INTRODUCTION

Meadow vipers (Vipera ursinii) form a group of five subspecies, but their distribution area is highly fragmented and covers Europe, Western and Central Asia. Karst viper, Vipera ursinii macrops (Vum) inhabits high mountain grasslands in Croatia, Bosnia and Herzegovina, Serbia, Macedonia, Montenegro and northern Albania. In Croatia it is a highly threatened viper species that appears only in five isolated localities, some of which are related to Bosna and Herzegovina: southern Velebit, Poštak, Dinara, Troglavl and Kamešnica mountains. Scientific literature on V. ursinii deals only with its morphology, ecology and distribution range due to the species’ conservation problems, while its venom composition and properties have not been investigated. The Karst Viper in Croatia favours high-mountain dry grasslands, on southern and south-eastern exposures, at altitudes from 1100 to 1900 m above the see level. Because of that, meadow vipers are medicinally less significant than other Viper species. Majority of envenoming generally display mild and negligible local symptoms only, which resolve spontaneously within a couple of days without any medical treatment or antivenom therapy. This may be associated with short length of their flanges (2-3 mm) and a very low amount of injected venom (1-4 mg in dry weight), which cannot cause serious systematic symptoms.

AIM

Here we investigate for the first time the composition and biological activity of the Vum venom, in comparison to the venom of Vipera ammodytes ammodytes (Vaa), the most venomous European snake.

RESULTS AND DISCUSSION

The Vum venom is less lethally toxic in mice than the Vaa venom (Table 1), however the pattern of mice dying indicates the presence of a strong neurotoxic component. Interestingly, 2D electrophoresis (Figure 1), as well as Western blot of non-reduced Vum venom with anti-Atx (Figure 2) and ELISA (Figure 3) revealed a lack of ammydotoxin-like proteins in Vum venom. These are well known neurotoxic components of Vaa venom with Mw of 14 kDa and highly basic pl. MS analysis (Figure 4) and ELISA (Figure 3) confirmed the presence of only non-toxic ammydotoxin-like phospholipases. Metalloproteinases are the most abundant components of Vum venom. Accordingly, Vum venom exhibited strong haemorrhagic activity, comparable to that of Vaa venom (Table 1). Antiserum specific for Vaa haemorrhagins recognised Vum haemorrhagins with comparable affinity. Moreover, Vum venom was shown highly instable when dissolved probably due to its very strong proteinases (Figure 5).

Table 1. The lethal toxicity (as LD50, in µg) and haemorrhagic potency (as MHD, in µg) of Vum venom in comparison to Vaa venom. Results are given as mean ± SE.

<table>
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<tr>
<th>Venom</th>
<th>LD50 [µg]</th>
<th>MHD [µg]</th>
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<tr>
<td>Vum</td>
<td>6.4 ± 1.7</td>
<td>37.0 ± 1.5</td>
</tr>
<tr>
<td>Vaa</td>
<td>21.6 ± 4.2</td>
<td>34.1 ± 4.8</td>
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</tbody>
</table>

The Vum venom was a pooled sample obtained by milking of 10 adult snakes, caught at the southern Velebit isolated locality and released afterwards. It was air dried and stored in the dark at 4 °C until use. An air-dried crude Vum venom was from Institute of Immunology Inc., Croatia. Commercial freeze-dried Vum venom (SInvac) was obtained from Serpentarium of the Central Trade Base “Zoolovershles” (private Morzne District, Russia) was the generous gift of Dr. Ivan Štiglic from NCPB, Tbilisi, Estonia.

Materials and methods

Antimal, snake venom(s) and antivenoms

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LITERATURE

Kurtz et al. Toxicon 79 (2014) 103-112
Leonard et al. J. Proteome Res. 11 (2012) 5496-5506

CONCLUSION

Taken all together, V. ursinii venom might be a good starting material for the discovery of novel neurotoxic component in Vipera venoms.