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Introduction

During the past several decades, a remarkable interest in rheumatoid arthritis has resulted in impressive developments. In the area of basic research, pathogenetic mechanisms are being unraveled and the role of immune complexes, complement amplification of immune phenomena and participation in the inflammatory reaction, and lymphocyte participation in the induction and maintenance of the inflammatory response are gradually being clarified. Biochemical events at the cellular level such as lysosomal activation and the elaboration of chondrolytic enzymes by rheumatoid synovium should eventually lead to an understanding of the events which can explain the disease. Yet to be discovered are the critical determinants which program the host to respond to as yet unidentified triggering events.

Despite these impressive advances, it has not been possible to design *specific* therapeutic interventions for identifiable and discrete aspects of this inflammatory response. While drug actions can be described, for example the effects on prostaglandin synthesis, it is still not possible to assess the significance of such actions in human rheumatoid arthritis.

Concurrent with these basic research efforts has been an intensive search for new therapeutic agents. Some drugs, such as cytotoxic compounds and D-penicillamine, which were developed for treating non-related diseases, have emerged as effective but toxic agents in rheumatoid arthritis. For practical reasons, the major outcome of targeted research has been the development of nonsteroidal anti-inflammatory agents. This group of drugs probably act by moderating the inflammatory response. Thus, they apparently provide symptomatic control. Whether these drugs have actions which can modify the course of rheumatoid arthritis remains to be demonstrated. Nonetheless the development of drugs which can make the patient feel better and function more effectively and which have minimal side effects and risks represents an important and worthwhile goal.

With respect to any new drug, the physician is faced with the task of searching out relevant studies on pharmacology, efficacy, tolerance and safety. This is not an easy task! Thus, it is hoped that this volume on tolmetin, a new non-steroidal inflammatory agent, will serve a real need. It is the outgrowth of a symposium held in Washington, D.C., April 5, 1975. The purpose of the meeting was to review the chemistry, pharmacology, clinical efficacy and safety of tolmetin. Every effort was made to present meaningful information in an unbiased fashion. No strong editorial license was taken. Rather, each investigator was permitted to present his data, and discussion

was encouraged. Hopefully this volume will permit each reader to develop his own conclusions based upon critical review of the evidence.

It is traditional to summarize the organization of the volume or perhaps even to make editorial comments on the significance of the reports. I will refrain from doing this as it is difficult enough to gain the attention of the reader for any significant period of time. Rather, I think it is important to set the philosophy of such a presentation as a mechanism for collating current available data on a new non-steroidal anti-inflammatory agent. While each reader will most certainly form his own opinion, one can generalize by stating that tolmetin would appear to be a well tolerated, safe, non-steroidal anti-inflammatory agent for the treatment of rheumatoid arthritis. While it lacks the capability of ''curing'' the disease, the addition of another agent to help control the clinical manifestations of the disease is welcomed. I am sure the readers will find many valuable papers in this volume.

It is hoped that the proceedings of this symposium will serve as a useful resource for investigators and physicians interested in non-steroidal anti-inflammatory agents.

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Pharmacology of Tolmetin:

1-Methyl-5-*p*-toluoylpyrrole-2-acetic Acid

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ABSTRACT

*Tolmetin, 1-methyl-5-*p*-toluoylpyrrole-2-acetic acid, has demonstrated anti-inflammatory activity in the acute rat hind paw edema tests, the sub-acute carrageenan abscess and cotton pellet granuloma tests, and chronic adjuvant arthritis (AA) tests. Tolmetin prevented development of AA and reversed the established paw volume changes. When skeletons of AA rats were evaluated using an Alizarin red staining technique, tolmetin was shown to contribute significantly towards preventing the ankylosing and degenerative changes.*

Since adjuvant arthritis is a chronic disease and its underlying changes in bone come about over a lengthy period of time, these changes cannot be halted abruptly. Greater beneficial effects were obtained when the treatment with tolmetin was prolonged. If treatment is initiated early, tolmetin has a greater ability to prevent and reverse the symptoms of the disease (both paw volume and bone changes). Tolmetin when combined with acetaminophen or aspirin always had a greater efficacy than tolmetin alone in suppression of inflammation and arthritic degenerations.

