Short communication

Beauvericin cytotoxicity to the invertebrate cell line SF-9

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Abstract. The cyclic hexadepsipeptide beauvericin, initially known as a secondary metabolite produced by the entomopathogenic fungus Beauveria bassiana and toxic to Artemia salina larvae, has been more recently recognized as an important mycotoxin synthesized by a number of Fusarium strains, which parasite maize, wheat and rice. Therefore, this mycotoxin may enter the food chain, causing yet unknown effects to the health of both domestic animals and humans. The cytotoxic effects of beauvericin on mammalian cells have been studied. We investigated the cytotoxicity of this compound in an in vitro invertebrate model, viz. the insect cell line SF-9 (immortalized pupal ovarian cells of the lepidopter Spodoptera frugiperda). Cultures of SF-9 cells in the stationary phase were exposed to beauvericin at concentrations ranging from 100 nM to 300 μM, for different periods of time (from 30’ to 120 h). The effects on cell viability were assessed by the trypan blue exclusion method. After 4 h of incubation no significant decrease in cell viability was recorded in SF-9 cell cultures exposed to low concentrations of beauvericin, i.e. 100 nM and 300 nM. However, a slight decrease in viability (3.9%) was seen already in cells exposed to the mycotoxin at the 1 μM concentration. This effect became gradually more evident at higher concentrations (≥ 28% at 30 μM, ≥ 50% at 100 μM, ≥ 68% at 300 μM). An even more pronounced reduction in cell viability was observed after a 24 h exposure. Under these conditions, 1 μM beauvericin caused an approx. 10% decrease in the number of viable cells, which became more significant at higher concentrations (≥ 23% at 3 μM, ≥ 47% at 10 μM, ≥ 65% at 30 μM, ≥ 90% at 100 μM, ≥ 99% at 300 μM). Therefore, the 50% cytotoxic concentrations (CC50) at 4 h and 24 h could be estimated as 85 μM and 10 μM, respectively. In time-course experiments, no effect of beauvericin (30 μM) on cell viability could be seen after exposure for periods of time as long as 30’, 1 h and 2 h, respectively. In contrast, when SF-9 cells were exposed to the mycotoxin for longer periods of time,
from 8 h to 120 h, we recorded a strong cytotoxic effect already in the low micromolar concentration range. Thus, the CC50 after both 72 h and 120 h exposure times was assessed as 2.5 μM. Higher concentrations caused a virtually 100% cell death. The data collected suggest that beauvericin exerts a substantial dose- and time-dependent cytotoxic effect on invertebrate cells, comparable to the effects described in mammalian cells.

**Key words:** biological control agents, insect cell lines, mammalian cells, mycotoxin cytotoxicity; *Spodoptera frugiperda*.

Beauvericin is a cyclodepsipeptide mycotoxin toxic to the brine shrimp *Artemia salina* (HAMIL et al. 1969), originally described as a secondary metabolite of the entomopathogenic fungus *Beauveria bassiana*. More recently, beauvericin has been recognized as an important toxic compound synthesized by several *Fusarium* strains, infecting maize, wheat and rice, worldwide (LOGRIECO et al. 1993a, 1993b, PLATTNER, NELSON 1994, MORETTI et al. 1995, DESJARDIN et al. 2000). Because of the occurrence of beauvericin producing fungi in such commodities, this mycotoxin might enter the food chain, causing so far unknown consequences to the health of domestic animals and humans (LOGRIECO et al. 2002). Beauvericin effects on mammalian cells have been studied (HARNOIS et al. 1997, QUE et al. 1997, LOGRIECO et al. 2002, CALO’ et al. 2004). We investigated the toxic effects of this mycotoxin in an in vitro invertebrate model, viz. the SF-9 cell line, made of immortalized pupal ovarian cells of the lepidopteran *Spodoptera frugiperda* (VAUGHN et al. 1977).

SF-9 cells were cultured at 27°C in the TNM-FH insect cell medium (Sigma, St. Luis, MO, U.S.A), supplemented with 10% (vol/vol) fetal calf serum (Biochrom KG, Berlin), penicillin (100 U/mL) and streptomycin (100 μg/mL). Antibiotics were from Sigma. Cultures in the early stationary phase (typical cell density: approx. 1.6 × 10^6 cells per ml; typical viability: approx. 80%) were passaged every 5 days with a seeding density of 4 × 10^5 cells per ml of culture.

