Anti-inflammatory Properties of Alkyl-pseudothioureas with Antibacterial and Antifungal Activity (35485)

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Various drugs were found to possess therapeutic properties quite different from their original indication: famous examples are tartar emetic (/parasiticidal), isoniazide (antitubercular/antidepressant), diguanidines (antidiabetic/antiprotozoan), the sulfonamides acetazolimide (antibacterial/diuretic), and tolbutamide (antibacterial/hypoglycemic). In the course of our studies, conducted in the last years on a series of alkyl-pseudothioureas to be used as topical anti-infective agents against both bacterial and fungal infections, we observed that they possess a considerable antiphlogistic effect.

A comparative evaluation of various compounds led to selection of 10 undecen-1-yl-thiopseudourea iodide (AHR-1911) as the agent to be investigated clinically. This compound, besides its therapeutic properties, showed some interesting pharmacological characteristics, which are being studied. We are presenting the most relevant aspects of our study concerning this drug to which we attribute clinical utility.

Material and Methods. AHR-1911 is a white, crystalline, water- and alcohol-soluble substance, with a 67-69° melting point. In powder form, it is irritating and induces itching. These properties were eliminated by using appropriate vehicles which permit the progressive liberation of the active component and/or its action as a complex formed with components of the vehicle. A vanishing type base prepared with triethanolamine, cholesterol, glycerine, stearic acid ("Base A") permitted the repeated topical application of doses as high as 1–2 g/kg of a 10% ointment.

Hospital Base (HB) (1) was tolerated up to 5% AHR-1911 concentrations. In polyethylene glycol, chronic irritation test by daily applications of 2% (well above the antimycotic concentration: 0.2–0.5%) caused no local reaction on the cornea and shaved skin of rabbits. Similar results were obtained in a stabilized, polymerized glycol preparation ("9200") developed by François Diconstanzo.

For the determination of antibacterial action *in vitro*, we used nutrient broth; for antifungal action, sporulating medium, as in previous studies on this type of structure (2). In addition to the dilution tests, experiments of the effect of the ointments on the growth of bacteria and fungi on agar plates were conducted. For *in vivo* determinations the classical method of the localized infection in mice was used (3), characteristic for the specificity of the local action (4, 5). Assays on isolated organs to establish the influence of AHR-1911 on various mediators and cold contraction were conducted in Tryode solution. The cold contraction of the colon (6) was induced with our routine method: rapidly cooling the bath from 37 to 4° (7).

Arthritis of the hind paw in rats was induced by the injection of 0.2 ml of 10% kaolin suspension in the plantar zone, of a 6% dextran solution (Macrodex), or of 0.1 ml of a 1% carrageenin solution. To induce skin burns the method of Wilhelm and Mason (8) was used: application of a copper plate of 1 cm^2 size heated to 54° for 20 sec. The topical application of 200–400 mg of ointment/rat was done 30 min before inflammatory challenge. The anti-inflammatory study carried out in the last 3 years is based on 1100 rats, ca. 30% of them representing untreated controls.

Results. Pharmacological findings. The in-

tradermal injection of 0.1 ml of a 1:1000 AHR-1911 solution into rats, rabbits, and guinea pigs, causes a histamine-like wheal. The oral LD_{50} in mice is 950 mg/kg, the intraperitoneal 75 mg/kg, the subcutaneous 600 mg/kg. In rats the intramuscular LD_{50} is 250 mg/kg. The spontaneous movements of rat and guinea pig colon are decreased by 7.5 μg/ml of AHR-1911 in the organ bath, but the vas deferens of the rat is not affected. The same drug concentration inhibits the contractions induced in guinea pig colon by acetylcholine (0.06 μg/ml), histamine (0.18 μg/ml), $BaCl_2$ (2 μg/ml) and by cold stimulation. On the vas deferens of rat, norepinephrine (0.6 μg/ml) contraction was not inhibited, though that induced by acetylcholine (0.2 μg/ml) remained totally blocked.

In dogs, intravenous doses of 0.5 mg/kg of AHR-1911 caused hypotension, potentiated by acetylcholine and histamine, counteracted with BP elevation by norepinephrine. Characteristically, the administration of 5 mg/kg of the antihistaminic mepyramine reverted the hypotension to hypertension.

