

SULFONAMIDES : A NOVEL APPROACH FOR ANTIMICROBIAL CHEMOTHERAPY**N. P. Shukla**Biotechnology Application Centre,
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ABSTRACT

The discovery of the sulphonamides marked the beginning of the chemotherapeutic era making possible a direct attack on microbial infections. Sulfonamides antibacterials, now in their fifth decade, continue to be used because they are effective, inexpensive and free of the superinfection problem of the broad spectrum antibiotics. They have been quite safe even when widely used in ambulatory patients, an application in which they are often preferred. After 40 years most of the original problems of toxicity have been taken care of and during the last three decades the sulfonamides have withstood critical evaluation along side the antibiotics, which have problem of their own. During this period active compounds with a wide range of choice in all the pertinent pharmacological properties and with lower toxicity have been synthesized and a wide spectrum of activity has been established. Thus, the activity of compounds acting as p-aminobenzoic acid antagonists extends from use against acute and chronic Gram negative Gram positive bacterial infections (Such as meningitis, intestinal tract infection or preoperative treatment of urinary tract infections and respiratory infections) through tuberculosis and leprosy to malaria and coccidiosis with certain structural specificities for each^{1-4,6,9,13,34,45,68,85,89,91}. The mechanically related diaminodiphenylsulfonamide is the well established drug of choice. The antibacterial action of these agents is by way of interference with a bacterial biochemical reaction lacking in man and represents the first magic bullet the sense visualized by Ehrlich.

Keywords : Sulfonamides, chemotherapy and antimicrobial activity.

INTRODUCTION

A major advance in sulfonamides therapy came with the proper appreciation of the role of pharmacokinetic studies in determining the dosage schedule of these drugs. The era of newer long acting sulfonamides started in 1956 with the introduction of sulfamethoxypyridazine having a half life of 37 hours which had to be administered only once a day^{12,16,17,56,86}.

A number of new sulfonamides have been described recently. An isomer of sulphadimethoxine. RO 4-4394 (4-sulphanilamido-5,6-dimethoxypyrimidine) is characterized by an extremely long persistence in the blood and other body fluids^{20,21,46}. Its activity against Gram positive and Gram negative organisms is comparable to that of sulphadiazine and sulphamethoxydiazine. A remarkably constant effect of a single oral dose in infections with both Gram positive and Gram negative bacteria is reported^{23,25,30,31,36,54}. Barbotin and Nguyen Trung-Luong successfully treated meningococcal meningitis with a single intramuscular injection of the drug. RO 4-4393 seems to exert remarkable action in the treatment

of trachoma. Milano¹⁹ obtained a cure rate of 83%. Barclay and his associates⁸ obtained favourable results in leprosy patients within a relatively short period of treatment with RO 4-4393. Similarly, number of investigators reported the therapeutic value of this sulfonamide against various types of leprosy^{24,27,39,41-44}.

The sulfonamides also succeeded in greatly reducing the number of deaths due to epidemic meningitis, thus in the city of Hamburg, mortality dropped from 43% in 1936 to 12% in 1941¹⁵. Similarly, it became possible for the first time to employ casual therapy of bacillary dysentery and numerous other infectious diseases⁸¹.

Since, long back Dapsone (DDS) is the drug of choice for the prophylaxis and treatment of human leprosy^{17,18,50,51,58,59}. Shephard⁷⁵ has shown that acedapsone (DADDS, 4-4diacetylamide diphenyl sulphone), a repository sulphone was effective in preventing growth of *Mycobacterium leprae* in mouse foot pad. Its value as an antileprosy drug has been demonstrated in Philippines^{76,77}. New Guinea^{69,70} and Micronesia^{71,72}. Administration of the drug to leprosy patients in dose of 225 mg (1.5 ml)

deep in at intervals of eleven weeks, produce release of DDS at a steady rate after the second injection^{53,79}. During regular treatment with acadapsons, plasma levels of DDS is maintained well above the MIC of DDS for *M. leprae*³⁵. Recently, a controlled study on chemoprophylaxis against leprosy with acedepsona was carried out by the central leprosy teaching and Research Institute, South India between 1976 and 1980. This study establish the fact that the chemoprophylactic and therapeutic value of acedepsona is more than that of dapsona^{52,60,61,63,64}.

