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Evaluation of the antibiotic Sinefungin as an antimicrosporidial drug

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Abstract. The anti-microsporidial activity of Sinefungin, a natural nucleoside isolated from *Streptomyces griseolus* and *S. incarnatus*, has been investigated. It is highly effective against *Nosema apis* infections in honey bees, *Apis mellifera*. Concentrations of 4.0 μg/ml and above completely eliminated *N. apis* infections from the midgut of young and overwintering bees. The drug was found to be less effective against *Nosema bombycis in vitro* and *in vivo*. In cultures of *Spodoptera frugiperda* cells, previously infected with *N. bombycis*, concentration of 500 μg/ml applied for 7 days reduced spore production by 70 % relative to control cultures. When 4th instar larvae of the lepidopteran *Helicoverpa zea* were fed with spores of *N. bombycis* concurrently with 10 μg/ml or 25 μg/ml of Sinefungin in the diet, reductions in the spore numbers harvested from the larvae on day 8 post-infection were 65.7 % and 77.1 % (10 μg/ml) and 73.5 % and 97.4 % (25 μg/ml) in separate experiments. The effect of Sinefungin is comparable to that of fumagillin, presently most effective drug against bee nosematosis.

Microsporidia, which are common parasites of insects, frequently become problematic in mass rearings. *Nosema apis* Zander, the widespread microsporidium of the honey bee, *Apis mellifera* L., is an example where combinations of the parasite, poor weather conditions and inappropriate managment of bees can severely diminish honey production and even destroy the bee colonies (Bailey 1981).

Microsporidia appear quite frequently in laboratory colonies of insects, where host density and closed colonies provide favourable conditions for the spread of infections. Temporary interruption of rearing schedules or complete destruction of insect colonies have been reported (e.g., Finney et al. 1947, Finney 1956, Bucher and Harris 1961, Gast 1966, Kawasaki 1970, Bordat et al.1984). The rearing of insects for use as predators and parasitoids in biocontrol may be disrupted and, even if the diseased colonies are severely depleted, the response of the insects to experimental treatments may be erratic, resulting in misleading conclusions (Allen and Brunson 1945, Marti and Hamm 1985).

Many drugs have been tested for anti-microsporidial activity, mainly for *Nosema apis* control in the bee-keeping industry (Bailey and Ball 1991). The therapy of microsporidioses has proved difficult because microsporidia are obligatory intracellular parasites with no metabolically active extracellular stage. So far only two drugs have shown pronounced activity. The secondary metabolite derived from *Aspergillus fumigatus* was found effective against *N. apis* by Katznelson and Jamieson (1952) and has since been widely used by bee-keepers under the name of Fumagillin DCH (Chinoin, Pharmaceutical and Chemical Works, Ltd., Budapest, Hungary) or Fumidil-B (Abbot Laboratories, North Chicago, Illinois, U.S.A.). The systemic fungicide Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate) was found to suppress microsporidian infection (*Nosema* sp.) in the alfalfa weevil (Hsiao and Hsiao 1973). Fumagillin and Benomyl have been tested for the suppression of other microsporidian infections but complete elimination of the parasites has not usually been achieved and the drug levels needed for full control of the parasites proved toxic to the hosts (Lewis and Lynch 1970, Flint et al. 1972, Wilson 1974, Armstrong 1976, Harvey and Gaudet 1977, Bordat et al. 1984, Briese and Milner 1986). Exceptionally, however, fumagillin prevented infection of the codling moth *Laspeyresia pomonella* L. with *Nosema carpocapsae* when incorporated into the diet at the dose of 800 ppm (Badowska et al. 1984).

Recently, three new drugs, Toltrazuril (Mehlhorn et al. 1988), Itraconazole (Liu and Myrick 1989) and albendazole (Blanshard et al. 1992, Haque et al. 1992 a,b) were reported to have antimicrosporidial activities. Unfortunately, we have found no effect either of toltrazuril or of intraconazole on *N. bombycis* (Haque, Canning and Hollister, unpublished) or of unbuffered toltrazuril on *N. apis* (Vávra and

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Šichtová 1992, Pohl 1991). Albendazole had a suppressive effect on growth of *N. bombycis* in *Spodoptera frugiperda* (Smith) cells *in vitro* and in *Helicoverpa* (syn. *Heliothis*) *zea* (Boddie) larvae *in vivo* and had a more pronounced effect on the mammalian microsporidian *Encephalitozoon cuniculi* Levaditi, Nicolau et Schoen *in vitro*. The levels of infection were markedly decreased in the presence of albendazole but infections were not entirely eliminated, once established (Haque et al. 1992 a,b).

Sinefungin, a natural nucleoside isolated from cultures of *Streptomyces griseolus* (Hamill and Hoehn 1973) and *S. incarnatus* (Rhône-Poulen 1976) has been reported as a broad-spectrum antiprotozoan agent. The drug has activity against malaria parasites (Trager et al. 1980, Messika et al. 1990), leishmaniae (Bachrach et al. 1980, Neal et al. 1985, Avila et al. 1990), trypanosomes (Nadler et al. 1982, Dube et al. 1983), coccidia (Ferrante et al. 1988) and amoebae (Ferrante et al. 1984, 1987, Gupta et al. 1986, 1987).

