Communications to the editor

TAENIACIDAL ACTIVITY OF STREPTOTHRICIN ANTIBIOTIC COMPLEX S15–1 (SQ 21,704)

Sir:

Antibiotic S15–1 (SQ 21,704), a member of the streptothricin family of antibiotics, was discovered by screening for agents active against Newcastle disease virus.[1,2] *In vitro,* the antibiotic proved to have broad antibacterial activity and moderate antifungal activity. We now wish to report that antibiotic S15–1 has taeniacidal activity.

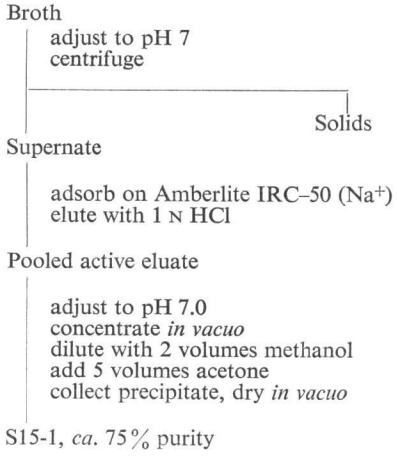
Antibiotic S15–1 was produced by deep tank fermentation of *Streptomyces purpeofuscus*[3] in a medium (pH 7.0) of the following composition, in %: soybean meal, 2.0; soluble starch, 3.0; $NaNO_3$, 0.4; K_2HPO_4, 0.2; KCl, 0.1; $MgSO_4 \cdot 7H_2O$, 0.1 and $FeSO_4 \cdot 7H_2O$, 0.002. The fermentation was allowed to proceed for about 96 hours at 29°C before it was harvested. The isolation scheme, outlined in Fig. 1, yielded S15–1 hydrochloride of approximately 75% purity. The isolated product was a light tan, hygroscopic powder decomposing at 161~171°C.

The taeniacidal activity was first noted in experiments in which the antibiotic was fed for 3 days at 0.2% in the diet to CF–1 female mice averaging *ca.* 18 g and infected with the tapeworm *Hymenolepis nana* and the roundworm *Nematospiroides dubius.* Although antibiotic S15–1 had no activity against *N. dubius,* it was 100% effective in removal of the cestode. The taeniacidal activity was subsequently confirmed in mice infected with another species of tapeworm, *Oochoristica symmetrica.* Since the first observations in mice, the extent of the taeniacidal activity has been shown to include tapeworms of cats, dogs and sheep.

In the cat, S15–1 was effective against the following species: *Taenia taeniaeformis, Spirometra mansonoides,* and *Dipylidium caninum.* In dogs, *D. caninum* and *Taenia pisiformis* and in sheep, *Moniezia expansa* and *Moniezia benedeni,* proved susceptible.

S15–1 was administered as a single oral dose in a gelatin capsule to naturally parasitized mongrel cats and dogs and to one sheep after overnight fasting. Seven days after medication, the animals were sacrificed and the intestinal contents and mucosal scrapings examined microscopically for the presence of tapeworms, including scolices. Single oral doses of S15–1 ranging from 15 to 37.5 mg/kg removed all *T. taeniaeformis* in 17 of 18 cats while single oral doses ranging from 15 to 45 mg/kg completely cleared *D. caninum* in 17 of 23 cats. *S. mansonoides* was removed by a single dose of 15 mg/kg. Similarly, single oral doses between 25 and 30 mg/kg consistently removed all *T. pisiformis* in 27 dogs and completely eliminated *D. caninum* from 16 of 18 dogs. Finally, one sheep was cleared of *M. expansa* and *M. benedeni* on treatment with 35 mg S15–1/kg body weight. The results of these experiments will be published in detail elsewhere.

Fig. 1. Isolation and purification of antibiotic S15-1



Antibiotic S15–1 was compared in mice with niclosamide and bunamidine, two taeniacides commonly used for removal of cestodes from animals. The three agents were fed to animals infected with *H. nana* with 4 mice per treatment group. All three agents removed the cestode when administered at 0.2% in the diet. At 0.1%, bunamidine cleared 3 of 4 mice while S15–1 and niclosamide destrobilized the cestodes but did not remove the scolices. At 0.05%, S15–1 and bunamidine cleared 2 of 4 mice while niclosamide was without effect. Further reduction of the concentration eliminated the taeniacidal activities of all three compounds. These data suggest that antibiotic S15–1 is approximately equivalent in

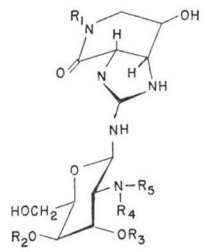
activity to the other two agents when administered to mice.

Although toxic to animals when administered systemically, S15–1 can be safely given for treatment of parasitic infections of the intestinal tract because it is not absorbed. For example, the acute toxicity (LD_{50}) of antibiotic S15–1 to male CD–1 mice weighing *ca.* 20 g was 55 mg/kg by the subcutaneous route and more than 1,000 mg/kg by the oral route. The animals were observed over a 6-day period, the prolonged observation period being necessary because of the delayed toxicity that is characteristic of the streptothricin antibiotics. Furthermore, it is significant that sufficient antibiotic to exert a taeniacidal effect survives passage through the sheep rumen in spite of potential destruction by the microbial flora.

