THE SPECIFIC RESISTANCE OF TUBERCLE BACILLI TO PARA-AMINOSALICYLIC ACID AND SULFONAMIDES

DIRAN YEGIAN AND ROBERT T. LONG

Ray Brook State Tuberculosis Hospital, Ray Brook, New York

Received for publication March 13, 1951

The observation by Youmans *et al.* (1947) that *para*-aminobenzoic acid (PABA) antagonizes the action of *para*-aminosalicylic acid (PAS) has been confirmed repeatedly. Since the role of PABA has come to assume such a central position in explaining the mechanism and site of action of the sulfonamides, the question now arises, Do PAS and the sulfonamides inhibit the same metabolic process?

The development of resistant strains of microorganisms provides a means of investigating this problem. If we assume that the two drugs act at similar sites, then an independently developed strain resistant to sulfonamides should, *a priori*, be resistant to PAS and a PAS-resistant strain should likewise be resistant to the sulfonamides.

Experiment 1. The development of a strain of *Mycobacterium tuberculosis*, H37Rv, highly resistant to PAS was accomplished by serial transfer through liquid sorbitan monooleate medium containing increasing concentrations of the drug. The resistant mutant grew well at a concentration of 500 μg of PAS per ml of medium, whereas the parent strain H37Rv is inhibited by 0.1 μg of PAS per ml.

These two strains were cultured in liquid sorbitan monooleate medium in the presence of different concentrations of sulfonamides and sulfonamidelike drugs. The results were recorded after 14 days of incubation (table 1). No differences were noted with regard to sensitivity to sulfonamides.

Experiment 2. An independently developed strain of *M. tuberculosis*, H37Rv, highly resistant to sulfathiazole (ST) was obtained by serial transfers in sorbitan monooleate medium to which ST was added in increasing concentrations. The resistant strain showed good growth in 200 μg of ST per ml, but the parent strain did not grow in 10 μg of drug per ml. These strains were cultured in concentrations of PAS ranging from 0.1 μg to 10 μg per ml of medium. The results were read after incubation for 14 days. Both strains were inhibited equally by PAS.

DISCUSSION AND SUMMARY

Although it is known that *para*-aminobenzoic acid antagonizes the action of both *para*-aminosalicylic acid and selected sulfonamides, it is our assumption that these drugs act upon different metabolic processes within the bacterial cell since there is no cross resistance between PAS-resistant and sulfonamide-resistant organisms.

Ivanovics (1950) has reported that a PAS-resistant strain of *Mycobacterium*

tuberculosis, in addition to being sensitive to the action of sulfamethylthiazole, showed a greater sensitivity than the parent strain. In our opinion the suggestion of increased sensitivity of the PAS-resistant strain shown in table 1 may be disregarded since a repeat test showed no appreciable difference between the PAS-resistant and the parent H37Rv strains with regard to sulfonamide sensitivity. Moreover, when the ST-resistant and parent strains were tested against PAS, even in very low concentrations, both were found to be equally sensitive.

The Woods-Fildes' theory claims that sulfonamides specifically block the growth stimulation by or the utilization of PABA. On the other hand, Sevag (1946) proposed that sulfonamides inhibit various oxidative-reductive enzyme systems. Sevag *et al.* (1942, 1945) reported that in *in vitro* enzyme systems PABA functions as an antagonist to sulfonamides or an analogous inhibitor. It has been shown that sulfonamides inhibit, among other enzymes, flavoproteins.

TABLE 1

PAS-resistant M. tuberculosis, H37Rv, cultured in the presence of sulfonamides and sulfonamidelike agents

	PAS-RESISTANT H37Rv				PARENT H37Rv			
	μg of drug per ml of medium				μg of drug per ml of medium			
	10	50	100	200	10	50	100	200
Sulfathiazole	0*	0	0	0	1	0	0	0
Sulfanilamide	4	4	4	0	4	4	4	1
Sulfadiazine	1	0	0	0	3	1	0	0
Sulfapyridine	4	0	0	0	4	4	2	1
Diaminodiphenyl sulfone	1	0	0	0	4	3	1	0
Promine	4	4	0	0	4	4	2	2
Para-aminosalicylic acid (control)	4	4	4	4	0	0	0	0

* Growth is reported arbitrarily as 0 to 4 according to turbidity.

