ANTIMYCOBACTERIAL ACTIVITY OF THE ANTIINFLAMMATORY AGENT DICLOFENAC SODIUM, AND ITS SYNERGISM WITH STREPTOMYCIN

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ABSTRACT

Diclofenac sodium, an antiinflammatory agent, exhibited remarkable inhibitory action against both drug sensitive and drug resistant clinical isolates of *Mycobacterium tuberculosis*, as well as other mycobacteria. This drug was tested *in vitro* against 45 different strains of mycobacteria, most of which were inhibited by the drug at 10-25 µg/ml concentration. When tested *in vivo*, diclofenac, injected at 10 µg/g body weight of a Swiss strain of white mice, could significantly protect them when challenged with 50 median lethal dose of *M. tuberculosis* H_{37} Rv 102. According to χ^2 test, the *in vivo* data were highly significant ($p<0.01$). Diclofenac was further tested for synergism with the conventional antimycobacterial drug streptomycin against *M. smegmatis* 798. When compared with their individual effects, synergism was found to be statistically significant ($p<0.05$). By the checkerboard assessment procedure, the fractional inhibitory concentration index of this combination was found to be 0.37, confirming synergism.

Key words: antiinflammatory drug, diclofenac sodium, antimycobacterial activity, streptomycin, synergism, non-antibiotic

INTRODUCTION

Mycobacteriosis, particularly tuberculosis, has become a global problem. The occurrence of multi-drug resistance among *Mycobacterium tuberculosis* in particular and mycobacteria in general needs surveillance and control. Failure to cure multi-drug resistant tuberculosis (MDR-TB) with the currently available antitubercular drugs leads to a search for newer and potent drugs to treat such cases, and thereby prevent an emerging multidimensional problem. Different studies aimed at discovering newer antimycobacterial agents have revealed moderate to powerful action in several compounds belonging to various pharmacological groups, e.g., promethazine (22), chlorpromazine (8), trifluoperazine (24), methdilazine (7), thioridazine (1) and other phenothiazines (17). Further studies have revealed the enhancement of antibiotic activity against MDR-TB by phenothiazines (25). Many of these agents have exhibited powerful inhibitory action against Gram positive and

Gram negative bacteria as well (18,20,21). Such compounds having antimicrobial properties in addition to their predesignated pharmacological action are entitled as "Non-antibiotics". The antiinflammatory drug diclofenac sodium was seen to possess powerful antibacterial activity against Gram positive and Gram negative bacteria (3,9). It also exhibited significant synergism with an antibiotic streptomycin (4) and a non-antibiotic trifluoperazine (11). The present paper describes the antimycobacterial action of diclofenac both through *in vitro* and *in vivo* tests, and potentiation of its activity by combination with known antitubercular drugs.

MATERIALS AND METHODS

Drugs

The drugs were obtained as pure dry powder from their respective manufacturers in India. Diclofenac sodium (Dc) and rifampicin (Rf) were obtained from Hindustan Ciba Geigy,

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streptomycin (Sm) from Sarabhai Chemicals, ethambutol (Eb) from Lyka Laboratories and isonicotinic acid hydrazide (INH) from Glaxo Laboratories. They were preserved at 4ºC.

Bacteria

Forty-five strains of mycobacteria were tested. The strains and their sources are given in Table 1. The strains were identified by Radiometric method (BACTEC 460) and biochemical tests (Niacin, Nitrate, Urease, Catalase, Tween80, Tellurite and 5% NaCl tests).

Media

Liquid medium used was Kirchner's Liquid medium (KLM) (16), which was used to grow and suspend the organisms.

Solid medium was Lowenstein Jensen Medium (LJM), prepared as described by the International Union Against Tuberculosis and Lung Diseases (IUT, 1955) (15).

Preparation of inocula for susceptibility tests

The bacterium was first grown in KLM. The inoculum was prepared by homogenizing the KLM culture with glass beads, spinning down the larger particles, and matching the supernate against Mc Farland's standard (23).

