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INTERACTION BETWEEN SULPHAMETHAZINE AND SULPHAPHENAZOLE AND SOME COCCIDIOSTATS IN CHICKENS OF DIFFERENT AGES

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Abstract

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Intramuscular administration of sulphamethazine and sulphaphenazole at a daily dose of 0.2 $g.kg^{-1}$ to cockerels aged 8, 22 and 36 days resulted in increasing blood levels of the two sulphonamides with advancing age, whereas on oral administration of sulphamethazine such differences between different age groups were not observed. The blood levels of sulphamethazine were invariably lower than those od sulphaphenazole given at the same dose rate. The coccidiostats used for feed medication were monensin (100g per ton feed), lasalocid (75 gt^{-1}) and salinomycin (60 gt^{-1}). The medicated feeds were fed to coccidia-free chickens from the 3rd post-hatching day. The medications exerted no negative effects on blood levels of the two sulphonamides. Sulphamethazine concentrations of the jejunum in the oldest group of birds after oral administration for 3 days were invariably lower than the blood levels in all medicated groups as well as in non-medicated controls. Body mass gains were largest after medication with lasalocid. AST and ALT activities were affected by the two sulphonamides in different ways: sulphamethazine reduced AST and ALT activity, whereas sulphaphenazole reduced ALT activity but produced a rise in AST activity in all medicated groups and non-medicated controls. The medication produced a decrease in both AST and ALT activity even when no sulphonamides were administered.

Gallus domesticus, age-dependence, interaction, monensin, lasalocid, salinomycin, sulphamethazine, sulphaphenazole.

Part of the results were presented in form of a poster at the 2nd National Pharmacological Congress and at the 6th National Conference on Pharmacology and Clinic of Bulgarian Drugs, Plovdiv, 1984

Investigations into interaction of drugs and biologically active substances in the broad sense of the word have been prompted by increasing intake of various chemical substances by farm animals particularly under intensive husbandry conditions and in our view constitute an integral part of pharmacology and toxicology. Some of our results concerning the interaction of sulphamethazine and phenobarbital (and also some central analeptics), sulphamethazine and some growth-promoting drugs, sulphamethazine and sodium nitrite, and sulphamethazine and ascorbic acid have been published (e.g. Šimůnek et al. 1980ab; 1982; 1983; 1985 abc; Siddique et al. 1979ab and Šimůnek and Jaroš 1985) and other investigations along this line are in progress. In these studies it was found that sulphamethazine increased acute toxicity of phenobarbital in chickens with advancing age, that under the conditions used in our experiments nitrovin, carbadox, olaquindox and cyadox affected the kinetics of injected sulphamethazine, which became manifested by a small rise of its blood levels and a more rapid decline of these levels particularly in younger age groups of domestic fowls and pigs, whereas its blood levels in cyadox-medicated chickens given this sulphonamide per os were lower, that sodium nitrite increased the intensity of suppressive action of phenobarbital on the CNS in chickens in dependence upon age and that administration of sulphamethazine enhanced the intensity of this suppressive action even further, and that ascorbic acid had only little positive effect on sulphamethazine blood levels of domestic fowls. In the light of the aforementioned results it became possible to specify guidelines for concurrent use of some drugs in the field.

Among drugs used continuously in large poultry flocks coccidiostats are, no doubt, of paramount importance. Since they are used in form of medicated feed on a long-term basis, other drugs such as chemoterapeutics are likely to be administered during the same period. Therefore studies on possible interaction between coccidiostats and other drugs are of practical value.

As to modern anticoccidials, Damron et al. (1977) found that the effects of lasalocid and monensin were not affected by methionine in the presence of the growth promotant roxarsone. Also Marusich et al. (1977) demonstrated the compatability of lasalocid with roxarsone, Zn-bacitracin and lincomycin in feeding experiments. Sulphonamides given concurrently with anticoccidial ionophorous antibiotics in water were reported to reduce water and feed intake and thus to reduce body mass gains (Henck en 1981).

The evidence cited above warrants experiments on possible interaction between coccidiostats and sulphonamides.

