Therapeutic Interference and Potentiation between Subtilin and Gold Sodium Thiomalate in Spirochetal and Streptococcal Infections.* (21049)

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The interference phenomenon in protozoan infections was widely studied by the school of Ehrlich and Morgenroth to explore the mechanism of chemotherapeutic action. Morgenroth and Rosenthal discovered that the trypanocidal activity of antimonyltartrate is inhibited by hexatantalate, a non-trypanocidal agent, which still induces drug resistance to antimonials(1). Browning—a pupil of Ehrlich—and Gulbransen, reported in 1922 the first case of interference between 2 effective drugs, by inhibiting the activity of acriflavin with pararosaniline, on a trypanosome strain resistant to the latter drug(2). Schnitzer(3) established the phenomenon in normal trypanosome strains and with his coworkers extended these findings to arsenicals and antimonials(4,5) and just recently reported(6) the original Browning-Gulbransen interference in the experimental *Trichomonas vaginalis* infection of mice. In bacterial infections the interference phenomenon between therapeutically effective drugs appeared only after the discovery of the antibiotics. We may refer to the studies of Price, Randall, Welch, and Chandler(7) and of Jawetz, Gunnison, Speck, and Coleman(8). Characteristic of the difficulties of interpretation of the experimental findings is the statement of Bliss, Warth, and Long(9): "Aureomycin and chloromycetin are usually reported as antagonistic toward penicillin (6 references quoted), but several authors have pointed out that the effect varies with the concentration of the agents (3 references quoted) and Spicer got different results with different bacteria."

The present study represents an example of antagonism between 2 chemotherapeutic agents which extends to both their anti-

TABLE I. Interference between Subtilin and Myochrysin in the β *hemolytic Streptococcus* (C-203) Infection of Mice. Subtilin was injected subcutaneously on the abdomen, myochrysin immediately after on the back, at the time of inoculation with 100 MLD = 0.5 cc 10^{-7} culture broth.

Exp.	Subtilin + myochrysin (mg/kg)		Survived total
I	—	—	0/10
	.5–1.0	—	8/20
	2	—	6/10
	2	5	7/10
	2	10	10/10
	2	20	4/10
	2	40–80	20/20
II	—	—	0/10
	4	—	6/10
	4	5	10/10
	4	10	6/10
	4	20	6/10
	4	40	5/10
	4	80	10/10
	—	20	0/10
	—	40	0/10
	—	80	3/10
	—	100	10/10

spirochetal and antibacterial activity.

Materials and methods. Mice were infected with the African strain of *Borrelia duttoni* and with *B. obermeieri.* For the *Streptococcus hem.* infection the C-203 strain was used; the intraperitoneal inoculum consisted of ca. 100 min. lethal doses. Drug treatment was given by the subcutaneous route and the min. effective doses were accurately established. In the case of combined treatment, when not otherwise specified, the 2 drugs, subtilin and gold sodium thiomalate (myochrysin), were injected at the same time, always at different subcutaneous sites.

Results. 1. *Streptococcus hemolyticus infection.* The average therapeutic dose of subtilin is 1.8 mg/kg, of myochrysine, 50 mg/kg. Two characteristic interference experiments are presented in Table I. There is an indication that low, sub-therapeutic myochrysin (5-10 mg/kg) reinforce the chemotherap action of subtilin, but a

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TABLE II. Interference between Subtilin and Myochrysin in the β *hemolytic Streptococcus* Infection of Mice.

Subtilin + myochrysin (mg/kg)		No. mice	Survivors, %
1.25–1.5	—	50	44
1.25–1.5	12.5–25	70	20
2.5	—	70	84
"	6.25–12.5	80	65
"	25	70	47
"	50	20	90
"	100	20	95
—	25	40	12.5
—	50	10	60
—	100	40	92.5

higher myochrysin dose (20-40 mg/kg) establishes interference. A further dose increase of the gold compound dose causes a synergistic action. Table II summarizes all other results and shows on a larger series of animals that the optimal interfering dose of myochrysin against 2.5 mg/kg subtilin is about 25 mg/kg, *i.e.*, 1/3-1/2 of the therapeutic dose. With a lower subtilin dose, 1.25-1.5 mg/kg, the interference phenomenon was very definite at the 12.5 mg/kg myochrysin level. Thus, it would seem that within certain limits, the higher the dose used, the higher is the dose of the other drug of the pair required for interference.

