Effects of SertaSil on wound healing in the rat

• **Objective:** SertaSil is a novel product for the topical management of wound exudate. The purpose of this study was to evaluate the ability of SertaSil to promote wound healing in a pre-clinical wound model.
• **Method:** An aseptic wound was induced in rats by administering 1ml 10% calcium chloride solution into the subcutaneous layer under local anaesthesia. Following opening of the abscess, animals were divided into a control group (no treatment) and either SertaSil or Gentaxane, which were applied topically to the wound every 24 hours until a clean wound was achieved, that is, free from necrosis, pus and fibrinogenous thickenings.
• **Results:** Rats (n=15 per group) receiving SertaSil reached the clean wound stage in 3.0±0.4 days compared to 7.0±0.4 days for Gentaxane and 10.0±0.4 days for the control. Time to wound closure was 13.9±0.3 days for SertaSil, 18.7±0.6 days for Gentaxane, and 23.0±0.4 days for the control. The surface area of the wounds were measured at day 1 and day 13. At day 1, the wound surface areas (mm^2) were similar in all three groups (157.4±8.9), but at day 13 the SertaSil group had significantly smaller wound areas (5.2±1.7) compared to the Gentaxane (38.0±1.5) and control groups (95.7±11.3). The study was conducted in young rats that are still growing and gaining weight. At day 19, only the rats receiving SertaSil exhibited a weight increase (271±5g) indicating good recovery, whereas rats receiving Gentaxane did not gain weight (249±5g) and rats in the control group lost weight (242±16 g).
• **Conclusion:** The study found that SertaSil reduced the time to reaching a clean wound by 60% compared to Gentaxane and promoted faster wound closure and better recovery. These findings suggest that SertaSil may be valuable for use in the treatment of wounds in patients.
• **Declaration of interest:** Dr. Bilyayeva, Dr. Neshta and Dr. Golub are inventors of SertaSil and Dr. Sams-Dodd is from Willingsford Ltd.

wound care; pre-clinical; healing; exudate; inflammation

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SertaSil (Willingsford, UK) is a novel first-in-class product for the topical management of wound exudate. It is intended for use in the treatment of wounds more than 24 hours old to heal by secondary intention. SertaSil is a powder product, composed of fumed silica with the proteolytic enzyme serratiopeptidase immobilised on its surface. SertaSil exerts its effects by adsorbing moisture, proteins and bacterial toxins in the wound exudate, which may delay healing, and by disrupting biofilm that may be present in the wound. Biofilm is a layer secreted by some types of bacteria on the wound surface to provide them with a protective environment. Serratiopeptidase disrupts this bacterially secreted biofilm by chemical hydrolysis[1] to facilitate an improved removal of exudate from the wound surface.

In the normal healthy wound[2] the exudate supports healing and its main role is in facilitating the diffusion of vital healing factors, such as growth and immune factors, and the migration of cells across the wound bed. It also promotes cell proliferation, provides nutrients for cell metabolism, and aids autolysis of necrotic or damaged tissue. As healing occurs, the amount of exudate produced usually decreases. In the non-healing wounds or wounds with delayed healing, the exudate increases its contents of pro-inflammatory factors, such as bradykinin, Substance P and activated proteolytic metalloproteinases, to the level where it may cause damage to the wound surface and the surrounding skin area. The exudate can in these conditions be almost corrosive to the wound surface. In one study[3] wound exudate was collected from the venous ulcers of six patients to investigate the effects of chronic wound fluid on the proliferation of human dermal fibroblasts, microvascular endothelial cells, and keratinocytes in culture. Results showed that chronic wound fluid inhibited or failed to stimulate the proliferation of all of these cells. The study also found that the chronic wound fluid was cytotoxic and that this may have contributed to the effect on cell proliferation. In contrast, fluid from acute wounds has been found to stimulate fibroblast proliferation.[3]

