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THE TREATMENT OF TUBERCULOSIS IN THE GUINEA-PIG WITH STREPTOMYCIN ALONE OR IN COMBINATION WITH DAPSONE, THIOSEMICARBAZONES, PAS, OR SODIUM IODIDE

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Combinations of antituberculous drugs are usually more effective than individual drugs alone. Thus Smith, Jackson, Junge, and Bhattacharya (1949) showed that some sulphones, given in combination with streptomycin, produced a greater therapeutic effect than streptomycin alone. Moeschlin and Schreiner (1949) showed that the same was true of 4:4′-diaminodiphenylsulphone (dapsone) and *p*-aminosalicylic acid (PAS). Bloch, Vennesland, Ebert, and Gomori (1949) found that a combination of streptomycin and PAS had a much more favourable effect on experimental tuberculosis in the guinea-pig than either drug used alone. Rees and Robson (1951) studied the effect of various treatments on the cornea of mice infected with tubercle bacilli. They found that combination of PAS and *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (EBT) showed no advantage over thiosemicarbazone alone. A combination of streptomycin and the thiosemicarbazone showed a definite additive effect, greater than that produced under the same experimental conditions by a combination of streptomycin and PAS. It was claimed by Woody and Avery (1948) that potassium iodide increased the antituberculous effect when given to guinea-pigs in combination with streptomycin, although Levaditi, Vaisman, and Lévy (1949), who used mice, did not confirm this observation.

In the work to be described the value of all these drugs and of *p*-acetylaminobenzaldehyde thiosemicarbazone (thiacetazone) was compared, when combined with streptomycin, under standard conditions.

METHODS

The method of intranasal instillation which produces pulmonary tuberculosis in monkeys (Francis, Spinks, and Stewart, 1950) was successfully applied to guinea-pigs. They were anaesthetized with ether, and, while lying first on one side and then on the other, had the human (Weybridge C) strain of tubercle bacilli, contained in 0.2 ml. of saline, introduced into the nostril. Previous experiments with a dye had shown that nearly all the colour passed into the lower lung when introduced in this way. In experiment 1 the dose was 10^{-4} mg. of moist bacilli grown on Löwenstein's medium for 16 days. In experiments 2 and 3 the dose of tubercle bacilli was increased to 2×10^{-4}. In every experiment treatment was begun one month after infection. During the first week of treatment in experiments 1 and 3 the guinea-pigs were given drug diet mixtures containing half the concentrations of the drugs shown in Table II, but in experiment 2 the concentrations shown were given from the start. The animals were killed one month after treatment began and examined *post mortem*. The convenient numerical assessment of lesions previously described was used (Francis, Spinks, and Stewart, 1950).

The individual scores were examined by Dr. O. L. Davies, who found that the variation in the scores between animals of the same group was greater for the higher mean scores. In order to equalize this variation it was necessary to use a square root transformation. This expedient was used in order to simplify the interpretation of the scores, although it did not alter the general trend of the results. The effect of the drugs on different organs was first added for each animal and then square roots derived. Left and right lungs, left and right bronchial lymph nodes, and the liver and spleen taken together made three groups which, added together, gave a modified score for each animal. The comparative effects of the drugs on the various organs appeared to be similar, so that the mean figure for each animal was used as a basis of assessment. Means of these scores for the three experiments are given in Table II. The differences required for significance were calculated from the variances of the modified scores within the groups of animals.

RESULTS

The actual scores of animals killed at the time treatment commenced in experiment 1 are shown in

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TABLE I

MAXIMUM POSSIBLE SCORES, AND ACTUAL MEAN SCORES OBSERVED IN CERTAIN GROUPS OF GUINEA-PIGS

Treatment	Experiment 1						
	Group I, Lung		Group II, Bronchial Lymph Nodes		Group III		Sum of Average Scores of Three Groups
	Right	Left	Right	Left	Liver	Spleen	
10 mg. streptomycin per kg. once daily, killed 2 months after infection ..	3·50	3·29	2·90	2·88	0·89	0·71	7·09
Untreated, killed 2 months after infection	4·63	5·00	2·74	2·78	1·63	1·38	9·08
Killed 1 month after infection, when treatment began	2·50	2·50	3·17	3·17	1·00	1·83	7·09
Maximum scores obtainable*	9·00	9·00	6·00	6·00	6·00	6·00	21·00

* The scores for cavitation (Francis, Spinks, and Stewart, 1950) have been omitted, because it was not observed in the present experiments and is rare in guinea-pigs.