2. *Borrelia duttoni infection.* Darkfield parasite counts were made 3, 5-6, 8, 22, and 40-44 hours after drug treatment. The dose of subtilin resulting in a temporary reduction of the spirochetes is 8-10 mg/kg, the average clearing dose, 16 mg/kg. The dose of myochrysine which clears the majority of the mice within 24 hours is 12.5 mg/kg. In one group of experiments 8 mg/kg subtilin alone induced a temporary (3-8 hours) parasite decrease of different magnitude in 18 of 31 (= 58%) mice; 22 hours after treatment, the spirochetes returned to the control level in 27/31 mice, and were only slightly reduced in the remaining 4. In the parallel group treated with 12.5 mg/kg myochrysine alone there was a significant early (5-8 hours) decrease of parasites in 8/21 mice; 22 hours after treatment, 20/21 were completely cleared and the remaining mouse showed an 80% reduction in the parasite count. The combined treatment with the above doses resulted in an initial parasite decrease in 17/21 mice (= 81%); 22 hours after treatment, 13/21 were completely cleared, 4 were reduced and 3 were at the control level. Thus, the delayed effect of the combined treatment seems lower than that of myochrysin alone.

Experiments involving 89 mice treated with doses of 4-8 mg/kg subtilin and 5-6 mg/kg myochrysin alone and in combination gave the impression that the combined treatment might perhaps have increased somewhat the early subtilin effect and possibly accelerated the clearing action of myochrysin without influencing the number of animals cleared. (The dose of myochrysin used cleared ca. half of the mice within only 48 hours.)

The difficulty in comparing the variables of the parasitemia curves in these experiments, *i.e.*, incidence, degree, time of appearance and duration of the reduction, did not lead to a definite conclusion on the effects of the combined treatment, but to the clue that under certain conditions interference might be possible. In fact, by varying time intervals between the administration of the 2 drugs and dosages, we finally obtained quite clear cut cases of reciprocal interference, as for instance those presented in Tables III and IV. The early action of subtilin and the delayed effect of myochrysin could be decreased by combined treatment. It would appear that in order to obtain the interference phenomenon it is necessary to keep an interval of at least one hour between drug administration, and one of the drugs has to be maintained below its therapeutic level.

On the other hand, by increasing the doses of both drugs to therapeutic levels or above, a synergistic action seems to prevail. For instance, combined treatment with 16 mg/kg subtilin and 12.5 mg/kg myochrysin doses give an effect which can be mainly visualized as the superposition of the early subtilin and the delayed (22 hours) myochrysin actions. However, the immediate action of subtilin can also be increased using for combination higher, 25-50 mg/kg, myochrysin doses as it would appear from the following results. Subtilin doses of 8 mg/kg alone—adding up our experiments—gave complete clearing

TABLE III. Interference between Subtilin and Myochrysin in the *Borrelia duttoni* Infection of Mice.

Exp. No.	1st treatment, mg/kg	Interval, hr	2nd treatment, mg/kg	No. spirochetes/25 fields ..... hr after treatment				
				Before	3	5–6	22	40–44
165F	Myo 6		—	25	110	130	60	3
	"		—	30	75	110	80	1
	"		—	25	100	1000	250	400
	"		—	25	100	125	20	0
	Myo 6	3	Subt 8	50	75*	25	2	35
	"	"	"	25	170*	20	50	0
	"	"	"	50	100*	7	60	0
	"	"	"	25	150*	0	0	0
	"	"	"	25	125*	25	0	175
	—		Subt 8	30	200*	0	0	10
	—		"	75	175*	0	50	25
	—		"	50	200*	0	10	125
	—		"	50	175*	0	0	450
	—		"	30	175*	0	0	80
167F	Control†	—	—	75	150	400	1000	1750
	Myo 5‡		—	75	125	150	75	50
	Myo 5	1	Subt 4	75	150	200	22	15
	"	"	"	50	175	200	20	18
	"	"	"	50	100	175	25	5
	"	"	"	100	75	100	150	10
	—		Subt 4	100	125	2	50	75
	—		"	75	0	4	60	80
	—		"	75	0	5	180	200
	—		"	100	125	160	250	275

