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CONTAMINATION OF LEG ULCERS

A Bacteriological and Clinical Study on the Effect of Treatment with an Ointment Containing Ristocetin A and Circulin

Peter Kamp and Axel Perdrup

From the Department of Dermatovenereology, Rudolph Bergh's Hospital, Copenhagen, Denmark

Abstract. In a series of 79 patients with 88 leg ulcers the bacterial contamination and the ulcerated areas themselves were examined before and at weekly intervals during treatment with an ointment containing the antibiotics ristocetin A and circulin. The microorganisms cultured from the ulcers were: *Staphylococcus aureus*, streptococci, pseudomonas, proteus, E. coli and *Klebsiella*. All except proteus were sensitive in vitro to either ristocetin A or to circulin. 70% of the *S. aureus* strains were resistant to tetracycline, none to neomycin. Both the number of contaminated ulcers and also the microorganism count in almost all ulcers decreased considerably during the first few days of topical treatment. Subsequently, some ulcers became transiently contaminated. In 61% of 62 ulcers that were observed for at least 4 weeks, half or more of the cultures yielded very few (at most, less than a hundred) colonies per plate. Resistance to ristocetin A was not found among the gram-positive cocci. One circulin-resistant pseudomonas strain appeared after 2 weeks of treatment. The ristocetin-circulin ointment gave a positive reaction in 2.3% of routine patch tests in 301 eczema patients. Circulin is probably the principal allergen. From an attempt to correlate the healing rate with the antibacterial effect of the treatment, no conclusion could be drawn.

Our purpose was to investigate the bacterial contamination of leg ulcers and to estimate the effect of topical treatment with a ristocetin–circulin-containing ointment on the growth of bacteria and on the healing time of leg ulcers.

Furthermore, a possible sensitizing property of the remedy will be studied.

Few major investigations into the bacterial contamination of leg ulcers exist (1, 4, 5, 7, 10). Ulcers, like bed-sores and burns (3, 15), usually contain such microorganisms as prevail in the environment, i.e. they function like biological Petri-dishes.

The seriousness of the bacterial contamination is debatable. Most investigators agree that it plays a minor role in the development and persistence of leg ulcers (2, 6, 9). Nevertheless most topical treatment media contain agents directed against the microbiological contamination. In most reports the effect of topical treatment is based upon estimations of the average healing time. Such methods including systematical measurement of the ulcer areas were published by Luger (8), Luger & Pavlik (9), Arma-Szlachcic & Strahler (2) and Perdrup (12, 13).

PATIENTS AND METHODS

The present investigation took place during the periods June–September 1970 (part 1) and November 1971 to April 1972 (part 2) at the Department of Dermatovenereology, Rudolph Bergh's Hospital, Copenhagen.

Patients were selected whose leg ulcers were considered to need a minimum of 4 weeks of hospitalization. The study comprised a total of 79 patients with 88 ulcers (range: 34–89 years of age, mean 70.6 years, male/female: 1 : 2.4) and was completed in 55 patients with 62 ulcers (range 42–89 years of age, mean: 71.3 years, male/female: 1 : 3.2).

The reasons for interrupting the planned treatment in 24 patients before the lapse of 4 weeks were: in one case because of eczema, in four the effect on necroses was inadequate, eight had healed, and 11 patients were transferred to other departments.

Topical treatment of ulcers

The remedy under investigation was an ointment containing two antibiotics: ristocetin A and circulin (RC-O) in concentrations of 5 mg ristocetin A and 25 000 units circulin per gram ointment in a lanolin-free o/w emulsion containing 0.11% parabenes.

Circulin (11, 16) is a basic polypeptide of the polymyxin group of antibiotics, its sulphate and hydrochloride salts are readily soluble in water and stable at pH values from 2 to 7. The effect is bactericidal mainly on gram-negatives,

Table I. *Antibiogram of 88 ulcers before treatment with RC-O*

33 ulcers in the 1971 group (first column, % of 33); 55 ulcers in the 1972 group (second column, % of 55)

Micro-organism	No. of contaminated ulcers		Number of strains resistant to															
			Neomycin		Bacitracin		Ristocetin		Circulin		Tetracycline		Chloramphenicol		Polymyxin		Fucidin	
	1971	1972	-71	-72	-71	-72	-71	-72	-71	-72	-71	-72	-71	-72	-71	-72	-71	-72
S. aureus	23	38	0	0	3	0	0	0	23	38	15	25	0	0	7	8	2	0
% of 23	(70%)		0%		13%		0%		100%		65%		0%		30%		9%	
% of 38		(69%)		0%		0%		0%		100%		66%		0%		21%		0%
Streptococci	3	11	0	0	0	0	0	0	3	11	1	1	0	0	1	5	0	0
	(9%)																	
		(20%)																
Pseudomonas	9	5	0	0	9	5	9	5	0	0	0	0	0	0	0	0	7	4
	(27%)																	
		(9%)																
Proteus	3	5	0	0	3	5	3	5	3	5	3	3	0	0	3	5	3	5
	(9%)																	
		(9%)																
E. coli/*Klebsiella*																		
	0	9		0		8		9		0		2		0		0		9
		(16%)																
Cocci	6	4																
	(18%)																	
		(7%)																
No growth	3	5																

including *Pseudomonas*. The antimicrobial activity of circulin is little affected by serum or pus, the absorption when given *per os* is minimal.