INTRODUCTION

The chemical structure of tolmetin is shown in Figure Ia. It is a toluoyl-pyrrole acetic acid with a methyl group at position one (Carson et al., 1971). Tolmetin lacks the indole nucleus present in indomethacin which is an indole acetic acid (Figure Ib). The α-methyl group that is present in almost all the phenylalkanoic acids in the clinical stage is also absent in tolmetin.

Rheumatoid arthritis (RA) is a chronic disease of the joints, usually polyarticular (Sokoloff, 1972; Boyle and Buchanan, 1971; Fassbender, 1971). It is marked by inflammatory changes in the synovial membranes and

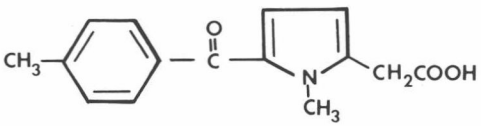

Figure 1a: Structure of tolmetin, TOLECTIN®, McN-2559, 1-Methyl-5-p-toluoylpyrrole-2-acetic acid.

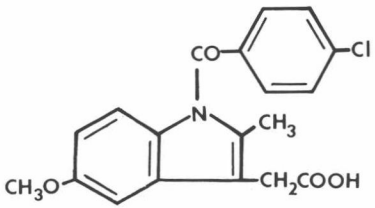

Figure 1b: Structure of indomethacin.

articular structure (Weissman, 1974) and by atrophy and rarefaction of the bones (Uehlinger, 1971; Harris, 1974). In the late stages of the disease, deformity and ankylosing develop (Sokoloff, 1972). Though the pathogenesis of RA has been reasonably well described, its etiology remains unproven (Bland and Phillips, 1972). Our inadequate knowledge of the cause of RA has deterred the development of specific pharmacologic tests (Swingle, 1974; Whitehouse, 1974; Wong, 1975) for antiarthritic agents.

Over the past decades, a number of acute antiphlogistic tests have been devised for the study of inflammation (Swingle, 1974). Even though inflammation is a common feature of these tests and arthritis, every type of inflammation will not lead to articular tissue damage that is associated with arthritis. Adjuvant arthritis (AA) in rats, induced by *Mycobacterium butyricum,* appears to be the most relevant model of RA that is currently available (Pearson, 1972; Swingle, 1974; Rosenthale, 1974).

The anti-inflammatory activity of tolmetin in the classical acute and subacute tests is considered in this paper, but major emphasis will be directed towards adjuvant arthritis test models.

PHARMACOLOGY

Effect of Tolmetin in Acute Anti-inflammatory Tests. Tolmetin and standard anti-inflammatory agents were administered to male Sprague-Dawley, Holtzman derived rats (Holtzman, Madison, Wisconsin), weighing 170-190 gm. Groups of ten animals each were used for each dose level in all tests.

All test compounds were administered by gavage. One hour later, each rat received a 0.1 ml s.c. injection of sterile 1.0% carrageenan or 10% kaolin suspension in the right hind paw. Paw volumes were determined by a modification of Van Arman's mercury displacement method (Winter et al., 1962; Van Arman et al., 1965). A strain gauge amplifier type 3126 (Hallmark Standards, Inc., Mt. Vernon, N.Y.) and Beckman model 3108 analog-digital converter were used to record data automatically on a modified TTY-ASR 33 teletype.

Dose-response relationships were evaluated by fitting a line to log dose versus mean percent inhibition of edema. Relative potency evaluations were made according to Finney (1952). ID_{30} values and their 95% confidence limits were calculated from the regression equations according to the method described by Goldstein (1964).

The positive slopes of the regression lines, as indicated by the regression coefficients ± 95% confidence limits (Table I, a & b), show that tolmetin and the standard agents were significantly effective in the acute kaolin- and carrageenan-induced rat hind paw edema tests. Using ID_{30} values, the various agents may be listed in order of relative potency for the kaolin test: indomethacin (2.1); flufenamic acid (4.3); tolmetin (7.8); phenylbutazone (21.0); aspirin (67.2); and for the carrageenan test: indomethacin (18.4); ibuprofen (19.2); tolmetin (33.3); aspirin (122); flufenamic acid (379); phenylbutazone (420).

