INVESTIGATIONS ON THE STREPTOMYCINS[1]

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Studies *in vitro* and *in vivo* with pure preparations of streptomycin, dihydrostreptomycin, mannosidostreptomycin and dihydromannosidostreptomycin have been continued. Unfortunately, up to the present only small amounts of the two latter antibiotics have been available in the pure form and for this reason *in vivo* studies have been carried out only with the two former.

Careful studies of the *in vitro* activity of these four streptomycins on nine species and 16 strains have been reported elsewhere (1). In general a pattern of activity was established which showed approximately equal activity of streptomycin and dihydrostreptomycin in terms of minimal inhibiting concentrations (M.I.C.) of antibiotic. Mannosidostreptomycin and dihydromannosidostreptomycin also showed M.I.C.'s equal to each other but significantly higher than streptomycin and dihydrostreptomycin, *i.e.* the mannosido-compounds were less active. The chief exceptions to this pattern were with *S. typhosa* and *S. schottmülleri* where dihydrostreptomycin was much less active than streptomycin but the mannosido- compounds showed activity similar to that of streptomycin.

Further *in vitro* studies have confirmed this general pattern of activity. In Table I are shown the data for nine species of which two have not been reported previously—*H. influenzae* and *D. granulomatis*. They follow the usual pattern. Of note is the fact that in both cases the dihydro compounds are slightly but significantly more active, and that *D. granulomatis* is by far the most sensitive strain we have tested so far.

In Table II are shown the *in vitro* results with 12 strains of seven species of *Salmonellae* and *Shigellae*. Although the two species of *Shigella* fit in with the pattern discussed above and shown by the strains in Table I, the *Salmonellae* are atypical as already indicated in the previous publication (1). Moreover, there are three distinct groupings with the *Salmonellae*. All strains show a higher resistance to dihydrostreptomycin than to streptomycin, this being least marked with *S. enteritidis* where the activity of the dihydro compound almost equals that of streptomycin while it is only one-third to one-fourth as active as the latter compound for the other species. The three distinct groupings appear when the activity of the mannosidostreptomycins is considered. With *S. enteritidis* the pattern resembles that of *Shigella* and the other microorganisms. With all strains of *S. typhosa* and *S. schottmülleri* the mannosidostreptomycins have an activity in terms of M.I.C. approximately equal to that of streptomycin while with *S. pullorum* and *S. gallinarum* the mannosidostreptomycins are more than twice as active. This activity of the mannosidostreptomycins against the *Salmonellae* is of particular interest in consideration of the mode of action of the different streptomycins. Although in the case of most organisms it might be hypothecated that the mannosidostreptomycins were only active after conversion to streptomycin, and that their lesser activity was due to incomplete conversion by the organisms involved, such cannot be the case with their activity on *S. pullorum* and *S. gallinarum* and the whole hypothesis must thus be put in question.

Unfortunately, at the present time insufficient amounts of the pure mannosidostreptomycins are available for *in vivo* tests. At this time further *in vivo* experiments with streptomycin and dihydrostreptomycin will be described and the results of different dosage schedules with these two compounds will be evaluated.

In Table III are seen the composite results of several tests comparing the activity of streptomycin and dihydrostreptomycin against *Rickettsia microti*—the vole rickettsia of Baker, in the egg. It will be noted that dihydrostreptomycin is twice as active as streptomycin itself. This finding is the reverse of those of Smadel, Jackson and Gauld (2) with other species. They found di-

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TABLE I

In vitro *activities of pure streptomycins against various organisms*

Test organism	Minimal inhibiting concentrations*			
	Streptomycin	Dihydro-streptomycin	Mannosido-streptomycin	Dihydromannosido-streptomycin
	μg/ml	μg/ml	μg/ml	μg/ml
Klebsiella pneumoniae (ATCC 9997)	1.76	1.76	6.39	6.59
Aerobacter aerogenes (ATCC 129)	2.71	3.27	10.8	11.1
Escherichia coli (D-56)	6.05	6.79	24.8	23.8
Hemophilus influenzae, Type B (D-68)	2.30	1.53	8.53	5.53
Donovania granulomatis (B-44A)	0.167	0.151	0.595	0.446
Staphylococcus aureus (209-P)	0.828	1.39	5.64	7.77
Streptococcus pyogenes (C-203)	11.7	15.9	82.9	87.9
Brucella abortus (Huddleson 1119 avirulent)	0.816	0.738	2.93	2.53
Mycobacterium tuberculosis:				
H-37Rv	2.0	2.2	5.5	6.5
Ravenel	0.58	0.62	2.5	2.2
BCG	0.52	0.55	1.9	1.7
N.†	0.54	0.56	2.5	2.1
T.†	0.55	0.54	2.2	2.0
P.†	0.62	0.85	2.3	2.2
O'D.†	0.63	0.75	2.3	2.6
K.†	1.0	1.7	3.9	3.9

