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Chemotherapeutic Activity of the Combination of Sulfachloropyridazine and Trimethoprim[1] against Experimental Colibacillosis of Chickens and Piglets and Demonstration of the Trimethoprim-induced Potentiation of Sulfachloropyridazine in Vitro and in Vivo

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1. Introduction

Sulfachloropyridazine (SCP)[3] is a sulfonamide in use for more than 20 years in human and veterinary chemotherapy [1–4]. It exhibits activity against a large bacterial spectrum, especially gram-negative strains. Its outstanding in vitro efficacy against pathogenic strains of E.coli derived from different animal sources has particularly been emphasized [5–13].

In these papers only a low frequency of resistance to SCP amongst bacterial isolates has been reported. These results led to controversies in the literature about the utility of SCP-therapy, since according to common belief a high percentage of resistance to sulfonamides is expected to occur in E.coli strains [10, 13–15].

Especially interesting is the report of Rhoades [13] who showed that 79% of 2658 isolates of E.coli were sensitive to SCP whereas only 28% were sensitive to Triple Sulfa.

Sieiro and *Meier* [16] reported on the activity of SCP against experimentally induced E.coli infections in broiler chickens. They used invasive E.coli aerosacculitis leading to generalized lesions such as pericarditis and perihepatitis and to septicaemia. This severe infection was entirely controlled by oral administration of SCP at dosage rates of 1.3 g/l drinking water (or 3.25 g/kg feed) during 3 days.

Excellent therapeutic success with SCP under practical conditions has been published covering a variety of infectious diseases of several animal species [5, 17–28].

The main range of application is the treatment of infections with predominant E.coli-aetiology (diarrhoea of piglets and calves, MMA-syndrome of sows, chronic respiratory disease of chickens). In these investigations SCP has been administered (p.o., i.m. and s.c.) in daily dosage rates extending from 30 mg/kg [18] to 400 mg/kg [23] with a median dosage range of 80–160 mg/kg.

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[1] A soluble Powder containing 10% Sulfachloropyridazine plus 2% Trimethoprim is available under CIBA-GEIGY trade name COSUMIX® Plus ad us.vet.

[2] Requests for reprints should be addressed to H. Heim

[3] Sulfachloropyridazine has mostly been used as sodium-salt.

Accordingly, the dosage recommendations retained for SCP were 50–150 mg/kg b.w. per day for injectable preparations[4] and 130 mg/kg b.w. per day by oral application[5].

More recently excellent therapeutic results of the use of SCP in combination with the sulfonamide-potentiator Trimethoprim (TMP) under field conditions have repeatedly been reported. The dosage rates used in these field trials are compiled subsequently and comprise the total of SCP + TMP in the ratio 5 + 1.

Weaned piglets with E.coli-enteritis were treated by daily doses of 20–40 mg/kg b.w. for 2–3 days with good results [29, 30].

In the therapy of E.coli-enterotoxaemia (edema disease) of weaned piglets medicated feed containing 400 ppm of active ingredients was administered for 2–3 weeks [31]. Good prophylactic and therapeutic efficacy against the same disease was achieved by using medicated feed (24–30 mg/kg b.w. daily) for 5 days [32].

Satisfactory results were obtained against enzootic pneumonia of pigs and MMA-syndrome of sows at daily doses of 30 mg/kg b.w. [33].

Colibacillosis in calves was controlled by supplying 30 mg/kg b.w. per day with the milk for 2–3 days [30, 34].

A broad range of poultry diseases was treated with good success by giving 37.5–56 mg/kg b.w. daily with the feed (septicaemia due to E.coli and S.aureus, chronic respiratory disease, sinusitis, coryza, fowl cholera, ulcerative necrotic enteritis, salmonellosis) [35–37].

Our own investigations were aimed at:

a) elucidating in vitro the extent of TMP-induced potentiation of the antibacterial efficacy of SCP.

b) manifesting the TMP-potentiation of SCP in vivo by means of the E.coli aerosacculitis model as described by *Sieiro* and *Meier* [16].

c) demonstrating the efficiency of the SCP-TMP (5 + 1) combination in the same infection model with a representative spectrum of field strains.

d) evidencing the efficacy of the SCP-TMP combination in an E.coli diarrhoea model of early weaned piglets.

2. Materials and Methods

2.1. Test Compounds

The sulfonamides and Trimethoprim were obtained from commercial sources. The sulfonamides were in the form of their sodium salts.

The SCP + TMP combination used in the chicken and piglet trials was a water soluble powder containing 10% SCP (as sodium salt) and 2% TMP[6].

The chicken trials with SCP alone were conducted using a water soluble formulation containing 65% SCP[7] (as sodium salt).

2.2. Determinations of Minimal Inhibitory Concentrations (MIC)

The sulfonamides and TMP were incorporated into DST-agar (Oxoid) containing 5% lysed horse blood [38, 39] either singly or in a variety of combination ratios (checkerboard pattern, 40,

[4] VETISULID®
[5] COSUMIX®
[6] COSUMIX® Plus
[7] COSUMIX®

41). Threefold dilution steps were chosen. 20 ml of the agar were poured into plastic Petri-dishes (9 cm diameter).

The bacterial strains were grown overnight in Eugon-broth (BBL). 1000-fold dilutions thereof were inoculated onto the plates by means of a Steers inocula-replicating apparatus [42].