Materials and Methods

Clinically healthy chickens (cockerels), H x SL line (Xaverov, farm Přemyslovice) were used in the experiments. They were divided into 4 groups of 120 birds each. Group 1 was medicated with monensin (100 g per ton feed), Group 2 with lasalocid (75 g per ton feed) and Group 3 with salinomycin (60 g per ton feed) starting from the 3rd post-hatching day. The medicated feed was prepared in the Research Institute of Feed Supplements and Veterinary Drugs, SPOFA. Birds of Group 4 were fed the same feed withou medication and served as controls. All four groups were fed *ad libitum* and had free access to drinking water. They were kept at a constant temperature of 28 °C and at a relative humidity of 75 % in a room equipped with thermostatically-controlled infra lamps and vacuum ventilators.

For determination of body mass, 25 chickens of each group were chosen

randomly twice a week. Chickens thus chosen were always birds that up to that time had not received sulphonamide.

At 8 days of age 60 chickens of each group were chosen for sulphonamide treatment. Of these 60 chickens 30 were treated with sodium salt of sulphamethazine and the remaining 30 birds with sodium salt of sulphaphenazole. Both treatments were given intramuscularly at 0.2 g per kg body mass once a day for 3 days using freshly prepared solutions of Sulfadimidin (SPOFA) pulv. ad. us. vet. and Plisulfan (R) inj. (PLIVA), respectively. The solutions for these 8-day-old chickens were prepared at 1 : 99 (w/w). At 22 days of age 30 chickens of each medicated group were chosen and divided into two groups of 15 birds for treatment with the two sulphonamides at the same rate per kg body mass for 3 days in 1 : 24 solutions. At 36 days of age the remaining 30 chickens of each medicated group were divided into two groups of 15 birds for treatment with the two sulphonamides for 3 days in 1 : 5 solutions using the same dose rate per kg body mass as was used in the younger birds.

For determination of blood sulphonamide levels by the method of B r a t t o n and M a r s h a l l as modified by W a g n e r (1950), blood samples were obtained from 8-day-old chickens by decapitation and from older chickens by repeated blood withdrawal from the *vena ulnaris* at 5 and 24 hours after sulphonamide administration on the third day and in the two older groups also at 8 hours.

For photometric determination of AST and ALT activity (in μkat per litre), blood serum was obtained from blood samples collected from 6 chickens sacrificed in each medicated group 24 hours after the last administration of sulphonamide. Blood samples from 6 control birds were obtained at the same time.

During the experiment systematic clinical observations of the chickens were made and their droppings were examined. Parasitological examination at the end of the experiment showed that the chickens were free from coccidia.

Assessment of the statistical significance of the differences between the mean values in the experimental groups for $P_{0.05}$ was based on computed confidence limits (W e b e r 1961) as follows:

$$L_{U,L} = \bar{x} \pm s_{\bar{x}} \cdot t$$

In extension of the foregoing investigations further similarly designed experiments were conducted on interaction between only two coccidiostats, monensin and lasalocid, given per os in feed medicated with sodium salt of sulphamethazine to 3 age groups of 18 chickens. Blood levels of sulphonamide were determined on each of the 3 days of its administration at 5 and 8 hours after administration of 0.2 gkg^{-1} in solution by tube into the crop. In the oldest group sulphonamide concentration was also determined in the jejunal contents.

Results

The results of the experiments were tabulated. Table 1 shows the results of body mass measurements at 6 selected intervals between 3 and 36 days after hatching. Table 2 shows the order of anticoccidials ranked according to per cent body mass gains. Table 3 shows blood levels of sulphamethazine and sulphaphenazole. Table 4 shows concentrations of sulphamethazine in the jejunal contents in comparison with its blood levels. Table 5 shows mean AST and ALT activity levels.

Table 1

Mean body mass gains (in g) of chickens in two experiments.
Letters indicate significant differences of the values as against those found in the corresponding controls

Day of experiment	Group (Experiments 1 and 2)						
	K1	K2	M1	M2	L1	L2	S1
3	37.0± 0.8 a	39.9± 1.0 A	37.6± 1.1 a,b	41.9± 0.9 B	37.3± 0.9 a,b	39.4± 1.5 A	38.0± 0.1 b
8	59.6± 2.2 a	55.2± 1.5 A	58.1± 1.6 a	53.3± 1.8 B	57.8± 1.7 a	56.2± 3.0 A	57.8± 1.9 a
12	65.0± 2.1 a	72.2± 2.3 A	67.6± 2.3 b	71.5± 1.9 A	73.3± 3.2 c	73.0± 3.3 A	67.8± 2.0 a
22	150.6± 6.0 a	138.2± 4.6 A	161.5± 7.5 b	139.4± 4.2 A	163.4± 8.2 b	142.5± 7.2 A	154.0± 7.4 a
33	218.8± 7.5 a	273.1±15.0 A	234.8±11.8 b	270.6±10.0 A	236.2± 7.8 b	279.8±14.0 A	235.2± 7.4 b
36	257.6± 9.3 a	295.6±15.3 A	277.2±10.5 b	299.5±11.4 A	290.0± 8.8 c	322.3±16.5 B	288.5± 9.2 c