* All figures given in terms of weight of the trihydrochlorides.
† Strains of *M. tuberculosis* freshly isolated from human cases.

hydrostreptomycin less than half as active as streptomycin in the egg against *R. rickettsii* and *R. akari.*

In seeking to obtain definitive data on the usefulness of different dose schedules both acute and chronic infections have been employed. In Table IV are shown the results with one of the acute infections used, namely, that with *S. schottmülleri.* It will be noted that in all cases a single daily dose schedule is better (*i.e.* the CD_{50} is less) than is a three dose schedule. This is more marked where a small bacterial inoculum is used but even with larger inocula the results are significant. In this table one should draw attention to a phenomenon mentioned before (1), namely, that dihydrostreptomycin is more active *in vivo* (better than half as active as streptomycin) than might be expected from its *in vitro* activity (see Table II).

With more chronic infections there is no actual advantage to the single dose schedule. Instead, one, two, or three dose schedules all give the same response. This is shown in Tables V and

TABLE II

In vitro *activities of pure streptomycins against various organisms*

Test organism	Minimal inhibiting concentrations*			
	Streptomycin	Dihydro-streptomycin	Mannosido-streptomycin	Dihydromannosido-streptomycin
	μg/ml	μg/ml	μg/ml	μg/ml
Salmonella typhosa (D-15)	12.2	51.0	12.4	12.9
Salmonella typhosa (D-14)	9.73	34.3	9.14	9.16
Salmonella typhosa (D-59 rough)	6.76	23.3	8.06	7.09
Salmonella schottmülleri (D-51)	10.1	36.5	14.3	14.4
Salmonella enteritidis (D-61)	4.14	5.50	12.7	13.6
Salmonella enteritidis (D-125)	3.26	5.76	12.1	14.0
Salmonella enteritidis (D-124)	1.71	2.25	7.09	6.64
Salmonella enteritidis (D-126)	4.21	5.72	15.6	16.5
Salmonella pullorum (D-123)	26.5	90.1	12.5	14.7
Salmonella gallinarum (D-122)	29.1	116	13.3	13.8
Shigella sonnei (D-116)	7.42	8.52	30.6	30.3
Shigella dysenteriae (D-105)	6.26	5.82	27.2	27.1

* All figures given in terms of weight of the trihydrochlorides.

TABLE III

Comparison of pure streptomycin and dihydrostreptomycin in vole rickettsial infections in embryonated chick eggs

	No. of tests	Total no. of eggs	Dead/ Total	% Dead
Controls 10^{-2}*	5	34	30/34	88.2
Controls 10^{-3}	5	40	25/40	62.5
Controls 10^{-4}	5	40	22/40	55.
Streptomycin				
0.001 mg/egg†	1	6	5/6	83.3
0.004 mg/egg†	5	34	24/34	70.6
0.016 mg/egg†	5	34	18/34	53.0
0.064 mg/egg†	5	34	11/34	32.4
0.25 mg/egg†	5	33	4/33	12.2
1.00 mg/egg†	3	22	0/22	0
CD_{50} = 0.02 mg/egg				
Dihydrostreptomycin				
0.001 mg/egg†	1	6	5/6	83.3
0.004 mg/egg†	5	34	21/34	61.7
0.016 mg/egg†	5	34	15/34	44.2
0.064 mg/egg†	5	34	5/34	14.7
0.25 mg/egg†	5	34	4/34	11.8
1.00 mg/egg†	3	22	1/22	4.5
CD_{50} = 0.01 mg/egg				

* 10^{-2} refers to dilution of yolk sac suspension used as inoculum. All treated eggs were inoculated with the 10^{-2} dilution.

† Dosage given in terms of weight of pure trihydrochloride.