The anti-microsporidial activity of Sinefungin was first reported by Vávra et al. (1992). The drug exerts its antiprotozoal activity by inhibiting transmethylation and DNA synthesis (Lawrence and Robert-Gero 1986, Paolantonacci et al. 1987, Messika et al. 1990). We here present expanded data on the activity of Sinefungin on *N. apis* and the results of tests using the drug against *N. bombycis*.

MATERIALS AND METHODS

Experiments with *Nosema apis* Zander

All experiments were made under laboratory conditions using bees which were either 5 days old (experiments A and B) or older, overwintering bees (experiment C). Each uninfected bee was fed by micropipette with 10 µl of 50 % sucrose, containing 1 x 10^5 spores of *N. apis*. Bees were kept in cages (14 x 7.5 x 5.5 cm) at 25°C and, after the initial infection, were fed with 50 % sucrose *ad libitum*. After the infection had been established on the 4th day p.i., groups of test bees were fed 50 % sucrose containing 100 µg/ml (experiment A), 76, 38 or 4.0 µg/ml (experiment B), and 4.0, 0.4 or 0.04 µg/ml (experiment C) of Sinefungin. Infected control bees were fed 50 % sucrose without the drug. Two bees from each group were examined daily for the presence of parasites. The midgut of each bee was removed and squashed in a drop of 75 % saline under a coverslip. The number of spores was estimated using an arbitrary scale: (0)= no spores; (1)= 1 to 10 spores per microscope field (spmf); (2)= 11 to 50 spmf; (3)= 51 to 100 spmf; (4)= more than 100 spmf; (5)= dense suspension of spores. Readings were made at 1000x magnification. Ten fields were read from different areas of each preparation.

Experiments with *Nosema bombycis* Naegeli *in vitro*

Spodoptera frugiperda (Smith) cells (SFc) were propagated in 25 cm^2 disposable plastic tissue culture flasks containing 5 ml TC-100 medium at 25 °C with 10 % heat-inactivated foetal calf serum (FCS) for growth and 5 % FCS for maintenance. Growth and maintenance media were supplemented with penicillin at 100 i.u./ml, streptomycin at 100 µg/ml and kanamycin at 100 µg/ml.

N. bombycis was obtained in a suspension culture of *Antheraea eucalypti* Scott cells. Infection was transferred to monolayers of SFc using the techniques of Kurtti et al. (1983).

For drug treatment, infected cells and uninfected cells were mixed 1:10 and 1 ml of the mixture containing about 1 x 10^5 cells was placed in each well of a 24-well tissue culture plate. Glass coverslips were introduced into the wells to reduce the surface area of plastic (to which the cells would naturally attach) and thus maintain many cells in suspension for further manipulation. Cells were allowed to grow for 4 days.

A stock solution of Sinefungin was obtained by dissolving 10 mg of the drug in 0.5 ml of distilled water to which 4.5 ml of TC-100 medium was added. The solution was sterilized by passing through a 0.22 µm millipore membrane filter. Drug concentrations of 100 µg, 250 µg, and 500 µg/ml in maintenance medium were obtained by dilution from the stock solution. For assays of cultures of drug activity, 4 day old cultures of infected SFc were exposed to medium containing these concentrations of Sinefungin for 3 and 7 days. Cell suspensions, harvested from the wells, were centrifuged at 150 g for 5 min. The pellet was resuspended in 500 µl of TC-100 medium and the cells spun on to glass slides using a cytospin centrifuge. The smears were air dried, fixed in methanol and stained with Giemsa. The percentage of infected cells was estimated by examining 200 cells in each of 5 randomly selected areas on the slide. Precisely the same areas were examined on all test and control slides with reference to the vernier scales on the microscope.

Experiments with *Nosema bombycis in vivo*

Larvae of the corn earworm, *Helicoverpa* (syn. *Heliothis) zea* (Boddie), reared on semi-synthetic diet were used as experimental hosts. For assessing the effect of Sinefungin, 4 mm^3 pieces of diet were first treated with 1x10^5 spores and then 5 µl drops of drug solution. Drug concentrations were 2.5, 5.0, 25.0, 50.0, and 100.0 µg/larva. For the control 4 mm^3 pieces of food were treated with 1x10^5 spores only. Each piece was transferred into 4.0 x 1.5 cm vials and newly emerged 3rd or 4th instar larvae, starved for 6 h, were individually introduced into the vials. The larvae consumed the whole piece of treated diet within several days and were transferred afterward into plastic cups containing drug-free diet. Twenty-five larvae were used for each treatment. After 8 days post-exposure to drug, 5 randomly selected larvae from each treatment were examined for the presence of spores in fat body and midgut tissues using phase-contrast microscopy. A further 10 larvae were macerated individually in distilled water and the number of released spores was estimated using a haemacytometer. The number of spores was expressed per mg of body weight.

