The Effect of Epidermin Produced by Local Isolate of *S. Epidermidis* on Antibacterial Activity of Some Agents Used in Wounds and Burns Treatment

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Abstract

Epidermin of local *Staphylococcus epidermidis* isolate was tested individually and in combination with some agents(that used in treatment of wounds and burns)against pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*), by using agar spot method , and agar diffusion method respictivelly . Results showed that, *S. epidermidis* isolate had the ability to produced epidermin and antibacterial activity toward *S. aureus*, whereas *E. coli* was not inhibited. Results of synergestic effect of pure epidermin with some agents, showed that, Samacyclin(with and without epidermin) was the most effective agent, Nifusin was the second and Silverin, acetic acid (6%) and iodine (10%) was the least effective agents.

Introduction

Epidermin:is atetra cyclic peptide produced and secreted by *S. epidermidis*. It is amember of the lantibiotic family, agroup of mostly plasmid-encoded, ribosomly synthesized and post trasitionally modified antimicrobial peptides that are secreted by *S. epidermidis* . (1, 2)

Epidermin is bactericidal to sensitive cells (of many Gram-positive bacteria). The bacteriocin inhibited simultaneously the synthesis of **DNA**, **RNA**, protein and polyssachariedes, leading to speculation that treated cells no longer have sufficient energy to carry biosynthetic processes, and that the energy-transducing cytoplasm membrane may be the primary biochemical target(3).

Epidermin, sem to affect the membrane permeabilrrier by forming water-filled membrane channels or pores, probably by a barrel-stave mechanism (4).

Epedermin is already used in medical therapy against pathogenic Gram-positive bacteria such as *Probonibacteriym* acne, *Streptococci* and hence is of therapeutic value in topical treatment of acne in humans and to treatment staphylococcal infections in mice (1).

The aim of the study: attempt to increase the capability of some materials which used in treatments of wounds and burns by mixing with the epidermin that produced from *S. epidermidis*.

Materials and Methods

Bacteral isolates*: S. epidermidis* isolate was obtained from patient with skin infections (Al-yarmook Hospital).

Drugs used: The drugs tested included: Aceticacid (6%), Iodine (10%), Samacyclin (tetracyclicHCL3% (Iraq), Nifucin (Nitrofurazon), Silverin (Silversulphadiae 1%) (Jordan), Soframycin (Framycelin sulphate+ Garamicidin) (Ireland).

Antibacterial activity of *S. epidermidis* isolate.

Crude culture of *S. epidermidis* isolate was tested for inhibitory activity towards the target isolates (*S. aureus* and *E, coli),* following the deferred antagonisim procedure (agar spot test). Overnight culture of *S. epidermidis* isolates were sootted onto the surface of agar plate of Brain-Heart Infusion agar (Biolif), and incubated for 24hr. at37C to allow colony development.

Approximately 0. 1 ml of 1. 5×10^8 cells/ml of the target isolates were inculated onto 7 ml of an appropriate soft agar (0. 7%) W/V, and poured over the plate onto which the producer *S. epidermidis* had been grown. . The plates were checked for inhibitory zones (inhibition was scored positive if the zone was wider than 2 mm). (5)

Purification of epidermin

The crude of epidermin was treated with $(NH_4)_2So_4$ (0-55)% saturated solution, (pH:7) . The resuspended ammonium sulfate precipitate was transferred into dialysis tube

and dialysis at 4 C against several changes 0. 01 m phosphate buffer (pH 7. 0), and antibacterial activity was determind by using agar diffusion method.

The third step of purification was done by gel filtration chromatography on sephadex G-70 column (1.6 × 70)cm (Elution was performed with 0. 05 m phosphate buffer (pH :6. 8) at flow rate 3ml/Fraction(0. 5 ml/min). (Fraction read at 280 nm and assayed for inhibitory activity). (6, 7)

Protein concentration determination

Protein concentration was determinated in the stages of purification by the method of Bradford, (1976), with coomassie brilliant blue-G250 (BDH)(8).

Determination of the antibacterial of purified epidermin with drugs used in treatment of wounds and burns.

Both the activity of drugs and the effect of purified epidermin with these drugs were determind by using modified agar diffusion method.