Nomenclature & Classification

The general term "Sulfonamides" have been used for derivatives of p-aminobenzene sulfonamide (sulfanilamide), whereas specific compounds are described as N¹ and N⁴ substituted sulfanilamides depending on whether the substitution is on the amino or aromatic amino group, respectively. Most of the sulfonamides used currently are N¹ derivatives. The generic name of a sulfonamide is built up by adding the prefix "sulfa-" to an abbreviated form of the chemical name of the N¹-residue. This is done in two ways, either the amid nitrogen is taken as a part of the "sulfa" residue (e.g. Sulfapyridine) or the amid nitrogen is taken as a part of the N¹-residue as in sulfaguanidine. The length of time that a sulfonamide remains in the body is normally expressed in terms of its half life i.e. the time required for half of the amount of drug introduced to be eliminated from the organism. There have been numerous attempts to classify the sulfonamides^{15,29,82}. One based on absorption and half life appears to be the most logical and clinically relevant. Sulfonamide that have a half life of less than 10 hr are termed short acting between 10 and 24 hr are considered to be medium acting and longer than 24 hr are long acting.

Mode of action

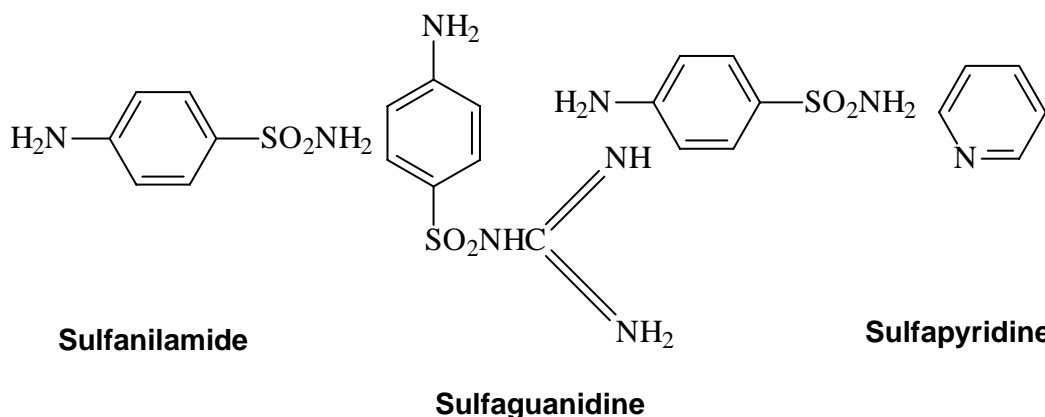
The mode of action of the sulfonamides is characterized by a competitive antagonism of certain vital factors in the metabolism of many bacteria. This antagonism, namely between p-aminobenzoic acid (PABA) and p-aminobenzene sulfonamide, was first mentioned in 1940 by Woods and Fildes. With regard to this antagonistic mode of action, a distinction can be drawn between the two classes of bacteria⁸¹

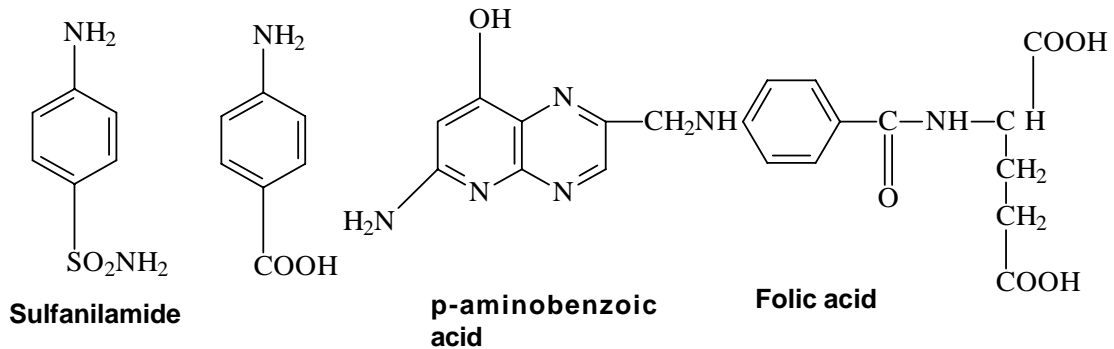
(i) This class accounts for the great majority of bacteria that require PABA as a nutrient or that can themselves synthesize PABA from the nutrient medium. The bacterium is so say, deceived by the structural similarity between the sulfonamide and PABA as a result of the latter is incorporated into the cell of the bacterium but does not play its expected part in the metabolism of the cell. Until 1946, no nutrient substance was known that contained PABA, it was only with the discovery of the vitamin, folic acid by American workers and with the identification of its structure that the first useful information on PABA metabolism became available.