Effect of Tolmetin in the Subacute Carrageenan Abscess Test. Female Holtzman rats, weighing 54-63 gm, were randomly divided into groups of ten animals each. Doses of tolmetin, standard anti-inflammatory agents and saline controls were randomly assigned. One hour after oral dosing, 0.5 ml sterile carrageenan (2%) was injected s.c. into the dorsal lumbar region by Cornwall syringe. Twenty-four hours later, all animals were sacrificed in CO_2 and the fresh weight of the abscess determined. Each individual value was expressed as percent inhibition relative to the saline control mean. Dose-response relationships, regression analyses and relative potency evaluations were made according to Finney (1952). ID_{50} values* and their 95% confidence limits were calculated from the regression equations according to the method described by Goldstein (1964).

Where data was insufficient for analysis according to Finney's Bioassay Procedure, results were plotted on semilogarithmic graph paper and the ID_{50} values estimated. Confidence limits were not included in such estimations.

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* ID_{50} refers to that dose of drug required to produce 50% antagonism of the carrageenan abscess induced s.c. by 0.5 ml 2% carrageenan.

TABLE Ia

Effect of Tolmetin and Various Standard Anti-inflammatory Agents in the Kaolin-Induced Rat Hind Paw Edema Test.

Anti-inflammatory Compounds	No. of Expts.	No. of Animals	Regression* Coefficient	ID_{30}** mg/kg (p.o.)	RP***
McN-R-1166 (Indomethacin)	8	350	34.3(26.4-42.1)	2.14(1.48-2.80)	3.29(2.31-4.58)****
McN-R-1238 (Flufenamic acid)	2	60	22.6(16.9-28.3)	4.29(——)	0.14(0.10-0.18)****
McN-2559 (Tolmetin)	18	440	47.4(44.3-50.4)	7.77(6.21-9.19)	1.0
McN-R-495 (Phenylbutazone)	7	210	44.8(42.8-46.8)	21.0(15.9-23.1)	0.36(0.26-0.50)****
McN-R-358 (Aspirin)	2	60	71.3(68.7-74.0)	67.2(53.8-77.3)	1.23(0.73-2.05)

*Regression coefficients, ID_{30} and their 95% confidence limits were obtained from the regression equations for each compound (Goldstein, 1964). Data for flufenamic acid was not sufficient to generate reliable 95% confidence limits for the ID_{30}.

**ID_{30} refers to the dose of drug required to produce 30% antagonism of the swelling caused by 0.1 ml of 10% kaolin.

***Relative potencies (RP) and their 95% confidence limits were calculated according to Finney (1952).

****Significant difference as compared to tolmetin, $p<0.05$.

TABLE Ib

Effect of Tolmetin and Various Standard Anti-inflammatory Agents in the Carrageenan-Induced Rat Hind Paw Edema Test.

Anti-inflammatory Compounds	No. of Expts.	No. of Animals	Regression* Coefficient	ID_{30}** mg/kg (p.o.)	RP***
McN-R-1166 (Indomethacin)	9	220	18.4(12.7-24.1)	18.4(9.2-37.1)	2.08(1.09-4.02)****
McN-R-1451 (Ibuprofen)	2	60	31.2(30.0-32.3)	19.2(15.8-26.4)	1.37(0.92-2.04)
McN-2559 (Tolmetin)	22	590	22.4(19.9-24.8)	33.3(25.6-50.9)	1.0
McN-R-358 (Aspirin)	2	60	41.7(41.0-42.4)	122(102-157)	0.20(0.13-0.30)****
McN-R-1238 (Flufenamic acid)	1	30	7.7(6.5-9.0)	379(——)	0.80(0.44-1.35)
McN-R-495 (Phenylbutazone)	2	60	17.2(16.9-17.6)	420(396-840)	0.10(0.06-0.16)****

*Regression coefficients, ID_{30} and their 95% confidence limits were obtained from the regression equations for each compound (Goldstein, 1964). Data for flufenamic acid was not sufficient to generate reliable 95% confidence limits for ID_{30}.