Cytotoxicity assays were performed by exposing early stationary phase cultures to beauvericin (Sigma), either ethanol or dimethyl sulfoxide (DMSO) solutions, at concentrations ranging from 100 nM to 300 μM. The reagent was added to 300 μL of mini-cultures in 96 well plates (Falcon, Bedford, MA, U.S.A) and the cultures were maintained at 27°C. The final concentration of the organic solvent in the cultures was 1% (3% in exceptional cases). Differences between groups were evaluated by the use of t-Student test. No difference in viability was seen when control cultures containing ethanol only (average viability after 24 h ± 70%; n = 3) were compared to control cultures containing DMSO only (average viability after 24 h ± 71.3%; n = 13; p = 0.7; Student’s t-test for unpaired data).

The cultures were examined by phase-contrast microscopy and cell density and viability were assessed by the trypan blue exclusion method at various time
points (from 30’ to 120 h), after the addition of beauvericin. The 50% cytotoxic concentration (CC50) was defined as the concentration of beauvericin that caused a 50% decrease in cell viability. Multiple observations are presented as arithmetic means.

Exposing SF-9 cultures in the early stationary phase to beauvericin at concentrations up to 300 nM did not provoke any decrease in cell viability even when the exposure time was prolonged up to 120 h. Clear effects, instead, were observed when the cells were exposed to concentrations as high as $1 \times 10^9$ M or above (Table 1, Figure 1).

After 4 h of incubation, in cultures exposed to 3 μM beauvericin an 8.7%, s.d. 12, reduction in cell viability was observed, compared to controls. This effect became more significant at higher concentrations: at 10 μM beauvericin, the cell viability was reduced by 16.9%, s.d. 12.3; beauvericin concentrations of 30 μM, 100 μM and 300 μM caused a reduction in cell viability of 28.4%, s.d. 19.1; 50.2%, s.d. 25.9; and 68.2%, s.d. 29.4, respectively (Table 1, Figure 1). The CC50 after 4 h was assessed as approx. 85 μM.

After the 24 h exposure, 1 μM beauvericin caused a decrease in viability of 10.2%, s.d. 11.4; beauvericin at the concentration of 3 μM reduced cell viability by 22.5%, s.d. 15.1. Cultures exposed to higher concentrations of the mycotoxin underwent even more drastic decrease in viability. Thus, beauvericin at 10 μM re-

### Table 1. Cell viability and % decrease in culture viability (arithmetic averages with s.d.) in SF-9 cell cultures after 4 h and 24 h of incubation at various beauvericin concentrations, respectively

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Cell viability</th>
<th>% decrease in cell viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Control</td>
<td>66.9%, s.d. 7.7; n = 13</td>
<td>71.6%, s.d. 5.9; n = 16</td>
</tr>
<tr>
<td>Beauvericin 100 nM</td>
<td>68.2%, s.d. 7.5; n = 4</td>
<td>72.3%, s.d. 7.5; n = 3</td>
</tr>
<tr>
<td>Beauvericin 300 nM</td>
<td>67.2%, s.d. 12; n = 4</td>
<td>70.9%, s.d. 3.3; n = 3</td>
</tr>
<tr>
<td>Beauvericin 1 μM</td>
<td>63.7%, s.d. 7.1; n = 12</td>
<td>63.2%, s.d. 9.3; n = 14</td>
</tr>
<tr>
<td>Beauvericin 3 μM</td>
<td>60.8%, s.d. 4.3; n = 13</td>
<td>55%, s.d. 11.8; n = 15</td>
</tr>
<tr>
<td>Beauvericin 10 μM</td>
<td>54.4%, s.d. 8.1; n = 14</td>
<td>36.5%, s.d. 17.1; n = 16</td>
</tr>
<tr>
<td>Beauvericin 30 μM</td>
<td>46.9%, s.d. 12.6; n = 18</td>
<td>26.6%, s.d. 20; n = 17</td>
</tr>
<tr>
<td>Beauvericin 100 μM</td>
<td>30.2%, s.d. 15.1; n = 13</td>
<td>9.2%, s.d. 14.9; n = 12</td>
</tr>
<tr>
<td>Beauvericin 300 μM</td>
<td>19%, s.d. 15.3; n = 7</td>
<td>4.8%, s.d. 8.5; n = 6</td>
</tr>
</tbody>
</table>
duced the cell viability by 47.1%, s.d. 23.3, while beauvericin at 30 μM, 100 μM and 300 μM caused a decrease in cell viability of 64.9%, s.d. 20.7; 88.6%, s.d. 13.7; and 98.8%, s.d. 1.1, respectively (Table 1; Figure 1). The CC50 after 24 h was estimated as approx. 10 μM (Figure 1).

