Inhibition of Rat Adjuvant Arthritis by a New Immunosuppressive Agent Rubidomycin

The effect on rat adjuvant disease of a cytotoxic compound of the anthracycline group, Rubidomycin[1-5], is reported. Adjuvant disease may be mediated by a delayed hypersensitivity reaction[6-12] and has therefore been used as a model for testing agents which might be of value in the treatment of human connective tissue diseases[6,13-21].

Methods. Twenty-four inbred male albino Wistar rats were each injected intradermally into the left hind paw with 0.05 ml of Freund's complete adjuvant. The local reaction and the polyarthritis which developed after 9–10 days were assessed visually on a 4-point scale and an integral arthritic score calculated (Figure). The reactions were also graded by caliper measurement of the thickness of the paws.

Blood counts were made regularly and body weights recorded.

Twelve animals (group 1) were injected daily from day 4 to day 9 with 1 mg/kg Rubidomycin (May and Baker) and 12 (group 2) with 10 mg/kg of the analogous antibiotic tetracycline (Tetracyn-Pfizer) at concentrations in sterile 0.9% NaCl w/v of 1 mg/ml and 10 mg/ml respectively. Tetracycline was used as control antibiotic since it is known from previous reports not to affect the rat response to adjuvant[6]. Treatment was started on the fourth day because the presence of intact regional lymph nodes until the fifth day is necessary for the development of polyarthritis[22].

Uric acid determinations[23] and micro-immunoelectrophoretic analyses[24] were made terminally on all sera. The rats were killed in paired groups of 4 at 17, 21 and 25 days. Paraffin-embedded sections of both decalcified hind paws were assessed microscopically and synovitis, periarthritis and bone disease were graded on a 5-point scale (Table).

Results. Rats treated with Rubidomycin remained in better condition throughout the experiment than those given tetracycline.

A slight reduction in the arthritic score for the injected paws accompanied a significant reduction in the score for the non-injected paws (Figure). A less marked increase in size in injected and non-injected paws in group 1 was detected but the difference between treated and control groups was more pronounced in the right, uninjected paws (Figure). In both groups there was a 12% reduction of red blood cell counts between day 11 and day 17. The counts of the rats treated with Rubidomycin rose to normal levels by day 25; those of rats given tetracycline dropped by a further 8%. White blood cell counts showed that the leucocytosis which normally accompanies the onset of adjuvant arthritis[25] was present only in animals of group 2. The mononuclear cells of group 1 animals appeared to be larger and to have a lower nucleo/cytoplasmic ratio than those of group 2. Uric acid levels (group 1: 3.03 ± 0.77 mg/100 ml, group 2: 4.16 ± 0.69 mg/100 ml) and microimmunoelectrophoretic analyses

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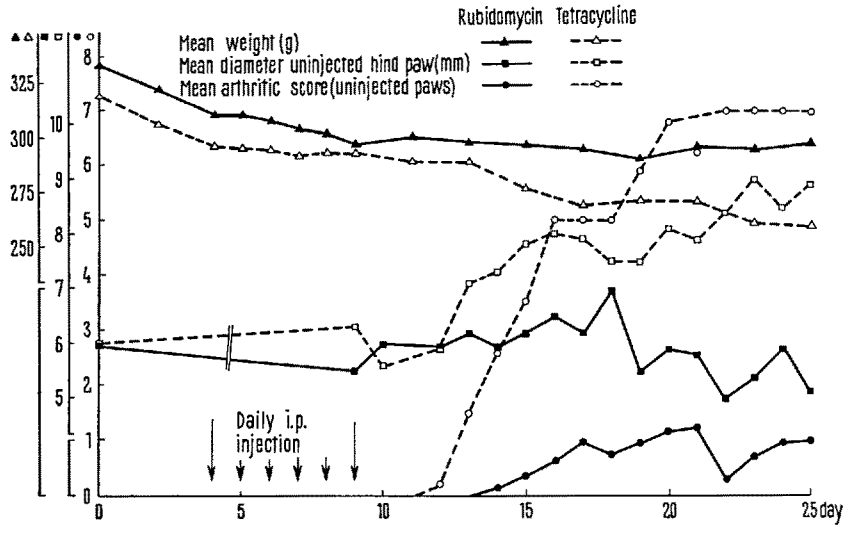

Mean weights, mean diameters of uninjected hind paws and mean arthritic scores for 12 rats given Rubidomycin from days 4–9 after a single intradermal injection of Freund's complete adjuvant and for 12 control animals treated for the same time with tetracycline.

revealed no significant differences. Microscopically, synovitis, pannus formation, bone destruction, new bone formation and periarthritis were identified in 11 of the 12 animals given tetracycline. In the Rubidomycin-treated animals the reaction at the site of injection was almost unchanged but the synovitis, cellulitis, periarthritis and bone destruction in the non-injected paws were greatly reduced in extent and intensity (Table).