The plates were incubated at 37 °C during 20 hours.

2.3. *Disc Sensitivity Testing*

Discs containing 23.75 mcg SCP and 1.25 mcg TMP (manufactured by Dr. Wild AG, Basle) were compared to commercial discs (BBL) containing corresponding amounts of Sulfamethoxazole and TMP.

Discs containing 300 mcg SCP (own manufacture) were also used.

Disc sensitivity testing was carried out using the Kirby-Bauer method described in the BBL-SENSI-DISC® brochure and discussed by Brown and Blowers [43].

2.4. *Experimental E. coli aerosacculitis in Broiler Chickens*

Three week old hybro-broiler chickens were randomly distributed to groups of 10 animals housed in battery cages. A commercial chicken feed without antibacterials and coccidiostats was used throughout the test period. The birds had free access to feed and water. The animals were infected with 0.25 ml of diluted overnight broth cultures of different pathogenic E. coli strains (Table 3). The number of viable germs injected into the airsacs was adjusted to the virulence of the strains.

The birds were medicated immediately after the infection either singly by direct application into the crop or continuously during 5 days via the drinking water. A water soluble formulation of SCP + TMP (5 + 1) containing 12% of active ingredients was used. SCP alone was applied as a 65% water soluble formulation.

Mortality was recorded twice daily. 8 days after infection the surviving animals were sacrified for inspection of lesions. Birds with heavy lesions presented all airsacs with copious and purulent exudation and extension of the infection to the adjacent tissues (perihepatitis, pericarditis, peritonitis). Slight lesions were confined to thoracal and abdominal airsacs. The animals were weighed in groups before being killed.

2.5. *E. coli Diarrhoea of Piglets*

In 3 trials the piglets (Large White) were randomly caged in groups of three (or two) in batteries immediately after being weaned at about 3 weeks of age. They were fed twice a day a commercial starter feed at a daily ration of 2 × 12 g per kg b.w.

SCP + TMP (5 + 1) was added to the feed in order to yield active ingredients concentrations of 750, 1000 and 2000 ppm. The feed was offered in a liquid form after addition of the threefold quantity of water. The piglets had free access to feed and water (nipples).

Each animal was infected with about $5 \cdot 10^9$ viable germs of an overnight culture of the enteropathogenic E. coli strain A45 (serogroup 0149). The feed medication was from 3 days before to 4 days after infection.

Rectal swabs were smeared on blood agar plates and on bromthymolbluelactose agar (Merck). After overnight incubation the proportion of the haemolytic to the total coliform flora was estimated in percent. A few haemolytic colonies per plate were picked and serologically checked for their identity with the infecting strain. When collecting the swabs, the consistency of the faeces was judged. Incohesive faeces from viscous to liquid were assessed as diarrhoeic.

3. Results

3.1. *Antibacterial Activity in Vitro*

Table 1 displays the essential data on the antibacterial activity of SCP and TMP singly and in combination. The TMP-induced potentiation of SCP was about tenfold. Although there were strain-specific variations of the best combination ratios, the

optimal median SCP + TMP proportions resulted to be 10 + 1 for gram-negatives and 5 + 1 for gram-positives.

With most SCP-resistant germs there was no synergism in vitro, however, with one resistant strain of E. coli and one of Diplococcus pneumoniae a true potentiation of the combination was found.

SCP used singly and combined with TMP compared favourably with corresponding efficacy data of Sulfamethoxazole and Sulfadiazine (Table 2).

Discs containing 23.75 mcg SCP and 1.25 mcg TMP led to inhibition zones on Müller-Hinton agar (Kirby-Bauer Method) [43] that correlated almost perfectly with corresponding values of Sulfamethoxazole + TMP (Cotrimoxazole) discs (23.75 mcg + 1.25 mcg).

With the 14 E. coli field strains of Table 3 and 8 laboratory strains (1 E. coli, 1 Aerobacter aerogenes, 1 Klebsiella pneumoniae, 1 Salmonella typhimurium, 2 Staph. aureus, 1 Sarcina lutea, 1 Bacillus subtilis) the mean diameters of inhibition zone were 19.86 ± 0.96 (standard error) for SCP + TMP and 20.43 ± 0.94 mm for Cotrimoxazole. The t-statistic revealed no significant differences between the two discs. The linear regression correlation was 0.978.

Highly resistant strains such as Pseudomonas aeruginosa and some Streptococci remained without inhibition zone at all.

Table 3 gives a characterization of the field strains of E. coli used for the chemotherapeutic work reported hereafter. Four of them turned out to be resistant to SCP (Disc sensitivity testing and MIC).

3.2. Chicken Aerosacculitis

The therapeutic activity of SCP in combination with TMP in the aerosacculitis model of chickens is presented on Tables 4–6.

After single oral administration into the crop a fivefold enhancement of SCP-efficacy by TMP was apparent on the base of a complete cure (no detectable lesions) and a tenfold potentiation was found upon analysing reduction of mortality and heavy lesions (Table 4).

Further aerosacculitis trials with 13 field strains of avian origin (characterization see Table 3) were run in order to test the efficacy of the SCP + TMP combination in drinking water. These results are displayed on Tables 5a + 5b and 6a + 6b.