The cytotoxic effects of beauvericin on SF-9 cells were also tested in experiments in which the cells were exposed for longer periods of time, i.e. 72 h and 120 h. Under these conditions the mycotoxin effects were more pronounced. Thus, beauvericin at 1 mM caused a 15.7%, s.d. 7.6 (n = 3), decrease in cell viability after 120 h, while beauvericin at 3 μM caused a 56.7%, s.d. 6 (n = 3), decrease already after 72 h of incubation. The effects on the cell viability at higher concentrations of beauvericin (10 μM and above) observed after 72 h exposure were similar to those observed after the 120 h exposure: 96.7%, s.d. 4.9 (n=3), and 99.3%, s.d. 0.6 (n = 3), decrease in cell viability at 10 μM, respectively. Beauvericin at 30 μM caused 100% cell death after 72 h. The viability of control was 73%, s.d. 4.4 (n = 3), after 72 h and 83%, s.d. 2.6, n = 3, after 120 h, respectively. The CC50 after 72 h and 120 h was assessed as approx. 2.5 μM, for both experimental times.

With three different time-course assays we also aimed at assessing the minimum exposure time required for beauvericin to cause cytotoxicity, as estimated by the trypan blue exclusion method. Therefore, SF-9 cells were exposed to beauvericin at 30 μM for 30’, 1 h, 2 h, 4 h, 8 h and 24 h. No evident reduction in cell viability was observed up to 24 h.
in viability was observed in cultures exposed to the mycotoxin for periods of time up to 2 h.

Beauvericin was characterized as an entomopathogenic mycotoxin in several invertebrate in vivo models, i.e. the brine shrimp *Artemia salina* (HAMILL et al. 1969), the mosquito *Aedes aegypti* (GROVE, POPLE 1980) and the Colorado potatoe beetles *Leptinotarsa decemlineata* (GUPTA et al. 1991). In 1973 VEY et al. (1973) first reported on the toxicity of beauvericin in an in vitro model, i.e. primary and continuous (LM 75) cultures of cardiac cells from the lepidopter *Leucophaea maderae*. We investigated the effects of this mycotoxin in another invertebrate in vitro model, i.e. the SF-9 cell line, made of immortalized pupal ovaric cells of the moth *Spodoptera frugiperda*. The data collected showed that beauvericin had pronounced cytotoxic effects, even at a low micromolar concentration. Moreover, time-course and dose-response experiments demonstrated that the cytotoxic action of this mycotoxin was dose- and time-dependent. Thus, the CC50 after 4 and 24 h of exposure to beauvericin was assessed as approx. 85 μM and approx. 10 μM, respectively. The CC50 underwent a further decrease after longer exposure times, being 2.5 μM after 72-120 h. Regarding the reproducibility of the results obtained, the relatively high standard deviation values calculated in certain cases are likely to be explained by possible differences in the toxic potency of the various commercial beauvericin batches used for this study.

These results, to our knowledge, are the first ones obtained in an in vitro invertebrate model after the pioneering observations by VEY et al. (1973). They confirm that beauvericin is toxic to insect cells and suggest future applications of this mycotoxin as a biological control agent against insects that parasite agriculturally important plants and agricultural commodities. However, beauvericin effects on the SF-9 cell line were similar to those observed in studies on mammalian cells (HARNOIS et al. 1997, QUE et al. 1997, LOGRIECO et al. 2002, CALO’ et al. 2004). Therefore, a large-scale employment of beauvericin as a biological control agent requires further studies. Finally, we suggest that the SF-9 cell line may be regarded as a suitable tool for studies on mycotoxins intended to be used in biological control agriculture.

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