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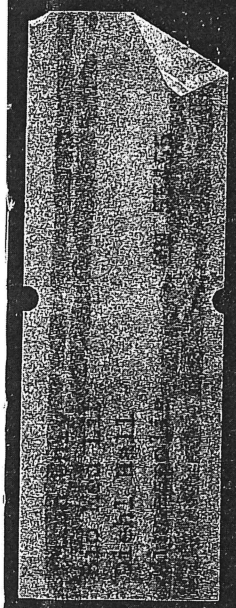

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ANTI-INFLAMMATORY EFFECTS OF ANTIMICROBIAL AGENTS: AN IN VIVO STUDY

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Antimicrobials are used emperically in a variety of inflammatory dermatoses such as rosacea and dermatitis herpetiformis, although these diseases are not believed to be of bacterial etiology. We have used potassium iodide ointment to induce follicular pustules and have found in this in vivo model properties of antimicrobials not related to their antibacterial actions.

Topical demethylchlortetracycline and erythromycin (5%) lead to suppression of KI-induced inflammation (erythema, pustules) and systemic use of these drugs as well as diaminodiphenylsulfone produces the same effect.

While investigating the inflammatory response due to patch tests with potassium iodide (KI), an inhibitory effect of concomitantly administered antibiotics was noted [1]. It was decided to conduct a larger series of in vivo experiments to clarify the role of antimicrobials in inhibiting intrafollicular abscess formation.

These experiments were designed to disclose properties of drugs not strictly related to their antimicrobial actions, a clinical observation known for many years in the treatment of inflammatory dermatoses such as dermatitis herpetiformis, acrodermatitis atrophicans Herxheimer, rosacea, acne, benign lymphocytic infiltration of the skin, pustular psoriasis [2], and follicular mucinosis [3].

MATERIALS AND METHODS

Experiments

Two types of experiments were performed:

Topical therapy. The effects of topically applied drugs were investigated. All patch tests were standardized: on the upper back a 4 × 4 cm field was outlined by tape (Leukosilk, 1¼ cm, Beiersdorf, Germany). The area was covered with liberal amounts of 40% KI ointment (w/w in Unguentum Cordes, Ichthyol Gesellschaft, Germany); KI = USP granular, Merck No. 4271, Rahway, N. J., USA, or KI = Merck No. 5044, Darmstadt, Germany). A woven gauze with 40% KI on one side was placed on top to ensure superhydration [4]. A 4 × 4 cm piece of plastic film covered the site and was occluded with impermeable tape (Dermiclear, transparent, Johnson & Johnson, USA). The opposite, symmetrical side of the back received a similar patch; however, the ointment consisted of a mixture of KI (40% w/w) and one of the drugs mentioned in Table I. If there were less than 5 pustules at 24 hr the patch was reapplied. This patch was then removed at 48 hr.

Systemic therapy. Orally administered drugs were investigated. In a screening test, 53 men were exposed to 10% and 40% KI concentrations in hydrophilic ointment (USP Torch Laboratories, USA). Seventeen of these 53 men reacted to both concentrations of KI and were given tetracyclines (tetracycline–HCl, 1500 mg; demethylchlortetracycline, 600 mg) for 10 days. The patch tests were then repeated with both concentrations of KI. Therapy was continued during patch testing.

Thereafter the effect of a variety of drugs given orally to patients undergoing KI patch tests was investigated (Tab. II). Patients were tested before systemic therapy and while receiving the drugs. Subjects were female and male inpatients and outpatients of the Department of Dermatology, aged 16 to 46 years. None had received systemic therapy for at least 4 weeks prior to this study. In all cases, control patch tests were done either simultaneously or a few days or weeks apart on the same side of the upper back. Test materials were kept at 4°C and not stored for more than 6 weeks.

Bacteriology

More than 40 pustules induced with 40% KI, 40% KI + topical antimicrobials, and 40% KI pustules raised in patients receiving systemic therapy were studied. Smears from pustules were Gram stained. A droplet of pus was collected into Triton X-100. Standard streak plates were prepared from the homogenate on trypticase soy agar (TSA) for aerobic incubation at 37°C for 48 hr and on casein yeast extract lactate glucose agar (CYLG) for incubation in N_2 + 10% CO_2 at 37°C for 7 days [5]. These cultures were done to identify Staphylococcus or Micrococcus or *Propionibacterium acnes* (*Corynebacterium acnes* group I) and *P. granulosum* (*C. acnes* group II) [5,6].

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* Martin R, Warr G, Yeager H, Couch R, Knight V: Effects of tetracycline on leukotaxis. Abstract, XII Interscience Conference Antimicrobial Agents Chemotherapy, Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, September 26–29, 1972.