These strains could easily be distinguished according to their virulence:

7 highly pathogenic strains caused heavy losses with relatively low infection loads (Table 5a) and 6 weakly virulent strains produced only low mortality even at high inocula (Table 6a). Concomitantly, the strains differed also in their growth depressing ability (Tables 5b and 6b).

Both SCP and SCP + TMP treatments reduced mortality almost completely. A comparison of untreated infected groups with the several medicated groups including all 13 strains according to the sign test of *Dixon* and *Mood* [44] demonstrates a highly significant reduction of lesions in the four medication groups at the 99% level, despite of the fact that a complete elimination of lesions was not achieved.

The same short cut statistics reveal no significant difference of lesion reduction between the low concentration applications of SCP + TMP and SCP alone (80 + 16 mg/l versus 650 mg/l respectively) but a significantly better effect at the 95% level of 160 + 32 mg/l SCP + TMP versus 1300 mg/l SCP. Thus a more than eightfold enhancement of the therapeutic effect of SCP by TMP could be demonstrated in these trials.

The influence of the SCP + TMP therapy on weight gain was dramatic, especially with concern to the highly virulent strains (Tables 5b and 6b) since the mean weight gain of the medicated groups reached almost the growth of the control animals.

On average of all treatment groups the medicated birds had a mean daily water intake of 186 ± 9.4 ml per kg bodyweight, corresponding to a mean SCP dosage rate of 121 and 242 mg/kg b.w. and a SCP + TMP dosage rate of 18 and 36 mg/kg b.w. respectively.

3.3. E. coli Diarrhoea of Piglets

The E.coli strain used in the diarrhoea trials was endemic in the pig unit from which the piglets derived. The stress of early weaning, change of feeding regimen and transfer to batteries caused a spontaneous outbreak of diarrhoea in the piglets of trials 1 and 2 (Table 7). In these cases the infection with the pathogen was only to reinforce a condition already established.

All piglets survived the massive infection even in the untreated controls, and a spontaneous cure occurred within 7 days of infection.

As evidenced on Table 7 the prophylactic administration of SCP + TMP strongly accelerated the curing process measured in terms of diarrhoeic piglet days and presence of the pathogen.

The piglets' growth was positively influenced by the SCP + TMP therapy (Figure 1), yet the weight gains remained modest due to the restricted feeding (2 × 12 g/kg b.w. daily).

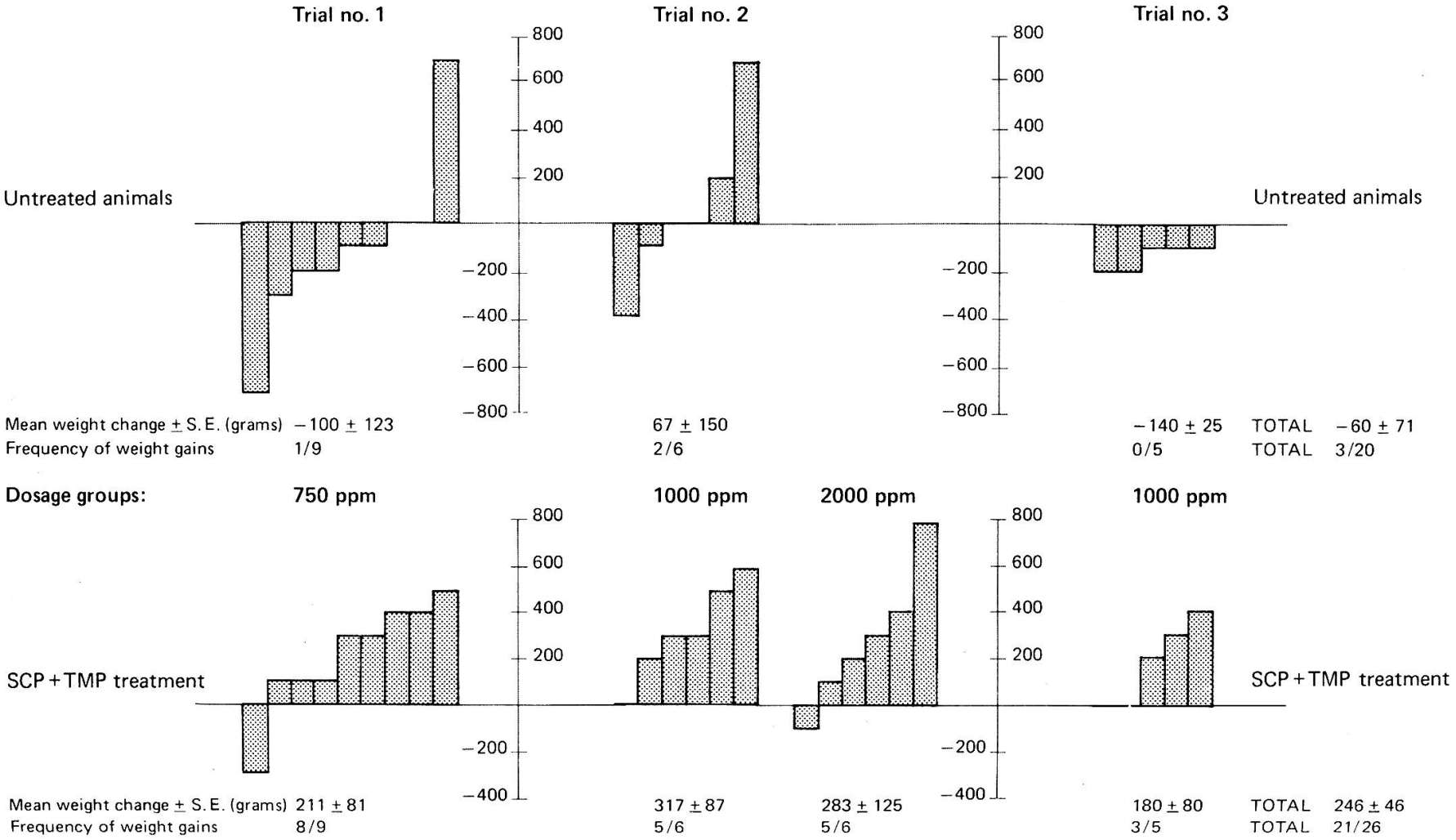
The comparison of the frequencies of positive weight change between control and treatment groups revealed a highly significant effect of SCP + TMP on growth ($P < 0.01$).