Objectives

The purpose of this study was to evaluate whether SertaSil in an *in vivo* model of wound healing reduced the time to reaching a clean wound, i.e. free from necrosis, pus and fibrinogenous thickenings, and the time to wound closure. The effects of SertaSil were compared to the topical antibiotic Gentaxane — the standard of care product for necrotic infected wounds at the hospital — and an unassist-

ed healing process (control). Gentaxane is used for treating wound infections and has been shown to promote wound healing.[4]

Methods

Animals

Forty-five white sexually mature rats of both sexes with an average weight of 230-250g were used for the study. The animals were kept in a vivarium at constant temperature (21±1°C), pressure and humidity. Food and drink were accessible *ad libitum*. The light cycle was 12:12 hours. All rats were kept under equal conditions. The animals were kept in individual cages to avoid any stimulatory effect of the saliva components from other rats that could influence the wound healing process.[5] According to generally accepted ethical standards, all manipulations that potentially could cause pain were carried out under local anaesthesia (0.5% Novocaine solution).

Induction of wound

Aseptic inflammation was modelled by the procedure of Shalimov et al.[6] The fur was shaved off on the back area, the area was treated with iodine alcoholic solution, and 1ml 10% calcium chloride solution was administered into the subcutaneous layer.

Treatment

The abscess that had formed under the skin was opened surgically 3–5 days after the injection. For the SertaSil and the Gentaxane groups, SertaSil was applied as a 3mm-thick layer and Gentaxane as a 1mm-thick layer, respectively, on top of the wound every 24 hours until the stage of a clean wound had been achieved, i.e. daily application until the wound was free of necrosis, pus and fibrinogenous thickenings. The control group was untreated.