Structural changes in developmental stages and spores of *N. bombycis* caused by Sinefungin were examined in 5th instar larvae. These had been infected with the microsporidia at the 2nd instar and exposed at the 5th instar to a piece of diet treated with 10 µg of Sinefungin. As soon as the drug-treated diet had been consumed, larvae were transferred to drug-free diet for 24 h. Pieces of midgut tissues were fixed in Karnovsky's fixative in cacodylate buffer and processed routinely for electron microscopy using OsO_4 post-fixation, acetone dehydration and embedding in Spurr's resin. Ultrathin sections, stained with uranyl acetate and Reynolds lead citrate, were examined with a Philips EM300 electron microscope at 80 kV.

RESULTS

Experiments with *Nosema apis*

Experiment A

Sinefungin at 100 µg/ml was highly effective in suppressing *N. apis*. Three days after the onset of therapy, the midgut of infected bees was completely free of spores (Fig. 1). However, at this concentration, the antibiotic was toxic to the bees, the mortality reaching 44 % 13 days post treatment compared with 9 % in the infected but untreated controls.

Experiment B

All three concentrations of Sinefungin (76, 38, and 4.0 µg/ml) cleared the infection from the midgut within 4-6 days of the onset of therapy (Fig.2). No clear difference was observed in the efficacy of the different concentrations of the drug, but all concentrations were toxic for the bees with mortality rates of 92 % by day 13 post treatment compared with 52 % for the infected, untreated controls.

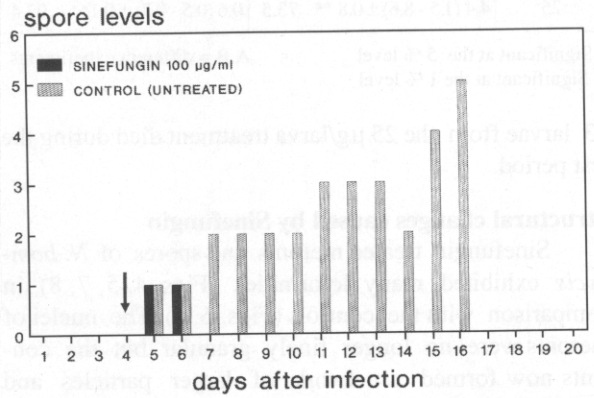


Fig. 1. Therapeutic effect of the antibiotic Sinefungin on the infection by *Nosema apis* in the honey bee *Apis mellifera* (young bees). Arrow - therapy start. For spore levels - see Methods.

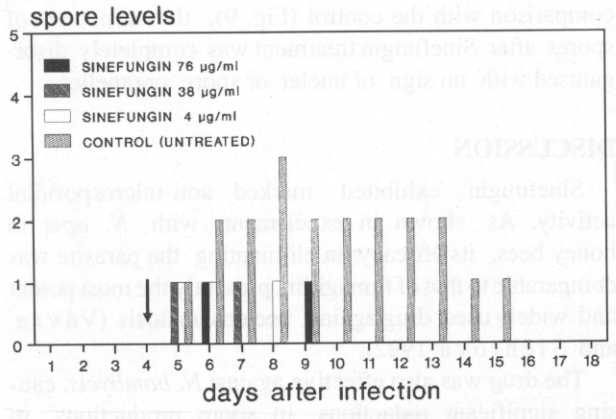


Fig. 2. Therapeutic effect of the antibiotic Sinefungin on the infection by *Nosema apis* in the honey bee *Apis mellifera* (young bees). Arrow - therapy start. For spore levels - see Methods.

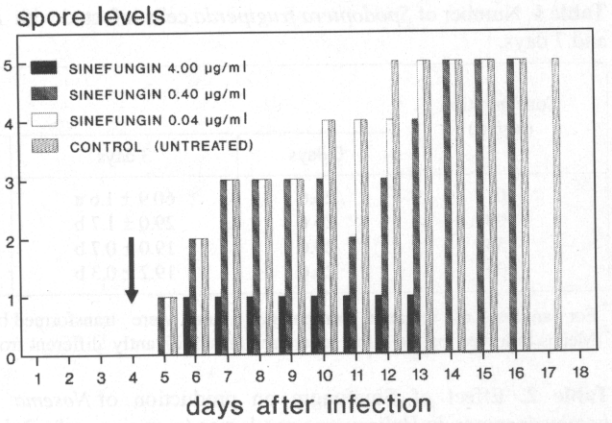


Fig. 3. Therapeutic effect of the antibiotic Sinefungin on the infection by *Nosema apis* in the honey bee *Apis mellifera* (old, overwintering bees). Arrow - therapy start. For spore levels - see Methods.

