METHODOLOGICAL PROBLEMS OF LAL ASSAY OF BACTERIAL ENDOTOXINS IN HEPARIN SUBSTANCE AND HEPARIN INJECTION

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INTRODUCTION

Bacterial endotoxins are, by chemical nature, lipopolysaccharides which are present in Gram-negative bacteria. During life-time, humans are exposed to minimal amounts of endotoxins which are released in the gastro-intestinal tract after bacteria are killed (mostly Escherichia coli Bergey). This small amount of endotoxins induces in the body the formation of moderate amount of antibodies to endotoxins. However, in case of gastro-intestinal crises (i.e. the obstruction of intestines with the occurrence of gangrene and rapid increase of the number of bacteria), a large amount of endotoxins can penetrate into blood inducing anaphylactic reaction resulting in severe shock (endotoxin shock) [1].

Due to the effect that bacterial endotoxins may have on human health, according to pharmacopoeic requirements, one of the conditions for release of drugs for parenteral use is their testing for bacterial endotoxins. Both compendial requirements (individual monograph in pharmacopoeias) and manufacturers' requirements for specified products or components (i.e. contact components) prescribe maximum bacterial endotoxin limit concentration (ELC) (in: EU/g or EU/mg or EU/IU/ml or EU/ml or EU/surface area/ml (for contact components) for contact components or a specified product. The ELC for Heparin substance and Heparin injection are 0.01 EU/1 IU of heparin [2,3] and 10 EU/1000 IU of heparin/ml [3]. The pharmacopoeial requirements for bacterial endotoxin level in Heparin injection contains the recommendation to use lysate with sensitivity not less than 0.06 EU/ml [3]. However, there is no similar recommendation regarding the level of lysate sensitivity for contains of bacterial endotoxins in Heparin substance [2,3].

This paper presents the results of bacterial endotoxin assays in Heparin substance and Heparin injection using lysates of different sensitivity.

MATERIAL AND METHODS

Bacterial endotoxins in Heparin substance (declared potency: 180.47 IU of heparin/mg) and Heparin injection (declared content: 5000 IU of heparin/ml) were assayed using LAL test, gel-clot method (the method of hard gel formation) [4], as well as the lysates having sensitivities 0.125 EU/ml and 0.06 EU/ml.

* Determination of maximal valid dilution (MVD)

a) Heparin substance: test solution working concentration: 180.47 IU of heparin/ml. With the lysate sensitivity of 0.125 EU/ml, MVD is 1:14.44 and with that of 0.06 EU/ml, MVD is 1:30.08.

b) Heparin injection: test solution working concentration: 1000 IU of heparin/ml. With the lysate sensitivity of 0.125 EU/ml, MVD is 1:80 and with that of 0.06 EU/ml, MVD is 1:166.67.
RESULTS AND DISCUSSION

Table 1
Heparin substance

<table>
<thead>
<tr>
<th>Lysate sensitivity (EU/ml)</th>
<th>0.125</th>
<th>0.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVD 1:14.44</td>
<td>1:30.08</td>
<td></td>
</tr>
<tr>
<td>Positive control of samples</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive control of lysate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Negative control of solvent</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2
Heparin injection

<table>
<thead>
<tr>
<th>Lysate sensitivity (EU/ml)</th>
<th>0.125</th>
<th>0.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVD 1:80</td>
<td>1:166.7</td>
<td></td>
</tr>
<tr>
<td>Positive control of samples</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive control of lysate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Negative control of solvent</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend:
Positive control of sample: the contents should coagulate (+).
Positive control of lysate: the contents should coagulate (+).
Negative control of solvent: the contents should not coagulate (-).
Sample: the coagulation of the contents occurs dependent on the amount of bacterial endotoxin in the tested sample. To pass the test, the contents should not coagulate.

The results show that if a lysate of the sensitivity less than 0.06 EU/ml (in this case 0.125 EU/ml), is used in assaying both Heparin substance and Heparin injection for bacterial endotoxins, THE POSITIVE CONTROL OF SAMPLE does not coagulate. The obtained result shows as if the tested sample contains coagulation-preventing inhibitor substances.

CONCLUSION

The presented results indicate that a lysate of the sensitivity higher than 0.06 EU/ml should be used in assaying both Heparin substance and Heparin injection for bacterial endotoxins. If a lysate with sensitivity less than 0.06 EU/ml is used the contents of the POSITIVE CONTROL OF SAMPLE does not coagulate resulting a false result showing the presence of coagulation-preventing inhibitor substances.

ACKNOWLEDGEMENT

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REFERENCES

[1] G.A. Guyton
Textbook of Medical Physiology