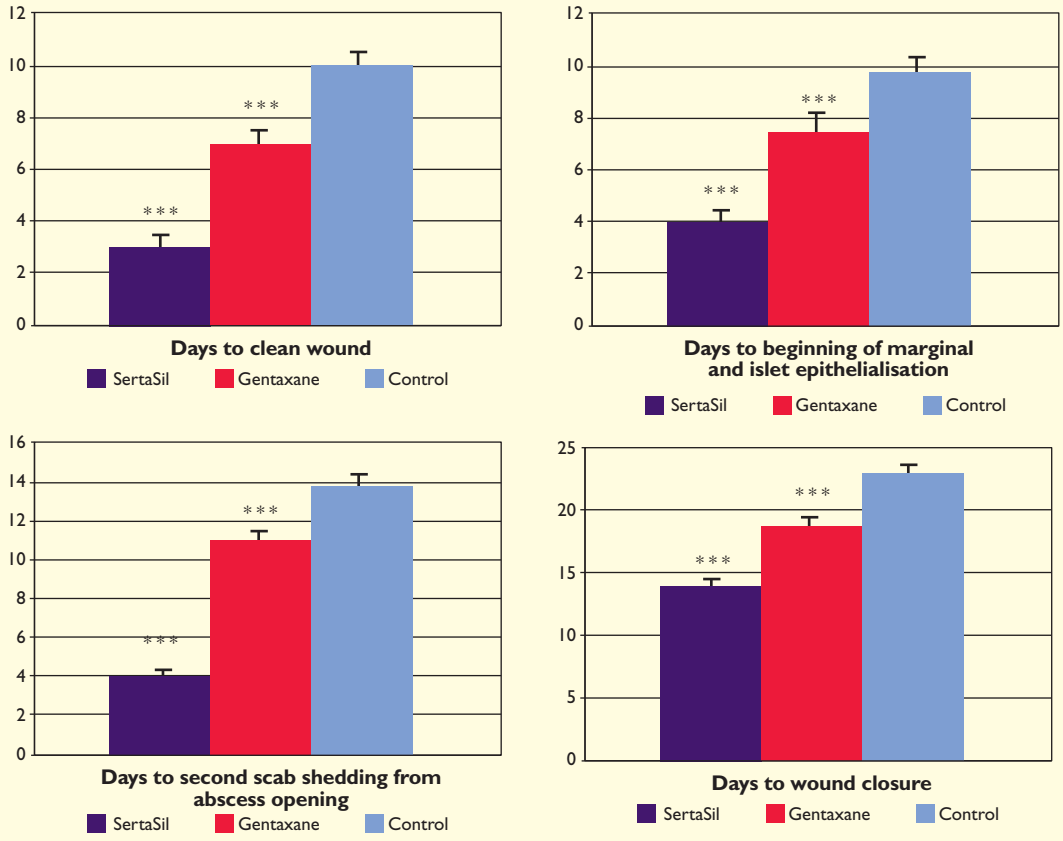
Experimental groups

The experiment included three groups, each with 15 rats; comprising SertaSil; Gentaxane (24mg/g gentamicin, L-tryptophan 14mg/g, zinc sulphate 10mg/g in a polymethylsiloxane powder ad 1g); and the untreated control.

Measurements

The animals were inspected daily and it was recorded which day they reached each of four stages in the

Fig 1. Days to reach four stages in the wound healing process for rats receiving SertaSil, Gentaxane or no treatment (control); n=15 per group, mean±SD. ***=p<0.001



wound healing process:
1. Clean wound, i.e. free of necrosis, pus and fibrinogenous thickenings
2. Second scab shedding from abscess opening
3. Beginning of marginal and islet epithelialisation
4. Wound closure, i.e. full epithelialisation.

The average wound surface area was measured on the first day after abscess opening and on day 13. The area of the wound defect, its perimeter, and average diameter were determined by applying polyethylene film over the wound. The wound circumference was copied onto the film and the lengths at all sides were measured in millimetres (mm). The data were entered into the computer programme package SigmaScan Pro and the surface area was calculated.

The body weight of the rats was measured on day 0 and day 19. The change in an animal body mass can be used as a non-specific index of how an organism is affected by various stress factors.

Samples of wound exudates were taken by gauze swab from the wound area. Native (naturally occurring) smears obtained from the gauze swabs were dried and imbued by Romanowsky-Gimza staining.[7] Cytological evaluations of microflora and immune response in the wound were made by studying smear-imprints using the method of Pokrovska and Makarova.[8] For the immune response, the distribution of neutrophils, monocytes and lymphocytes were calculated based on 100 fields of view using a Goriainov's Camera. Measurements were made 6, 12, 24 and 48 hours after opening of the abscess.

Statistical analysis

Data were analysed statistically using ANOVA with Fischer's Least Significant Difference post-hoc test (Systat 8.0). Measurements are given as mean±SD.

Animal ethics

The study was conducted in accordance with the Ukrainian Regulation for Animal Experimentation: the Law of Ukraine No. 3447-IV (2006) on animal welfare; and the order of the Ministry of Health of the USSR No. 755 (1977) and No. 701 (1978) on the use of experimental animals.

Results

Following the injection of 1ml 10% calcium chloride solution into the subcutaneous layer of the rat, a purulo-necrotic process developed in, on average, 3–4 days. After lancing of the abscess, topical application of SertaSil, Gentaxane, or no application (control) were initiated.

Fig 1 summarises the wound measurements for each of the three groups. There were significant difference between the three groups for days to reach a clean wound ($F(2,42)=1295.0$; $p<0.001$); days to scab removal ($F(2,42)=1317.0$; $p<0.001$); days to beginning of marginal and islet epithelialisation ($F(2,42)=408.15$; $p<0.001$); and days to wound closure ($F(2,42)=1567.21$; $p<0.001$).

The SertaSil group reached the stage of a clean wound after only 3.0±0.4 days compared to 7.0±0.4 days for Gentaxane and 10.0±0.4 days for the control group. SertaSil and Gentaxane were applied daily until the clean wound stage was reached and SertaSil was therefore only applied for 3 days, whereas Gentaxane was applied for 7 days.

The SertaSil group also reached the subsequent wound healing stages faster than the two other groups and demonstrated wound closure after 13.9±0.3 days compared to 18.7±0.6 days for Gentaxane and 23.0±0.4 days for the control group.

Measurements of wound surface area (Fig 2) showed that the wound surface area at day 1 was similar for all groups ($F(2,42)=0.07$; p=not significant (NS)), but at day 13 there was a significant difference ($F(2,42)=708.15$; $p<0.001$) with the SertaSil group having the smallest wound surface followed by the Gentaxane group. At day 13 all the wounds

Fig 2. Wound surface area at day 13 following opening of the abscess (n=15 per group).