Determination of minimum inhibitory concentration (MIC) of antibiotics / non- antibiotics against different strains of mycobacteria

While determining MIC by tube dilution method (12), Sm, Rf, INH and Eb each were used in the following concentrations (μg/ml) in KLM: 0 (Control), 0.25, 0.5, 1, 2, 4 and 8. Dc was used in KLM in concentrations of 0 (Control), 5, 10, 15, 20, 25 and 50

μg/ml. For some selected strains, the drug was tested in concentrations ± 2 of its MIC value, in order to find out its mean ± standard deviation values with respect to those organisms. Amount of inoculum used to inoculate each tube above was 0.01 ml. Incubation was done at 37ºC for 10-20 days as required.

The MIC of each organism was defined as the lowest concentration of antibiotic where the growth obtained was reduced to 1% or less when compared to the control slopes. All the tests were run in duplicate. The resistant strains were further processed for determining the resistant break point by using 8, 16, 32, 64 and 128 μg/ml of the drug in LJM. The following were the MIC levels of primary antitubercular drugs indicating resistance - streptomycin (Sm) $\geq$ 32 mg/l, isoniazid (INH) $\geq$ 1 mg/l, rifampicin (Rf) $\geq$ 128 mg/l and ethambutol (Eb) $\geq$ 8 mg/l.

Determination of synergism between Dc and Sm by disc diffusion tests (5)

0.5 ml of inoculum was applied on LJM, poured in plates. Filter paper discs (Whatman No.1) containing 50 μg of Dc and 10 μg of Sm were placed on the LJM, and incubation was done at 37ºC. The clear zones of inhibition around each disc individually or in combination were measured in three different directions to obtain the mean values of each test. The increase or decrease of surface area (πr^2) due to a particular combination as well as those due to single effects was statistically evaluated by χ^2 test (6) for the level of significance of alteration. The occurrence of mutual influence /interference when drugs were used in combination was assessed as (i) indifference, when both tangential circles of inhibition were unaffected, (ii) antagonism, when the circles receded and assumed kidney shape, (iii) synergism (24), when the circles enlarged. The

Table 1. Source of mycobacterial strains tested.

Mycobacteria	Source
Reference strains *M. tuberculosis* H$_{37}$ Rv102, H$_{37}$Ra16	Tuberculosis Research Center, Chennai, ICMR, Govt. of India
M. marinum 50, *M. scrofulaceum* 1323, *M. gordonae* 1324, *M. flavescens* 1541, *M. xenopi* 160, *M. avium* 724, *M. intracellulare* 1406, *M. terrae* 1450, *M. trivate* 1453, *M. smegmatis* 798, *M. smegmatis* 1546, *M. fortuitum* 1529, *M. phlei* L1	Central JALMA Institute for Leprosy, ICMR, Agra, India
Clinical strains *M. tuberculosis* Bajaj, J15, N23, 912042, 911928, 905574, 911454,910708,911831,905358,911447,911884,912234,91 1677,90657,912359,911337,912073,912447,912056,91105 3,906909	Tuberculosis Research Centre, Chennai, India
M. tuberculosis BTA1, BTA2, BTA3, BTA4, BTA5, BTA6, BTA7, BTA8	Bengal Tuberculosis Association (BTA), Calcutta, India

combined effects of two drugs were further determined by the checkerboard dilution technique for derivation of the Fractional Inhibitory Concentration (FIC) indices (19). The checkerboard was such arranged that in the first horizontal row, all the tubes had 80 µg/ml of Dc in KLM against 0, 0.25, 0.5, 1, 2, 4 and 8 µg/ml of Sm in a final volume of 2 ml. The same was followed in the next 6 rows with 40, 20, 10, 5, 2.5 and 0 µg/ml of Dc, respectively. The index was calculated as -

$$\frac{\text{Agent A}}{\text{MIC of A}} + \frac{\text{Agent B}}{\text{MIC of B}} = \text{FIC A} + \text{FIC B}$$

Bactericidal activity

It was measured as the average reduction in $\log_{10}$ colony forming units (CFU)/ml/day when exposed to the respective concentration of the drug. In this test, MIC of an antibiotic or an antimicrobic agent was taken to be the lowest concentration that inhibited visible bacterial growth *in vitro* after incubation upto 7 days at 37ºC, using an initial inoculum of *ca*.10^5 CFU/ml. The minimum bactericidal concentration (MBC) of these agents was determined by subculturing from the tube of MIC dilution to antibiotic free solid medium (LJM) and determining the % kill [(CFU survivors / 10^5) x 100] and incubating at 37ºC for 3 weeks for colonies to develop (6). Similar culture inoculum from the drug free medium provided the control.

