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The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis

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Abstract

Most strains of Gram-positive and Gram-negative bacteria were inhibited by 50–100 mg/l of the anti-inflammatory agent, diclofenac sodium (Dc). In vivo test using 30 or 50 μg Dc per 20 g mouse (Swiss Albino variety) significantly ($P < 0.001$) protected the animals when challenged with 50 MLD of a virulent *Salmonella typhimurium*. The anti-bacterial action of Dc was found to be due to inhibition of DNA synthesis which was demonstrated using 2 μ Ci (^{3}H) deoxythymidine uptake.

Keywords: Non antibiotic; Diclofenac sodium; Anti-bacterial action; Inhibition of DNA synthesis

1. Introduction

Chemotherapeutic agents are usually designated and used according to their most predominant pharmacological action. There are very few drugs, however, with a single specific function. It is well known that aspirin and metronidazole are administered to human beings for a variety of ailments. Systematic studies among various pharmaceutical compounds have revealed that the anti-histamines, bromodiphenhydramine [1], diphenhydramine [1], methdilazine [2], promethazine [3], trimeprazine [4], triprolidine [5], the anti-psychotics, chlorpromazine [6,7], fluphenazine [8,9], alimemazine [7], the tranquilizer, promazine [10], the anti-hypertensives, methyl-DOPA [11] and propranolol [12] and even the local anaesthetics, procaine and lignocaine [13] possess anti-microbial action. These studies have clearly indicated that the tricyclic phenothiazines, comprise a unique class of compounds, endowed with significant anti-bacterial action. Further collaborative work between Japanese and Hungarian scientists, proved the potential of the phenothiazines as anti-tumor [14], anti-viral [15,16] and anti-plasmid [17,18] agents. All these chemical compounds possessing moderate to powerful anti-microbial properties, have been grouped together under the common term 'non-antibiotics'. In an intensive search for anti-microbial action among the non steroidal anti-inflammatory drugs, diclofenac sodium (Dc) exhibited significant potential against both Gram-positive and Gram-negative bacteria, while piroxicam, mefenamic acid, naproxen and oxyphen butazone were found to have mild to moderate activity [19]. When tested in vivo, diclofenac at 1.5 and 3.0 μg/gm body weight of Swiss strain of white mice, could protect the animals when challenged with 50 MLD of *Salmonella typhimurium* NCTC 74. The data were analyzed statistically and accordingly subjected to χ-square test; the data on protection were found to be highly significant ($P < 0.001$). Diclofenac sodium further demonstrated significant clearance of the challenged pathogenic bacteria from liver and spleen [19]. This paper illustrates the mechanism of anti-bacterial action of diclofenac is by inhibition of bacterial DNA synthesis.

2. Materials and methods

2.1. Bacteria

Escherichia coli K12 C600 was obtained from S. Palchoudhuri, Detroit and *Staphylococcus aureus*

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NCTC 6571 from S.P. Lapage, London. Both of these were maintained in stab agar at 4°C and in the freeze-dried state.

2.2. Detection of bacteriostatic/bactericidal activity of diclofenac

Both *E. coli* C600 and *S. aureus* 6571 were grown in nutrient broth (NB; Oxoid, UK) overnight at 37°C, from which 2 ml amount was added to 4 ml of fresh broth and incubated for 2 h to obtain the logarithmic phase of growth. After a bacterial count was performed from the culture tubes, diclofenac was then added at a concentration higher than the minimum inhibitory concentration (MIC) and bacterial counts were again determined at 2, 4, 6 and 18 h.

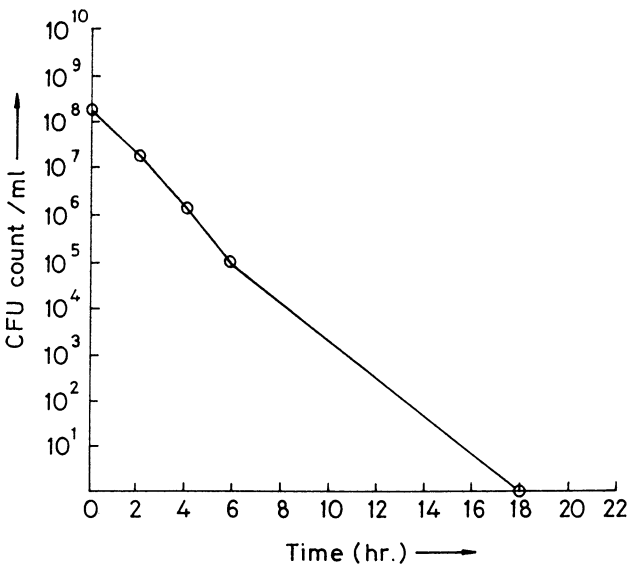

Fig. 1. Bactericidal action of diclofenac (100 mg/l) on *Staphylococcus aureus* NCTC 6571.