Initially and for 3 days, sterile water dressings or Carbowax® 1500 was applied locally. From the fourth day RC-O was applied under linen dressing every morning and afternoon for 4 to 7 weeks. Finally, for 1 week the ulcers were again treated solely with sterile water dressings or Carbowax® 1500. Systemic antibiotic treatment was omitted during the investigation.

Culture

A total of 654 swabs were taken: just before the first application of the antibiotic ointment and subsequently once a week in the morning (16 hours after the latest ointment application). An additional swab was taken after 2 days of RC-O treatment. A sterile white cotton swab of standard size was rolled firmly over the whole surface of the ulcer until filled with as much material as possible, put into a sterile glass tube and immediately brought to the microbiological laboratory of H. Lundbeck, Copenhagen, and within 2 hours cultivated on three agar plates, of which one was used for semiquantitative evaluation of the number of microorganisms and the other two for determination of sensitivity to eight antibiotics. Ristocetin and circulin at a concentration of 300 mg/ml and 2 500 U/ml resp. are dissolved in phosphate buffer (pH 6) and absorbed into paper discs (ϕ: 12.6 mm, 100 μl per disc). The discs are placed on the seeded plate. Organisms exhibiting a zone spread halting 16 mm around the impregnated paper disc are regarded as sensitive to the antibiotic in question.

Sensitivity to the other six antibiotics is determined by means of the tablet method (Neo-sensitabs®, Rosco). Usual diagnostic substrates and biochemical methods were employed for identification.

Ulcer measurement

Measurement of the ulcer area was done weekly. Each ulcer is outlined on transparent paper and the area calculated by means of an Ott-Compensation-Polarplanimeter. All calculations were performed by the same person (chief medical secretary).

Healing time of leg ulcers and the "healing rate"

The logarithmic values of the measured ulcer areas are plotted against time. In this way straight lines are usually obtained (12). The regression of the curve expresses the rapidity of healing. A "broken" curve indicates an acceleration in the speed of healing during the period of observation. We express the regression of a curve as the number of weeks required for the curve to cover one decade on the logarithmic paper, i.e. the time, in weeks, it takes to reduce the ulcer area to one-tenth of its original size. This graphically calculated value is called "the healing rate".

Sensitization

In order to evaluate the possible sensitizing effect of the antibiotic ointment, the areas surrounding the ulcers were carefully observed for signs of irritation or eczematous reactions. The ointment was included in our routine standard patch test series and furthermore ristocetin and circulin were tested separately.

Table II. *Number of contaminated ulcers (> 100 microorganisms per plate) during treatment*

Percentage values given in parentheses

Micro-organism	Before treatment (% of 88)	After 2 days (% of 88)	After 1 week (% of 88)	After 2 weeks (% of 80)	After 3 weeks (% of 71)	After 4 weeks (% of 62)	After 5 weeks (% of 53)	After 6 weeks (% of 34)	After 7 weeks (% of 3)
Staph. aureus	61 (69)	28 (32)	16 (18)	12 (15)	12 (17)	9 (15)	8 (15)	7 (21)	0
Streptococci	14 (16)	4 (5)	1	3	0	1	0	0	0
Pseudomonas	14 (16)	2	1	2	3	0	1	1	0
Proteus	8 (9)	6 (7)	5 (6)	4 (5)	3	3 (5)	4 (8)	1	0
E. Coli/Klebsiella	9 (10)	1	1	0	1	0	0	0	0

RESULTS

Contamination of the leg ulcers before treatment

Table I shows that during the 18 month interval from 1971 to 1972 the occurrence of staphylococci and of proteus remained stable, that streptococci and *E. coli/Klebsiella* became more frequent, while pseudomonas contaminations decreased by 18%. The high incidence of staphylococci—in 70% of the ulcers—and the large number of strains resistant to tetracycline (66%) tallies with the results of our routine cultures during recent years. None of the bacteria were resistant to neomycin; none were resistant to ristocetin and circulin, except for proteus.

Effect of treatment on the contamination of leg ulcers

Before treatment most ulcers yielded innumerable colonies on the plates. For the sake of clarity Table II shows the number of ulcers which give rise to over 100 colonies per plate. We feel that less than 100 colonies per plate—which in most cases actually means very few colonies—is in practice of little or no importance. Table II shows that the number of ulcers under observation at the start was 88, after 4 weeks 62 and that 3 ulcers were followed for 7 weeks. The number of ulcers yielding over 100 colonies per plate decreased conspicuously after only 2 days of treatment. Maximum reduction is reached after 1 week's treatment. *Staph. aureus* was more persistent than the other microorganisms except for proteus, which remained unaffected by treatment.

During the treatment of 62 ulcers which were observed for 4 weeks or more, unimportant growth (less than 100 colonies per plate) was registered in 38 (61%).

