenson et al. [10] 10^2 *S. aureus* bacteria introduced into the skin incision of rats significantly accelerated the rate of gain in breaking strength, but this effect was not as great as when 10^9 organisms

**Fig. 2.** Wound fluid PGE$_2$ concentration in control implants and implants inoculated with different amounts of viable *S. aureus* organisms. Each value indicates mean ± SE of 6 rats. o = Control; △ = *S. aureus* (10^2); ■ = *S. aureus* (10^5).

**Table I.** Effects of various sizes of *S. aureus* inoculum on blood flow and albumin extravasation in granulation tissue and different skin sites in the rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood flow (ml × min$^{-1}$ × 100 g$^{-1}$ dry weight)</th>
<th>Albumin extravasation (μl plasma/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>granulation skin</td>
<td>overlaying skin</td>
</tr>
<tr>
<td>Control</td>
<td>203.3 ± 17.8 (6)</td>
<td>68.9 ± 8.3 (4)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>320.0 ± 34.8 (7)**</td>
<td>56.9 ± 8.4 (6)</td>
</tr>
<tr>
<td>(10^2)</td>
<td>256.6 ± 17.1 (6)</td>
<td>61.6 ± 4.8 (7)</td>
</tr>
<tr>
<td>(10^5)</td>
<td>303.3 ± 17.8 (6)</td>
<td>68.9 ± 8.3 (4)</td>
</tr>
</tbody>
</table>

The mean ± SEM with the number of rats in parentheses is given. The analyses were carried out 7 days after implantation. **p < 0.01 (two-way analysis of variance, Student's t test with the Bonferroni correction).
were introduced. This accelerating effect was evident 4 days postoperatively, maximal at 7–10 days and still present at 28 days. Laato et al. [9] studied the effects of inoculated *S. aureus* microorganisms on granulation tissue formation in rats using subcutaneous cellulose sponge implants. Implants inoculated with $10^3$ or more organisms developed infection, which delayed healing, while implants inoculated with $10^2$ bacteria showed no infection and were able to clear themselves from the organisms in 6 out of 8 cases within 14 days. In the latter implants the number of wound fluid monocytes and macrophages was markedly elevated and the mean amount of collagen exceeded the control level by 55%.

It is well known that PMNs accumulate at sites of inflammation especially when the tissue is infected with various bacteria. It has been suggested that PMN accumulation enhances blood flow and exudation [1, 6, 8]. This effect can be exerted through release of inflammatory mediators from emigrating and/or phagocytizing PMNs [8, 13]. Inflammatory exudate contains large amounts of PGE$_2$ which is a potent vasodilator [25]. Prostaglandins of the E type and other vasodilatory agents have been found to enhance protein exudation [23, 24].

In the present study the number of wound fluid PMNs and the wound fluid PGE$_2$ concentration correlated significantly with each other in infected implants and the highest mean values were observed 14 days after implantation. In a previous study [11], it was suggested that the PMN is not the source of PGE$_2$ in a healing open wound. In that study, however, the wounds were not infected and the concentration of wound PMNs was low. It is thus possible that the source of PGE$_2$ in uninfected wounds is different from that in infected wounds. No marked differences were seen in albumin extravasation between the control and infected implants.

In implants contaminated with $10^2$ organisms the content of wound fluid PMNs showed no change from normal but the wound fluid PGE$_2$ concentration rose significantly from the initial level 7 days after implantation. During this phase the blood flow of these implants was also significantly elevated. The specific reason why the blood flow was measured on day 7 was that the occurrence of newly formed blood vessels in the developing granulation tissue gradually increases up to day 7 after wounding and then slowly subsides [14, 22].

In conclusion, most of the inflammatory parameters in experimental wounds responded to inoculation with an infective dose of pathogenic organisms, whereas in contaminated but not infected wounds the most prominent finding was the enhanced blood flow 7 days after implantation. This might serve to explain some of the mechanisms leading to *S. aureus*-induced acceleration of wound healing.

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Abstract.

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Preliminary Report

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