Experiment C

Using older bees, Sinefungin at 4.0 µg/ml proved to be the threshold value for the control of bee nosematosis. At this concentration isolated spores were still present in the gut up to the 9th day after the onset of therapy but then disappeared completely (Fig. 3). Also at 4.0 µg/ml of Sinefungin the mortality of the bees was lower (24 % at 21 days) than that of the infected but untreated controls (44 % at 21 days). The level of infection was slightly suppressed by 0.4 µg/ml of Sinefungin between days 11 and 13 but thereafter was comparable with the controls. The 0.04 µg/ml concentration of the drug did not influence the course of infection (Fig. 3). At the two lower concentrations the drug showed slight toxicity for the bees (mortality of 56 % at 21 days). Apparently in this case the toxic effect of the drug combined with the pathologic effect of the microsporidian infection.

Experiments with *Nosema bombycis in vitro*

All concentrations of Sinefungin (100-500 µg/ml in culture medium) significantly reduced the percentage of infected cells ($P < 0.01$) (F test). At 250 µg/ml and 500 µg/ml the percentage of infected cells decreased respectively from the initial level of 26.6 % to 19 % and 19.2 % at 3 days and to 21.7 % and 20.2 % at 7 days. At 100 µg/ml the drug prevented increase above the initial level throughout the experimental period. In untreated control cultures, the level of infection rose to 60.9 % and 73.2 % by 3 and 7 days, respectively (Table 1). No adverse effects of Sinefungin on the host cells were observed at 3 days with any of the doses. However, on the 7th day post-exposure the 250 µg/ml and 500 µg/ml doses caused clumping of cells. This was noted in uninfected cultures used as controls for drug effects as well as in the infected cultures.

Table 1. Number of *Spodoptera frugiperda* cells infected with *Nosema bombycis* before and after treatment with Sinefungin for 3 and 7 days.

Concentration (µg/ml)	Percentage of infected cells* (Mean ± S.E.)				
	0 days	3 days	% Reduction	7 days	% Reduction
0	26.6	60.9 ± 1.6 a		73.2 ± 0.3 a+	
100	26.6	29.0 ± 1.7 b	52.4	26.4 ± 0.4 b	63.9
250	26.6	19.0 ± 0.7 b	68.8	21.7 ± 0.2 b	70.4
500	26.6	19.2 ± 0.3 b	68.5	20.2 ± 1.0 b	72.4

* For anylysis of variance, percentage figures were transformed by arcsin $\sqrt{\%}$
+ Means followed by the same letter are not significantly different from each other

Table 2. Effect of Sinefungin on production of *Nosema bombycis* spores in *Helicoverpa zea* larvae (exposing early 3rd instar larvae to drug and spores concurrently) - 18th day p.i.

Sinefungin (µg/larva)	Mean (range) ± S.E./mg x 10^5	% Reduction
0	4.1 (1.8 - 7.3) ± 0.5	
2.5	3.8 (2.3 - 7.0) ± 0.5	7.3
5	Toxic	
25	Toxic	
50	Toxic	
100	Toxic	

Table 3. Effect of Sinefungin on production of *Nosema bombycis* spores in *Helicoverpa zea* larvae (exposing early 4th instar larvae to drug and spores concurrently) - 8th day p.i.

Sinefungin (µg/larva)	A		B	
	Mean (range) ± S.E./mg x 104	% Redn.	Mean (range) ± S.E./mg x 104	% Redn.
0	16.6 (10.1 - 27.5) ± 1.8		22.7 (9.6 - 38.4) ± 3.2	
5	9.8 (4.3 - 22.7) ± 1.7*	41.0	-	
10	5.7 (0.8 - 12.7) ± 1.2**	65.7	5.2 (1.4 - 9.5) ± 1.0**	77.1
25	4.4 (1.5 - 8.6) ± 0.8 **	73.5	0.6 (0.5 - 0.7) ± 0.1**	97.4

* Significant at the 5 % level
** Significant at the 1 % level
A,B = different experiments

Experiments with *Nosema bombycis in vivo*

When early 3rd instar larvae of *H. zea* were exposed to diet containing 5-100 µg/ml of Sinefungin, all larvae died between the 2nd and 4th days, even before the whole drug-treated piece of diet had been consumed. No mortality was observed with Sinefungin at 2.5 µg/larva. The dose reduced the mean number of spores harvested from the larvae on the 18th day post-exposure by 7.3 % compared to control values (Table 2). However, the reduction was not significant (Student *t*-test). Sinefungin was less toxic when given to early 4th instar larvae. On the 8th day post-exposure all doses (5.0, 10.0, and 25.0 µg/larva) significantly reduced the mean number of spores/larva (Student *t*-test) (Table 3-A). At 10 µg and 25 µg/larva there were spore reductions of 65.7 % and 73.5 %, respectively, compared to control values, and the differences were significant at the 1 % level. At 5 µg/larva there was a percentage reduction of 41.0 % compared with the controls and the difference was significant at the 5 % level. In the 25 µg/larva treatment, 3 out of 25 larvae died during the test period but no deaths were recorded in the other groups.