4. Discussion

Neander [45] investigated the efficacy of SCP + TMP (20 + 1) in vitro on E.coli and S.aureus strains of avian origin and demonstrated a synergistic activity with the majority of strains. By comparison with Sulfadiazine and Sulfamethoxazole on a large bacterial spectrum we showed in this study, that the activity of SCP is potentiated by TMP to a similar extent as has been described for other sulfonamides [38, 39, 46–52].

The synergism described for the combined action of sulfonamides and TMP relies on a sequential double block in the bacterial folic acid metabolism, a process essential to many bacterial species [53]. The degree of TMP-potentiation amongst sulfonamides of approximately equal activity may therefore be expected to be similar. It

Figure 1: **Effect of the SCP + TMP feed medication on growth of early weaned piglets infected with Enteropathogenic E. coli**



has been claimed that maximal synergism is observed with equipotent combinations of TMP and sulfonamides e.g. with equal fractions of MIC's of the drugs used singly [46]. For the SCP + TMP combination we found a large range of combination ratios to act synergistically. Similarly, *Seydel* [54] reported on synergism attained by Sulfamethoxazole + TMP within widely varying combination ratios. The same extent of in vitro potentiation was observed in vivo in the chicken E.coli aerosacculitis model that proved to be a very sensitive chemotherapeutic tool to check the SCP + TMP combination. A pecularity of this model is its strong distinctive capacity between pathogens of high and low virulence. The high inocula of low virulence strains necessary to produce severe lesions were finally more difficult to cure than the slight inocula of the highly pathogenic germs. The observable airsac lesions in the medicated groups remained localized and were non purulent. Such a degree of curing was generally achieved when less than 50% of the birds exhibited observable lesions. The normalized weight gains may be interpreted as complete recovery even though slight localized airsac lesions still persisted.

Particularly interesting are the results obtained with the sulfonamide resistant strains (73/576, 73/563, 2298 and 403): whereas with SCP alone at 1300 mg/l only faint curative effects were noticed, a clear therapeutic efficacy was achieved by SCP + TMP at 160 + 32 mg/l. These results confirm the findings of *Acar* et al. [52] who emphasized the utility of the combination of Sulfamethoxazole and Trimethoprim in the treatment of human urinary tract infections attributable to sulfonamide resistant E.coli.

Our trials with chicken aerosacculitis allow the conclusion that, despite a heavy challenge with E.coli, water medication with SCP + TMP (5 + 1) at 192 mg/l perfectly controls the disease. This dosage is 6.8 times lower than the SCP dose of 1300 mg/l found to be effective by *Sieiro* and *Meier* [16] in the same model infection.

If the same potentiation factor is applied to the dosage rate recommended for oral use of SCP alone (130 mg/kg b.w. daily) a dose of 19 mg/kg results for the SCP + TMP combination. The efficacy of this rather low dosage rate has been confirmed in the diarrhoea trials with piglets where even the lowest dose of 750 ppm via the feed (corresponding to approx. 18 mg/kg b.w.) led to satisfactory improvement.

In this partly spontaneous, partly controlled enteritis model a clear dose response dependence could not be established. An interesting feature of the piglet diarrhoea model was that the clinical symptoms disappeared more rapidly than the pathogen (Table 7), thus indicating that the therapy may interfere with enterotoxin production but also that immunity is built up quickly.

The literature on the kinetics and elimination data of SCP has been reviewed by *Buntenkötter* [55]. SCP is rapidly absorbed and eliminated with an elimination half life of 3.9 hours (mean of several species). TMP has, on the basis of the same species, a mean elimination half life of 2.7 hours [56]. The two drugs seem therefore to fulfil the requirement of similar pharmacokinetic properties to a good extent.

This and the excellent microbiological activity of the combination are felt to be the main reasons for the good clinical efficacy under controlled as well as under field conditions.