**ID_{30} refers to the dose of drug required to produce 30% antagonism of the swelling caused by 0.1 ml of 1% carrageenan.

***RP and their 95% confidence limits were calculated according to Finney (1952).

****Significant difference as compared to tolmetin, $p<0.05$.

Relative potency values were estimated on the basis of the ID_{50} values. Results are presented in Table IIa. Using the ID_{50} values, the various agents may be listed in order of relative potency: indomethacin (8.0); tolmetin (47.1); flufenamic acid (125); ibuprofen (137); phenylbutazone (188); aspirin (1416).

TABLE IIa

Effect of Tolmetin and Various Standard Anti-inflammatory Agents in the Carrageenan-Induced Abscess Test.

Anti-inflammatory Compounds	No. of Expts.	No. of Animals	ID_{50}* mg/kg (p.o.)	RP
McN-R-1166 (Indomethacin)	2	60	8.0(6.3-9.8)	5.99(4.39-8.53)
McN-2559-21-98 (Tolmetin)	3	90	47.1(39.9-60.1)	1.0
McN-R-1238 (Flufenamic acid)	1	40	125	0.18(0.11-0.27)
McN-R-1451 (Ibuprofen)	1	40	137	0.11(0.06-0.17)
McN-R-495 (Phenylbutazone)	1	30	188(83.7-410)	0.31(0.20-0.46)
McN-R-358 (Aspirin)	1	40	1416	0.01(0.007-0.02)

*ID_{50} refers to the dose of drug required to produce 50% antagonism of the abscess caused by 0.5 ml of sterile carrageenan (2%) injected subcutaneously. All ID_{50} values with 95% confidence limits were obtained from the regression equations for each compound (Goldstein, 1964). ID_{50} values presented without 95% confidence limits were estimated.

Effect of Tolmetin in the Subacute Cotton Pellet Granuloma Test. Cotton pellets were prepared by cutting cotton dental rolls (Johnson and Johnson, No. 1) into 5 mm sections. Only pellets of identical weight (nearest milligram) were used in any given experiment. All pellets were soaked in carrageenan (1%), then dried under infrared lamps for 24 hours. Pellets were autoclaved and just prior to implantation, 0.2 cc tetracycline was applied to each side.

Male Holtzman-derived Sprague-Dawley rats weighing 140-170 gm were anesthetized with ether and the pellets aseptically implanted s.c. in the lower ventral thoracic region. Ten rats were used per dose level of tolmetin, standard anti-inflammatory agent, or control. All rats were dosed once daily (p.o.) for one week. On the day of termination, animals were sacrificed with an overdose of ether and the pellets were removed. The fibrous capsule was stripped off and discarded. The pellets were cleaned, dried overnight at 60°C, then weighed after proper cooling.

The increment in dry weight of the pellets was used as a measure of granuloma formation. Data was evaluated statistically as described in the carrageenan abscess test. Results are presented in Table IIb.

The regression coefficients, including the upper and lower 95% confidence limits for all agents were positive. These agents were all significantly active in the cotton pellet granuloma test. The low regression coefficient values indicate that the dose-response curves are very flat. Using the ID_{30} values, the various agents may be listed in order of relative potency: indomethacin (1.7); tolmetin (6.0); phenylbutazone (24.5); aspirin (112).

Induction of Adjuvant Arthritis (AA) in Rats. Adjuvant arthritis was induced in female Wistar, Lewis rats (from Charles River) weighing 160-190 gm by a single subcutaneous injection of 0.75 mg *Mycobacterium butyricum* (Difco) into the left hind paw. The non-injected hind paw remained a normal size for the first seven days. Swelling began to appear in the non-injected paw of the first rats on Day 8. Using the paw volume determination technique as described under acute anti-inflammatory tests, the time progress curve of volume changes in the non-injected paw may be followed (Figure 2).