K - non-medicated control
M - monensin
L - Lasalocid
S - salinomycin

Table 2

Body mass of chickens in the course of medication with monensin (M,m), lasalocid (L,l), or salinomycin (S) in two series of experiments (non-medicated groups K and k)

Sequence of groups	based on body mass in g on day		based on body mass in % of day 3 on day				
	3	36	8	12	22	33	36
1	41.9 m	322 l	K 161	L 196	L 438	l 710	l 818
2	39.9 k	300 m	L 155	M 185	M 430	K 684	L 777
3	39.4 l	295 k	M 155	K 181	K 407	m 646	S 758
4	38.0 S	290 L	S 152	S 180	S 405	L 633	k 741
5	37.6 M	289 S	l 142	l 176	l 362	M 625	M 738
6	37.3 L	277 M	k 138	k 171	k 346	S 618	m 715
7	37.0 K	258 K	m 127	m 168	m 333	K 591	K 695

Table 3

Blood levels of sulphonamide (in mmol/l) in anticoccidial-medicated chickens of different ages after i.m. or oral administration of sulphamethazine (SM) and sulphaphenazole (SP) for 3 days.

a = mean from all measurements; b = mean from all measurements at 5 hours after adimistration

Age (days)		8 - 10			22 - 24			36 - 38		
Sulphonamide		SM	SP	SM	SM	SP	SM	SM	SP	SM
administration		i.m.	i.m.	oral	i.m.	i.m.	oral	i.m.	i.m.	oral
n		30	30	18	15	15	18	15	15	18
non-medi-	a	-	-	-	0.2157	0.4149	0.2277	0.2518	0.5526	0.2834
cated	b	0.2712	0.6629	0.3449	0.3054	0.5518	0.2378	0.3819	0.7257	0.2960
monensin	a	-	-	-	0.2949	0.5551	0.2295	0.2892	0.6503	0.2892
	b	0.2777	0.6779	0.3499	0.3373	0.6654	0.2400	0.4239	0.8015	0.3032
lasalocid	a	-	-	-	0.2407	0.4868	0.2475	0.330.5	0.6316	0.3151
	b	0.3700	0.7423	0.3337	0.3366	0.5641	0.2414	0.4742	0.8540	0.3122
salinomy-	a	-	-	-	0.2389	0.4617	-	0.3018	0.5946	-
cin	b	0.3097	0.9503	-	0.2536	0.5123	-	0.4157	0.7286	-

- = Not examined

Table 4

Sulphonamide concentration in the jejunal contents and sulphonamide blood levels in monensin- or lasalocid-medicated 36- to 38-old chickens after oral administration of 0.2 g sulphamethazine per kg body mass for 3 days.

Group (J - jejun.) n =	Sulphonamide in mmol/l hours after administration/days						Total means at hours	
	5/36	8/36	5/37	8/37	5/37	8/38	5	8
Non-medicated controls 6.6	0.2982 ±0.017	0.2766 ±0.035	0.2479 ±0.35	0.2227 ±0.071	0.3413 ±0.032	0.3125 ± 0.035	0.2946 ±0.114	0.2838 ±0.046
J 6.2	0.6593	0.9575	0.8307	0.5102	0.7505	0.7674	0.7473	0.7437
monensin 5.6	0.3018 ± 0.61	0.2622 ± 0.035	0.3054 ± 0.025	0.2982 ± 0.017	-	0.2802 ± 0.007	0.3018 ± 0.017	0.2874 ± 0.021
J 6.6	0.4390	0.6532	0.5896	0.5773	0.7042	0.6866	0.5784	0.6395
lasalocid 5.6	0.3018 ±0.071	0.3161 ±0.053	0.3233 ±0.057	0.3413 ±0.043	-	0.2910 ±0.028	0.3125 ±0.136	0.3161 ±0.025
J 6.6	0.4491	0.8256	0.5888	0.7509	0.8695	0.7627	0.5173	0.7796