Table I, together with the scores of animals treated for one month with streptomycin and the untreated control animals. The maximum scores which could be allocated to each organ are also shown. The results in the three experiments were similar, so Table I shows the range of the severity and the distribution of the lesions. There were six guinea-pigs per group in experiments 1 and 2 and eight in experiment 3. The results of these experiments have been combined in Table II. In experiments 1 and 3, in which a group of animals were killed when treatment began, the disease progressed in the controls but regressed in nearly all the other groups: in some groups the regression was large.

In experiment 1 dapsone, 0.05% in the food, produced the greatest increase in effect, followed closely by EBT and thiacetazone. Increasing the amount of dapsone from 0.05% to 0.3% decreased the effect, but this was restored in the presence of 0.05% sodium iodide.

In experiment 2, 0.05% dapsone again produced the best effect of any single drug given with 10 mg. streptomycin per kg. It was ineffective at a concentration of 0.01%, and in this experiment EBT 0.1% and thiacetazone 0.2% produced the next lowest effects; but thiacetazone combined with 0.05% dapsone produced the best effect, and the mixture seemed less toxic than thiacetazone alone. When given with 40 mg. streptomycin per kg. both thiacetazone and dapsone increased the effect produced by streptomycin alone, and several drugs combined with 10 mg. streptomycin per kg. produced a better effect than 40 mg. streptomycin per kg. given alone. There was a clear indication in experiment 1 that 0.05% dapsone was superior to 0.3%; in experiment 2, 0.05% dapsone again produced a good effect, but 0.01% was ineffective. In experiment 3 there was little difference in the effects produced by 0.025, 0.05, or 0.1% dapsone. In this experiment 40 mg. streptomycin per kg. produced a smaller effect than usual, and all drugs except 0.25% PAS combined with 10 mg. streptomycin produced a better effect than 40 mg. streptomycin per kg. alone. EBT 0.1% produced the best effect, but was the most toxic. In all

TABLE II

MEAN SCORES* OF LESIONS IN TREATED AND UNTREATED GROUPS OF TUBERCULOUS GUINEA-PIGS IN EXPERIMENTS 1, 2, AND 3†

Streptomycin (mg./kg.) Once Daily	Other Treatments: Percentage of Drug in Food	Mean Scores	Change in Weight per Day (g.)
10	EBT, 0·1 and 0·2‡	3·58	−1·72
10	Thiacetazone, 0·1 and 0·2 ..	4·19	+0·55
10	Thiacetazone, 0·1+PAS, 0·25 and 0·5§	3·90	−1·93
10	PAS, 1·0	3·35	−1·76
10	,, 0·5	4·35	−1·06
10	,, 0·25	5·23	−0·59
10	Dapsone, 0·01	4·45	+2·18
10	,, 0·025, 0·05, and 0·1	3·79	−0·12
10	,, 0·3	4·39	−0·22
10	Thiacetazone, 0·1 and 0·2+ dapsone, 0·05	3·69	−1·97
10	Dapsone, 0·05+PAS, 1·0, 0·5, and 0·25	3·99	−1·03
10	Sodium iodide, 0·05	4·98	+2·20
10	,, ,, 0·05+dapsone, 0·3	3·78	−0·21
10	Sodium iodide, 0·05+EBT, 0·2	3·79	+0·94
10	None	5·53	+0·54
40	Thiacetazone, 0·1 and 0·2 ..	3·66	−0·91
40	Dapsone, 0·05	3·47	−0·69
40	PAS, 0·05	3·76	−1·86
40	None	4·57	+0·84
None	Sodium iodide, 0·05	6·66	−0·97
Controls		6·86	−1·32
Animals killed one month after infection, when treatment began		5·52	

* After square root transformation.

† The difference in mean scores required for significance between groups in experiments 1 and 2 was 1.2 and in experiment 3 0.77. In preparing this Table the results of experiment 3 have been given twice the weight of either experiments 1 or 2.

‡ The average result of one group given 0.1% and another given 0.2% of EBT in addition to streptomycin.

§ The average result of one group given thiacetazone 0.1%+PAS 0.25%, and another group given 0.1% thiacetazone+PAS 0.5% in addition to streptomycin. A similar nomenclature is adopted in the rest of the Table.

experiments 10 mg. streptomycin per kg. produced a significant reduction in the extent and number of the tuberculous lesions. This effect was significantly increased by all the drugs used except sodium iodide.