Scoring

The number of pustules in each 4 × 4 cm patch test field was recorded at 24 and 48 hr. In the case of severe pustular reactions, patches were removed at 24 hr.

Statistical analysis

As every patient served as his own control in both experimental designs, paired comparisons of the number of pustules were made. The Wilcoxon matched-pairs signed-rank test was used [7]. This is a nonparametric test which is roughly equivalent to the paired *t*-test but does not require the assumption of a normal probability distribution. In the experimental design I the number of pustules from the 40% KI patch vs the 40% KI + antimicrobials, and in experimental design II the number of pustules from the 40% KI patches before and during systemic therapy were compared.

Controls

Controls with ointment base alone, plastic occlusion, and 5% antimicrobial ointment without KI were used.

RESULTS

Patch tests with KI produced follicular pustules, usually within 24 hr. Pustules were often on an erythematous and edematous base. Erythema and edema without pustule formation also occurred in a few subjects. The reaction was usually confined to the patch test areas, although spillage under the occlusive tapes sometimes caused erythema and papulopustules outside the patch test areas beneath the strips of tape.

The pustules were not caused by bacteria [1]. Smears did not reveal bacteria except when a microcomedo or seborrheic filament was incidentally removed, which contained rods, most likely *P. acnes*. The majority of pustules was sterile. Micrococci were found in less than 10% of the pustules (streak plates). Small numbers of *P. granulosum* were grown in a few cases. Similarly *P. acnes* was rarely recovered. *C. acnes* group I and II were identified according to morphologic criteria on streak plates [6].

The most prominent finding was the suppression of erythema and pustules by either topically or systemically administered antibiotics or chemotherapeutics. Representative clinical pictures are presented in Figures 1 and 2.

Topical application. Diminution of pustules was seen with tetracyclines, erythromycin, and long-acting sulfonamides. Erythema and edema were also decreased. Questionable or no inhibition was achieved with gentamycin, penicillin G-Na, ampicillin, and DDS.

Quantitative data for DMCT and sulfamethoxypyridazine are given in Tables III and IV. A marked reduction (number of reactors, degree of reaction) of about 50% was achieved with DMCT (Tab. III) after 24 hr, and over 60% after 48 hr in a group of 15 subjects. Thirteen of 15 subjects had less reaction in the KI + antibiotic-containing patch test as compared to the KI patch. Topical application of sulfamethoxypyridazine led to a similar, but less pronounced depression of pustules (Tab. IV), statistically, though of marginal significance. The mean number of pustules at 24 and 48 hr was halved. Seven of 10 subjects had either little or no reaction in the sulfonamide-containing patch vs the KI patch.

Systemic treatment. From the screening test with 17 men exposed to antibiotics, qualitative and quantitative anti-inflammatory properties of antibiotics could be expected (Tab. V, Fig. 2). The number of reactors with pustules due to KI declined from 75 to 26% and even more so with the higher KI concentration (Tab. V). In those still reacting to KI patch tests while receiving systemic antibiotics, the degree of reaction (0 to 4+) was suppressed (Fig. 3).

Tetracyclines, erythromycin, and the sulfone definitely suppressed the inflammatory reaction.

TABLE I. *Antibiotics and chemotherapeutics tested topically (all concentrations were 5% (w/w) in a KI (40%) ointment)*

Drugs	No. of patients	No. of trials
Tetracycline-HCl	12	25
Oxytetracycline	10	20
Demethylchlortetracycline	15	15
Erythromycin	10	20
Gentamycin	16	30
Penicillin G-Na	8	16
Ampicillin	14	28
Sulfamethoxypyridazine	10	10
Diaminodiphenylsulfone (DDS)	8	16

TABLE II. *Antibiotics and chemotherapeutics given orally at time of 40% KI patch testing (range of therapy 7 to 30 days at time of patch testing)*

Drugs	Range of dosage at patch testing (*mg or U/day*)	No. of patients	No. of trials	% Inhibition
Tetracycline-HCl	1000–1500	8	10	>80
Oxytetracycline	100–200	8	8	>80
Demethylchlortetracycline	600–900	13	13	>80
Erythromycin	1000–1500	8	8	>80
Penicillin G-Na	1.5×10^6	6	6	none
Ampicillin	2000–4000	6	10	none
Diaminodiphenylsulfone (DDS)	100–200	20	20	>90

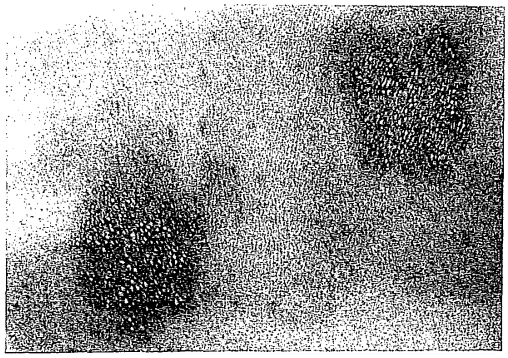

FIG. 1. Typical KI-induced follicular pustules 48 hr after occlusive application of 40% KI. These are two 4 × 4 cm sites paravertebrally on the upper back.