- = Not examined

Table 5
AST and ALT activity (in μkat) in variously medicated chickens of different ages

Age (days) / Group	11		25		39	
	AST	ALT	AST	ALT	AST	ALT
K without SA	1.32±0.09	0.18±0.04	1.32±0.50	0.22±0.20	1.22±0.06	0.30±0.13
SM	1.24±0.07	0.12±0.06	1.08±0.60	0.11±0.08	=0.97±0.13	0.25±0.16
SP	1.37±	0.11±0.08	1.60±0.50	0.11±0.09	=1.36±0.07	=0.13±0.06
M without SA	*1.12±0.09	0.18±0.09	1.00±0.30	x	1.07±0.18	0.23±0.09
SM	1.14±0.09	* 0.13±0.05	1.20±0.54	x	* 1.17±0.15	*0.23±0.05
SP	*1.25±0.06	* 0.20±0.07	*1.06±0.23	x	* 1.16±0.18	*0.18±0.07
L without SA	*1.10±0.13	* 0.05±0.01	0.90±0.05	x	* 0.88±0.22	*0.11±0.04
SM	*0.98±0.05	0.12±0.07	*1.19±0.50	x	0.99±0.14	*0.17±0.10
SP	*1.06±0.07	=0.10±0.01	=*1.50±0.45	x	* 1.07±0.16	*0.12±0.06
S without SA	*0.90±0.06	* 0.03±0.04	*0.77±0.10	x	* 0.89±0.09	*0.09±0.05
SM	*0.90±0.05	* 0.03±0.05	=*0.90±0.09	x	0.94±0.03	*0.20±0.10
SP	*1.00±0.10	* 0.05±0.05	=*0.95±0.05	x	=1.21±0.16	0.13±0.04

K - non-medicated controls; M - monensin; L - lasalocid; S - salinomycin;
SM - sulphamethazine; SP - sulphaphenazole; SA - sulphonamide.
x - not measured because of a technical defect.
= - significant difference as against equally medicated group without SA.
* - significant difference as against non-medicated controls.

Discussion

Blood levels of directly reacting sulphonamide after both i.m. and oral administration of sodium salt of sulphamethazine were lower (regardless of the premedication with coccidiostats) than those found after i.m. administration of sulphaphenazole in the same dose of $0.2 g.kg^{-1}$ and, for the most part, were no longer measurable 24 hours after administration. This difference between the two sulphonamides is not surprising because the experiments were not made with therapeutic doses; these are substantially lower for sulphaphenazole owing to its protracted action.

The experiments with i.m. administration of sodium salt of sulphamethazine confirmed our previous reported observations (e.g. Šimůnek 1974) that sulphonamide blood levels of the domestic fowl increased with advancing age. In the present experiments this phenomenon was observed even in chickens premedicated with coccidiostats and became particularly conspicuous on comparison of the youngest and the oldest age groups. Administration of sulphonamides by injection makes it possible to assess potential manifestations of interaction of ionophorous anticoccidials in the body (tissues) in the pharmacokinetic phase and to exclude possible interaction due to incompatibility on administration. Ionophorous anticoccidials themselves operate by intracellular intervention (Pressman in McQuistion and McDougald 1981). Therefore further experiments were designed to look for manifestations of possible interaction of the pharmaceuticals under study on oral administration. However, the aforementioned difference in the blood levels was not observed on oral administration of sulphamethazine in either controls or medicated groups. This finding was not surprising; also in our previous experiments with various modes of sulphamethazine administration (Šimůnek et al. 1973) the kinetics after i.m. and oral administration differed by a substantial decrease of age differences in the blood levels. The medications exerted no marked negative affects on blood levels of directly reacting sulphonamide on either i.m. or oral administration of sulphamethazine or on administration of sulphaphenazole.

In 38-day-old chickens given sodium salt of sulphamethazine *per os* on three preceding days sulphonamide concentrations were invariably higher in the jejunum than in the blood even in non-medicated controls. Evidently differences in the kinetics depending on the route of administration were involved and any effects of medication with the coccidiostats can be excluded.

Body mass gains were largest on medication with lasalocid in both experiments, whereas those obtained with monensin showed little difference from the results recorded in non-medicated controls. This is essentially a confirmation of the experience to date because the growth-promoting effect of monensin in the domestic fowl is closely related to its anticoccidial action which could make itself felt in our experiments with coccidia-free chickens. McDougald (1982), using coccidia-free chickens, demonstrated even an evidently negative effect of monensin on their body mass gains.