Animal Experiments

Swiss albino mice maintained in our animal house were used in this study; the animals were maintained at standard conditions at 21 ± 1ºC and 50-60% relative humidity with a photoperiod of 14:10 h of light-darkness. Water and a dry pellet diet were given *ad libitum*. *M. tuberculosis* H_{37} Rv 102 was the test bacterium as it was naturally virulent to mice. The median lethal dose (MLD/LD_{50}) of the strain (after repeated passage through mice) was determined by using graded challenges in batches of mice and recording the mortality upto 30 days. The LD_{50} was not affected by freeze-drying and reconstitution. Reproducibility of the challenge dose was ensured by standardizing its optical density at 640nm in a Klett-Summerson colorimeter to obtain the desired CFU on KLM.

Systemic infections were produced in groups of 20 inbred Swiss Albino male mice (*ca*. 18-20 g). Each mouse was administered intraperitoneally 0.05 ml of a suspension (containing 0.5 mg homogenized KLM culture deposit, representing c < 9 x 10^9 CFU) (13); of these, 10 were protected by Dc (dose 10 µg/g body weight/day x 6 weeks) while the other 10 did not receive any drug and served as the control. The viscera from the animals autopsied 6 weeks after infection were obtained, taking strict precaution respecting sterility and examined for macroscopic lesions of systemic infections, e.g., tubercles and caseation, both for the treated and untreated groups (27). Portions of each organ were processed for histological study of the lesions, while the remainder were homogenized aseptically in sterile glass homogenisers in saline, examined under the microscope as stained smears (Hematoxylin and Eosin, as well as, Ziehl-Neelsen stains) for presence of acid fast bacilli (AFB)/ and contaminants, and inoculated onto nutrient/blood agar plates to determine rapid growth, if any. Sterile specimens (as well as contaminated specimens after adequate decontamination by Petroff's method) were plated out on LJM in 0.1 ml amounts and examined for growth of the infecting *M. tuberculosis*. The growth was confirmed by Radiometric method.

RESULTS

Minimum inhibitory concentration (MIC) of Sm/Rf and Dc against different strains of mycobacteria

The MIC of different agents with respect to 45 strains of mycobacteria tested is given in Table 2. The MIC of Dc is much higher (5-6 times) than the MIC of the conventional antimycobacterial drugs (Sm/Rf). It can also be seen that the MIC of Dc as well as Sm and Rf is higher against the MDR strains as compared to the sensitive strains.

Out of 45 strains of mycobacteria tested, 5 strains (*M. tuberculosis* Bajaj, J15, N23, H_{37}Rv102 and H_{37}Ra16) were inhibited by diclofenac at 10 µg/ml, while 13 strains (*M. marinum* 50, *M. scrofulaceum* 1323, *M. gordonae* 1324, *M. flavescens* 1541, *M. xenopi* 160, *M. avium* 724, *M. intracellulare* 1406, *M. terrae* 1450, *M. trivate* 1453, *M. fortuitum* 1529, *M. phlei* L1*, M. smegmatis* 798, *M. smegmatis* 1546) were inhibited at 15 µg/ml of Dc. These 18 strains were highly to moderately sensitive with respect to conventional antitubercular drugs. Eight strains (*M. tuberculosis* BTA1, BTA2, BTA3, BTA4, BTA5, BTA6, BTA7, BTA8) were found to be multidrug resistant. They were inhibited by Dc at 20 µg/ml. Finally, *M. tuberculosis* 912042, 911928, 905574, 911454, 910708, 911831, 905358, 911447, 911884, 912234, 911677, 90657, 912359, 911337, 912073, 912447, 912056, 911053, 906909 were inhibited by Dc at 25 µg/ml. These strains were polydrug resistant. The susceptible strains like *M. tuberculosis* H_{37}Rv102 were inhibited at lower doses of conventional antitubercular agents (0.5 to 2 µg/ml), while the single-, poly- and multidrug resistant clinical isolates (like *M. smegmatis* 798, *M. tuberculosis* 912042 and *M. tuberculosis* BTA8 and so on) were inhibited at much higher concentrations, and some were even resistant. MIC of Dc against *M. tuberculosis* H_{37}Rv102 was 10 µg/ml, while it was 25 µg/ml for the drug-resistant strains. The MIC values of Dc in terms of mean ± standard deviation with respect to five strains (*M. tuberculosis* H_{37}Rv102*, M. intracellulare* 1406*, M. smegmatis* 798*, M. tuberculosis* BTA1 and *M. tuberculosis* 912042) are given in Table 2. It was noticed that even the multidrug resistant strains like were susceptible to diclofenac, although at a higher concentration (25 µg/ml).