Table 1: Spectrum of antibacterial activity of SCP and TMP singly and in optimal combination

	Minimal inhibitory concentrations (MIC) mcg/ml		
	SCP	TMP	SCP + TMP in optimal synergistic combination
Pseudomonas aeruginosa	>10	>1	no synergism
E. coli (5 strains)	2 – >10	0.06 – 0.2	0.2 + 0.02
E. coli	>10	0.06	no synergism
Aerobacter aerogenes	2	0.06	0.2 + 0.02
Enterobacter cloacae	>10	0.6	no synergism
Klebsiella pneumoniae	10	0.06	0.2 + 0.02
Proteus vulgaris	0.6	0.06	0.2 + 0.02
Proteus morganii	2	0.6	0.6 + 0.06
Salmonella typhimurium	6	0.06	0.2 + 0.02
Salmonella gallinarum	6	0.2	0.2 + 0.02
Salmonella pullorum	2	0.02	0.06 + 0.006
Salmonella cholerae-suis	2	0.06	0.2 + 0.002
Arizona	6	0.06	2 + 0.02
Bordetella bronchiseptica	0.2	>1	0.06 + 0.6
Brucella abortus	2	>1	0.2 + 0.2
Pasteurella multocida (2 strains)	2	0.01	0.6 + 0.0006
Haemophilus influenzae	0.2	0.06	0.06 + 0.006
Median MIC (gram-negative strains)	2	0.1	0.2 + 0.02
Micrococcus flavus	2	0.6	0.2 + 0.02
Staph. aureus (4 strains)	0.6 – 6	0.2	0.2 + 0.02
Sarcina lutea	0.06	0.2	0.06 + 0.2
Diplococcus pneumoniae	>10	>1	0.6 + 0.06
Streptococcus pyogenes	6	0.6	0.6 + 0.06
Streptococcus agalactiae	>10	0.2	no synergism
Streptococcus faecalis	>10	0.2	no synergism
Corynebacterium bovis	0.2	>1	0.06 + 0.06
Listeria monocytogenes	0.6	0.006	0.2 + 0.002
Erysipelothrix rhusiopathiae	0.2	0.2	0.06 + 0.06
Bacillus subtilis	6	0.02	0.6 + 0.006
Median MIC (gram-positive strains)	2	0.2	0.2 + 0.04

DST-agar with 5% lysed horse blood

Table 2: Median values of MIC's of sulfonamides used singly and in optimal combination with TMP

	Gramnegative strains (n = 22)			Grampositive strains (= 14)		
	Compounds singly		Combination	Compounds singly		Combination
	Sulfa (mcg/ml)	TMP (mcg/ml)	Sulfa + TMP (mcg/ml)	Sulfa (mcg/ml)	TMP (mcg/ml)	Sulfa + TMP (mcg/ml)
Sulfachloropyridazine	2	0.1	0.2 + 0.02	2	0.2	0.2 + 0.04
Sulfamethoxazole	8	0.1	0.5 + 0.02	1.5	0.2	0.3 + 0.02
Sulfadiazine	9	0.1	0.8 + 0.02	6	0.2	0.6 + 0.05

Table 3: Characterization of E. coli field-strains used to test the chemotherapeutic activity of SCP + TMP

E. coli strains	SCP + TMP 10 + 1 MIC mcg/ml	SCP + TMP 23.75 + 1.25 mcg/disc Zone of inhibition, mm	SCP MIC mcg/ml	SCP 300 mcg/disc Zone of inhibition, mm	Animal origin and virulence	Geographical origin	Serogroup
2301	0.1 – 0.3	20.5	3 – 10	18		UK[1]	01
Flanigan	0.1 – 0.3	21	1 – 3	19		Australia	01
Appinfi	0.1 – 0.3	21	1 – 3	20		Australia	02
935	0.3 – 1	23	3 – 10	19	High virulence	UK[1]	069
E 38	0.3 – 1	22	1 – 3	23	broiler strains	Denmark[2]	078
RP 45410	0.3 – 1	18	3 – 10	19		USA[3]	088
73/576	1 – 3	(9)	>300	(11)		UK	0100
73/563	1 – 3	21	>300	(15)		UK	02
U9/41	0.3 – 1	22	3 – 10	24		Denmark[2]	02
Banano Lac$^-$	1 – 3	18	1 – 3	19	Low virulence	Australia	011
2298	1 – 3	(11)	>300	(15)	broiler strains	UK[1]	011
403	1 – 3	11.5	>300	(12)		UK[1]	078
1935	0.3 – 1	20	3 – 10	17		Denmark[2]	0150
A 45	0.1 – 0.3	22	1 – 3	22	Pig enteritis	Switzerland[4]	0149
	DST + 5% lysed horse blood	Müller-Hinton	DST + 5% lysed horse blood	Müller-Hinton			

[1] Houghton Poultry Research Station – E. G. Harry
[2] Statens Seruminstitut Copenhagen – I. Ørskov
[3] Ralston Purina
[4] Ciba-Geigy SA, CRA, St-Aubin
( ) = overgrowth in inhibition zone

Table 4: Comparative chemotherapeutic activity of SCP + TMP and SCP singly. *Aerosacculitis of chickens*

		n	Birds with heavy lesions or perished %	Birds with slight lesions %	Birds without lesions %	Efficacy conclusions
Uninfected, untreated control		30	3	0	97	
Infected untreated control		90	92	8	0	
SCP+TMP 5+1						
	1	30	97	3	0	– Mortality and heavy lesions reduced to half at 6 mg/kg b.w. – 50% of the birds completely cured at 65 mg/kg b.w.
	3	30	63	37	0	
Dosage	10	30	37	53	10	
mg/kg b.w.	30	30	13	57	30	
	100	30	3	37	60	
SCP singly						
	1	30	93	7	0	– Mortality and heavy lesions reduced to half at 60 mg/kg b.w. – 50% of the birds completely cured at 300 mg/kg b.w.
	3	30	67	33	0	
Dosage	10	30	80	20	0	
mg/kg b.w.	30	30	70	27	3	
	100	30	33	60	7	
	300	10	0	50	50	

The animals were infected with about 10^4 viable germs of E.coli RP 45410 into the left thoracic airsac. Medication was by single oral application.