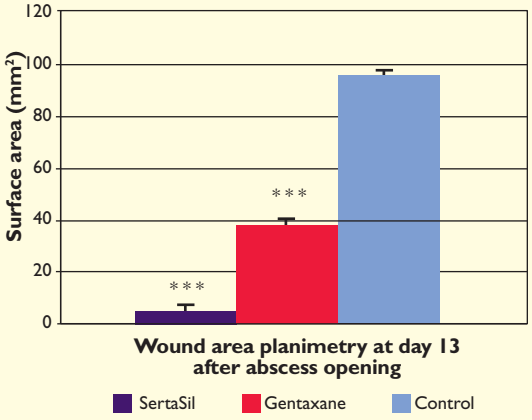


Fig 3. Body weight at day 1 and day 24 (n=15 per group).

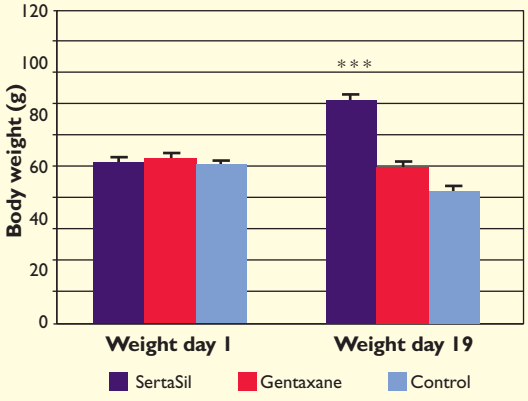


Table 1. Presence of microflora in the wound at three time points following opening of the abscess

Parameter	SertaSil n=15	Gentaxane n=15	Control n=15
Wound at 6 hours	Present in 25% of group	Present in 15% of group	Present in 25% of group
Wound at 24 hours	Present in 85% of group	Present in 25% of group	Present in 80% of group
Wound at 48 hours	Present in 90% of group	Present in 30% of group	Present in 90% of group

in the SertaSil group were almost closed.

Measurements of body weight (Fig 3) showed that on day 1 the three groups had the same weight ($F(2,42)=0.08$; p=NS), but at day 19 ($F(2,42)=29.62$; $p<0.001$) it was only the SertaSil group that had gained weight, whereas the Gentaxane group had remained on its starting weight and the control group had lost weight.

Swabs of wound exudates were taken following opening of the abscess and at different time points after commencement of SertaSil or Gentaxane application. An analysis of the microflora in the wound (Table 1) demonstrated that the SertaSil group was comparable to the control group. In the Gentaxane group, the microflora was reduced as expected owing to the antibiotic effects of gentamicin.

The presence of neutrophils, monocytes and lymphocytes were measured in the wound at four time points following opening of the abscess (Table 2). The relative distributions of immune cells as well as their overall numbers were essentially similar in the Gentaxane and the control group. In contrast, the SertaSil group had considerably more immune cells at all time points and the levels of monocytes and lymphocytes were highly elevated compared to the Gentaxane and the control group.

Discussion

SertaSil was compared to Gentaxane and an untreated control for its ability to promote wound healing in a pre-clinical model of wound healing. The SertaSil group reached the stage of a clean wound in three days compared to seven and ten days for Gentaxane and the control group, respectively. Wound closure was reached after 14 days in the SertaSil group compared to 19 and 23 days for the Gentaxane and the control group, respectively. Measurements of wound surface area demonstrated the same trends. The study therefore showed that SertaSil, compared to Gentaxane and unassisted wound healing, substantially reduces the time to reaching a clean wound and promotes an overall acceleration of the wound healing process, which results in faster wound closure.

Cardinal et al.[9] have shown that the early healing rates of wounds are predictive for the time to closure and the present findings are in accordance with this. The wounds in the SertaSil group more rapidly reached the clean wound stage compared to the wounds in the Gentaxane and control group, and this acceleration of the early wound healing rate also resulted in faster wound closure.