Commercially available 60 mm Petri dishes containing Brain-Haert infusion agar were inoculated with (0. 1ml of 1.5×10 cell/ml) target isolate (*S. aureus*) by using sterile swabs. Three evenly spaced holes 3mm in diameter were made in the agar of each plate with sterile cork borer. To identify the intrinsic drugs activity of the diluents, two control wells were filled with epidermin, drug alon (100 ul) and saline respectively. Test well contained 50% of epidermin+50% of drug. An equal volume of each agent was expressed into each well (two replica plate were prepared for each agent). Test plates were then incubated at 37C for 72 hrs, and zone of inhibition were measured using aruler in millimeter. A clear area test will indicates that the agent had retained its antibacterial activity. This method was repeated to each drugs. (9)

Results and Discussion

Antibacterial activity of *S. epidermidis*.

S. aureus isolate was inhibited by the crude extract produced by *S.* e*pidermidis* isolate. *E. coli* isolate was not inhibited. This confirms by the results obtained by (10, 11).

Epidermin don't have the same lytic activity like lysostaphin and dosent have the same spectrum of activity like other bacteriocins from Gram-positive microorganisms, it dosent inhibit any Gram-negative bacteria, but dose inhibit several other genera of Gram-positive. (12, 13).

Purification of epidermin

The purification procedure is summarized in Table (1) and Fig. (1), the crude supernatant was heated at 70c for 15 min. before precipitation with ammonium sulfate to sterilize the supernatant and inactivate any heat labile enzymes that might reduce the recovery of antibacterial substance during purification (14).

The first step of purification (precipitation with ammonium sulfate), eliminated most of the contaminating proteins and removed water to obtain apartially purified epidermin. Results showed that the activity of epidermin was at it highest level at saturation ratio of (55)% of ammonium sulfate.

The second step of purification was achieved by gel filtration through sephadex G-75.

The result show in Fig. (2) indicates the presence of two major peaks, (number of fraction 1-16 was pooled to represented the first peak, however other fraction 19-36 represented the second peak). Estimation of the epidermin activity in all eluted fractions showed that first peak don't contain epidermin activity, while epidermin activity was found in the second peak. (The fractions of the second peak were then all collected and concentrated with sucrose).

Method of purification seems to have accomplished in the final product, epidermin activity could be identified in the final product, and this result was previonsly reported by Sahl and Brandis, 1981;Vescovo et al., 1993 (15, 7).

Table (1)
Yiled and specific activity of the epidermin at various stages of the purification procedures.

Stsge of purification	*Volume (ml)*	*Protein (mg/ml)*	*Activity (Au/ml)*	*Total activity*	*Specific activity*	*Recovery (%)*	*Fold of purification*
1-Culture Supernatant fluid (crude).	500	16	20	10000	1. 25	100	1
2-Ammonium sulphate precipitation	70	3. 5	40	2800	1. 33	28	~ 2
3-Dialysis against phosphate buffer	50	0. 8	40	2000	250	20	40
4-Sephadex G-75 column	30	0. 2	50	1500	250	15	200

The final specific activity of pure epidermin was about 250-fold greater than that in the culture supernatant with afinal recovery of 15%, these results were similar to those reported by Patinn and Richard, 1995 (11) Fig. (2).

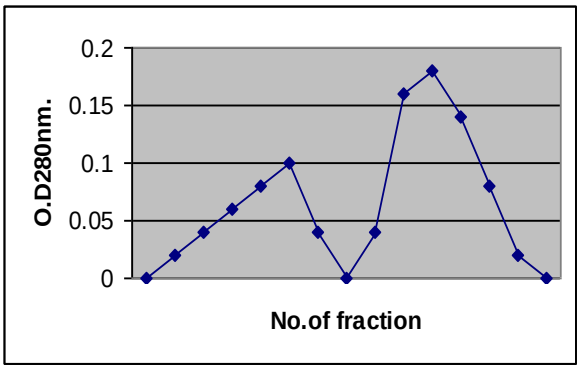


Fig. (1) Gel filtration chromatography on sephadex G-75 column (1. 6×70)cm. Elution was achived with 0. 05 M phosphate buffer Ph6. 8-7, flow rate of 3ml/fraction (0. 5 ml\min).