Table 2. Minimum inhibitory concentration (MIC) of diclofenac, rifampicin and streptomycin.

Strains	MIC (μg/ml)		
	Dc	Sm	Rf
Highly sensitive			
M. tuberculosis Bajaj, J15, N23, H_{37} Rv102, H_{37}Ra16	l0	0.25-2	0.5-2
Moderately sensitive			
M. marinum 50, *M. scrofulaceum* 1323, *M. gordonae* 1324, *M. flavescens* 1541, *M. xenopi* 160, *M. avium* 724, *M. intracellulare* 1406, *M. terrae* 1450, *M. trivate* 1453, *M. fortuitum* 1529, *M. phlei* L1	15	0.5- 2	1-2
M. smegmatis 798, *M. smegmatis* 1546	15	2	2
Multidrug Resistant			
M. tuberculosis BTA1, BTA2, BTA3, BTA4, BTA5, BTA6, BTA7, BTA8	20	4-8	4-8
Polydrug resistant			
M. tuberculosis 912042, 911928, 905574, 911454, 910708, 911831, 905358, 911447, 911884, 912234, 911677, 90657, 912359, 911337, 912073, 912447, 912056, 911053, 906909	25	>8	>8

Activity of Dc against *M. tuberculosis* H_{37}Rv 102

The MIC and MBC of Dc against *M. tuberculosis* H_{37}Rv 102 were 10 and 40 μg/ml respectively, i.e., the MBC value was 4 times higher than the MIC value for a complete killing of the population in the initial inoculum. The bactericidal activity was 0.33 with 40 μg/ml of Dc on day 3; it was 0.27 with 40 μg/ml and 0.16 with 20 μg/ml on day 7 (Table 3, Fig. 1).

Synergism between Dc and Sm by disc diffusion tests

The synergism between Dc and Sm with respect to *M. smegmatis* 798 is shown in Fig. 2. The individual average inhibition zone for Sm was 17.2 mm and for Dc was 16.6 mm, while their combined activity was synergistic; the inhibition zone for Sm increased by 0.8 mm while that of Dc increased by 0.5 mm. The percentage increase of surface area of inhibition zones was 9.52 and 6.12 for Sm and Dc, respectively (Table 4). Statistical analysis of these values by Student's 't' test showed the result was significant ($p<0.05$). The FIC index for *M. smegmatis* 798 was 0.37, thus confirming synergism between Dc and Sm. (Fig. 3).

***In vivo* assessment**

Table 5 shows that of the 10 animals in the untreated group, all developed minute tubercles in the liver, 5 in the spleen, 5 in the lungs and 9 in the peritoneum and intestines; microscopic necrosis suggestive of caseation was found in the liver of 3 animals and in the spleen, peritoneum and intestines each in one animal. Smears for acid-fast bacilli by Z-N stain, from centrifuged deposits (for 100 fields) of tissue homogenates,

Table 3. Activity of Dc against *M. tuberculosis* H $_{37}$ Rv102.

Conc. (μg/ml)	Viable counts ($\log_{10}$ CFU/ml) on day		
	0	3	7
Nil	4.60	6.16	8.39
40	4.60	3.60(0.3)*	2.66(0.27)
20	4.60	4.94	4.23(0.16)
10	4.60	5.58	6.03