Summary

The potentiated efficacy of the combination of sulfachloropyridazine and trimethoprim has been confirmed in a series of in vitro and in vivo tests.

The minimum inhibitory concentrations (MICs) of the components alone and in combination have been ascertained for 21 strains of 16 gram negative and 14 strains of 11 gram positive species of bacteria. The comparison of the mean MICs for components alone and in combination revealed that the trimethoprim-induced potentiation of the efficacy of sulfachloropyridazine was about tenfold.

In chickens artificially infected with E.coli (13 strains) chemotherapeutic tests demonstrated an increase in efficacy by a factor of 5 to 10. In comparison with untreated controls the various treatments produced significant reductions in mortality, frequency and severity of lesions as well as depression of growth. The best results were obtained with a dosage of 192 mg combined active ingredient (160 mg sulfachloropyridazine + 32 mg trimethoprim) per liter of drinking water. The treatment was started on the day of infection and maintained for 5 days.

In model tests with artificial air sac infection with E.coli clear differences between strains of high and low virulence could be seen.

Treatment of piglets weaned at 3 weeks of age and artificially or naturally infected with E.coli with 750, 1000 or 2000 ppm in feed resulted, in 3 trials, in a highly significant reduction in the number of piglet-diarrhoea-days as well as in the total of rectal swabs with more than 50% of bacteria isolated as the infective strain ($P < 0.01$).

Table 5a: Comparison of the chemotherapeutic activity of SCP + TMP and SCP singly administered via the drinking water against E. coli aerosacculitis of chickens (virulent strains only)

E. coli strain	Untreated control groups				Infected medicated groups								Inoculum (viable germs)
	Uninfected		Infected		SCP singly 650 mg/l		1300 mg/l		SCP + TMP 80 + 16 mg/l		160 + 32 mg/l		
	% M	% L	% M	% L	% M	% L	% M	% L	% M	% L	% M	% L	
2301	0	20	50	100	0	0	0	20	0	30	0	20	10^3
Flanigan	0	0	100	100	0	50	0	50	0	40	10	40	$3 \cdot 10^2$
Appinfi	0	10	45	100	0	15	0	15	5	20	0	0	10^3
935	0	10	70	100	0	20	0	0	0	10	10	10	$2 \cdot 10^3$
E 38	0	5	40	95	45	55	5	65	30	70	0	60	$2 \cdot 10^7$
RP 45410	0	0	70	100	0	0	0	0	0	0	0	20	$3 \cdot 10^4$
73/576	0	0	40	100	10	100	30	100	0	70	0	60	$3 \cdot 10^4$
Mean ± S.E.	0	6.4 ± 2.8	59.3 ± 8.3	99.3 ± 0.7	7.9 ± 6.3	34.3 ± 13.7	5.0 ± 4.2	35.7 ± 14.2	5.0 ± 4.2	34.3 ± 10.4	2.9 ± 1.8	30.0 ± 9.0	$2 \cdot 10^3$ (median inoculum)

% M = percent mortality
% L = percent of birds with lesions (slight and heavy including dead birds)
S.E. = standard error

Table 5b: Effect of SCP + TMP water medication on growth of chickens infected with E.coli into the airsacs (virulent strains only)

	Mean weight gain of living birds 8 days after infection (g/bird)			
	Untreated control groups		Infected medicated groups SCP + TMP	
E.coli strain	Uninfected	Infected	80 + 16 mg/ml	160 + 32 mg/ml
2301	192	10	180	209
Flanigan	260	0*	249	223
Appinfi	219	14	226	220
935	234	29	247	245
E 38	233	100	178	203
RP 45410	260	0	257	256
73/576	216	181	222	224
	A	B	C	D
Mean ± S.E.	230.6 ± 9.2	47.7 ± 25.8	222.7 ± 12.2	225.7 ± 7.1
Relative performance	100	20.7	96.6	97.9

A = C = D
B ≠ C
B ≠ D
Link and Wallace short cut analysis of variance (44) P = 0.01

* = 100% mortality

The synergism observed in vitro and in vivo and similar pharmacokinetic properties of the two components are the most important factors which give rise to the excellent clinical activity observed in extensive field trials under practical conditions.

Zusammenfassung

Die überadditive Wirkung der Kombination von Sulfachloropyridazin mit Trimethoprim wurde in-vitro und bei experimentellen Infektionen klar nachgewiesen.

Die minimalen Hemmkonzentrationen (MHKs) der Einzelkomponenten und deren Kombination wurden für 16 gram-negative (21 Stämme) und 11 gram-positive (14 Stämme) Bakterienarten bestimmt. Aus dem Vergleich der mittleren MHKs der Einzelkomponenten und der Kombination ergab sich eine ungefähr zehnfache Potenzierung der Wirkung von Sulfachloropyridazin durch Trimethoprim.

