Journal of Animal and Veterinary Advances 5 (12): 1067-1072, 2006

The Wound Healing Effects of Some Topical Antiseptic Creams in Dogs

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Abstract: The wound healing effects of five broad-spectrum topical antiseptic creams namely TCP ® (Pfizer), Dettol® (Reckitt and Colman), Savlon® (Novartis), Drapolene® (Warner Lambert) and Bacidin® (Xepa-soul Pattison) were evaluated on experimentally infect wounds in Nigerian local dogs. Wounds treated with sterile physiological saline solution 0.9% (Aestus Pharmaceuticals Corp) served as controls. The aim of this study is to evaluate the effects of five broad-spectrum topical antiseptics creams on wound contraction, epithelization and reduction in wound bacterial load in dogs and also to advocate their suitability for Clinical application in Veterinary practice. Six wounds measuring 2×3 cm were created bilaterally (6 identical pairs of wounds/dog) on the dorsolateral aspect of the trunk of the dogs. A pure culture of beta-hemolytic coagulase positive *Staphylococcus aureus* of dog skin origin at concentration of 10^9 organisms mL^{-1} was used for the wound infection A total of seventy-two wounds were evaluated and treatment was carried out in a random fashion. All treatments were carried out 2 h after wound creation on day 0 and subsequently to day 28. The mean rate of wound contraction, epithelization and wound bacterial colony count (10^5 organism mL^{-1}) of the various topical antiseptic cream treated wounds were assessed and compared statistically using the Duncan's multiple range tests. Dettol® antiseptic treated wounds, has shown a statistically significant (0.004±0.01; 8.0±0.0, *$p<0.05$) greater rate of wound contraction, epithelization and reduction in wound bacterial colony count, which was below 10^5 organism mL^{-1} by day 21, as compared to Drapolene® (0.08±0.11; 8.0±0.0), TCP® (0.14±0.13; 8.0±0.0), Savlon® (0.16±0.16; 8.0±0.0) and Bacidin® (0.21±0.17; 7.8±0.18) (*$p<0.05$). However the physiological saline treated control wounds has a more statistical significant values (1.0±0.28; 5.5±0.45, *$p<0.05$) and the wound continued to discharge exudates till day 28, as compared with the topical antiseptic creams used in the study. The results indicate that, there are beneficial effects in the use of topical antiseptic creams for wound management in dogs.

Key words: Wound healing, effects, topical antiseptic creams

INTRODUCTION

Almost everyday, animals are presented with wounds, making wound evaluation and treatment an integral part of Veterinary practice. Many wounds heal without complicated intervention, but wounds that are large, have extensive tissues damage, or infected are often treated as open wounds by Veterinarians, that is allowing healing to occur by contraction and epithelization[1-4].

When treating such wounds, the goal is to provide an optimal environment for wound contraction and epithelization through the use of medications and bandages[5-8]. Antiseptics have been widely used in surgical procedures and wound management, but their benefits in clinical practice remains controversial, because the method of evaluation of antiseptic use varies[9-12]. Antiseptics have been used as first aid and as definitive therapy in wounds[12,13]. In addition they have also been used to disinfect the surgical site on the patient, hospital surfaces, the hands of surgeons and some instrument in surgical operations[14,15].

Wound antiseptics are non-antibiotic antimicrobial drugs, which are suitable for application on living tissues[10,7]. But when not properly applied may injure host tissues[16-18]. Therefore an ideal wound antiseptics should be effective against likely contaminants and pathogens, fast acting with prolonged residual activity after a single dose, non toxic, non carcinogenic and non tetratogenic to

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host cells, non allergenic, widely available, incapable of promoting bacterial resistance and should have a minimal systematic absorption[19-21].

Although a lot of wound antiseptics are available commercially, most of these antiseptics have not been properly evaluated before being used in our environment. Thus, the objective of this study is to evaluate their effect on wound contraction, epithelization and reduction in bacterial wound count in dogs.

MATERIALS AND METHODS

The Ahmadu Bello University Institutional Animal Care and Use Committee approved all animal-related procedures of this study.

Six Nigerian local dogs (3 males and 3 females) 8 months of age, with mean average weight of 12.5±3.5 kg were used for this study. They were housed and fed adlibbed, in the kennels of the Small Animal Unit of the Veterinary Teaching Hospital, Ahmadu Bello University Zaria. Thereafter a Pre-experimental evaluation was carried out.