In another experiment Sinefungin at 10 µg/larva and 25 µg/larva were tested again on early 4th instar larvae (Table 3-B). Both the doses significantly reduced the number of spores compared to the control on the 8th day post-exposure to drug ($P < 0.01$) (Student *t*-test), the reduction for the 10 µg and 25 µg concentrations being 77.1 % and 97.4 %, respectively. In this experiment, Sinefungin was toxic to the larvae. Out of 25 larvae, 2 larvae from the 10 µg/larva treatment and 23 larvae from the 25 µg/larva treatment died during the test period.

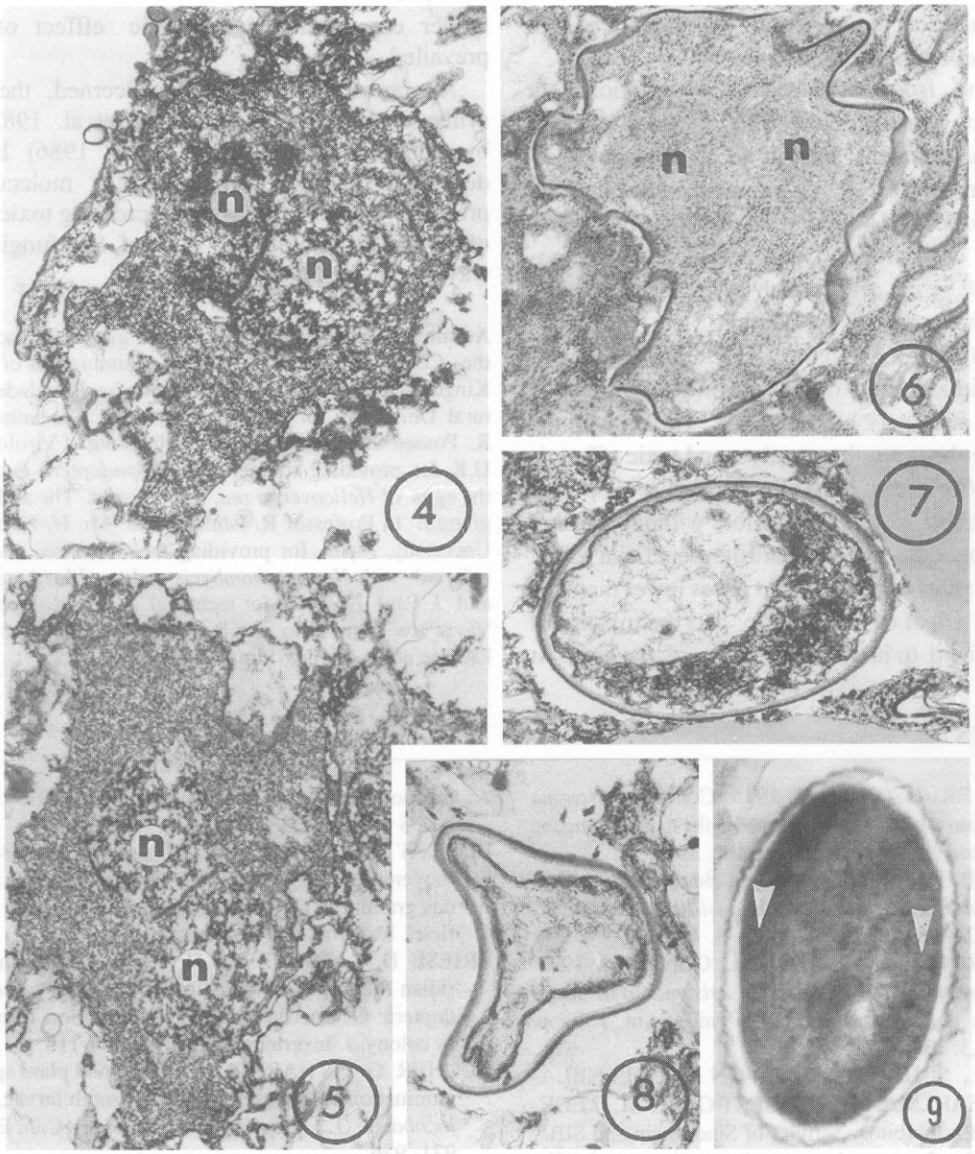
Structural changes caused by Sinefungin

Sinefungin treated meronts and spores of *N. bombycis* exhibited many deformities (Figs. 4, 5, 7, 8) in comparison with the controls (Figs. 6, 9). The nuclei of meronts were no longer finely granular but the contents now formed a network of larger particles and threads. The changes involved the entire volume of the nuclei. The cytoplasm still contained abundant ribosomes but there were signs of development of clear spaces (Figs. 4, 5). Most of the spores were irregular in shape, either semi-lunar, triangular or ovoid (Figs. 7, 8). In comparison with the control (Fig. 9), the structure of spores after Sinefungin treatment was completely disorganised with no sign of nuclei or spore organelles.

DISCUSSION

Sinefungin exhibited marked anti-microsporidial activity. As shown in experiments with *N. apis* in honey bees, its efficacy in eliminating the parasite was comparable to that of fumagillin, presently the most potent and widely used drug against bee nosematosis (Vávra and Šichtová 1992).

