The Bacterial Endotoxins Specification – Points to Consider

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Learning Objectives

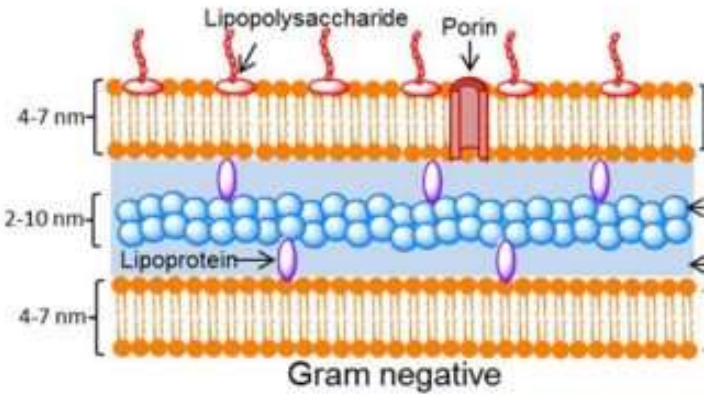
- What are bacterial endotoxins and why they are a patient risk?
- How do you control endotoxins in injectable drug products?
- How do you set acceptance criteria for release/stability endotoxins specifications?

Why am I here?

- People in my office assess the information submitted in Abbreviated New Drug Applications
  - Release/stability specification for bacterial endotoxins
- Too high endotoxins levels in drug products – patient risk
- The release/stability acceptance criterion for endotoxins can be hard to set!

What are endotoxins?

- Endotoxins are a component of the cell wall of Gram-negative bacteria (like *E. coli*)
- Interact with the human immune system to cause an inflammatory response
- Patient risk from endotoxins exposure
  - Fever
  - Shock
  - Death



Adapted from https://www.europeanpharmaceuticalreview.com/article/87191/endotoxin-definition-and-standardisation/

How do we control endotoxins in drugs?

- Endotoxins are not inactivated by most sterilization methods
- Quality by design
  - Control of API/excipients
  - Bioburden control
  - Depyrogenation of container closure system
  - **Release/stability testing**

The endotoxins specification

- Specification = test + method + acceptance criteria
- Test – for bacterial endotoxins
- Method – most typically USP <85> “Bacterial Endotoxins Test”
  - Gel clot
  - Turbidimetric
  - Chromogenic
  - Product-specific suitability testing should be performed
- Acceptance criteria

Setting the acceptance criteria

- USP <85> - Injectable products

$$\textbf{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

Okay so…what are K and M?

The secret to finding K

USP <85> - LAST PAGE!!!

TEENY TINY writing

K – as listed in USP <85>

- 0.2 EU (endotoxin units)/kg body weight/hour (intrathecal)
- <u>5 EU/kg body weight/hour (any route other than intrathecal)</u>
- 100 EU/m^2 body surface area/hour (products administered per meter of body surface area)
- Additional limits for radiopharmaceuticals

Setting the acceptance criteria

$$\textbf{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

- When considering M:
  - Consider the product label
  - Co-packaged products should not collectively exceed the limit

Setting the acceptance criteria

$$\textbf{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

- What weight?
  - OPMA considers 70 kg as the standard adult weight for endotoxins calculations
  - Pediatric weight should be considered – recommend CDC Weight-for-Age Charts

Setting the acceptance criteria

- Consider justifying your endotoxins specification in eCTD section 3.2.P.5.6.
- Discuss your rationale for your K and your M.

Case study 1 (Simple)

- Leuprolide acetate injection
  - Treatment for prostate cancer

- “The recommended dose is 1 mg (0.2 mL) administered as a single daily subcutaneous injection”

- Subcutaneous injection – not intrathecal
- No pediatric indications listed in label
- No kit – just drug product

Case study 1 (Simple)

- Leuprolide acetate injection

$$\textit{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

$$\textit{Endotoxins Acceptance Criteria} = \frac{5\ \frac{EU}{kg}}{\frac{1}{70}\ \frac{mg}{kg}}$$

Taken directly from USP <85> - this product is not administered intrathecally

1 mg dose for a 70 kg adult patient

$$\textit{Endotoxins Acceptance Criteria} = 5\ \frac{EU}{kg}\ x\ \frac{70}{1}\ \frac{kg}{mg}$$

$$\textit{Endotoxins Acceptance Criteria} = 350\ EU/mg$$

Case Study 2 (Complex)

- Levetiracetam
  - Anti-epileptic drug, many formats including injectable (intravenous)

This tells us that
K will be 5 EU/kg

Partial-Onset Seizures (monotherapy or adjunctive therapy)

- 1 Month to < 6 Months: 7 mg/kg twice daily; increase by 7 mg/kg twice daily every 2 weeks to recommended dose of 21 mg/kg twice daily (2.1)
- 6 Months to < 4 Years: 10 mg/kg twice daily; increase by 10 mg/kg twice daily every 2 weeks to recommended dose of 25 mg/kg twice daily (2.1)
- 4 Years to < 16 Years: 10 mg/kg twice daily; increase by 10 mg/kg twice daily every 2 weeks to recommended dose of 30 mg/kg twice daily (2.1)
- Adults 16 Years and Older: 500 mg twice daily; increase by 500 mg twice daily every 2 weeks to a recommended dose of 1500 mg twice daily (2.1)

Myoclonic Seizures in Adults and Pediatric Patients 12 Years and Older

- 500 mg twice daily; increase by 500 mg twice daily every 2 weeks to recommended dose of 1500 mg twice daily (2.2)

Primary Generalized Tonic-Clonic Seizures

- 6 Years to < 16 Years: 10 mg/kg twice daily; increase by 10 mg/kg twice daily every 2 weeks to recommended dose of 30 mg/kg twice daily (2.3)

Case Study 2 (Complex)

- Levetiracetam
  - Worst-case exposure – 1500 mg in 12 yo patient

  - CDC – 144.5 month <u>female</u>, 50th percentile weight = 41.8 kg
  - 1500 mg/41.8 kg = 36 mg/kg

Case Study 2 (Complex)

Yes – 36 mg/kg is our highest dose. Now we have our M!

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$$\textbf{Endotoxins Acceptance Criteria} = \frac{5\ \text{EU/kg}}{36\ \text{mg/kg}} = \mathbf{0.14\ EU/mg}$$

Resources

- United States Pharmacopeia Chapter <85> - Bacterial Endotoxins
  - Remember – K is on the last page in teeny tiny writing
- United States Pharmacopeia Chapter <771> - Ophthalmics Products – Quality Tests
- CDC Weight for Age charts
  - https://www.cdc.gov/growthcharts/html_charts/wtage.htm
- FDA Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers (June 2012)
- FDA Guidance for Industry: Controlled Correspondence Related to Generic Drug Development (March 2024)
  - Explain your rationale

Recap

- Exposure to excessive endotoxins is a risk to patients.
- Process design AND end-product testing are important for the control of endotoxins.
- The United States Pharmacopeia chapter <85> contains information on appropriate endotoxins acceptance criteria.
- The release/stability acceptance criteria should be set using information from the product label.

Challenge Question 1

- The acceptance criterion for the endotoxins specification for a subcutaneous injection should be calculated from the formula provided in USP <85>.

1. True
2. False

Challenge Question 2

- K is the same for all injectable products
  1. True
  2. False

Thank you!

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