Pre-operative, operative and post-operative procedures: Atropine sulfate was administered intramuscularly, at a dose rate of 0.05 mg kg^{1} body weight and chlorpromazine intravenously at dose rate of 3.3 mg kg^{1} body weight as pre-anaesthetics. Anesthesia was achieved by intravenous administration of Thiopentone BP® (Thiopentone sodium, Rotex/Medica Gmbh, Trittau, Germany) at a dose rate of 25 mg kg^{1} body weight and oxygen was administered through an endotracheal tube throughout the surgery. Lactated Ringers solution was administered (10mL kg h IV) during anesthesia. Each dog was positioned on sternal recumbency. Hair on the dorsum of the trunk from the cranial thoracic to the coxofemoral region extending to the flanks and the ventral midline was clipped. The clipped areas were scrubbed with soap solution using a sterile cotton wool. The surgical site was isolated using sterile quarter drapes and this was followed by application of alcohol as a sterile wipe, which was allowed to dry up.

A sterile x-ray film template was constructed so that the 2×3cm wounds to be created lie 4 cm from the dorsal midline and 5 cm apart as describe by other workers[12,22]. Template were centered between the caudal border of the scapular and the tubae coxae and a sterile skin scribe were used to outline the wounds. Six full thickness skin wounds were created bilaterally (6 identical pairs of wounds/dog), using a No.15 scalpel blade. Hemostasis was achieved by applying direct pressure to the wound surface using of sterile gauze pads.

For open wounds with the subcutis removed, there was a tendency for the overlying skin to shift or slide over the underlying fascia. To prevent this, we loosely applied tacking sutures of 3-0 nylon were placed in the corners of the square wounds. Sutures were placed with small bites at skin edge and in the underlying fascia to promote adhension to the fascia and were tied loosely using large suture loops. The effect was to restrict the skin from excessive sliding over the subcutis, thereby keeping the defects in the skin and subcutis aligned, while allowing the skin to remain freely moveable for contraction. Tacking sutures were removed at 7 days to further minimize opportunity for inhibition of wound contraction and epithelization.

A pure culture of beta-hemolytic, coagulase positive, *Staphylococcus aureus* of dog's skin origin, at concentration of 10^{9} organisms mL^{-1} was used for the wound infection, one mL was instilled into each wound and the site was covered with sterile a drape and left for two h to allow bacteria to invade and infect the tissue before commencement of treatment[2,22].

Antiseptic cream preparations were used for the wound treatments. The antiseptics formulations used were labeled A = TCP ®, B = Dettol®, C = Drapolene®, D = Salvon®, E = Bacidin® and F = PSS (Physiological saline) was used as control Table 1. Each dog in the study (n = 6) had six identical pairs of wounds totaling seventy-two wounds, which were assessed. The cranial most wound on left hand sides of the animals was identified as wound number one and, subsequently the wounds were numbered up to twelve in anti clockwise fashion, such that each antiseptic was applied to a pair of wound on each dog. This also applied to the paired control wounds on each experimental animal. The antiseptics treatment was randomized to eliminate bias. Sterile non-adherent gauze pads, which were cut into 5×5 cm, were placed over each wound. Pads placed over wounds had approximately 2.5 mL of the antiseptic medication applied on them, with a sterile 3-mL syringe. The controls, had sterile gauze soaked in physiological saline solution, placed over them, this was done every other day through out the treatment day of 28 days.

To maintain a sterile wound environment, the entire wound area were then wrapped with a semi-thin layer of sterile cotton wool and secured with a bandage. A 10-cm,

Table 1: Shows the various topical antiseptics creams used in the study and label with their identification marks

Experimental Identification	Trade Name	Manufactures Name
A	TCP®	Pfizer pharmaceuticals
B	Dettol®	Reckitt and Colman Product Ltd
C	Drapolene®	Warner Lambert
D	Savlon®	Novartis Consumer Health
E	Bacidin®	Xepa-soul Pattison (M) Sdn. Bhd.

adhesive tape was used as cast padding for the entire wound area. This was to prevent wound contamination and maintain a moist wound environment. Caudal bandage slippage was prevented by securing crisscross bandage strips to the body, extending over the shoulder and between the fore limbs. A cotton-padded material was placed as a neck brace and in between the fore-limb on each dog to prevent self-inflicted bandage wounds or wound disruption, while neck collars were used to prevent the animal from removing the wound dressings. After bandaging, animals were moved to an anesthetic recovery room, where they were under continuous observation for post operative pain during the next 24 h. Opioid analgesia was administered post operatively.