The most pronounced anti-inflammatory response was seen with DDS. The penicillins showed little suppression. Representative data are given in Tables VI and VII. In the subjects receiving tetracyclines, the mean number of pustules was reduced from almost 15 to 3 at 24 hr, and from 20 to 1 at 48 hr (Tab. VI).

Following systemic therapy with DDS, the pustular response was reduced at 24 and 48 hr (Tab. VII). Thirteen subjects showed either a marked suppression or no reaction at all when receiving DDS. Erythema and edema were suppressed within the confines of the patches, even if there were no pustules.

DISCUSSION

Various antibiotics, sulfonamides, and sulfones have been used in the past to successfully treat a

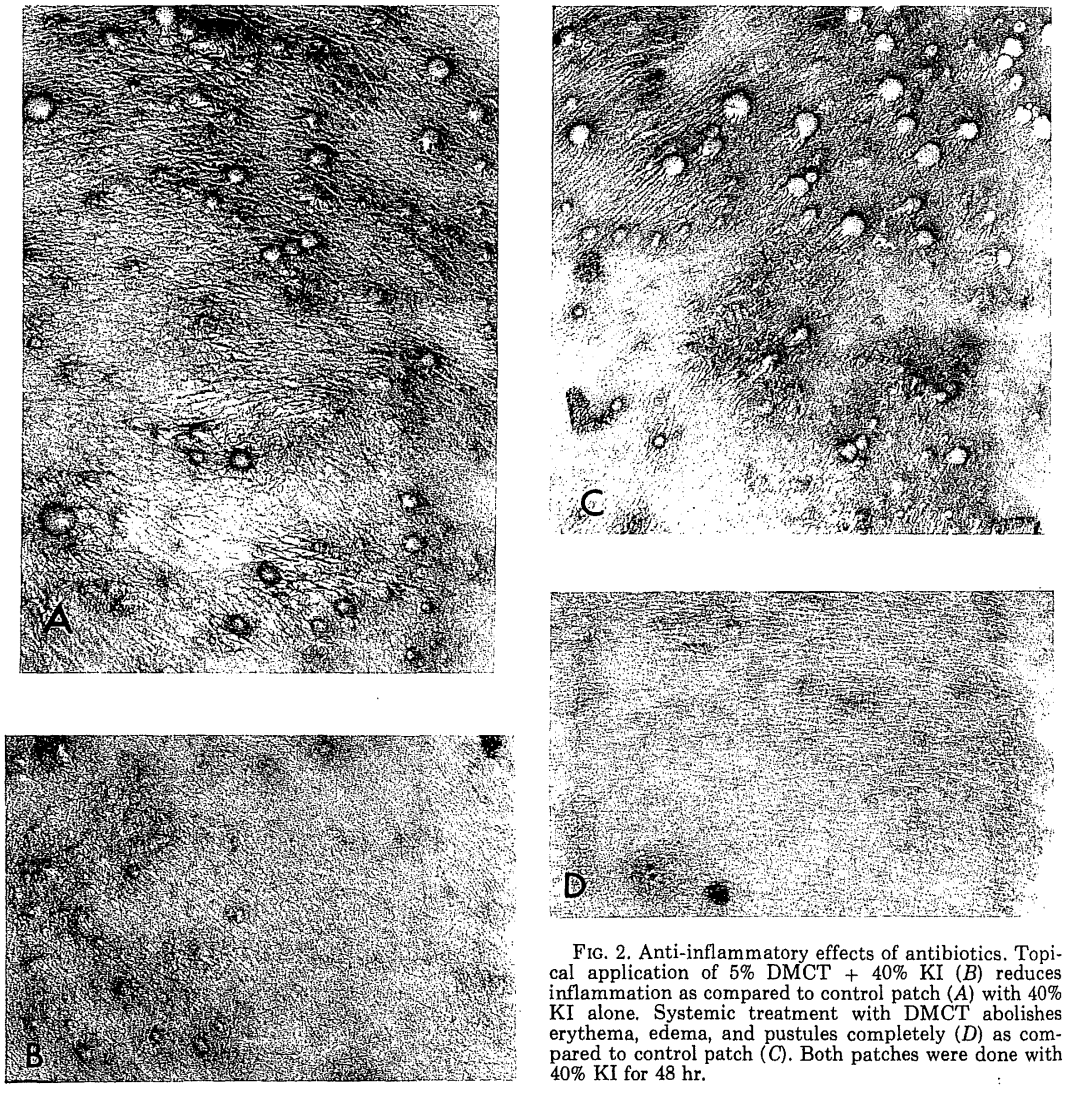

FIG. 2. Anti-inflammatory effects of antibiotics. Topical application of 5% DMCT + 40% KI (*B*) reduces inflammation as compared to control patch (*A*) with 40% KI alone. Systemic treatment with DMCT abolishes erythema, edema, and pustules completely (*D*) as compared to control patch (*C*). Both patches were done with 40% KI for 48 hr.