Discussion. Rats injected with Freund's complete adjuvant intradermally develop a severe, delayed polyarthritis[25]. The polyarthritis is greatly diminished when the rats are given Rubidomycin from day 4 to day 9 after the injection but is not influenced by tetracycline.

These results are comparable with those obtained with antilymphocytic serum and with anti-inflammatory and immuno-suppressive drugs. Rubidomycin cuts short the anaemia and prevents the leucocytosis normally associated with adjuvant disease[26]: after concluding treatment there is no evidence of the 'escape' characteristic of steroid administration[13]. The normality of uric acid levels suggests that white blood cell destruction does not persist after treatment stops. The absence of qualitative differences in serum proteins of treated animals does not exclude the possibility that quantitative differences may be present[27].

Histological assessment on 5-point scale of response of joints of right and left feet to Rubidomycin and to tetracycline

	Left foot			Right foot		
	Cellulitis	Synovitis	Bone destruction	Cellulitis	Synovitis	Bone destruction
Tetracycline	4.00	3.70	3.33	3.08	2.73	2.20
Rubidomycin	3.75	3.01	2.90	0.75	0.67	0.16

Résumé. Un nouveau composé anthracyclique à propriétés antibiotiques et cytotoxiques, la Rubidomycine (Daunomycine), a été administré à des rats peu après injection intra-dermique d'adjuvant complet de Freund. Par comparaison avec un groupe témoin qui a été traité avec un autre antibiotique, la tétracycline, non réputé cytotoxique, on a constaté une réduction marquée des lésions systémiques et de l'arthrite de la maladie provoquée par l'adjuvant.

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Über die Hemmung des Wachstums von *Lactobacillus casei* durch einige Antiphlogistica[1]

Wir stellten fest, dass Verbindungen einer neuen, von uns beschriebenen Klasse von Antiphlogistica der 1,2-Malonyl-4-phenyl-1,2-dihydrocinnolin-Reihe[2] eine Hemmwirkung auf das Wachstum des *Lactobacillus casei* ausüben. Die Resultate des Vergleiches mit bekannten Antiphlogistica zeigt Tabelle I.

Die Substanzkonzentration, welche das Wachstum um 50% hemmt (ID_{50}) bestimmen wir im allgemeinen im Riboflavin Assay Medium «Difco», mit Zusatz von 0,03 µg Riboflavin/ml. Für die Hemmungsversuche mit den Antimetaboliten 5-Bromuracil, 6-Chlorpurin, 6-Mercaptopurin, 2,6-Diaminopurinsulfat, 8-Azaguanin und 6-Thioguanin (Tabelle II) verwendeten wir das halbsynthetische Medium nach Landy und Dicken[5] unter Hinzufügen der entsprechenden Wuchsstoffe und Metaboliten. Bebrütung: 24 h bei 37 °C. Trübungsmessung: im Lumetron mit Filter 530 nm gegen unbeimpftes Medium als Leerwert.

Für die Herstellung der Hemmstofflösungen werden 50 mg Substanz in 1 ml Wasser bzw. 1 *N* NaOH (5 ml 1 *N* NaOH für Scha 306) gelöst und mit Wasser auf 50 ml aufgefüllt. Die 10fache Verdünnung mit Nährlösung ergibt die Ausgangskonzentration von 100 µg/ml.

Unser *L.-casei*-Stamm ATCC 7469 zeigt unbeeinflusstes Wachstum bis zu einem pH von 7,1. Nur bei einem Gehalt von über 50 µg Scha 306/ml werden die Versuche durch die Alkalität der Lösung beeinträchtigt, nicht aber im Bereich niedrigerer Konzentrationen. Bei den restlichen Verbindungen ist auch in den höchsten geprüften Konzentrationen keine Hemmung des Wachstums durch Alkali zu befürchten, wie Kontrollversuche mit pH-Bestimmung gezeigt haben.

Aus Tabelle I ist zunächst ersichtlich, dass Salicylsäure, Phenylbutazon, Indomethacin und Azapropazon (Mi 85)[3] praktisch unwirksam, Mefenaminsäure sehr wenig sowie Meclofenaminsäure und Flufenaminsäure mittelmässig wachstumshemmend sind. In der Reihe der 1,2-Malonyl-4-phenyl-1,2-dihydrocinnoline (Scha-Nummern) finden wir von R' = Propyl bis Hexyl stetig ansteigend eine Hemmwirkung des Wachstums von *L. casei*. Am

[1] Alexander von Muralt zum 65. Geburtstag gewidmet.

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