Staph. aureus appeared in nine ulcers during the first 2 weeks of treatment; in nine other ulcers, infected from the beginning, the staphylococci changed resistance pattern. Of these 18 strains, apparently acquired in the ward, 13 (72%) were resistant to tetracycline. Although sensitive to ristocetin A, the staphylococci persisted in about 16% of the ulcers.

Pseudomonas disappeared very rapidly. Four ulcers were contaminated in the ward for a short period during treatment. In three strains the resistance pattern changed and in one of these the sole case of resistance to circulin appeared.

E. coli and *Klebsiella* disappeared almost at once. Transient contamination was later seen in two ulcers only. Change in resistance pattern was not observed.

Proteus, resistant to the antibiotics of the ointment, remained in half of the original eight contaminated ulcers.

Effect of treatment on the healing rate

In 59 of the 62 ulcers, healing rates could be calculated. The healing rates of 24 more permanently contaminated ulcers were compared with the healing rates of 35 ulcers showing half or more of the cultures with unimportant growth. No significant difference between the two groups was found (Mann-Whitney rank sum test, $p > 0.10$).

Table III. *Frequency of "broken" curves in 24 more contaminated and 35 less contaminated ulcers*

	Group of 24 ulcers	Group of 35 ulcers	
Number of "broken" curves	7	13	20
Number of not "broken" curves	17	22	39
	24	35	59

We furthermore examined the frequency of "broken" curves in the two groups, since a curve break indicates acceleration of healing (13). From Table III it appears that no significant difference in the number of "broken" curves in the two groups was found.

Sensitization

In 3 out of 79 patients we observed eczema in the surroundings of the ulcer and patch tests were positive to RC-O. In 88 patients three patch tests were positive to circulin, none to ristocetin A. Because polymyxin could be an alternative to circulin this antibiotic was included in the 88 patch tests. One was positive to polymyxin.

Furthermore, RC-O was included in our standard patch tests applied during 10 months on 301 contact-allergy suspect patients. Seven (2.3%) were positive to RC-O. Of these, 3 were also positive to neomycin. Positive patch tests to neomycin were found in 23 (7.6%) of the series. The concentrations of circulin, of ristocetin A, and of neomycin, were 20 times the concentrations in the ointments.

DISCUSSION

The microorganisms found in leg ulcers are, as expected, those which predominate in most dermatologic wards. The resistance to tetracycline found in 70% of our *Staph. aureus* strains is extraordinary when compared with the finding of only 23% of 18 393 *S. aureus* strains isolated in the State Serum Institute from Danish hospitals during 1971 (14). The high incidence of tetracycline resistance may be a consequence of a superabundant topical use of tetracycline (and polymyxin). On the other hand no neomycin-resistant strains were found, probably due to exclusion of neomycin from our ward for more than 10 years because of its allergenic properties.

Most microorganisms disappear amazingly rapidly after the inception of treatment. This effect must be ascribed to the antibiotics and not to the vehicle, since the ulcers had been pretreated for 3 days with dressings applied by a most experienced personnel and since proteus which is resistant to the antibiotics remains in half of the proteus-infected ulcers. During treatment several ulcers were transiently contaminated. A pseudomonas strain may have acquired resistance to circulin after 2 weeks of treatment; this strain was found in three consecutive cultures from the same ulcer. All the cocci were and remained sensitive to ristocetin A.

The allergenic properties of the ristocetin–circulin ointment are not negligible. Three cases of contact dermatitis in 79 treated patients and 2.3% positive patch tests is rather less than is seen in neomycin. Circulin seems to be the allergen of the ointment and could be replaced by polymyxin B which has equal antibiotic and less antigenic properties.

In spite of the obvious effect on contamination we could not demonstrate any significant difference in the healing rates between more or less successfully cleaned ulcers. This is in agreement with an investigation of Haeger (6) who, in a series of 116 topically treated ulcers, compared the effect of four different agents: (i) Katadyn® colloidal silver spray, (ii) Hexetidine ointment, (iii) Dianabol cream, and (iv) Hirudoid®, two of which possessed bacteriostatic properties (i and ii), on the healing time of leg ulcers. He found no difference in the speed of healing between the four groups, nor did he find any effect on the growth of bacteria during treatment. The same conclusions were reached by Luger & Pavlik (9) who, in a study of 22 leg ulcers, found no difference in the healing time between a group of 12 ulcers treated with antibiotic ointments (containing: gentamycin, chloramphenicol or rifamycin) and a placebo group of 10 ulcers. 7 out of 12 ulcers in the former group were sterile at the end of treatment, whereas the placebo ointment had no influence on the bacterial contamination.

Our inability to register any effect of antibiotic treatment on the healing rate may be due to the fact that the rapidity of healing is influenced by a large number of pathogenic factors. The authors have in an unpublished series of 233 patients tried to demonstrate if any of the factors: sex, age,

anemia, arterial insufficiency, body weight, blood pressure or arthrosis, were of statistical significance to the healing rate, but in vain. The role played by bacterial decontamination may likewise be a statistically insignificant detail in the complicated process of healing.

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A. Perdrup, M.D.
Department of Dermatovenereology
Rudolph Bergh's Hospital
Tietgensgade 31
DK-1704 Copenhagen
Denmark