TABLE III. *Topical 5% DMCT in 40% KI ointment*

Subjects	Number of pustules			
	24 hr		48 hr	
	40% KI	40% KI + 5% DMCT	40% KI	40% KI + 5% DMCT
1	28	35	*	*
2	4	6	7	16
3	11	4	50	7
4	30	0	*	*
5	19	6	50	30
6	28	16	*	*
7	33	9	*	*
8	7	2	50	11
9	0	0	18	2
10	21	3	32	18
11	0	0	13	0
12	2	0	12	0
13	3	0	15	1
14	1	0	9	3
15	0	0	4	1
Mean	12.5	5.4	23.6	8.1
T	9.0		3.0	
P	.02		.005	

* Patch not repeated at 48 hr because of severe reaction at 24 hr.

TABLE IV. *Topical 5% sulfamethoxypyridazine (SMP) in 40% KI ointment*

Subjects	Number of pustules			
	24 hr		48 hr	
	40% KI	40% KI + 5% SMP	40% KI	40% KI + 5% SMP
1	6	0	12	0
2	0	1	4	8
3	50	40	*	*
4	25	15	*	*
5	0	0	10	2
6	8	8	4[a]	4[a]
7	0	0	5	3
8	3	0	8	4
9	0	0	2	1
10	0	0	0	0
Mean	9.2	6.4	5.6	2.8
T	1		3.5	
P	.08		.14	

* Patch not repeated at 48 hr because of severe reaction at 24 hr.

[a] Several pustules broke from 24 to 48 hr interval, therefore the smaller numbers of lesions at day 2.

variety of inflammatory diseases showing either erythema, vesicles, pustules, or granulomas. These diseases are believed of nonbacterial origin. Examples include erythema chronicum migrans, acrodermatitis chronica atrophicans, dermatitis

TABLE V. *Percent of subjects who react to KI*

Systemic therapy with antibiotics suppresses pustules induced by KI patch tests. Fifty-three subjects (controls) were exposed to two concentrations of KI ointment, 17 of these were subsequently given tetracycline-HCl 1500 mg, or DMCT 600 mg for 10 days.

	Controls (N = 53)	Systemic antibiotics (N = 17)
10% KI	74.9	25.6
40% KI	80.7	23.6

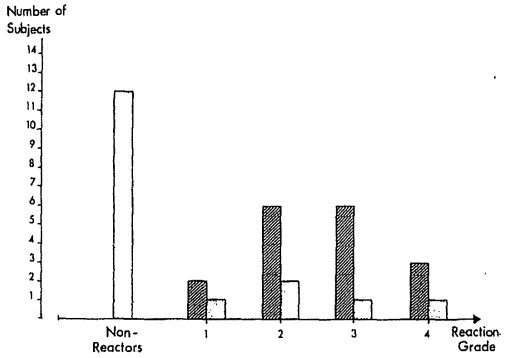

FIG. 3. Systemic antibiotics suppress KI-induced follicular pustules. Screening experiment with good reactors to KI (N = 17). *Darker, striped bars* indicate pronounced reactions prior to exposure to antibiotics (tetracycline–HCl and DMCT). *Stippled bars* indicate the suppressed reaction in these 17 subjects following systemic tetracycline treatment. Twelve subjects were made nonreactive to KI patch tests.

TABLE VI. *Systemic antibiotic administration; effects of DMCT given orally*

Subjects	Number of pustules in the 40% KI patch test			
	24 hr		48 hr	
	before Rx	during Rx	before Rx	during Rx
1	0	0	3	0
2	9	0	29	0
3	9	5	32	8
4	8	5	33	6
5	0	3	4	8
6	0	2	4	4
7	21	0	32	0
8	8	0	4	0
9	5	0	*	*
10	10	0	*	*
11	50	2	*	*
12	50	3	*	*
13	9	3	30	10
Mean	13.8	1.8	19.0	4.0
T	3.5		2.5	
P	.005		.02	

* Patch not repeated at 48 hr because of severe reaction at 24 hr.

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