VI where a smaller and larger total daily dosage are compared in a standardized tuberculous infection in mice. It will be seen that when the average survival time in days is compared there is no significant difference in one type of dosage schedule over another whether the total daily dose is 10,000 or 50,000 units per kg or whether treatment is maintained for seven, 14 or 21 days.

In general, it can be said then that no benefit can be expected in setting up any standardized test for the streptomycins with a dosage schedule using greater frequency than one dose a day. One would presume that the same would hold for the mannosidostreptomycins when they become available.

TABLE IV

Effect of dosage schedule on streptomycin and dihydrostreptomycin action in Salmonella schottmülleri *infection in mice*

Antibiotic preparation	No. of tests	Average inoculum* bacterial cells/mouse	Therapy schedule	CD_{50} mg/kg
Streptomycin (650 u/mg)	5	60	Single dose	4.2
Streptomycin (650 u/mg)	5	60	3 doses in 1 day	8.6
Streptomycin (850 u/mg)	3	1100	Single dose	24.0
Streptomycin (850 u/mg)	3	1100	3 doses in 1 day	38.5
Dihydrostreptomycin (850 u/mg)	3	1100	Single dose	40.0
Dihydrostreptomycin (850 u/mg)	3	1100	3 doses in 1 day	69.0

* One to two bacterial cells per mouse were lethal to all control mice.

TABLE V

Effect of schedule of treatment with streptomycin in mice infected with* Mycobacterium tuberculosis (*Ravenel*)

No. of mice	Total daily dose† Units/kg/ Day	Schedule	Duration of treatment (days)	Average survival time (days)	% Surviving longer than 34 days
8	None (Controls)	—	—	19.8	0
8	10,000	3333 t.i.d.	7	21.9	0
8	10,000	5000 b.i.d.	7	23.5	0
8	10,000	Single dose	7	22.8	0
8	10,000	3333 t.i.d.	14	26.0	0
8	10,000	5000 b.i.d.	14	25.6	0
8	10,000	Single dose	14	25.9	0
8	10,000	3333 t.i.d.	21	27.6	12.5
8	10,000	5000 b.i.d.	21	28.5	12.5
8	10,000	Single dose	21	27.5	0

* Partially purified streptomycin, 400 units/mg.
† Treatment given via the subcutaneous route.

TABLE VI

Effect of schedule of treatment with streptomycin in mice infected with* Mycobacterium tuberculosis (*Ravenel*)

No. of mice	Total daily dose† Units/kg/ Day	Schedule	Duration of treatment (days)	Average survival time (days)	% Surviving longer than 34 days
8	None (Controls)	—	—	19.8	0
7	50,000	16,667 t.i.d.	7	31.7	14.3
7	50,000	25,000 b.i.d.	7	28.7	14.3
8	50,000	Single dose	7	30.1	28.6
8	50,000	16,667 t.i.d.	14	37.4	87.5
8	50,000	25,000 b.i.d.	14	37.9	87.5
8	50,000	Single dose	14	40.5	75.0
8	50,000	16,667 t.i.d.	21	52.0	100
8	50,000	25,000 b.i.d.	21	59.0	100
8	50,000	Single dose	21	59.4‡	100

* Partially purified streptomycin, 400 units/mg.
† Treatment given via the subcutaneous route.
‡ One mouse survived through 118 days, at which time it was sacrificed.

SUMMARY

In vitro studies continue to reveal unique behavior on the part of the various strains of *Salmonellae* in their response to the four streptomycins. Where with most other species the activity of dihydrostreptomycin equals that of streptomycin, in this genus all strains tested are less sensitive to the former than to the latter, all comparisons being made on a weight basis. On the other hand, for most genera studied the mannosidostreptomycins are less active than is streptomycin. In contrast to this for the *Salmonellae,* the mannosido compounds are often as active as streptomycin, and for two species are even more active than the latter. The characteristics of dihydrostreptomycin mentioned above do not hold for dihydromannosidostreptomycin which approximately equals mannosidostreptomycin in activity for every species tested thus far *in vitro*.

In vivo studies in acute as well as in chronic experimental infections show that streptomycin or dihydrostreptomycin given in a single dose daily gives results as good as, or better than, those obtained by giving two to three divided doses daily. Against *S. schottmülleri* dihydrostreptomycin is more active than might be expected from the *in vitro* findings. In embryonated eggs infected with *Rickettsia microti* dihydrostreptomycin is somewhat more active than streptomycin.

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