It is interesting to speculate on the extent to which taeniacidal activity is a property of the streptothricin class of antibiotics. The class appears to be a large one with over 40 different streptothricin antibiotics having been reported in the literature. Unfortunately, a great deal of replication exists as the antibiotics usually occur in fermentation broths as mixtures of compounds of closely related structures, so that antibiotic complexes with differing designations apparently vary only in the proportions of the individual components present.[4~9] As a consequence, the number of structurally unique entities is much smaller. A generalized structural formula for the streptothricin class is presented in Fig. 2[7,9], the major difference in structure of the various streptothricins being the nature of the substituent R_5. Seven of the streptothricins differ in the number of β-lysine units present at R_5. In addition, five streptothricins lacking the β-lysine substituent have been reported: LL–AB541, LL–AB664, LL–BL136, glycinothricin[10] and sclerothricin[11]. The first four differ from the more commonly encountered streptothricins in having an N-formininoglycine or N-methylglycine replacing the β-lysine units. The structural details of sclerothricin have not been reported. Acetal and possibly ketal derivatives of the streptothricins are also known *e.g.* racemomycin B[12] and R4H[13], respectively. Whether taeniacidal activity is associated with all variations of the streptothricin structure is not known.

Antibiotic S15–1 is a complex of several components of which the major ones have been isolated[14]. Two of the three components proved to be streptothricins F and E, with one and two β-lysine units, respectively. Streptothricin F constituted about 60% of the weight of the antibiotic complex while streptothricin E constituted about 30%. By agar diffusion assay with *Bacillus subtilis* PCI 219, these two components were approximately equal in bioactivity. A third component, S15–1C, on the other hand, had about one tenth the antibacterial activity of the other two. The taeniacidal activities of the three components were determined against *H. nana* in the mouse. The streptothricin F (S15–1A) component was clearly taeniacidal. To date sufficient quantities of the other two components have not been available for final determination of activity although preliminary data suggest both are inactive or have low activity against *H. nana*.

Fig. 2. Structures of the streptothricins



where R_1=H (except CH_3 for LL–AB664 and glycinothricin)

R_2, R_3=H, $-\overset{\overset{O}{\|}}{C}NH_2$

R_4=H (except CH_3 for LL–AB664, glycinothricin, LL–AC541, and LL–BL136)

$R_5=(\overset{\overset{O}{\|}}{C}CH_2\underset{\underset{NH_2}{|}}{C}HCH_2CH_2CH_2NH)_n$–H where

n	streptothricin
1	F
2	E
3	D
4	C
5	B
6	A
7	X

$=\overset{\overset{O}{\|}}{C}$–$CH_2\overset{\overset{NH}{\|}}{N}HCH$ for LL–AB664 and LL–AC541

$=\overset{\overset{O}{\|}}{C}$–$CH_2NHCH_3$ for LL–BL136 and glycinothricin

Attempts to extend these observations to other streptothricins have met with limited success because of the unavailability of appropriate samples. The taeniacidal activity of streptothricin F against *H. nana* has been confirmed in the mouse with various preparations when administered in the feed at 0.2%: the streptothricin F component of the S15–1 complex (S15–1A), streptothricin F (Lederle) and racemomycin A (Prof. TANIYAMA). A crude preparation of LL–AC541 isolated from a fermentation of *Streptomyces hygroscopicus* NRRL 3111 in the form of an IRC–50 resin eluate was also taeniacidal. Another streptothricin antibiotic, 24010–1[15,16], also known as 156B–1, proved to be taeniacidal in the mouse and in the dog. Since the identity of antibiotic 24010–1 is as yet unknown, the activity could be due to the presence of streptothricin F. In contrast with these results, an unidentified streptothricin antibiotic preparation, EM 201, obtained by fermentation of a *Streptomyces* soil isolate and yielding β-lysine on hydrolysis, was inactive under the test conditions. TANIYAMA *et al.*[4] have reported that the antimicrobial activities and animal toxicities of the racemomycins (*i.e.* streptothricins) increases as the number of β-lysine residues increases from one to four. The preliminary data we have obtained do not appear to support a corresponding relationship between number of β-lysine units and taeniacidal activity. Consequently, we propose that taeniacidal activity is a property common to some but not all of the individual streptothricin antibiotics.

There is no doubt that many of the streptothricin antibiotic complexes reported in the literature such as the racemomycin complex[4], the yazumycin complex[8], the pleocidin complex[5], the nourseothricin complex[6], *etc.*, will have demonstrable taeniacidal activity because of the presence of streptothricin F. Determination of the activity of individual components of these complexes awaits the availability of samples of sufficient quantity.

Taeniacidal activity is a property of only a limited number of antibiotic classes. Several aminoglycoside antibiotics, including paromomycin, the gentamicin complex, antibiotic G–418, neomycin and kanamycin exhibit taeniacidal activity against a variety of cestodes in mice, cats, lambs, and even man[17~21]. In addition, the axenomycins, particularly axenomycin D, have attracted attention for their potential utility as taeniacides[22]. Other antibiotics for which taeniacidal activity has been reported include the solvent extractable thiamycins[23], the phenothiazine analog myxin[24] and the anticancer agent actinomycin D[25]. None of the antibiotics have achieved commercial utility for this indication with the exception of paromomycin which is recommended for therapy of man under some circumstances[20].

Finally, it is interesting to speculate on how the taeniacidal-active streptothricins exert their effect. The antibiotics have been shown to be strong inhibitors of protein synthesis in microorganisms[26] and consequently it is assumed that they also interfere with protein synthesis in cestode metabolism.

In conclusion, we have demonstrated a new biological property for a member of the streptothricin class of antibiotics. Antibiotic S15–1 complex was active as a taeniacide for the common tapeworms found in cats, dogs, and sheep when administered at a single oral dose of *ca.* 50 mg/kg. The antibiotic was well tolerated causing neither diarrhea nor vomiting. The taeniacidal activity appears in large part to be associated with the presence of streptothricin F, the first of the streptothricin antibiotics to be described by WAKSMAN and WOODRUFF in 1942[27].

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