PABA also has been shown to inhibit flavoproteins (Altman, 1946; Hellerman *et al.*, 1946). Sevag *et al.* (1950), in a review, reported that sulfonamides inhibit pyruvate dismutation, oxidation, and the synthesis of amino acids, succinate, lactate, bacterial dehydrogenases, cytochrome reductase, cytochrome oxidase, glucose-6-dehydrogenase (flavoprotein), bacterial luciferase, staphylococcal coagulase, yeast sucrose, amylase, and many other mammalian and plant tissue enzymes. These investigators have also reported that sulfonamide inhibitions are antagonized by PABA, cocarboxylase, flavine-adenine dinucleotide, riboflavin, methylene blue, etc.

Since sulfonamides are capable of blocking so many varieties of enzyme systems, it is evident that the antagonistic action of PABA to sulfonamides could not be assumed to be limited to a single specific site. It seems reasonable, therefore, to conclude that PAS and sulfonamides may act on different enzyme systems, the inhibition of which by either drug, as shown, can be antagonized by PABA. These relationships appear to be capable of explaining the ability of

PABA to antagonize both PAS and sulfonamides, and also account for the demonstrated absence of cross resistance between these two drugs.

The assumption based on the antagonism exercised by PABA that if resistance develops to one drug of such a series there will also be resistance to the remaining members of the series cannot be supported by the results obtained with PAS and sulfonamides and the observations discussed above.

REFERENCES

ALTMAN, K. I. 1946 The effect of sulfonamides on respiratory enzymes. J. Biol. Chem., **166**, 149–160.

HELLERMAN, L., LINDSAY, A., AND BOVARNICK, M. R. 1946 Flavoenzyme catalysis. Inhibition of *d*-amino acid oxidase by competition with flavine-adenine-dinucleotide of atabrine (quinacrine), quinine, and certain other compounds. J. Biol. Chem., **163**, 553–570.

IVANOVICS, G. 1950 Über die *p*-Aminosalicylsaure-Festigkeit von Tuberkel-bazillen. Verlag Birkhauser, **6**, 108.

SEVAG, M. G. 1946 Enzyme problems in relation to chemotherapy, "adaptation," mutations, resistance and immunity. Advances in Enzymol., **6**, 33–127.

SEVAG, M. G., GOTS, J. S., AND STEERS, E. 1950 Enzymes in relation to genes, viruses, hormones, vitamins and chemotherapeutic drug action. *In* Sumner, J. B., and Myrback, K., The enzymes, I, part I, 115–186. Academic Press, New York.

SEVAG, M. G., HENRY, J., AND RICHARDSON, R. A. 1945 Studies on the action of sulfonamides on the respiration and growth of bacteria. A. Factors controlling the inhibition by sulfonamides of carboxylases. II. Antagonism between *p*-aminobenzoic acid and sulfathiazole. J. Bact., **49**, 71–77.

SEVAG, M. G., SHELBURNE, MYRTLE, AND MUDD, S. 1942 The action of sulfonamides on the respiration of bacteria and yeast. Inhibition of bacterial and yeast carboxylases by sulfonamide drugs structurally related to cocarboxylase. J. Gen. Physiol., **25**, 805–817.

SEVAG, M. G., SHELBURNE, MYRTLE, AND MUDD, S. 1945 Studies on the action of sulfonamides on the respiration and growth of bacteria. A. Factors controlling the inhibition by sulfonamides of carboxylases. I. Antagonism between cocarboxylase and sulfathiazole. J. Bact., **49**, 65–70.

YOUMANS, G. P., RALEIGH, G. W., AND YOUMANS, A. S. 1947 The tuberculostatic action of *para*-aminosalicylic acid. J. Bact., **54**, 409–416.