AST and ALT activity in non-medicated chickens decreased after administration of sulphamethazine, whereas the administration of sulphaphenazole produced a rise in ALT activity and a decrease in AST activity. The medication with anticoccidials alone without administration of sulphonamides reduced the activities of both enzymes. The administration of sulphonamides to medicated chickens produced the same effect as in non-medicated birds. Analysis of the differences between the effects of the two sulphonamides on AST and ALT activity in medicated birds revealed no consistent trend.

The results of our experiments confirmed once more the existence of the effect of age on the kinetics of sulphonamide depending on the route of ad-

ministration and demonstrated that the medications exerted no adverse effects either on the blood levels of directly reacting sulphonamide after administration of sulphamethazine and sulphaphenazole or on the persistence of sulphamethazine in the gastro-intestinal tract after its oral administration. Therefore other routes of administration of sulphonamides in the field are not required because negative effects on their action in this respect are unlikely. The varying results on assessment of AST and ALT activity allow us only to suggest that the activities of the two enzymes may have been decreased by medication with the drugs used in our experiments and further reduced by sulphamethazine.

Interakce sulfadimidinu a sulfafenazolu s některými kokcidiostatiky u kuřat různého věku

Při třídenním podávání uvedených sulfonamidů v denní dávce 0,2 gkg^{-1} rozdílně starým kohoutkům (8, 22 a 36 dní) se zvyšují jejich koncentrace v krvi po nitrosvalové aplikaci, a to se zvyšujícím se věkem; po orální palikaci sulfadimidinu takovéto rozdíly mezi věkovými skupinami nejsou. Krevní hladiny sulfadimidinu jsou při shodném dávkování nižší než sulfafenazolu. Z kokcidiostatik byl použit monensin (100 g na tunu krmiva), lasalocid (75 gt^{-1}) a salinomycin (60 gt^{-1}) k medikaci krmiva podávaného od 3. dne stáří kuřat, která byla prosta kokcidií. Tato medikace v žádném případě neovlivnila negativně výši krevních hladin aplikovaných sulfonamidů. Koncentrace sulfadimidinu v jejunu u nejstarší skupiny kuřat po třídenní orální aplikaci jsou vesměs vyšší než krevní hladiny, a to u všech skupin medikovaných i u nemedikované kontroly. Hmotnostní přírůstky jsou největší po medikaci lasalocidem. Aktivita AST a ALT je použitými sulfonamidy ovlivněna rozdílně - sulfadimidin ji snižuje, po sulfafenazolu dochází k poklesu ALT, ale k vzestupu aktivity AST, a to ve skupinách medikovaných i nemedikovaných kontrolních. Za medikace dochází k poklesu aktivity AST i ALT i bez aplikace aulfonamidů.

Взаимнодействие сульфаметазина и сульфафеназола и некоторых кокцидиостатиков на петушков разного возраста

В ходе трехдневной дачи приведеных сульфонамидов суточной дозой 0,2 $г/кг^{-1}$ петушкам разного возраста (8, 22 и 36 суток) повышается их концентрация в крови после внутримышечного применения, а именно с увеличивающимся возрастом; после оральной дачи сульфаметазина такая разница между возрастными группами не наблюдалась. Уровень крови сульфаметазина при тождественной дозировке большей частью ниже сульфафеназола. Из кокцидиостатических препаратов применяли монензин (100 г на тонну кормов), ласалоцид (75 $г/т^{-1}$) и салиномицин (60 $г/т^{-1}$) для медикации кормов, подаваемых с трехсуточного возраста цыплят, лишенных кокцидий. Приведенные препараты не оказали никакого отрицательного влияния на уровень крови используемых сульфонамидов. Концентрации сульфаметазина в тощей кишке у самой старшей группы цыплят трехдневной оральной дачи большей частью выше уровня крови, а именно у всех групп, получающих препараты и у контрольной фруппы без препаратов. Наибольший прирост массы был установлен после дачи ласалоцида. Активность AST и ALT используемыми сульфонамидами проявляется по-разному. Сульфаметазин её понижает, после сульфафеназола понижается ALT, но увеличиваетса активность AST, а именно во всех потребляемых препараты группах и в контрольной

группе без медикации. В ходе дачи препаратов понижается активность AST и ALT также без применения сульфонамидов.

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