The drug was also effective against *N. bombycis*, causing significant reductions in spore productions in both experimental systems used. However, complete elimination of *N. bombycis* was not achieved. Similar differences in response of microsporidia have been obtained with fumagillin, viz., complete elimination of



Figs. 4, 5 and **7, 8.** Ultrastructural changes of *Nosema bombycis* in *Helicoverpa zea* larvae treated with Sinefungin. **Figs. 4, 5.** Meronts with coarsely granular diplokaryon nuclei - n (Fig. 4: x20 000), (Fig. 5: x15 000). **Figs.7, 8.** Aberant and distorted spores with no trace of internal organells (Fig. 7: x25 000), (Fig. 8: x23 000). **Figs. 6, 9.** Normal appearance of *Nosema bombycis* in *Helicoverpa zea* larvae untreated with Sinefungin. **Fig. 6.** Meront with diplocaryon nuclei - n (x20 000). **Fig. 9.** Spore (arrowheads indicate the polar filament threads) (x29 000).

N. apis infections in bees compared with suppression only of microsporidia in Lepidoptera (Lynch and Lewis 1971, Wilson 1974, Toguebaye and Bouix 1982, Vávra and Šichtová 1992). One possible explanation is that the absorptive cells of the bee gut epithelium assure efficient transport of the drug to the parasites which are located in these cells. In the case of microsporidia of Lepidoptera, where the target tissue is principally the fat body, the drug has to pass from the gut to the haemolymph before entering the infected cells. Another factor contributing to the relatively high efficacy of Sinefungin in bee nosematosis is probably the high regeneration and turnover of cells in the bee digestive epithelium. This, combined with the inhibition of the multiplication of the parasite by the drug, leads to the rapid clearance of the parasite from the gut.

The relatively low efficacy of Sinefungin in the tissue cultures infected with *N. bombycis* was unexpected since the host cells were directly exposed to the drug. In the cultures the same amount of drug (100 µg/ml) which caused rapid disappearance of *N. apis* from the bees permitted some growth of the *N. bombycis* and maintenance of spore production at the initial level. It is possible that the *Spodoptera frugiperda* cells, used in the *in vitro* experiments, lacked proper receptors or other mechanisms for the efficient transport of the drug into the cells. The *in vitro* and haemocoele systems have also in common that spores and other infective stages of

N. bombycis are not removed from the target cells in contrast to *N. apis*, as mentioned above.

It is evident from ultrastructural observations that Sinefungin acts already on developmental stages of microsporidia and that the meront nuclei are among targets of the drug. This is in agreement with data that Sinefungin blocks DNA synthesis in some organisms (Blanchard et al. 1986). Deformed spores were probably produced by the cells which were influenced by the drug at the onset of sporulation.

The good (and in the case of *N. apis*, excellent) anti-microsporidial activity of Sinefungin is unfortunately vitiated by its relatively high toxicity. This is why the threshold value between the curative and toxic effect is very narrow. With bees a dose of 4.0 µg/ml was optimal since it eliminated *N. apis* infection without causing mortality. In this case the anti-parasite beneficial effect balanced the toxicity of the drug. At doses lower than this the pathologic effect of the parasite and the toxicity of the drug combined to increase mortality of the bees. At higher concentrations the toxic efffect of the drug prevailed.

As far as the toxicity is concerned, the successful synthesis of Sinefungin (Geze et al. 1983)) and its biosynthesis (Blanchard et al. 1986) have provided opportunities for modifying the molecule so as to preserve drug efficacy while decreasing toxicity. Testing of several structural analogues of Sinefungin is under way in our laboratories.

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REFERENCES

ALLEN H. W., BRUNSON M. H. 1945: Control of *Nosema* disease in potato tuberworm, a host used in the mass production of *Macrocentrus ancylivorus*. Science 105: 394.

ARMSTRONG E. 1976: Fumidil B and Benomyl: chemical control of *Nosema kingi* in *Drosophila willinstoni*. J. Invertebr. Pathol. 27: 363-366.

AVILA J. L., ROJAS T., MONZON H., CONVIT J. 1990: Sinefungin as treatment for American *Leishmania* in sensitive BALB/c and resistant C57BL/6 mice. Am. J. Trop. Med. Hyg. 2,3: 139-145.

BACHRACH U., SCHNUR L. F., EL-ON J., GREENBLATT C. L., PEARLMAN E., ROBERT-GERO M., LEDERER E. 1980: Inhibitory activity of Sinefungin and SIBA on the growth of promastigotes and amastigotes of different species of *Leishmania*. FEBS Letters 121: 287-291.

BADOWSKA-CZUBIK T., ZEMNIACKA J., LIPA J. J., SUSKI Z. W. 1984: Effect of fungicides Benlate and Bavistin and antibiotic Fumagillin on incidence of *Nosema carpocapsae* Paillot (Protozoa, Microsporidia) in rearing of codling moth (*Laspeyresia pomonella* L.) Prace Naukowe Instytutu Ochrony Roslin 26: 189-196. (In Polish.)