(ii) Bacteria that can neither synthesize PABA nor utilize it but must rely on a supply of folic acid. This group includes for example, enterococci, which like the cells of the human organism, must rely on a supply of vitamins of the folic acid group and are accordingly unaffected by sulfonamides^{19,65-67,95}.

More specifically the action of the sulfonamides is generally understood by the following:

The sulfonamides permeate is non-ionized from into the bacterial cell. Once dissociation equilibrium has been established, the ionized form of the sulfonamide antagonizes the biosynthesis of folic acid which is necessary as a coenzyme for the formation of purines such as adenine and guanine,





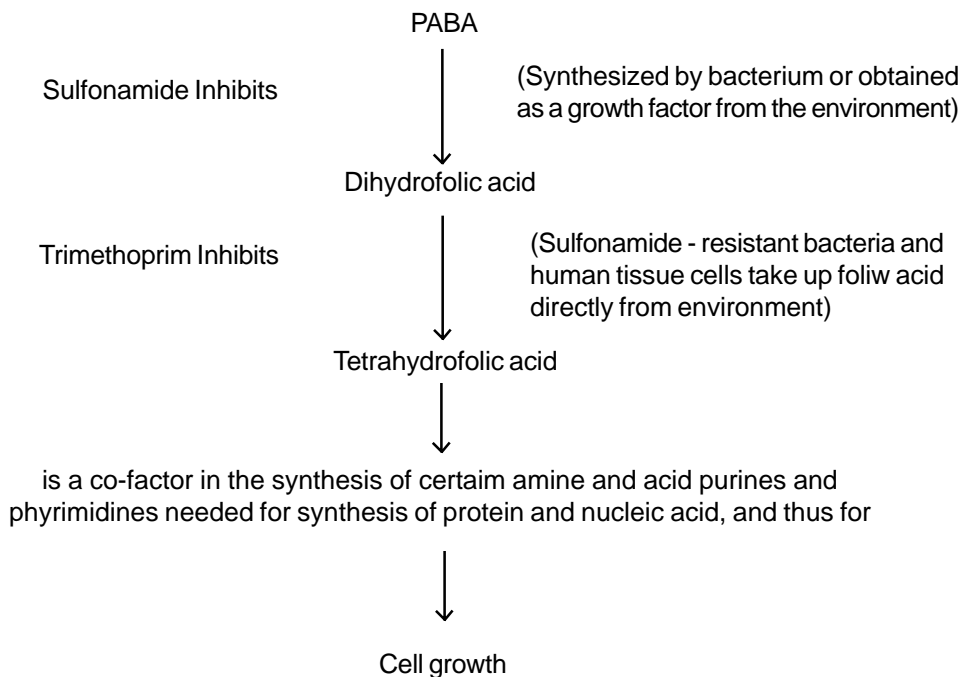
the pyrimidine base thymine, and also the amino acids methionine, serine and histidine. The sulfonamide ions inhibit the enzyme that condenses PABA with dihydropteridine. This inhibition has the practical effect that the dihydrofolic acid synthesis does not take place. Dihydrofolic acid is a preliminary stage in the formation of a coenzyme. Under normal conditions the penultimate stage in the formation of this coenzyme is the reduction of dihydrofolic acid to tetrahydrofolic acid. This reduction is accomplished with the aid of the enzyme dihydrofolic acid reductase^{37,74,89}.

The last stage in the formation of the complete coenzyme consists in the condensation of the latter with one carbon compound to give one of the carbon tetrahydrofolic acids. The function of the holoenzyme consists in a one-carbon transferase action. The enzyme, in other words

cause the incorporation of a single carbon atom in the substrates *i.e.* uracil, serine etc^{26,34}.

The lack of transferase activity inhibits the synthesis of thymine from uracil which is an essential constituent of DNA. This inhibition of DNA synthesis, finally explains the action of the sulfonamides. Since no DNA synthesis takes place in the quiescent stage of the bacteria, the sulfonamides have no effect during this stage even when used in very high doses. The sulfonamides exert their bacteriostatic action during the multiplication stage after a given "log period". This log period is dependent on the quantity of stored PABA^{73,80,81}.

If sulfonamides are compared with other antibacterial substances, e.g. penicillin, an important difference becomes apparent, whereas



the bactericidal effect of the antibiotic depends primarily on high concentrations rather than on the contact time, the opposite is true of the bacteriostatic concentrations for a period of time sufficient for the normal defence mechanism of the organism to become active against the bacteria³⁸.