Evaluation of wound healing: Wounds were treated for 28 days and were photographed on each treatment day (on day 0 and days 1,2,3,4,6,9,12,15,18,21,25 and 28). Bandages were changed daily for the first 4 days, then every other day, for the total treatment period of 28 days. Exudates present over wounds at the time of bandage change were cleaned removed gently using sterile cotton wool or sterile saline- soaked gauze sponges.

Wound epithelization was scored as none = 0 (-), onset = 2 (+), progressive = 4 (++), excellent = 6 (+++) and complete = 8 (++++). As the wound dressings were being changed, wound dimensions were measured and recorded on each treatment day using a sterile 30 cm ruler.

Wound infection assesment: A presumptive diagnosis of wound infection was made based on the presence of erytherma, swelling and purulent exudates. Quantitative bacteria culturing of wounds was performed on alternate days, so as not to disturb the wound environment, after creating wounds on day 1, 3, 6, 9, 15, 21 and 28. Swabs specimens for bacterial cultures were collected before cleaning of wounds by the use of sterile sticks, which were rolled over the wound each time it is aseptically placed in a tube containing 5 mL of saline.[22,24] Using a 100-fold dilution, finally 0.1 mL were pipetted and inoculated on nutrient Agar plate and grown aerobically at 37°C for 24 h. Bacterial colonies were counted at $x10^5$ bacterial organism mL^{-1}[24,25].

Data analysis: Duncan' multiple range test, was used to compare the statistical significant difference between mean values of wound contraction, epithelization and bacterial colony counts. For all comparisons, differences between groups were considered significant at $p<0.05$. All statistical analyses were conducted using SAS software (Proprietary software release version 8.2 SAS Institute, Cary. NC).

RESULTS AND DISCUSSION

Generally, all the wounds were enlarged during the first three days of treatment, which was due to wound retraction as well as splinting of wound edges by the bandaging, In addition, experimental wounds developed exudates within the first four days which decreased as the wound healing process progresses, except for the control wounds, which show exudation up to day twenty-eight.

Granulation tissue became visible in wounds treated with various topical antiseptic creams between days 4-6 and with those of the controls appearing between days 9-12.

The mean values for wound contraction, rate of epithelization and reduction in wound bacterial colony counts of the various topical antiseptic cream treated wounds, including that of the control were compared statistically using Duncan multiple range test, to determine the level of their significance, at a 5% confidence interval ($p<0.05$).

Wounds treated with Dettol® cream antiseptic showed a statistical significant reduction in mean wound size, epithelization (0.004±0.01; 8.0±0.0 *$p<0.05$) Table 2 and 3 and tremendous reduction in wound bacterial colony count by day 21 (below 10^5 organism mL^{-1}) Table 4. And interestingly, by day 21, all wounds in this treatment groups have achieved almost 100% rate of wound epithelization (7.8±0.0 *$p<0.05$) Table 3.

Drapolene® treated wounds follow closely to Dettol® cream with mean wound size, epithelization and reduction in bacterial wound counts. (0.08±0.11; 8.0±0.0*$p<0.05$). TCP® had a mean wound size and epithelization of 0.14±0.13; 8.0±0.0*$P<0.05$ which was followed by Salvon® (0.16±0.16; 8.0±0.0) and Bacidin ®(0.21±0.17; 7.8±0.18) (*$p<0.05$) antiseptic creams, respectively Table 2 and 3.

However we noticed a significant statistical difference between the antiseptic creams treated wounds and control wounds. The physiological saline treated control showed slower rate of wound contraction, epithelization and reduction in bacterial wound counts, which was evident up to day 28 with a statistical significant values of 1.0±0.28; 5.5±0.45, *$p<0.05$ Table 2-4.