*Bactericidal activity.
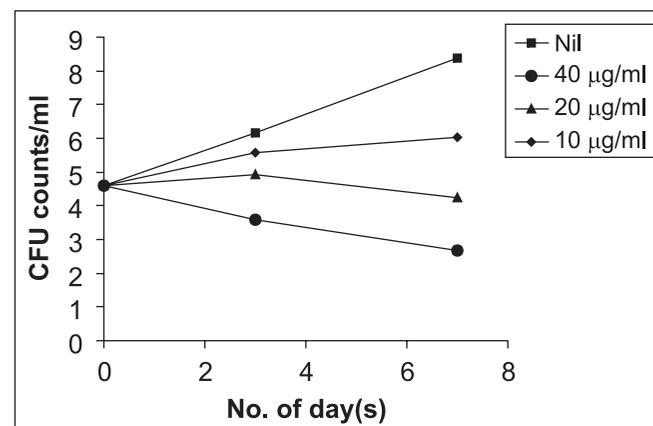
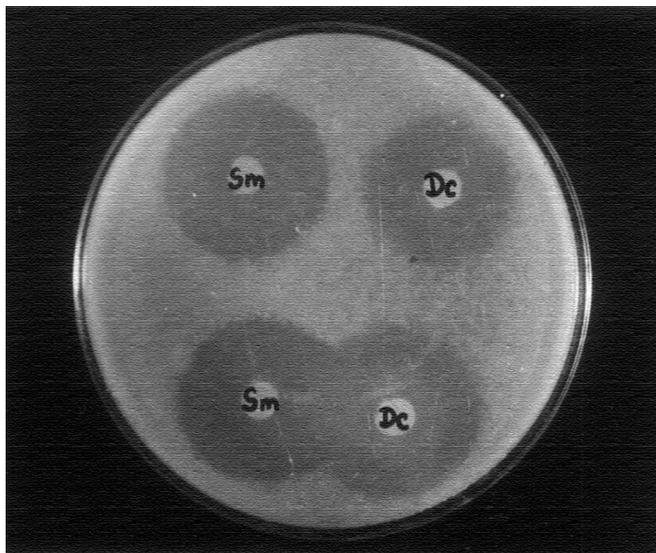**Figure 1.** Bactericidal activity of diclofenac sodium against *Mycobacterium tuberculosis* H_{37} Rv102.

Figure 2. Synergism between diclofenac (50 µg/ml) and streptomycin (10 µg/ml) against *Mycobacterium smegmatis* 798.

Figure 3. Assessment of antimycobacterial combination by checkerboard.

showed all 10 animals to be smear positive at the time of autopsy, which suggested successful infections in these animals. In contrast, macroscopic examination of the treated group (10 animals) showed tiny tubercles to be present in some of the liver specimens (2) and in the spleen, peritoneum, as well as in the intestine (3 each), but in the lungs, Z-N stained smears showed presence of AFB only in 4 cases (Table 5). In 5 animals of the untreated group, *M. tuberculosis* $H_{37}Rv$ 102 could actually be recovered on subculture (as confirmed by BACTEC test) in comparison with only one of the treated groups, which appeared to be significant ($p<0.01$). The failure to recover the bacterium in other untreated animals was probably due to a non-viability of these bacilli, although these could readily be detected in smears in all cases. The histopathological sections of liver also revealed a considerable decrease in number of infiltrations in infected mice treated with Dc as compared to the untreated ones.

DISCUSSION

The non-steroidal antiinflammatory drug Dc had proved to be a powerful bactericidal antimicrobic agent, when tested against a large number of Gram positive and Gram negative bacteria, the MIC ranging from 25-100 µg/ml in most of the instances, and even lower in some cases. This bactericidal agent could also offer significant protection to mice, when challenged with a virulent bacterium (3,9). Moreover, the antibacterial activity of Dc was found to be due to its inhibition of bacterial DNA synthesis, which was demonstrated using 2 µ Ci (^{3}H) deoxythymidine uptake (10). Further studies on the combined
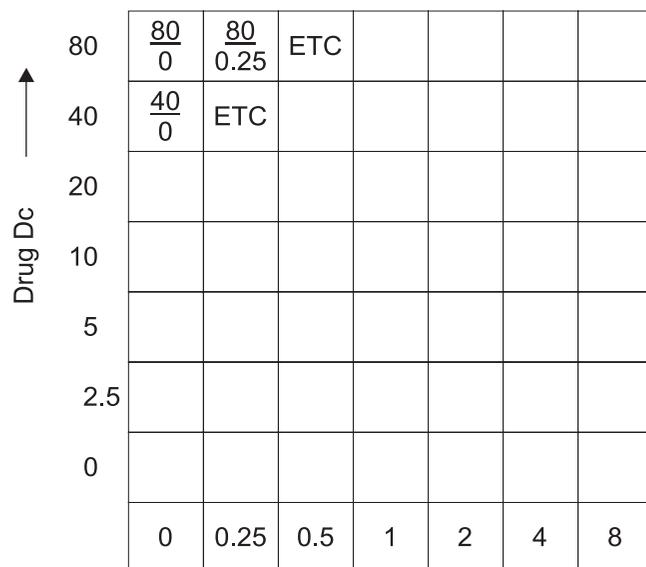
effects of Dc and Sm, as well as Dc and the non-antibiotic trifluoperazine on Gram positive and Gram negative bacteria provided significant synergism both through *in vitro* and *in vivo* experiments (4,11).