Synergism and Combination

Examination of mixtures in vitro suggests that sulfonamides and bacteriostatic drugs have an additive effect and that sulfonamides and bactericidal antibiotics have an antagonistic or a supra-additive effect on bacteria. Thus, it can be said that combinations of sulfonamides and certain antibiotics and other anti-infective drugs have a wide antibacterial spectrum and that such mixtures might be more effective than either constituent alone. One well authenticated case of an additive effect is the combined treatment of *H. influenzae* meningitis with a mixture of sulphadizine and chloramphenicol. Polymyxin acts synergically with sulfonamides. A considerable amount of work has been carried out to potentiate the sulfanilamides, bacteriostasis by using them in combination with a compound that blocks a later step in the sequence of folic coenzyme syntheses. Sequential blocking has been achieved with "antibiotics" which have a 2,4-diamino 1,3-diazo structure and a bulky aromatic group at the 5 position. Such compounds are trimethoprim and the earlier pyrimethamine (2,4-diamino-4-ethyl-5-p-chlorophenyl pyrimidine) and 2,4-diamino-5-diamethy,6-p-chlorophenyl-5,6-dihydro-5-triazine.

In vitro tests show that the bacteriostatic drugs, trimethoprim and sulphamethoxazole combination (1:5) acts synergistically to produce a bactericidal effect at most levels achieved in clinical therapy. Both components exert their antimicrobial action by blocking folate metabolism. Trimethoprim inhibits the enzyme dihydrofolate reductase and thereby prevents the reduction of dihydrofolate to tetrahydrofolate. This leads to an inhibition of the synthesis of bacterial DNA. Sulphamethoxazole acts as a typical sulfonamide and inhibits the process whereby bacteria from dihydrofolate from para-amino benzoate and from pterate. Both of these bacterial actions have a high therapeutic index relative to folate metabolism in man. The combination is active against a broad spectrum of infecting organisms which include *Streptococci*, *Staphylococci* (including those resistant to penicillin), *Salmonellae*, *Shigellae*, Coliform bacteria, *Neisseriae* some strains of *Proteus* and of *Brucella*, *Diplococcus pneumoniae*, *H. influenzae* and *Klebsiella pneumoniae*.

Drug Resistance

The development of resistance is considered to arise because of over production of PABA⁸⁸. Altered permeability of the organisms to sulfonamides⁶², or altered sensitivity to H₂-pterate synthetase enzyme from resistant cells (which can bind PABA more tightly and sulfonamide less tightly than the corresponding enzyme from the sensitive cells) are also possible cause of resistance^{96,92,93}. Difference sulfonamides show cross resistance, but there is no cross resistance to other antibacterial. It has been found that sulfonamide resistant strains are also resistant to pyrimethamine, that presumably can utilize the reduced forms of folic acid available in the host erythrocytes^{10,11}.

It was noticed that multiple drug resistance involving Streptomycin, Chloramphenicol, Tetracycline and Sulfonamides could be transferred between *Shigella* and *E. coli* in mixed cultivation. Now this fact is established that the transfer of R-factors is carried out through conjugation by plasmids this transfer is noticed in both conditions i.e. in vitro as well as in the alimentary tract. Drug resistance acquired in this manner can be transferred to other sensitive strains indefinitely. It has been shown that in *E. coli* R-plamid transmitted resistance is the most common mechanism of sulfonamide resistance^{51,83,90,91}.

Toxicity and side effects

Sulfonamides should be administered with caution to pregnant woman near term, and to premature and new born infants since detoxifying mechanisms do not develop fully until later in infancy. The sulfonamides carry the risk of numerous toxic effects, which are particularly severe in the case of the long acting drugs. The problem of crytalluria with the possible consequence of hematuria and oliguria, was common with the older preparations and is still a risk with sulfadiazine and sulfamethoxazole. Precautions should be taken to avoid crytalluria, particularly in dehydrated patients, concomitant alkalinization of the urine is advisable^{22,96}. Sulfonamides should be used with great care or avoided in patients with impaired renal functions. Acute haemolytic anaemia is a rare but serious complication sometimes occur early in treatment. it may occur in subjects with a glucose-6-phosphate dehydrogenase deficiency. the foetus and premature infants normally have low levels of this enzyme. A secondary anaemia accompanied by fatigue and mental depression often follows the administration of sulfonamides over a long period.

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