When immediate reconstruction of a wound is deemed inadvisable, an opened wound must be managed until surgery can be performed or until the wound has healed. [22, 26] Many types of antiseptic have been used in wound management with different varying results. The major role of antiseptics in wound management is to prevent further microbial invasion of the tissues, thus giving the body a chance to repair itself[11,12,21,].

Table 2: The Mean values of wound area (cm^2) of experimental canine wound following treatment (in days) with various topical antiseptic creams,±values represent standard error, ($p<0.05$)

Topical antiseptic creams	Days of treatment												
	D0	D1	D2	D3	D4	D6	D9	D12	D15	D18	D21	D25	D28
PSS	6.0±0.0	8.6±0.38	9.5±0.43	10.5±0.46	11.5±0.76	11.1±0.93	8.5±0.82	7.0±0.79	5.1±0.68	3.6±0.57	2.5±0.46	1.5±0.39	1.0±0.28
Tcp®	6.0±0.0	8.5±0.27	9.6±0.36	10.2±0.34	10.1±0.48	7.7±0.69	5.8±0.70	3.7±0.63	2.0±0.49	1.0±0.34	0.5±0.21	0.2±0.14	0.14±0.13
Dettol®	6.0±0.0	8.2±0.20	9.5±0.27	10.1±0.31	9.9±0.41	7.3±0.51	4.8±0.54	2.4±0.47	1.2±0.35	0.4±0.26	0.1±0.16	0.03±0.02	0.004±0.01
Savlon®	6.0±0.0	8.3±0.34	9.6±0.35	10.7±0.36	11.4±0.60	9.1±0.79	6.7±0.71	4.2±0.65	2.5±0.49	1.3±0.40	0.7±0.30	0.2±0.21	0.16±0.16
Bacidin®	6.0±0.0	8.3±0.36	9.7±0.39	11.1±0.40	11.4±0.64	7.8±0.85	7.7±0.73	5.2±0.70	3.3±0.59	1.9±0.54	1.0±0.48	0.4±0.31	0.21±0.17
Drapolene®	6.0±0.0	8.4±0.29	9.5±0.31	10.4±0.34	10.5±0.45	8.3±0.63	5.7±0.71	3.4±0.61	1.8±0.45	1.0±0.31	0.4±0.19	0.1±0.12	0.08±0.11

Table 3: The Mean values of wound epithelization of experimental canine wound following treatment (in days) with various topical antiseptic creams,±values represent standard error, ($p<0.05$)

Topical antiseptic creams	Days of treatment												
	D0	D1	D2	D3	D4	D6	D9	D12	D15	D18	D21	D25	D28
Pss	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0.7±0.28	1.5±0.26	2.2±0.17	3.8±0.29	3.9±0.48	4.0±0.30	4.3±0.73	5.5±0.45
Tcp®	0±0.0	0±0.0	0±0.0	0±0.0	0.5±0.30	2.2±0.17	2.9±0.30	3.5±0.26	5.2±0.30	5.8±0.30	6.5±0.30	7.3±0.62	8.0±0.0
Dettol®	0±0.0	0±0.0	0±0.0	0.7±0.28	1.7±0.22	3.2±0.30	3.7±0.22	5.0±0.38	6.0±0.17	6.8±0.30	7.8±0.26	8.0±0.0	8.0±0.0
Savlon®	0±0.0	0±0.0	0±0.0	0±0.0	0.2±0.36	1.9±0.26	2.8±0.29	3.3±0.42	4.9±0.37	5.6±0.28	6.2±0.31	7.1±0.57	8.0±0.0
Bacidin®	0±0.0	0±0.0	0±0.0	0±0.0	0.1±0.34	1.7±0.38	2.3±0.17	3.0±0.29	3.8±0.42	5.3±0.37	5.9±0.17	6.8±0.68	7.8±0.18
Drapolene®	0±0.0	0±0.0	0±0.0	0±0.0	0.7±0.28	2.8±0.22	3.2±0.27	3.8±0.41	5.6±0.26	5.9±0.26	7.0±0.30	7.5±0.26	8.0±0.0

Table 4: The mean values of bacterial colony count at x 10^5 from experimental wounds, at each bandage change, following treatment (in days) with various topical antiseptic creams.±Figures represent standard error, ($p<0.05$)

