Effect of povidone iodine and hydrogen peroxide on fracture healing: a histomorphometric study on rats

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ABSTRACT

Purpose. To evaluate the effect of povidone iodine and hydrogen peroxide on fracture healing in a rat model.

Methods. The middle section of the right femur of 36 male Sprague Dawley rats was osteotomised with a saw. In the control group (n=9), the wound was irrigated with 100 ml 0.9% saline. In the 10% povidone iodine (n=9), 1% povidone iodine (n=9), and 3% hydrogen peroxide (n=9) groups, the wound was completely soaked with the respective solution for 2 minutes, and then irrigated with 100 ml saline. The osteotomy was fixed with an intramedullary Kirschner wire. Rats were euthanised at week 1, 2, and 5. In each femur, the percentage area of osseous, cartilaginous, and fibrous tissue in the callus was evaluated in 3 slides (one median and 2 paramedian).

Results. The control group differed significantly to the other 3 groups (p=0.023 to p<0.001) in weeks 1, 2, and 5, except for the 1% povidone iodine group in weeks 1 and 5 (p=0.999) tissue. The healing process was similar in the 1% povidone iodine group and the control group, whereas healing was impaired in the 10% povidone iodine group and 3% hydrogen peroxide group, as indicated by the lower percentage of osseous tissue, higher percentage of fibrous tissue, and increased percentage of cartilaginous tissue between weeks 2 and 5 (delayed bone healing).

Conclusion. The 1% povidone iodine solution is recommended as the irrigation adjuvant in fracture surgery.

Key words: anti-infective agents, local; fracture healing; hydrogen peroxide; povidone-iodine

INTRODUCTION

Adequate irrigation and debridement to remove foreign bodies, non-vital tissues, and contaminating microorganisms is the most important means to prevent infection in open fractures. Antiseptics (povidone iodine and hydrogen peroxide) and antibiotics are used as an adjuvant to destroy
microorganisms and prevent infection, despite being toxic to the host cells. Although povidone iodine destroys microorganisms in contaminated bone in vitro, its effects on cells, tissues, and fracture healing were not studied. A high concentration (stock solution) of povidone iodine, sodium hypochlorite, and hydrogen peroxide can destroy 100% cultured fibroblast cells in vitro. A diluted 1% povidone iodine solution is safe to use on wounds to reduce infection in children with appendicitis. Toxicity in vitro is not always the same as in vivo; although povidone iodine has a potent cytotoxic effect in vitro, its in vivo effect is weak.

Hydrogen peroxide has effervescent and oxygen-releasing effects. The effervescent effect lifts debris from the wound bed to the surface; released oxygen may also kill anaerobic bacteria. It is toxic to giant cell tumour metabolism and osteoblasts in vitro. A 3% hydrogen peroxide solution is used as a local adjuvant to intralesional curettage of giant cell tumour of the bone. This study evaluated the effect of povidone iodine and hydrogen peroxide on fracture healing in a rat model.

METHODS

This study was approved by the ethics committee of the Faculty of Medicine Universitas Indonesia. Male Sprague Dawley white rats were used. The rats were anaesthetised by intraperitoneal injection of ketamine 80 mg/kg and xylazine 10 mg/kg. Through the anterolateral approach, the space between the vastus lateral and biceps femoris of the right femur was dissected bluntly, with the periosteum undisturbed. The middle section of the femur was osteotomised with a saw.

In the control group (n=9), the wound was irrigated with 100 ml 0.9% saline. In the 10% povidone iodine (n=9), 1% povidone iodine (n=9), and 3% hydrogen peroxide (n=9) groups, the wound was completely soaked with the respective solution for 2 minutes and then irrigated with 100 ml saline.