In the present study, Dc has shown a remarkable antitubercular activity against a number of mycobacteria. Streptomycin and rifampicin are known conventional antitubercular drugs, and their MIC ranged from 0.5 to 8 µg/ml with respect to most of the strains tested. The susceptible strains like *M. tuberculosis* $H_{37}Rv102$ were inhibited at lower doses

Table 4. Individual and combined (synergistic) effects of Sm and Dc on *M. smegmatis* 798.

Strain	Diameter of the inhibition zone in mm^2					
	Single (A) Drug Effect		Combined (B) Drug Effect		Percentage increase on the basis of πr^2	
.	Sm 10*	Dc 50	Sm 10	+ Dc 50	Sm	Dc
M. smegmatis 798	17.2	16.6	18	17.1	9.52	6.12

*Amount (μg) of the drug /disc; Mean surface area of the inhibition zone (mm^2) was calculated as πr^2 on the basis of their mean diameter (2r) and % increase was calculated as (B-A)/A x 100, which was statistically significant (p<0.05).

Table 5. Effects of Dc[a] on *M. tuberculosis* H$_{37}$ Rv 102[b] infection in mice.

	Liver	Spleen	Peritoneum/ Intestine	Lung	Cumulative value	Recovery of H$_{37}$Rv102 in Smears	Culture
Untreated (10)							
Smears AFB (+)	10/10	10/10	10/10	10/10	40/40		
Tubercles	10	5	9	5	29	10[c]	5
Caseation	3	1	1	0	5		
Treated (10)							
Smears AFB (+)	0/10	2/10	2/10	0/10	4/40		
Tubercles	2	3	3	0	8	4[d]	1
Caseation	0	0	0	0	0		

Untreated, did not receive Dc; Treated, received Dc; [a] 10 μg/g body wt./day; [b] 4.5 x 10^9 CFU/mouse i.p.; [c] from at least one viscera of 10 animals, all viscera did not yield (+) culture; the viable counts (CFU) varied:10^3-10^6/ml; [d] only from 4 animals, recovery from even single organ being counted as positive, other organs did not yield any growth; CFU10^1-10^3/ml in the positive samples.

(0.5 to 2 μg/ml), while the drug-resistant varieties were inhibited at much higher concentrations, and some were totally resistant. A similar pattern was noticed in the *in vitro* activity of Dc as well – while its MIC against *M. tuberculosis* H$_{37}$Rv102 was 10 ± 0.4 μg/ml, it was 25 ± 0.4 μg/ml for the drug resistant strains. It was noticed that even the multidrug resistant strains like those obtained from BTA and Tuberculosis Research Centre were susceptible to Dc, although at higher concentrations (20-25 μg/ml). The MIC of Dc seems to be high against mycobacteria in the *in vitro* studies. However, the antimycobacterial chemotherapeutics like INH and pyrazinamide also have quite high MIC values against mycobacteria; such high doses are often toxic to liver and other organs.

Dc was found to be bactericidal in action against *M. tuberculosis* H$_{37}$ Rv 102.

The activity of Dc against *M. smegmatis* 798 was enhanced in the presence of an antitubercular drug Sm (Fig. 2).