Im Chemotherapieversuch an künstlich mit 13 Stämmen von E.coli infizierten Mastküken konnten Wirkungssteigerungen um das 5- bis 10-fache nachgewiesen werden. Im Vergleich mit unbehandelten Kontrollen bewirkten die verschiedenen Behandlungen signifikante Reduktionen bezüglich Mortalität, Häufigkeit und Schwere der Läsionen, sowie der Wachstumsdepressionen. Die besten Resultate wurden mit der Dosierung von 192 mg Gesamtwirkstoff (160 mg Sulfachloropyridazin + 32 mg Trimethoprim) pro Liter Trinkwasser erzielt. Die Medikation begann am Infektionstag und dauerte 5 Tage.

Am Modell der künstlichen Luftsackinfektion mit E.coli kann deutlich zwischen hoch- und schwachvirulenten Stämmen unterschieden werden.

Die Behandlung von natürlich und künstlich mit E.coli infizierten, im Alter von 3 Wochen abgesetzten Ferkeln mit verschiedenen Dosen (750, 1000 und 2000 ppm im Futter) der Kombination von Sulfachloropyridazin und Trimethoprim führte in drei Versuchen zur Senkung der Ferkeldurchfalltage sowie der Anzahl von Rektalabstrichen mit einem Anteil des Infektionskeimes an der

Table 6a: Comparison of the chemotherapeutic activity of SCP + TMP and SCP singly administered via the drinking water against E. coli aerosacculitis of chickens (weakly virulent strains only)

E. coli strain	Untreated control groups				Infected medicated groups								Inoculum (viable germs)
	Uninfected		Infected		SCP singly 650 mg/l		1300 mg/l		SCP + TMP 80 + 16 mg/l		160 + 32 mg/l		
	% M	% L	% M	% L	% M	% L	% M	% L	% M	% L	% M	% L	
73/563	10	10	25	100	25	80	20	75	10	55	5	65	$1 \cdot 10^4$
U9/41	0	0	10	100	10	90	0	80	0	80	0	70	$5 \cdot 10^7$
Banano Lac$^-$	0	0	0	100	0	40	0	60	0	60	0	50	$2 \cdot 10^7$
2298	10	10	10	100	0	80	0	90	0	100	0	50	10^7
403	0	0	0	80	0	70	0	50	0	50	0	20	$4 \cdot 10^7$
1935	0	0	20	90	0	80	0	50	0	50	0	30	$2 \cdot 10^7$
Mean ± S.E.	3.3 ± 2.1	3.3 ± 2.1	10.8 ± 4.2	95 ± 3.4	5.8 ± 4.2	73 ± 7.1	3.3	67.5 ± 6.8	1.7	65.8 ± 8.2	0.8	47.5 ± 7.9	$2 \cdot 10^7$ (median inoculum)

% M = percent mortality
% L = percent of birds with lesions (slight and heavy including dead birds)
S.E. = standard error

Table 6b: Effect of SCP + TMP water medication on growth of chickens infected with E. coli into the airsac (weakly virulent strains only)

	Mean weight gain of living birds 8 days after infection (g/bird)			
	Untreated control groups		Infected medicated groups SCP + TMP	
E. coli strain	Uninfected	Infected	80 + 16 mg/l	160 + 32 mg/l
73/563	203	122	183	197
U9/41	223	106	167	194
Banano Lac$^-$	252	138	210	201
2298	223	154	192	205
403	238	204	229	246
1935	252	147	208	222
	A	B	C	D
Mean	231.8 ± 7.8	145.2 ± 13.7	198.2 ± 9.0	210.8 ± 8.1
Relative performance	100	62.6	85.5	90.9

A = C = D
B ≠ C
B ≠ D
Link and Wallace short cut analysis of variance (44) P = 0.05

Gesamtflora von mehr als 50%. Die Unterschiede zu den unbehandelten Kontrollen erwiesen sich als hoch signifikant ($P < 0.01$).

Der in-vitro und in-vivo nachweisbare Synergismus sowie die weitgehende Übereinstimmung der beiden Komponenten im pharmakokinetischen Verhalten sind wichtige Voraussetzungen für die gute klinische Wirkung, wie sie in ausgedehnten Praxisversuchen beobachtet worden ist.

Résumé

L'efficacité accrue résultant de l'association de la sulfachloropyridazine avec le triméthoprime a été démontrée dans de nombreux essais in vitro et in vivo.

Les concentrations inhibitrices minimales (CIM) des composants pris isolément et de leurs associations ont été déterminées pour 27 espèces de bactéries dont 16 gram-négatives (21 souches) et 11 grampositives (14 souches). Il en résulte en moyenne une augmentation d'activité de la sulfachloropyridazine d'un facteur 10.

Des essais chimiothérapeutiques sur poulets infectés artificiellement avec 13 souches de E. coli ont mis en évidence des augmentations d'efficacité de 5 à 10 fois. Sous l'influence des différents traitements, la mortalité ainsi que la fréquence et gravité des lésions ont été réduites de manière significative par rapport aux témoins infectés non traités et les gains de poids ont atteint le niveau des témoins non infectés. Les meilleurs résultats furent obtenus avec un dosage de 192 mg de substance active totale par litre d'eau d'abreuvage (160 mg de sulfachloropyridazine + 32 mg de triméthoprime) en commençant la médication le jour de l'infection pour une durée de 5 jours.