When the results of the three experiments are combined (Table II) it will be seen that, except for 0.25% PAS, 0.01% dapsone, and 0.05% sodium iodide, which were probably ineffective, there was little to choose between the various drugs given in combination with streptomycin. If the scores of the lesions and the changes in weight are considered together, dapsone was the best drug when combined with 10 or 40 mg. streptomycin per kg. and PAS was inferior to EBT or thiacetazone.

Discussion

It will be seen that the maximum tolerated dose of dapsone did not produce a better effect on tuberculosis in the guinea-pig than smaller doses, and the same is true in the treatment of leprosy in man (Lowe, 1951). Sodium iodide increased the body weight of the guinea-pigs, but had no therapeutic effect, a result differing from that reported by Woody and Avery (1948) in guinea-pigs, but in accord with the findings of Levaditi, Vaisman, and Lévy (1949) in mice.

It is of interest to compare the result of treatment in guinea-pigs with that in other species. The fact that streptomycin causes regression of established lesions in guinea-pigs and man is well recognized. The author's unpublished observations show that marked regression occurs in dogs and monkeys, but macroscopic and histological examination of mice with established experimental tuberculosis indicated that although streptomycin, alone or combined with other drugs, retarded the development of lesions it did not cause regression. This is rather surprising, as the mouse is generally considered to be more resistant to tuberculosis than the monkey or guinea-pig. None of the drugs which increased the total therapeutic effect when given to guinea-pigs in combination with streptomycin produced a similar effect in tuberculous dogs.

Martin (personal communication) found that the thiosemicarbazones produced a much greater therapeutic effect on tuberculosis in mice, when given in combination with streptomycin, than did PAS, and dapsone produced little or no additive effect.

In the M.R.C. trial (Report, 1950) carried out in patients with acute progressive bilateral tuberculosis the differences between the groups treated with streptomycin alone and streptomycin plus PAS were not great. Most of them did not satisfy tests for statistical significance. The improvement was, on the whole, somewhat greater in the patients who received PAS as well as streptomycin than in those who received streptomycin alone. The big difference between the groups was, of course, in the development of streptomycin-resistance. Strains with an eightfold increase in resistance to streptomycin were isolated in 33 of 49 streptomycin cases and in only 5 of 48 streptomycin-plus-PAS cases. In two of these 5 cases there was only a single resistant culture, and deterioration in the streptomycin group was related to the emergence of streptomycin-resistance.

Streptomycin-resistance has not been observed in dogs, and it would appear that, if the three-month period of treatment in man and dogs is compared, the results in dogs are very similar to those in man, i.e., in both species the combined treatment with PAS and streptomycin produced little or no greater therapeutic effect than streptomycin alone, although this combination does produce an increased effect in mice and guinea-pigs. It seems possible, therefore, that the thiosemicarbazones may produce less additive effect in man than in mice or guinea-pigs. These drugs have been in clinical use for several years, but there seems to be no clear evidence of their value when given in combination with streptomycin. It has been claimed that the therapeutic effect in man is increased when dapsone is given in addition to streptomycin (Rist and Cottet, 1949) and that dapsone reduces the emergence of streptomycin-resistant strains (Bernard, Nouvion, Coletsos, and Kreis, 1950). On the other hand, no beneficial effect was observed by Edwards, Penman, and Cutbill (1952); moreover, toxic effects were produced, a result which is again similar to that in tuberculous dogs but different from that in guinea-pigs.

Summary

1. Human tubercle bacilli were instilled intranasally into guinea-pigs, which were one month later treated with streptomycin alone or in combination with other drugs. Treatment was continued for one month, when post-mortem examinations were performed. Most treatments caused a significant regression of lesions when these were compared with lesions in guinea-pigs killed at the time treatment began.

2. *p*-Acetylaminobenzaldehyde thiosemicarbazone (thiacetazone), *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (EBT), 4:4′-diaminodiphenylsulphone (dapsone), or *p*-aminosalicylic acid (PAS) given in combination with 10 or 40 mg. streptomycin

per kg. increased the total therapeutic effect. There was little to choose between the value of these drugs at suitable doses, but dapsone produced the best effect. In two experiments the foregoing drugs, given in combination with 10 mg. streptomycin per kg., produced a greater effect than 40 mg. streptomycin per kg. given alone. Sodium iodide did not increase the total therapeutic effect when given in combination with 10 mg. streptomycin per kg.

3. These results are discussed, and it is pointed out that, although the various drugs increased the effect produced by streptomycin in guinea-pigs and mice, *p*-aminosalicylic acid produces little increased effect on pulmonary tuberculosis in man or dogs.

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