The osteotomy was fixed with a retrograde intramedullary 1.4-mm Kirschner wire through the intercondylar femur. The fascia and skin were sutured, and the wound was bandaged. Prophylactic antibiotic (ampicillin 50 mg/kg/day) and analgesic (paracetamol 50 mg/kg/day) was given for 3 days. Rats were euthanised with phenobarbital injection 75 mg/kg intraperitoneally on week 1, 2, and 5. The right femur was harvested with the Kirschner wire in situ and fixed in 10% neutral buffered formalin for 48 hours. The samples were decalcified in Plank Rychlo solution (Wako Pure Chemical Industries, Osaka, Japan) and then embedded in paraffin and sliced longitudinally with a 5-μm microtome, and stained with haematoxylin and eosin. In each femur, 3 slides (one median and 2 paramedian ±250 μm from the median slice) were evaluated histomorphometrically with a digital microscope to determine the percentage area of osseous, cartilaginous, and fibrous tissue in the callus using the ImageJ program (National Institutes of Health, USA); osseous tissue of the original bone

![Figure 1](image-url)  
**Figure 1** Histomorphometric measurement using the ImageJ: (a) borders of fibrous, cartilaginous, and osseous tissue of the callus are outlined (H&E, 40x); (b) tissue areas are measured after the original image is subtracted.
was not included (Fig. 1).14

The percentage area of osseous, cartilaginous, and fibrous tissue in the 4 groups were compared separately using the one-way ANOVA with repeated measurement test. The control group was compared with the other 3 groups using the Dunnett post hoc test. A p value of <0.05 was considered statistically significant.

RESULTS

Using the one-way ANOVA with repeated measurement test, not all the 4 groups were equal in terms of percentage of osseous tissue [F (3, 16)=15.407, p<0.001], cartilaginous tissue [F (3, 16)=7.901, p<0.001], and fibrous tissue [F (3, 16)=7.769, p<0.001] (Table). Using the Dunnett post hoc test, the control group differed significantly to the other 3 groups (p=0.023 to p<0.001) in weeks 1, 2, and 5, except for the 1% povidone iodine group in terms of percentage of osseous (p=0.349) and fibrous (p=0.999) tissue.

The healing process was similar in the control and 1% povidone iodine groups, whereas healing was impaired in the 10% povidone iodine and 3% hydrogen peroxide groups, as indicated by a lower percentage of osseous tissue, higher percentage of fibrous tissue, and increased percentage of cartilaginous tissue between weeks 2 and 5 (delayed bone healing) [Fig. 2].

DISCUSSION

The iodine element in povidone iodine is carried in aggregates (or micelles) and is released gradually into the solution to achieve its antiseptic effect15,16. Povidone iodine exposure causes cell death by damaging the cell wall, cell membrane, and cytoplasm.17 10% povidone iodine solution is widely used and does not cause resistance by

<table>
<thead>
<tr>
<th>Group</th>
<th>% area of fibrous tissue</th>
<th>% area of cartilaginous tissue</th>
<th>% area of osseous tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 5</td>
</tr>
<tr>
<td>Control (0.9% saline)</td>
<td>70.9±2.44</td>
<td>57.2±5.08</td>
<td>37.9±3.25</td>
</tr>
<tr>
<td>10% povidone iodine</td>
<td>91.7±3.8</td>
<td>84.3±3.11</td>
<td>65.0±5.85</td>
</tr>
<tr>
<td>1% povidone iodine</td>
<td>78.8±4.1</td>
<td>53.1±6.09</td>
<td>33.3±8.05</td>
</tr>
<tr>
<td>3% hydrogen peroxide</td>
<td>78.6±3.4</td>
<td>72.5±2.83</td>
<td>63.4±3.08</td>
</tr>
</tbody>
</table>

Figure 2  Histological image of the callus in week 5: (a) the control group, (b) the 10% povidone iodine solution group, (c) the 1% povidone iodine solution group, and (d) the 3% hydrogen peroxide solution group. Borders of fibrous, cartilaginous, and osseous tissue are outlined (original bone and void area is not included). In the control group, the osteotomy is united by little amount of callus largely composed of osseous tissue, whereas in the other 3 groups the fracture is united by large amount of callus composed of various amounts of fibrous, cartilaginous, and osseous tissue.
microorganisms.\textsuperscript{17,18} The effect of povidone iodine is maximum at a dilution of 1:100 (0.1%) and remains effective at a dilution of 1:10 000 (0.001%).\textsuperscript{19,20} The increasing bactericidal activity in diluted solution is due to an increased level of free iodine (up to 26 ppm in 1% to 0.1% povidone iodine solution).\textsuperscript{16} Povidone iodine may be deactivated by organic substances and protein, but 0.2% and 0.5% povidone iodine solution have been reported to eradicate microorganisms totally in a medium-containing serum at concentrations of 5% and 10%.\textsuperscript{18}