* Time of 2nd treatment. † Avg of 3 mice. ‡ Avg of 4 mice.

within 3 and 5 hours in 19% of 78 mice. Myochrysin doses of 25-50 mg/kg alone did not clear any of 6 mice during this period, but only after 20 hours, while the combination treatment cleared all 6 mice within 5 hours.

3. *Borrelia obermeieri infection.* This infection is more sensitive to subtilin and less to myochrysin than the duttoni strain. The reducing dose is 1.5 mg/kg subtilin and 25 mg/kg myochrysin (in 24 hours), the clearing doses, 2.5 and 37.5 mg/kg, respectively(10).

Table V summarizes the results of the combined treatment. Both possibilities, interference and potentiation, were encountered in this infection too. It would also appear that the interfering combinations are formed around the range of effective dosages, which once surpassed, lead to synergism. It should be noted that the effects of the combination treatment may vary according to the observation period considered.

Discussion. From a quantitative viewpoint, the interference between myochrysin and subtilin in the streptococcal infection is in line with previous observations on this phenomenon. There is an optimal interfering dose which is overcome by an excess of the drugs(5,11).

In retrospect, we understand the reasons which made difficult the establishment of the interference in the spirochetal infection: (a) First, the effect may vary at different time intervals and therefore a careful comparison of the whole curve of parasitemia is required. (b) Combined treatment with sub-therapeutic dosages remains mostly ineffective. (c) Therapeutic dosages of *both* drugs generally reinforce each other's action, thus at this level the interference no longer appears. (d) The interference is most marked when a sub-therapeutic dose of one drug is followed by a therapeutic dose of the other. (e) The subtilin effect is somewhat variable and therefore the same dose may or may not provoke interference according to whether it was subeffective or effective. Consequently, the interference phenomenon between the 2 drugs studied is contained within very narrow margins.

TABLE IV. Interference between Subtilin and Myochrysin in *Borrelia duttoni* Infection of Mice.

Exp. No.	1st treatment, mg/kg	Interval, hr	2nd treatment, mg/kg	No. spirochetes per 25 fields ..... after treatment							
				Before	3 hr	5 hr	8 hr	1 day	2 days	3-4 days	5-8 days
102F	Myo 12		—	25	75	50	15	0	0	0	25
	"		—	40	80	50	19	0	0	0	30
	"		—	25	75	50	10	0	0	0	3
	—		Subt 10	25	150*	1	0	4	40	0	5
	—		"	40	125*	2	5	10	150	2	3
	—		"	25	125*	10	19	8	200	0	1
	Myo 12	3	"	25	80*	25	10	0	0	0	2
	"	"	"	25	50*	50	3	0	0	0	1
	"	"	"	25	75*	50	19	0	0	0	3
	"	"	Subt 16	25	75*	4	0	0	0	0	3
	"	"	"	30	100*	1	0	0	0	0	2
	"	"	"	25	100*	12	7	0	0	0	3
	—		"	25	125*	0	0	2	15	5	25
	—		"	25	110*	0	0	0	0	10	25
	—		"	25	125*	1	0	1	10	6	4
112F	Subt 4	3	Myo 12.5	20	60*	40	3	3	75	350	
	"	"	"	30	75*	30	125	7	110	250	
	"	"	"	30	80*	60	150	50	80	350	
	"	"	"	35	110*	60	200	75	110	180	
	—		"	25	170*	100	75	0	0	0	
	—		"	35	175*	80	150	0	0	0	
	—		"	40	150*	90	250	0	0	0	
	—		"	25	180*	75	200	0	0	0	
118F	Subt 1	3	Myo 12.5	25	80*	75		2	0	1	
	"	"	"	25	100*	50		3	25	7	
	"	"	"	25	75*	60		0	0	2	
	Subt 4	"	"	18	25*	20			Died		
	"	"	"	25	25*	25		1	0	0	
	"	"	"	25	25*	25		1	0	0	
	—		"	25	75*	75		0	0	0	
	—		"	25	100*	75		0	0	0	
	—		"	25	80*	100		0	0	0	

* Time of treatment (second).