In vitro *activity*. The antibacterial properties of the alkyl-pseudothioureas were observed first by Bandelin and Tuschhoff (9) the antimycotic ones by Volini *et al.* (2). These properties assume for AHR-1911 the following magnitude: In the serial dilution test with *Trychophyton mentagrophytes*, the minimal inhibitory concentration (MIC) of 11 different experiments gave an average of 4.8 μg/ml for AHR-1911 and 7 μg/ml for griseofulvin; against *Candida albicans* 9.1 μg/ml for AHR-1911, and 7.8 U/ml (about 2.2 μg/ml) for nystatin. Using very heavy inocula of *C. albicans*, the MIC of AHR-1911 was 12.5 μg/ml and its fungidical concentration 25 μg/ml. The activity of AHR-1911 against *Torula cremoris* (11.5 μg/ml), *Hormodendrum sp.*, *Helminthosporium sp.* (5.8 μg/ml), *Microsporum canis*, *Microsporum gypseum* (11.5 μg/ml) was of the same order as the activity against *Trichophyton* and *Candida*. In the serial dilution test against bacteria, the MIC varied from 2.9 to 12.6 μg/ml for *E. coli*, *S. aureus*, *B. subtilis*, *S. thyphimurium*. *Proteus vulgaris*, *K. pneumoniae*, *Ps. aeruginosa* required higher doses, 46.5 μg/ml. Against *E. coli*, streptomycin had the same order of bacteriostatic activity (2.9 μg/ml), but it was less bactericidal (37.5 μg/ml) than AHR-1911 (9 μg/ml). In general, the bactericidal action of AHR-1911 is close to the bacteriostatic one; mostly they are one tube apart in the dilution test. This accounts for the observation that against a number of organisms (*K. pneumoniae*, *P. vulgaris*, *Ps. aeruginosa*) AHR-1911 was less bacteriostatic but more bactericidal than the antibiotics chloromycetin and aureomycin.

The optimal pH for antistaphylococcal activity is 8.0: at this pH, AHR-1911 (iodide) is bacteriostatic at 1 μg/ml concentration, its bromide analogue at 2 μg/ml.

On agar plate the halo of inhibition depends on the vehicle: lowest in Base A, highest in Base 9200, confirming the difference in release and tolerance. On Sabouraud agar a 5% HB ointment gave 1.7-cm diameter inhibition of *C. albicans*, in comparison to 2 cm obtained with 0.5% in Base 9200. In the same conditions a commercial nystatin ointment (ca. 3%), containing 100,000 U/g gave a 1.4-cm diameter inhibition zone.

Anti-infective activity in vivo. All untreated controls inoculated with 100 infective doses (ID_{50} for *S. aureus* = 3.10^7, for *C. albicans* = 10^6 organisms), sacrificed 24 hr later, developed subcutaneous abscesses and external ulcerous-necrotic lesions, giving positive cultures. The protective action of AHR-1911 with its dose–effect relationship appears from Table I. The majority of mice are cured by 100 μg of AHR-1911, the range needed for nystatin against *C. albicans* and for tetracycline against *S. aureus*, but lower than that of penicillin, which has a reduced local effectiveness (4).

Anti-inflammatory activity. Table II illustrates the protective action against burns (8). The degree and incidence of the protection of rats injected 0.5 hr before injury is dose dependent: 50 mg/kg (im) gives complete protection, similar to the topical application of a 10% ointment in Base A.

In kaolin-induced arthritis, the intravenous application of 15 mg/kg of AHR-1911 reduced the weight difference between inflamed and normal paw from 38.9% of the control group, to 13.8% in animals sacrificed 24 hr after challenge (Table III).

TABLE I. Therapeutic Action of AHR-1911 in the Local Infections of Mice with *S. aureus* and *C. albicans.*

Infection	Untreated controls	AHR-1911 (μg) 30	62	125	250	500		Antibiotics (μg) 50	100	200	500
C. albicans	0/25[a]	0/10	4/18	18/21	18/18	18/18	N[b]	0/10	3/5	5/5	—
S. aureus	0/24	—	0/6	14/15	5/5	—	T	1/5	4/5	5/5	—
							P	—	0/5	1/5	7/10

[a] Number of mice cured/total infected.

[b] N = nystatin; T = tetracycline; and P = penicillin.

In carrageenin-induced arthritis, 2 hr after challenge the inflammation measured plethysmographically was 44% higher in the 10 control rats than in the 10 animals receiving by topical application 10% AHR-1911 in Base A. This effect persisted in the determination made after 24 hr, when only the untreated animals showed edema and reddish color of the carrageenin-injected foreleg.

In dextran-induced arthritis, the same preparation reduced in the first two determinations made after challenge, *i.e.,* after 15 and 30 min, the inflammatory response by 23–28.5%. At 60 min, when the inflammatory response reached its peak in the controls, its inhibition by the drug become even higher: 41% reduction. In Base 9200 the corresponding therapeutic effect was obtained with 0.5–1.0% drug concentrations.

Discussion. Undoubtedly, different types of mechanism, or receptors, are involved in the pharmacological effects of AHR-1911: some are blocked by antihistamines; another, after antihistamine blockade, causes blood pressure elevation; a different one must be responsible for the interference with the effects of acetylcholine, histamine, $BaCl_2$ and of the thermal stimulation on the smooth muscle. The last type of action may be affecting depolarization: the agents inhibited cause contraction with depolarization, while norepinephrine, which causes contraction with hyperpolarization, is not inhibited.