Hydrogen peroxide facilitates wound healing. It is produced in acute wound to attract neutrophils.\textsuperscript{21} Both hydrogen peroxide and superoxide anions have been shown to be present in a wound model in rats.\textsuperscript{22} Application of a low concentration of hydrogen peroxide improves wound healing.\textsuperscript{23,24} It has been used as an adjuvant in irrigation and debridement of wounds and/or open fracture,\textsuperscript{4,7,10,25} in curettage of bone neoplasm,\textsuperscript{12,13} and as a haemostatic agent to improve contact between trabecular bone and bone cement.\textsuperscript{26} Its effervescence effect lifts debris and neoplastic cells from the wound bed to the surface.\textsuperscript{12,13,25} A 3% hydrogen peroxide solution is toxic to cultured human fibroblasts, but does not interfere with wound epithelialisation.\textsuperscript{10} A 1.5 to 3.5% hydrogen peroxide solution has been shown to increases healing of ischaemic ulcers in guinea pigs through increased angiogenesis.\textsuperscript{24,27–29} Hydrogen peroxide stimulates macrophages to release vascular endothelial growth factor,\textsuperscript{29} resulting in an increase in angiogenesis.\textsuperscript{24}

Quantitative analysis of bone histology (histomorphometry) is a good method to evaluate bone healing.\textsuperscript{14,30} as is the cartilage resorption time index.\textsuperscript{14} Histomorphometry measurement on a longitudinal section of fracture callus enables description of the composition of callus tissue with fewer slices. Heterogeneity of tissue proximal and distal to the fracture site can be seen more clearly.\textsuperscript{30} Transverse sections can describe the tissue composition better, but multiple (6–10) slices within a certain distance of the fracture site are needed.\textsuperscript{14,30} The original cortical bone and intramedullary haematopoietic tissue should not be included; only the fracture callus characterises the fracture healing process. In rats, the peak of cell proliferation, the beginning of the chondrogenesis period and endochondral ossification occur at the end of week 1.\textsuperscript{31,32} Soft callus formation, cartilage resorption, woven bone formation, and neoangiogenesis occur at the end of week 2, during which the osteogenesis process is most active.\textsuperscript{31,32} Solid union with woven bone occurs at the end of week 5, followed by remodelling and lamellar bone formation.\textsuperscript{32} From week 1 to week 5, the percentage of fibrous tissue is decreasing and the percentage of osseous tissue is increasing, indicating fracture healing.\textsuperscript{30} In our study, at week 5 the percentage of fibrous tissue was highest (most impaired fracture healing) in the 10% povidone iodine group, followed by 3% hydrogen peroxide group, control group, and 1% povidone iodine group.

Cartilage resorption is calculated by the difference in the percentage of cartilaginous tissue in the callus between week 2 (the peak of chondrocyte proliferation)\textsuperscript{31,33} and week 5 (the checkpoint in the remodelling phase in which the cartilaginous tissue had been replaced by woven bone).\textsuperscript{32} In our study, cartilage resorption (the difference in the percentage of cartilaginous tissue) was highest in the control and 1% povidone iodine groups (both 9.9%), followed by the 10% povidone iodine and 3% hydrogen peroxide groups (-2.7% and 1%, respectively). A greater difference in decreased percentage of cartilaginous tissue from week 2 to week 5 indicates a more favourable remodelling process. Calcified cartilage is resorbed by osteoclasts and bone is formed by osteoblasts.\textsuperscript{31,33} Povidone iodine has a toxic effect on fibroblasts\textsuperscript{7,10} and osteoblasts\textsuperscript{34,35} in vitro, but it has no negative effect on surgical wound healing in vivo.\textsuperscript{8,36} Irrigation with low concentration (0.35%) povidone iodine in spinal surgery does not affect the wound healing, fusion rate, or clinical outcome.\textsuperscript{15} Low concentration povidone iodine also increases angiogenesis.\textsuperscript{36} A 1% povidone iodine solution increases mineral deposition in vitro, especially when combined with BMP-2.\textsuperscript{34}

CONCLUSION

The 1% povidone iodine solution is recommended as the irrigation adjuvant in fracture surgery.

DISCLOSURE

No conflicts of interest were declared by the authors.

REFERENCES

1. Anglen JO. Comparison of soap and antibiotic solutions for irrigation of lower-limb open fracture wounds. A prospective,