BAILEY L. 1981: Honey Bee Pathology. Academic Press London, New York, Toronto, Sydney, San Francisco, 124 pp.

BAILEY L., BALL B. 1991: Honey Bee Pathology, 2nd edition Academic Press London, New York, Toronto, Sydney, San Francisco, 208 pp.

BLANCHARD P., DODIC N., FOUREY J. L., GEZE M., LAWRENCE F., MALINA H., PAOLANTONACCI P., VEDEL M., TEMPETE C., ROBERT-GERO M., LEDERER E. 1986: Sinefungin and derivatives: synthesis, biosynthesis and molecular target studies in *Leishmania*. In: Borchardt R. T., Creveling C. R., Ueland P. M. (Eds.), Biological Methylation and Drug Design. Humana Press, New Jersey, Clifton, pp. 435-446.

BLANSHARD C., ELLIS D. C., TOVEY D. G., DOWELL S., GAZZARD B. G. 1992: Treatment of intestinal microsporidiosis with albendazole in patients with AIDS. AIDS 6: 311-313.

BORDAT D., COQUARD J., RENAND M. 1984: Quelques moyens de lutte pour enrayer les nosémoses de trois foreurs des graminées élevés en laboratoire sur milieu nutritif artificiel. L Agronomie Tropicale 39: 275-285.

BRIESE D. T., MILNER, R. J. 1986: Effect of the microsporidian *Pleistophora schubergi* on *Anaitis efformata* (Lepidoptera: Geometridae) and its elimination from a laboratory colony. J. Invertebr. Pathol. 48: 107-116.

BUCHER G. E., HARRIS P. 1961: Food plant spectrum and elimination of disease of cinnabar moth larvae, *Hypocrila jacobaeae* (L.) (Lepidoptera: Arctiidae). Can. Entomol. 93: 931-936.

DUBE D. K., MPIMABAZA G., ALLISON A. C., LEDERER E., POVIS L. 1983: Antitrypanosomal activity of Sinefungin. Am. J. Trop. Med. Hyg. 32: 31-33.

FERRANTE A., LJUNGSTROM I., HULDT G., LEDERER E. 1984 : Amoebocidal activity of the antifungal antibiotic Sinefungin against *Entamoeba histolytica*. Trans. Roy. Soc. Trop. Med. Hyg. 78: 837-839.

FERRANTE A., ABELL T. J., ROBINSON B., LEDERER E. 1987: Effects of Sinefungin and difluoromethylornithine on pathogenic free-living amoebae *in vitro*. FEMS Microbiology Letters 40: 67-70.

FERRANTE A., LJUNGSTROM L., LEDERER E. 1988: Antitoxoplasmosis properties of Sinefungin in mice. C. R. Ac. Sci. III, Sciences de la Vie 306: 109-113.

FINNEY G. L. 1956: A fortified carrot medium for mass culture of the Oriental fruit fly and certain other Tephritids. J. Econ. Entomol. 49: 134.

FINNEY G. L., FLANDERS S. E., SMITH H. S. 1947: Mass culture of *Macrocentrus ancylivorus* and its host, the potato tuber moth. Hilgardia 17: 437-483.

FLINT H. M., EATON J., KLASSEN W. 1972: The use of Fumidil B to reduce microsporidian disease in colonies of the boll weevil. Ann. Entomol. Soc. Am. 65: 942-945.

GAST R. E. 1966: Control of four diseases of laboratory reared boll weevils. J. Econ. Entomol. 59: 793-797.

GEZE M., BLANCHARD P., FOURREY J. L., ROBERT-GERO M. 1983: Synthesis of Sinefungin and its C-6'epimer. J. Am. Chem. Soc. 105: 7638-7640.

GUPTA S., KISHORE P., SHUKLA O. P. 1986: Effects of phenothiazine compounds and Sinefungin on growth of *Acanthamoeba culbertsoni*. Ind. J. Parasitol. 10: 177-179.

GUPTA S., KISHORE P., SHUKLA O. P. 1987: Polyamine metabolism as a target for growth inhibition of *Acanthamoeba culbertsoni*. Am. J. Trop. Med. Hyg. 37: 550-555.

HAMILL K. L., HOEHN M. M. 1973: A9145, a new adenine-containing antifungal antibiotics - discovery and isolation. J. Antibiotics 26: 463-465.

HAQUE Md. A., COLBOURN M. I., HOLLISTER W. S., CANNING E. U. 1992a: The anti-microsporidial activity of albendazole XXVth Annual meeting Soc. Invertebr. Pathol., Heidelberg, Germany, Aug. 16-21, 1992. Abstracts of reports, p. 319.

HAQUE Md. A., HOLLISTER W. S., WILLCOX A., CANNING E. U. 1992b: The anti-microsporidial activity of albendazole. J. Invertebr. Pathol. (submitted).