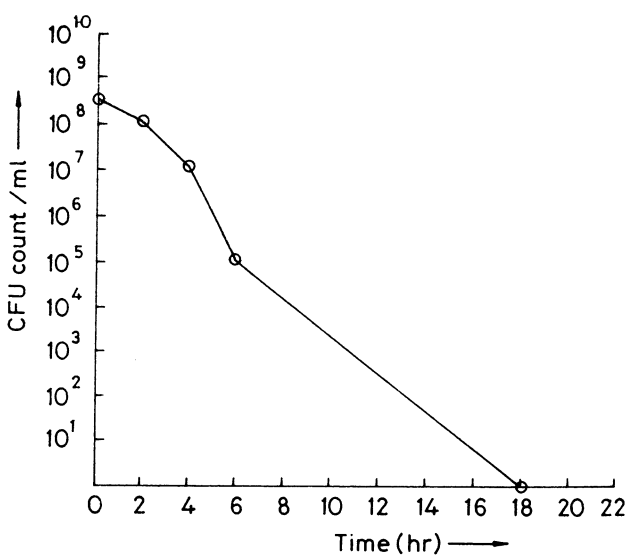

Fig. 2. Bactericidal action of diclofenac (100 mg/l) on *Escherichia coli* K12 C600.

2.3. Assay of DNA synthesis

The test bacteria was grown at 37°C in nutrient broth; after 18h incubation, 1 ml of growth was mixed with 6 ml of fresh broth containing 2 μ Ci (^{3}H) deoxythymidine (specific gravity of 13.5 Ci/m mole). The mixture was shaken at 37°C to accelerate growth. After 2 h, 1 ml aliquot was removed, mixed with 100 μl of trichloroacetic acid (TCA) and kept on ice for determining the initial counts. To the remaining 6 ml broth culture was added diclofenac at 2x MIC of the test strain and the mixture was incubated with shaking at 37°C. At intervals of 30 min, a 1 ml sample was removed, mixed with 100 μl of TCA and was kept on ice. After 5 h, all the deposits were washed twice individually with 5 ml of 10% TCA and filtered through a millipore filtration system. The filter pads were then dried at 70°C and radioactivity was measured in a scientillation counter. A broth culture treated with 2 μ Ci(^{3}H) deoxythymidine, but containing no diclofenac was used as control.

3. Results

3.1. Mode of action of diclofenac on bacteria

The MIC of diclofenac against *S. aureus* NCTC 6571 was 50 mg/l. The initial number of organism when 100 mg/l of Dc was added, was 2.2×10^8 cfu. After 2 h, the bacterial count was 3.9×10^7 cfu; after 4 h, 6.6×10^6 cfu; after 6 h, 1.0×10^5 cfu; and at the end of 18h it was 0 (Fig. 1). A similar bactericidal action was observed using *E. coli* C600 (Fig. 2).

3.2. Determination of radioactive count in the bacterial culture

The breakdown of cellular DNA after incorporation of diclofenac was measured by loss of TCA-precipitable radioactivity. At 30 min intervals, after the addition of diclofenac, the TCA-precipitable radioactivity, was found to exhibit a gradual decline in the counts/min Fig. 3.

No degradation of cellular DNA was observed when *E. coli* C600 cells were not treated with diclofenac.

4. Discussion

Several groups of workers have repeatedly reported on the existence of moderate to powerful anti-microbial property in a variety of non antibiotic compounds,

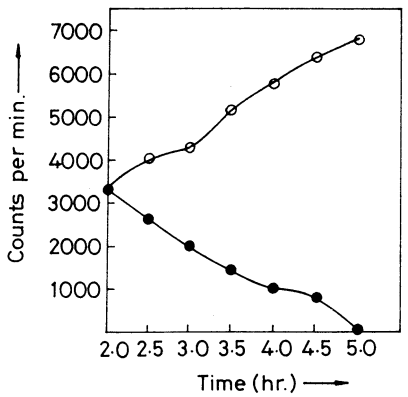

Fig. 3. Effect of diclofenac on DNA synthesis using *E. coli* C600; ○, control; ●, Dc treated cells.

particularly the phenothiazines. However, the structurally and functionally different drug diclofenac has also been proved to possess anti-microbial action [19,20]. The present study showed that this drug is highly bactericidal to both Gram-positive and Gram-negative bacteria. Moreover, the cellular DNA of diclofenac-treated *E. coli* K12 C600, could undergo appreciable degradation, while the DNA of the cells of the same strain were not degraded when they were not treated with diclofenac.

All bacterial cells depend on the synthesis of DNA for their growth, while RNA is required for transcription and provision of information on which the synthesis of proteins and various enzymes depend. Thus, any interference with the synthesis of DNA or RNA can block the growth of a bacterium. The compounds which bind to DNA or RNA in such a manner that their message cannot be read, are also inhibitory. The quinolones and fluoroquinolones inhibit microbial DNA synthesis by blocking DNA gyrase, while the antibiotic rifampicin inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase, thereby inhibiting bacterial RNA synthesis. Trimethoprim acts by inhibiting dihydrofolic acid reductase in bacteria, leading to reduced formation of purines. Although this work clearly indicated that diclofenac can inhibit DNA synthesis, further work is necessary and is in progress to determine whether diclofenac binds directly to the DNA of actively multiplying cells or that it induces DNA degradation.

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