In the animal experiments with *M. tuberculosis* H$_{37}$ Rv 102 in mice, several minute tubercles were observed in the liver, spleen, lungs, peritoneum and intestines of infected mice. However, there was a definite reduction in these macroscopic lesions in Dc-treated animals. The tubercle bacilli could not be recovered from all the untreated animals (Table 5), possibly because of the relatively few bacilli that mouse lesions had during autopsy, with even fewer survivors some weeks after infection, since it is known that compared to mice, guinea pig is a better animal model for producing experimental tuberculosis infection.

Although Dc is reported to be a rather toxic agent for human consumption, this drug could be tolerated by mice for the entire period of 6 weeks when this was administered intraperitoneally everyday in the dose of 10 μg/g body weight. Protection at such a low concentration could be achieved possibly due to the fact that Dc is rapidly and completely absorbed after oral administration. There is a substantial first pass effect, such that only about 50% of the drug is available systematically. Its half-life in plasma is 1 to 2 hours. Dc produces side effects in only 20% of patients when used as an antiinflammatory agent, and only 2% of them discontinue therapy as a result (14). This depends upon genetic factors, nutritional factors and physiological state of the patient.

Earlier studies by Amaral and Kristiansen (2) had proved the efficacy of chlorpromazine in combating tuberculosis *in vivo*, along with a significant *in vitro* action. Apparently, the drug Dc has remarkable structural correlation with chlorpromazine in having two complete benzene rings attached to each other as phenyl acetic acid derivative through an NH group, and two halogen (Cl) atoms.

Most antimycobacterial non-antibiotics reported so far have shown *in vitro* MIC values ranging from 10 to 25 μg/ml, which seems to be in accordance with that of Dc. Phenothiazines such as chlorpromazine (8), thioridazine (1) and promethazine (22) have been shown to have *in vitro* activity against clinical strains of *M. tuberculosis*. This activity required concentrations that are beyond those that are clinically achievable (like 1 mg/l). However, such antitubercular non-antibiotics may be concentrated more than 10-fold by macrophages that have phagocytosed *M. tuberculosis*. Thus, clinically acceptable dosing of a tuberculosis patient might result in an inhibitory effect *in situ* intracellularly similar to that observed *in vitro*.

This suggests that such drugs, including Dc, might be used as adjuvants to current regimens used for the management of freshly diagnosed tuberculosis.

An elaborate study on the effects of combination of Dc plus Sm proved synergism, which could be further substantiated by carrying out tests for FIC index. Both these drugs have been in use satisfactorily in clinical medicine with known toxicity limits. The combination of Sm-Dc may prove to be a breakthrough in the treatment of tuberculosis. Furthermore, in course of time, it may be possible to obtain compounds with much greater synergistic effect with the help of suitable structural modification, thereby making a new generation of potential non-antibiotic antitubercular drugs. The actual factors responsible for attributing antimycobacterial activity to Dc are yet to be ascertained. QSAR studies may reveal the actual moieties responsible for conferring antimycobacterial activity to Dc.

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RESUMO

Atividade antimicobacteriana do agente antiinflamatório diclofenac sódico e seu sinergismo com estreptomicina

Diclofenac sódico, um agente antiinflamatório, mostrou ação inibitória marcante contra isolados clínicos de *Mycobacterium tuberculosis* sensíveis e resistentes à drogas, bem como contra outras micobactérias. A droga foi testada *in vitro* contra 45 cepas diferentes de micobactérias, sendo que a maioria foi inibida pela droga na concentração de 10-25 μg/ml. Quando testado *in vitro*, diclofenac injetado em ratos albinos da linhagem Swiss, na proporção de 10 μg/g de peso corporal, provocou proteção significativa dos animais desafiados com metade da dose letal de *M. tuberculosis* H$_{37}$ Rv 102. De acordo com o teste χ², os dados *in vivo* foram altamente significativos (p ≤ 0,01). Diclofenac foi posteriormente testado quanto ao sinergismo com a droga antimicobacteriana convencional estreptomicina, frente a *M. smegmatis* 798. Quando comparado aos seus efeitos individuais, o sinergismo foi estatisticamente significativo (p ≤ 0,05). Através da análise checkerboard, o índice fracional de concentração inibitória para essa combinação foi 0,37, confirmando o sinergismo.

Palavras-chave: droga antiinflamatória, diclofenac sódico, atividade antimicobacteriana, estreptomicina, sinergismo, não-antibiótico

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