HARVEY G. T., GAUDET P. M. 1977: The effects of benomyl on the incidence of microsporidia and developmental performance of eastern spruce budworm (Lepidoptera : Tortricidae) Can. Entomol. 109, 987-993.

HSIAO T. H., HSIAO C. 1973: Benomyl: a novel drug for controlling a microsporidian disease of the alfalfa weevil. J. Invertebr. Pathol. 22: 303-304.

KATZNELSON H., JAMIESON C. A. 1952: Control of *Nosema* disease of honey bees by fumagillin. Science 115, 70-71.

KAWASAKI H. 1970: Some pathogens and pests associated with tephritid flies in the laboratory. J. Econ. Entomol. 63: 1353-1355.

KURTTI T. J., TSANG K. R., BROOKS M. A. 1983: The spread of infection by the microsporidian, *Nosema disstriae*, in insect cell lines. J. Protozool. 30: 652-657.

LAWRENCE F., ROBERT-GERO M. 1986: Mode of action of Sinefungin, an antiparasitic nucleoside. Bull. Soc. Fr. Parasitol. 4: 193-196.

LEWIS L. C., LYNCH R. 1970: Treatment of *Ostrinia nubilalis* larvae with Fumidil-B to control *Perezia pyraustae* J. Invertebr. Pathol. 15: 43-48.

LIU T. P., MYRICK G. R. 1989: Deformities in the spore of *Nosema apis* as induced by intraconazole. Parasitol. Res. 75: 498-502.

LYNCH R. E., LEWIS L. C. 1971: Reoccurence of the microsporidian *Perezia pyraustae* in the European Corn Borer, *Ostrinia nubilalis,* reared on diet containing Fumidil-B. J. Invertebr. Pathol. 17: 243-246.

MARTI O. J., HAMM J. J. 1985: Effect of *Vairimorpha* sp. on the development and survival of larvae and pupae of *Calleida decora*. J. Agric. Entomol. 2: 285-291.

MEHLHORN H., SCHMAHL G., HABERKORN A. 1988: Toltrazuril effective against a broad spectrum of protozoan parasites. Parasitol. Res. 75: 64-66.

MESSIKA E., GOLENSER J., ABU-ELHEIGA L., ROBERT-GERO M., LEDERER E., BACHRACH U. 1990: Effect of Sinefungin on macromolecular biosynthesis and cell cycle of *Plasmodium falciparum*. J. Trop. Med. Parasitol. 41: 273-278.

NADLER J. P., LEDERER E., WITTNER M., BAUM S. G., TANOWITZ H. B. 1982: The effect of adenosine analogues on the *in vitro* growth of *Trypanosoma cruzi*. Trans. Roy. Soc. Trop. Med. Hyg. 76: 285-287.

NEAL R. A., CROFT S. L., NELSON D. J. 1985: Anti-*Leishmania* effect of allopurinol ribonucleoside and the related compounds, allopurinol, thiopurinol, thiopurinol ribonucleoside, and of formycin B, Sinefungin and the lepidine WR 6026. Trans. Roy. Soc. Trop. Med. Hyg. 79: 122-128.

PAOLANTONACCI P., LAWRENCE F., NOLAN L. L., ROBERT-GERO M. 1987: Inhibition of leishmanial DNA synthesis by Sinefungin. Biochem. Pharmacol. 36: 2813-2820.

POHL F. 1991: In-vitro und in-vivo Untersuchungen zu Microsporidien der Honigbiene. Diplomarbeit, Ruhr- Univers ität Bochum, Germany, 78pp.

RHÔNE-POULENC INDUSTRIES FRANCE 1976: Patent 761114

TOGUEBAYE B.S., BOUIX G. 1982: Effets de la fumagilline et du bénomyl sur une microsporidiose expérimentale chez *Heliothis armigera* (Hübner,1908) (Lepidoptera: Noctuidae). Cot. Fib. Trop. 37: 259-264.

TRAGER W., TERSHAKOVEC M., CHIANG P.K., CANTONI G. 1980: *Plasmodium falciparum:* antimalarial activity in culture of Sinefungin and other methylation inhibitors. Exp. Parasitol. 50: 83-89.

VÁVRA J., ŠICHTOVÁ M. 1992: Experimental chemotherapy of the honey bee nosematosis. 1st European Congress of Protozoology, Reading, U. K., April 5-10, 1992. Europ. J. Protistol. 28: Abstracts, p. 360.

VÁVRA J., ŠICHTOVÁ M., ROBERT-GERO M. 1992: The antibiotic Sinefungin is effective in suppressing the honey bee nosematosis caused by *Nosema apis*. Abstracts of the 44th Annual Meeting, Society of Protozoologist, June 28-July 2, 1991, Bozeman, Montana, USA. J. Protozool. 39: Suppl., abstr. No. 46.

WILSON G. G. 1974: The use of Fumidil B to suppress the microsporidian *Nosema fumiferanae* (Lepidoptera: Tortricidae). Can. Entomol. 106: 995-996.

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