It was determined that whenever an animal showed edema $\geq$ 0.25 ml, further increase in the contralateral paw size always followed and the swelling developed rapidly during the next two to three days. Using 0.25 ml edema as criterion of arthritis onset, the mean onset time for 100 rats was determined and found to be 11.6 days with a standard deviation of 1.9 days. The frequency of onset time values were distributed normally between Days 8 and 19 (see frequency distribution curve superimposed on the time progress curve in Figure 2).

Phases of Adjuvant Arthritis. The whole time progress of AA development can be divided into four phases (Figure 2). Phase I (Days 0-10) is the incubation period. Phase II (Days 10-18) is characterized by the rapid development of paw swelling. Phase III (Days 18-25) is established AA and Phase IV is the phase of osteogenic changes. This report contains experimental results from Phases II, III and IV.

TABLE IIb

Effect of Tolmetin and Various Standard Anti-inflammatory Agents in the Cotton Pellet Granuloma Test.

Anti-inflammatory Compounds	No. of Expts.	No. of Animals	Regression* Coefficients	ID_{30}** mg/kg/day p.o.	RP***
McN-R-1166 (Indomethacin)	7	210	28.0(15.1-40.9)	1.7(0.8-2.6)	3.41(1.6-13.9)
McN-2559-21-98 (Tolmetin)	15	430	10.2(7.1-13.2)	6.0(4.8-21.9)	1.0
McN-R-495 (Phenylbutazone)	1	28	34.6(32.3-36.9)	24.5(16.6-28.8)	0.25(0.15-0.64)
McN-R-358 (Aspirin)	1	29	26.6(24.2-29.0)	112(92.0-279)	0.038(0.018-0.067)

*Regression coefficients, ID_{30} values and their 95% confidence limits were obtained from the regression equation for each compound.
**ID_{30} refers to the dose of drug required to produce 30% antagonism of the granuloma produced in the water treated control.
***RP and their 95% confidence limits were calculated according to Finney (1952).
****Significant difference as compared to tolmetin, $p<0.05$.

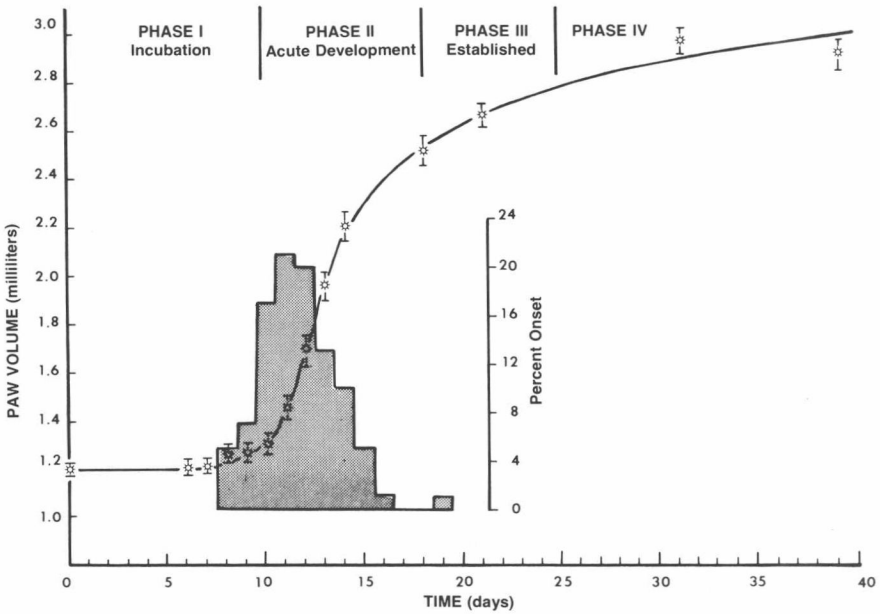

Figure 2: Development of Adjuvant Arthritis–Time Progress Curve with Frequency Distribution of Onset and Phases of AA.