Though AHR-1911 in powder form is irritating, this property is not considered directly related to its anti-inflammatory effect, since no irritation was demonstrable by the repeated application of the 10% ointment in Base A, which experimentally and clinically is anti-inflammatory, nor of the 2% ointment in Base 9200, effective in 0.5% concentrations. While we do not consider a counterirri-

TABLE II. Protective Action of AHR-1911 Against Cutaneous Burns in Rats.

AHR-1911 (mg/kg, im)	No. of rats injected	Primary lesion[a]	Necrosis and escar in: 10 hr	24 hr
Controls (0)	10	10/10	10/10	10/10
AHR-1911				
12.5	10	10/10	10/10	10/10
25.0	10	0/10 (delayed 90 minutes)	0/10	10/10
50.0	10	0/10	0/10 erythema	2/10
100.0	10	0/10	0/10 (edema in 3 hr)	1/10
Topical 10% in Base A	10	0/10	0/10 (edema in 3 hr)	1/10

[a] Erythematous = vascular reaction within 5 min.

TABLE III. Effect of 15 mg/kg Intravenous AHR-1911 on Kaolin-Induced Arthritis of Rats. Autopsy: 24 hr after challenge.

Group	No. of rats	Paw av wt (g)		Difference ± SE inflamed — normal paw (g)
		Inflamed	Normal	
Control	6	2.354	1.696	0.658 ± 0.010
Treated	6	1.976	1.736	0.240 ± 0.084

tant mechanism responsible for the anti-inflammatory effect, we assume that liberation of histamine, or of a similarly acting mediator, at the cellular or subcellular level may be involved. Interestingly, the antiphlogistic effect extends to lesions caused by various mediators: histamine following kaolin, serotonin following dextran, bradykinin following carrageenin injections, and those produced by burns.

Considering structural analogies, it is recalled that the amino-alkyl-pseudothioureas are radio-protective and the simplest of the series, 2 amino-ethyl-pseudothiourea (AET) may be the most effective (10). The terminal C of the alkyl-pseudothiourea radical, R–S–C–NH_2·NH, can be looked upon as an amidine. In fact, some of these, as reviewed by Fastier (11), liberate histamine and show other analogies with our pharmacodynamic observations.

The observation just published by Saunders and Saunders (12) that 2,2′-(9, 10-anthrylenedimethylene) bis (2-thiopseudourea), an antileukemic substance, acts on bacteria by influencing nucleic acid synthesis, could also be valid for the thiopseudoureas studied by us.

The topical activity of AHR-1911 has been clinically confirmed by Di Prisco (13) in cutaneous inflammatory lesions and by Riobueno (14) in 20 cases of contact dermatitis. We applied the 10% A Base with favorable impression in 12 arthritic–rheumatic cases; the latter observation lacks statistical force and calls for an accurate follow-up.

Summary. From a series of alkyl-pseudothioureas studied, 10-undecen-1-yl-thiopseudourea iodide (AHR-1911) was selected as antifungal–antibacterial agent for topical use. The preparation possesses *in vitro* and in the local infection of mice an anti-infective activity on the order of the antibiotics. This activity is associated with a considerable antiphlogistic action demonstrated in experimental arthritis of various types and in burns induced on rat skin.

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1. Formulary Massachusetts General Hospital Boston, 1951.

2. Volini, M., Stubbs, R., and Ercoli, N., Antibiot. Chemother. **6**, 603 (1956).

3. Morgenroth, J., and Abraham, L., Deut. Med. Wochenschr. **46**, 57 (1920).

4. Guzzon, V., and Ercoli, N., Bull. Ist. Sieroter. Milanese **36**, 543 (1951).

5. Grunberg, E., and Schnitzer, R. J., Ann. N.Y. Acad. Sci. **82**, 114 (1959).

6. Ercoli, N., and Guzzon V., Science **115**, 672 (1952).

7. Arbona, J., Proc. Soc. Exp. Biol. Med. **127**, 359 (1968).

8. Wilhelm, D. L., and Mason, B., Brit. J. Exp. Pathol. **41**, 487 (1960).

9. Bandelin, J. F., and Tuschhoff, J. V., J. Amer. Chem. Soc. **74**, 4271 (1952).

10. Maisin, J. R., and Doherty, D. G., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **19**, 564 (1960).

11. Fastier, F. N., Pharmacol. Rev. **14**, 37 (1962).

12. Saunders, P. P., and Saunders, G. F., Mol. Pharmacol. **6**, 335 (1970).

13. Di Prisco, J., Curr. Ther. Res. **11**, 634 (1969).

14. Riobueno, C. J., Pan-Amer. Congr. Professional Risks, 3rd Bogotá, Colombia, 1969, Curr. Ther. Res. **12**, 718 (1970).

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