Le modèle d'infection artificielle des sacs aériens par E. coli permet de distinguer nettement entre souches de forte et faible virulence.

Des porcelets sevrés, âgés de 3 semaines, naturellement ou artificiellement infectés par une souche entéropathogène d'E. coli furent traités par l'association de sulfachloropyridazine et triméthoprime (750, 1000 et 2000 ppm dans l'aliment). Cette thérapie diminua d'une manière hautement significative ($P < 0.01$) la fréquence des jours diarrhéiques et la présence des germes pathogènes dans les fèces.

Table 7: Therapeutic activity of SCP + TMP administered in the feed to early weaned piglets infected with enteropathogenic E. coli

	Trial No. 1				Trial No. 2						Trial No. 3				Total Trials No. 1–3			
	Untreated animals		SCP + TMP 750 ppm 18 mg/kg b.w.		Untreated animals		SCP + TMP 1000 ppm 24 mg/kg b.w.		SCP + TMP 2000 ppm 48 mg/kg b.w.		Untreated animals		SCP + TMP 1000 ppm 24 mg/kg b.w.		Untreated animals n = 20		SCP + TMP all treatments n = 26	
															frequency in %			
	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149	Piglets with diarrhoea	Rectal swabs with ≥ 50% haemolytic 0149
3 days before infection	0/9	0/9	0/9	0/9	2/6	0/6	2/6	0/6	2/6	0/6	1/5	0/5	2/5	0/5	15	0	23	0
Day of infection	7/9	7/9	3/9	2/9	4/6	4/6	1/6	3/6	2/6	0/6	0/5	0/5	0/5	0/5	55	55	23	19
1 day after infection	8/9	8/9	3/9	4/9	4/6	6/6	0/6	3/6	1/6	3/6	5/5	5/5	0/5	2/5	85	95	15	46
2 days after infection	5/9	8/9	1/9	3/9	3/6	6/6	1/6	4/6	3/6	0/6	4/5	5/5	0/5	0/5	60	95	19	27
3 days after infection	3/9	8/9	1/9	2/9	3/6	6/6	0/6	2/6	3/6	0/6	3/5	4/5	0/5	0/5	45	90	15	15
7 days after infection	0/9	1/9	0/9	0/9	0/6	1/6	1/6	0/6	1/6	0/6	0/5	0/5	0/5	0/5	0	10	8	0

Chi² statistic of table 7

1–3 days after infection

	Diarrhoeic piglet-days	Non-Diarrhoeic piglet-days	
	Rectal swabs with ⩾50% haemolytic 0149	Rectal swabs with <50% haemolytic 0149	
Untreated animals	38	22	60
	54	6	60
SCP + TMP All treatments	13	65	78
	23	55	78
	51	87	138
	77	61	138

Diarrhoea Chi²: 29.7
Haemolytic 0149 Chi²: 47.9
} highly significant $p<0.01$

Le synergisme mis en évidence in vitro et in vivo, ainsi que la similarité pharmacocinétique des composants sont des conditions importantes de la bonne efficacité clinique enregistrée pour l'association de sulfachloropyridazine et triméthoprime dans des essais de pratique courante.

Riassunto

L'effetto di addizione della combinazione Sulfacloropiridazina con Trimetoprima è stato dimostrato chiaramente in vitro e con infezioni sperimentali.

La concentrazione minima inibitrice (MHKs) delle singole componenti e della loro combinazione è stata determinata per 16 specie batteriche gram negative (21 ceppi) e per 11 specie gram positive (14 ceppi). Dal confronto della media MHKs delle singole componenti e della combinazione è risultato un potenziamento di circa dieci volte dell'effetto della Sulfacloropiridazina attraverso la Trimetoprima.

Nell'indagine chemioterapica condotta su pulcini di razze da carne infettati sperimentalmente con 13 ceppi di E.coli, si è potuto stabilire un incremento di effetto da 5 a 10 volte. In confronto con i controlli non trattati, le diverse terapie hanno ridotto in modo significativo la mortalità, la frequenza e la gravità delle lesioni e il periodo di depressione della crescita. I migliori risultati sono stati ottenuti con un dosaggio di 192 mg di sostanza attiva totale (160 mg di Sulfacloropiridazina + 32 mg di Trimetoprima) per litro di acqua da bere. La somministrazione dei farmaci è iniziata il giorno dell'infezione e si è protratta per cinque giorni.

Con l'uso del modello della infezione dei sacchi aerei con E.coli si possono differenziare chiaramente i gradi di virulenza dei ceppi.

Suinetti svezzati all'età di tre settimane infettati per via naturale o sperimentalmente con E.coli, sono stati trattati con dosi diverse (750, 1000, 2000 ppm nel mangime) della combinazione Sulfacloropiridazina e Trimetoprima. Tale terapia ha condotto in tre esperimenti alla riduzione dei giorni di diarrea e degli strisci rettali con una quota di germi infettivi isolati superiore al 50% della popolazione batterica totale. Le differenze rispetto ai controlli non trattati sono risultate altamente significative ($P<0.01$).

Il sinergismo dimostrabile in vitro ed in vivo e l'ampia consonanza delle due componenti nel comportamento farmacocinetico sono le più importanti premesse per il buon effetto clinico osservato nelle nostre estese prove di campo.

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