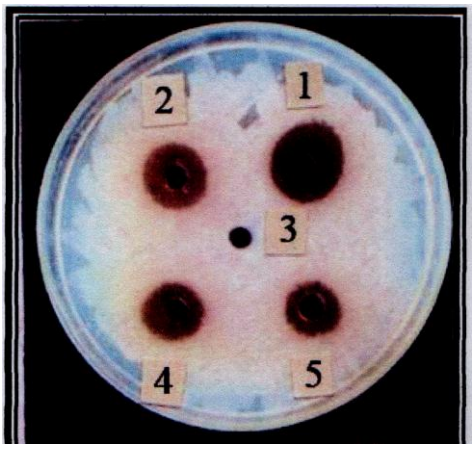


Fig. (2) Epidermin activity during purification steps against S. aureus.
1-Pure epidermin (gel filteration.
2-Pure epidermin(dialysis step).
3-Parsial purified epidermin (ammonium selfate).
4-Culture supernatant fluid (crude).
5-Saline (negative control).

Determination the effect of purified epidermin with some drugs and agents that used in treatment of wounds and burns.

Table (2) showed that the *in vitro* effect of purified epidermin on the antibacterial activity of some drugs and agents that used in treatment of wounds and burns. Results showed, samacyclin was the most effective agent inhibiting *S. aureus*. Nitfusin was the second, silverin, acetic acid (6%) and iodine(10%) were lest inhibitory respectivity. While epidermin was exhibit activity to those of acetic aced.

Samacyclin has the greater inhibitory effect against microorganisms, because it has

better aqueous solubility and skin permeability (16). Silverin has been commonly used as atopical antimicrobial agent after burn injury (17). Silverin activity due to the active compound(silver sulphadiazine). Nitrofurazone is asynthetic antibacterial agent that is routinely used in the topical treatment of burn and wounds. It is active against avariety of Gram-positive and negative bacteria.

The reason for the antibacterial activity of acetic acid may be due to the used in the concentration 6%.

Results showed: Siliverin, Nifusin with epidermin produced mean zones larger than that produced by undiluted agents or as an epidermin only. This may be due to the ability of absorption of siliverin and nifusin by agar (*in vitro)* or by skin or eschar(*in vivo*) during the treatment, and their ability had increased by using the epidermin compounds. Samacyclin and acetic acid 6%

Diluted with epidermin produced approximation zones them that produced by undiluted agents. This result probably due to the chemical structure and contents of these agents such as acids which interact with compounds (protein and polypeptide) of epidermin and inhibited theres activity. The activity loss of bacteriocins (epidermin)upon treatment with acidic andalkaline pH values is avane is likely to be due to denaturation of the protein or to loss of cofactor(12, 7).

Iodine 10%withe epidermin was exhibited increase inhibition zone size. Any antibacterial agents used for burns or wounds treatment should be tested in some manner for its ability to penetrate the skin or eschar.

Table(2)
Activity of full-strength and diluted agents with epidermin against S. aureu.

Agents (active compound)	*Inhibition zone diameter(mm) of:*	
	(100%) agent	*(V\V) Agent+Epidermin*
1-Samacyclin (tetrcyclin Hcl 3%)	35 39	37 40
2-Nifusin (Nitrofurazon)	20 25	26 27
3-Silverin (Silver sulphadiazine 1%)	15 18	23 26
4-Acetic acid 6%	14	14
5-Iodine 10%	9	12

- **Inhibition zone diameter of pure epidermin = 19 mm**

Bacteriocin have some limitation, which reduce their effectiveness as drugs treatment for wounds and burn infections. First, they are not effective against Gram-negative bacteria, second, they are not effective against all Gram-positive bacteria and even in sensitive Gram-positive strains, there are a sensitive variant cell which can multiply in the presence of bacteriocin. Aprevious paper described that bacteriocins can be combined to have abetter antibacterial effect against Gram-positive bacteria (18). Results of our study reports that epidermin and some agents (used in wound and burns treatment), can be combined to have abetter activity against *S. aureus.* The variants of activity may be due to the difference adsorption both epidermin and agents.

Agent may be associated with the mechanism by which epidermin enter the cell following binding to the cell surface. It has been proposed that following of binding abacteriocin to the surface, the barrier functions of the cell wall of sensitive cells are impaired. This disruption of cell wall of functions allow other molecules (agents) to pass through the wall, come in contact with the cytoplasm membrane and destabilization its function (19).

The result presented here indicated the epidermin combination with some agents (Samacyclin and Silverin) will have agreater antibacterial activity against *S. aureus* and probably against other bacteria. This

consideration will be important in the treatment of wounds and burn infections.

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