The present study was conducted in young rats that are still growing and gaining weight. Only rats in the SertaSil group gained weight during the course of the study, whereas the Gentaxane group remained on its starting weight and the control group lost weight. Reduced growth is generally a very strong

Table 2. Presence of neutrophils, monocytes and lymphocytes in the wound at four time points following opening of the abscess. The last column shows the average range of number of cells in each field of view using the Goriainov's Camera (n=15; mean ± SD)

Parameter	% Neutrophils	% Monocytes	% Lymphocytes	% Other	Cells in view (range)
SertaSil wound					
6 hours	70.0 ± 1.51	5.0 ± 0.76	25.0 ± 1.36	0	3 to 5
12 hours	75.0 ± 0.93	5.0 ± 1.07	20.0 ± 1.00	0	6 to 8
24 hours	65.0 ± 1.37	10.0 ± 1.69	20.0 ± 1.07	5.0 ± 2.2	11 to 13
48 hours	60.0 ± 1.31	12.0 ± 1.13	21.0 ± 1.07	7.0 ± 1.5	14 to 17
Gentaxane wound					
6 hours	95.0 ± 1.00	1.0 ± 0.0	4.0 ± 1.00	0	1 to 3
12 hours	94.0 ± 0.65	2.0 ± 0.65	4.0 ± 1.00	0	1 to 3
24 hours	94.0 ± 1.51	2.0 ± 1.00	3.0 ± 0.85	1.0 ± 0.5	5 to 7
48 hours	92.0 ± 0.93	1.0 ± 0.53	6.0 ± 1.00	1.0 ± 0.8	6 to 9
Control wound					
6 hours	98.0 ± 0.76	1.0 ± 0.38	1.0 ± 0.65	0	1 to 2
12 hours					
24 hours	96.0 ± 0.85	1.0 ± 0.53	2.0 ± 0.65	1.0 ± 0.5	3 to 5
48 hours	88.0 ± 1.60	2.0 ± 1.36	8.0 ± 0.76	2.0 ± 0.9	7 to 10

indicator of stressors affecting an organism and these data show that SertaSil appeared to limit the consequences of having a wound, thereby leading to faster recovery. SertaSil did not cause any adverse events.

The analysis of wound smears showed that the microflora in the wounds in the SertaSil and the control group were comparable, whereas it was reduced in the Gentaxane group. Gentaxane is an antibiotic and this effect was expected. For SertaSil, the data indicate that it lacks direct bactericidal or antibiotic-like effects, since the level of microflora was similar to the control group.

The immunological cell counts showed highly increased levels of immune cells in the SertaSil group compared to the Gentaxane and the control group, indicating a more effective and faster recruitment of immune cells to the wound. The percentage distribution of neutrophils was lower in the SertaSil group compared to Gentaxane and control, whereas the levels of monocytes and lymphocytes were elevated. However, if the actual cell numbers are considered, then the level of neutrophils was elevated in the SertaSil group as well but the numbers of monocytes and lymphocytes were very highly increased. Neutrophils are the predominant cell type for the first 48 hours after injury and are known to protect wounds from invading pathogens, cleanse the wound site of necrotic matter and release inflammatory mediators, whereas macrophages and lymphocytes are required for the subsequent proliferation phase.[10-12] The findings therefore suggest that SertaSil facilitates the transition from the inflammatory phase to the proliferative phase, which would be consistent with the accelerated wound closure that was seen. However, further studies are needed to evaluate these effects.

Conclusions

SertaSil assists the wound healing process by removing wound exudate and by breaking down biofilm to facilitate the removal of exudate. Furthermore, the powder format of SertaSil allows it to penetrate into all openings and crevices of the wound surface to remove wound exudate to a much greater degree than for example absorbent dressings and topical negative pressure. The present study has shown that SertaSil reduces the time to reaching a clean wound by 60% and results in 30% faster wound closure compared to a topical antibiotic and has even more pronounced effects when compared to the untreated control group. These findings in a pre-clinical model are supported by observations in patients,[13] and together they indicate that SertaSil can be an important new first-in-class approach to advanced wound care. ■

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