On the basis of the foregoing it can be explained also why the *absolute* doses required to obtain the phenomenon depend on the sensitivity of the spirochetal strain to the interfering drugs.

The lack of additive action of sub-therapeutic doses of myochrysin and subtilin may be interpreted in various ways: it could be the result of an interference in the "undetectable" range, but it could also indicate that the therapeutic action depends on independent cell receptors each of which has to be attacked up to a certain threshold effect before the 2 lesions can integrate each other to influence the vitality and the reproductive rate of the spirochetes. We are inclined to believe that in both infections myochrysin and subtilin act by mechanisms which are not identical. The BAL reversibility of myochrysin in both infections, in contrast to that of subtilin(12), gives a certain support to our hypothesis. It has been said(13) that the antibacterial action of subtilin is due to its surface activity, "damaging the cell membrane, rather than by blocking a particular metabolic step," while for the Au compound its thiol binding capacity is generally accepted as the mode of action.

In a previous case of interference between antibiotics and arsenoxide we have analyzed the arguments in favor of the interpretation that the interference involves other systems than the final receptor sensitive to the biochemical lesion produced by the drugs(11). The same considerations should be valid in the present case. It may be noteworthy from this standpoint that among the antispirochetal agents assumed to act by similar

TABLE V. Effects of Combined Subtilin and Myochrysin Treatment in the *Borrelia obermeieri* Infection of Mice. 8 mice in each series.

Exp. No.	mg/kg		Hr after treatment			
	Subtilin	Myochrysin	2-3	5-5½	22-23	29
9	1.0	—	++	++	+	±
	—	15.0	0	0	0	±
	—	20.0	0	0	±	+
	1.0	15.0	Ind	Ind	Ind	Syn
	1.0	20.0	Intf	Sl. intf	Ind	Sl. syn
	1.5	—	+++	+++	+++	++
	1.5	15.0	Ind	Ind	Syn	Syn
	1.5	20.0	Sl. intf	Ind	Syn	Syn
7	1.5	—	+++	+++	++	++
	—	12.5	0	±	0	0
	1.5	12.5	Ind	Ind	Sl. syn	Sl. syn
	3.0	—	+++	+++	++	++
	3.0	12.5	Ind	Syn	Syn	Syn

Effect of single treatment: 0 = ineffective, ± = slight effect, + = moderate effect, ++ and +++ = marked effects.
Effect of combination: Ind = indifference, Syn = synergism, Intf = interference.

mechanisms on the basis of their *dithiol reversibility* only one (chloromycetin) interferes with arsenoxide, while the others (penicillin, bacitracin) remain indifferent(14). Further, other *non dithiol reversible* antibiotics, such as aureomycin and terramycin, interfere also with arsenoxide(12).

The necessity for a certain interval between treatments to determine interference depends most likely on the period required for a sufficient uptake of the interfering drug to block the absorption-fixation and/or the passage of the second drug to the final receptor.

In a broad sense, this differentiation between systems responsible for interference and for the chemotherapeutic lesion is parallel to the "primäre" and "sekundäre giftbindende Kerne" in the frame of the chemoceptor theory of Ehrlich(15).

Summary. 1. Myochrysin interfered with the therapeutic activity of subtilin in the experimental β hemolytic streptococcus infection of mice. 2. The myochrysin dose required for interference increased with the amount of subtilin used (it varied between 12.5-40 mg/kg). 3. In the *Borrelia duttoni* infection of mice, subtherapeutic doses of one drug interfered with the therapeutic action of the other, while therapeutic doses of both drugs acted synergistically. 4. Analogously, in the *Borrelia obermeieri* infection, both interference and synergism were noted. 5. The effect of the combined treatment in the spirochetal infection may vary at different time intervals following treatment.

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