Topical antiseptic creams	Days of treatment						
	D1	D3	D6	D9	D15	D21	D28
Pss	631.2±54.2	534.4±38.6	436.1±26.7	249.8±21.3	189.3±19.1	147.6±8.7	98.1±6.8
Tcp®	228.4±29.1	156.4±15.6	72.5±9.1	41.4±6.2	18.4±2.3	8.1±0.42	0.0±0.0
Dettol®	141.8±22.6	68.8±11.1	34.6±4.6	20.8±1.4	4.6±0.28	0.0±0.0	0.0±0.0
Savlon®	206.3±36.9	167.2±18.1	98.3±11.3	51.6±8.6	23.7±3.7	10.1±1.2	0.0±0.0
Bacidin®	264.7±40.6	174.6±27.2	121.4±15.6	86.9±10.2	47.4±5.3	19.1±2.3	0.4±0.12
Drapolene®	197.5±26.1	115.4±13.4	82.6±7.8	31.2±5.1	15.8±1.4	3.9±0.19	0.0±0.0

These study, compared the effects of Dettol®, TCP®, Savlon®, Drapolene ® and Bacidin ® which are topical antiseptic creams commonly used in Zaria [Nigeria] for the management of clinical wounds in dogs Table 1.

Dettol® antiseptic cream proves to be more superior to the other antiseptic creams used in the study. Although, the major difference of this study with other previous works is that, it employed the use of topical antiseptic creams rather than aqueous preparations used in earlier studies[2,25,20,27].

Dettol® contains chloroxylenol and triclosan as active ingredients, which are both derivatives of phenol compounds. Chloroxylenol is broad-spectrum germicide with an objectionable odour. It has been use extensively as a preservative, disinfectant and a topical antiseptic[28-30]. Triclosan has a broad-spectrum bactericidal activity, a 0.5% tincture has been reported to be superior to 4% chlorhexidine emulsion or 60% isopropanol for disinfection of hands[28-30]. These two combinations probably accounts for the superiority of Dettol® antiseptic cream.

Drapolene® which was used for the treatment of burns, followed closely to Dettol® in enhancing wound healing contains a quaternary ammonium compound Benzalkolonium chloride in combination with cetrimide and cetylalcohol. The incorporation of alcohol in this topical cream antiseptic consequently potentiates the action of Benzalkolonium chloride[30]. He also reported that tinctures are more potent than aqueous preparations, hence this may have accounted for the effectiveness of this antiseptic cream.

TCP® followed closely to Drapolene ® in enhancing wound contraction, epithelization and reduction in bacterial wound counts, from day 4 to day 21. Possibly this could be due to the combine action of chlorine, iodine and phenol which are incorporated in TCP® to give it a wider spectrum of activity. Savlon® and Bacidin® contain chlorhexidine gluconate, but savlon® also contain cetrimide as an additional ingredient. Chlorhexidine has a wide spectrum of antimicrobial activity as well as minimal deleterious effects on wound healing[30,31]. Cetrimide (cationic detergent) has been reported to be toxic to fibroblasts at low concentrations[20,21]. However, this account for the slow rate of healing recorded in wounds treated with savlon® cream. However, high concentration of chlorhexidine has been reported to compromised wound epitheliztion, granulation tissue formation, wound contraction and decrease tensile strength[32-33].

CONCLUSION

On the basis of the results of this study, there is beneficial effect of the use of topical antiseptic in wound management. Thus, randomized controlled studies to evaluate the effect of each antiseptic on the different kinds of wounds (acute, venous, diabetic or pressure ulcers) are indicated to provide greater evidence regarding the benefits of antiseptic use on wounds. However, in general, the use will depend on the clinician sense of judgment. Efforts to develop superior antiseptic formulations and delivery systems are likely to and should continue. Vehicles that contribute to the maintenance of an optimal moist environment may be more appropriate as delivery systems of antiseptics than the current ones, since moist environment result in both increased wound healing rate and enhancement of antimicrobial penetration of wounds. The superiority of Dettol® topical antiseptic cream over other antiseptic creams used in the study is a new finding in this area and so it's used, in wound management in dogs should be advocated.

However, more research work and clinical investigation should be carried out to determine the host tissue reaction to various topical antiseptic creams used in the study. This may consequently broaden their acceptability as wound antiseptics.

ACKNOWLEDGMENT

We wish to thank the Ahmadu Bello University Board of Research for the provision of funds for this research.

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