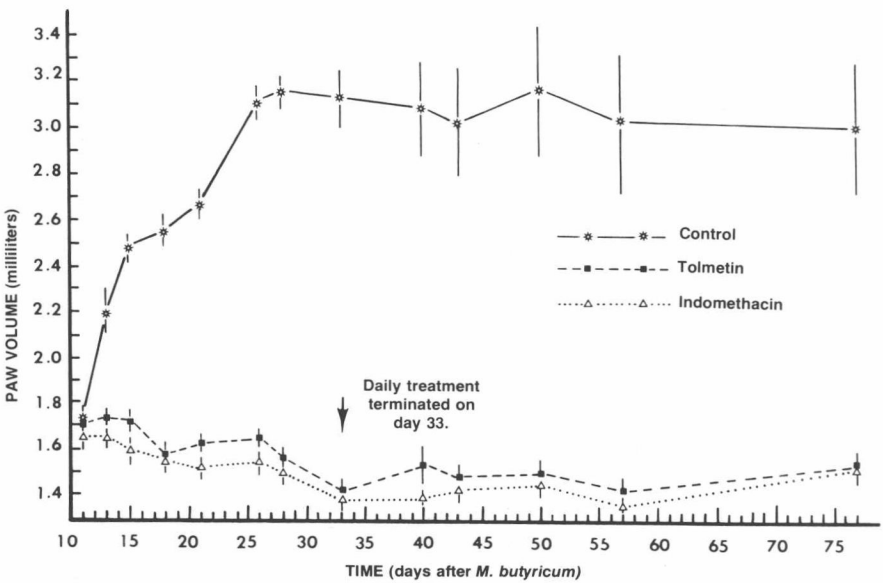

Figure 3: Effect of Prolonged Treatment with Tolmetin and Indomethacin in the Development of Adjuvant Arthritis.

Effect of Tolmetin and Standard Anti-inflammatory Agents in the AA Phase II Test. Adjuvant arthritic rats with early but significant signs of arthritis in the contralateral paw were selected on Day 11 and evenly distributed into groups of 10 rats each. Saline control and treatments with tolmetin or standard agents were coded and randomly assigned to the various groups. All animals were dosed once daily (p.o.) on Days 11 to 14 inclusive. Non-injected paw volumes were determined initially on Day 11 and again on Day 15. Rat paws were dipped to the hairline to avoid variations in marking at the lateral malleolus.

Normal paw volume for all AA rats were determined from a standard curve relating normal paw volume to body weight. Each individual rat with a paw volume greater than the normal volume was utilized in the analysis of the data. Values for treated animals were expressed as a percent inhibition of the paw volume change relative to the mean value for saline controls. Data was evaluated statistically as described in the acute edema tests. Results are presented in Table III.

Tolmetin and the standard agents were all found to be active in the AA Phase II Test. The maximal effectiveness exhibited by any anti-inflammatory agent was 75% under conditions of this test procedure. Using ID_{50} values, the various agents may be listed in order of relative potency: indomethacin (0.67); ketoprofen (2.03); flufenamic acid (5.48); naproxen (14.5); phenylbutazone (15.3); tolmetin (42.1); fenoprofen (80); ibuprofen (124); aspirin (452).

Effect of Tolmetin and Indomethacin in the AA Phase II Test with Prolonged Treatment. In another experiment, AA rats were selected on Day 11 as described above. Saline control and treatments with tolmetin (100 mg/kg/day, p.o.) and indomethacin (2.0 mg/kg/day, p.o.) were randomly assigned and animals were treated daily to Day 33. Results are presented in Figure 3.

Both tolmetin and indomethacin totally suppressed further increases in paw volume. The small amount of edema present before the experiment commenced was also suppressed. After treatment was terminated on Day 33, no signs of ''flare-up'' could be observed between Days 33 and 77. These results showed that total prevention of arthritis development could be accomplished with 21 days of treatment.

Effect of Duration of Treatment with Tolmetin on the Development of Adjuvant Arthritis. Adjuvant arthritic rats with early but significant signs of arthritis in the non-injected hind paw were selected on Day 11 and evenly distributed into four groups of 5 rats each. Saline control and treatments with tolmetin (100mg/kg/day, p.o.) for 7, 14, or 21 days were assigned randomly