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In vitro **Toxicology**

In vitro Hemolysis

Background Information

'For excipients intended for injectable use, an *in vitro* hemolysis study could be performed at the intended concentration for I.V. administration (bolus and/or infusion) to determine the hemolytic potential.'

FDA Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients (May 2005)

- Drug-induced hemolysis is a relatively rare but serious toxicity liability.

  It occurs by two mechanisms[1]:

  - Toxic hemolysis — direct toxicity of the drug, its metabolite, or an excipient in the formulation.
  - Allergic hemolysis — toxicity caused by an immunological reaction in patients previously sensitised to a drug.

  Cyprotex's *in vitro* hemolysis testing is designed to screen for toxic hemolysis as recommended by the US FDA[2].

- Although the majority of normal individuals may suffer toxic hemolysis at sufficiently high concentrations of hemolytic drugs, for most drugs toxic hemolysis involves lower doses given to individuals who are genetically predisposed to hemolysis[1].

- The US FDA recommends that for excipients intended for injectible use, an *in vitro* hemolysis study should be performed at the intended concentration for IV administration to test for hemolytic potential[2].

- The *in vitro* hemolysis assay evaluates hemoglobin release in the plasma (as an indicator of red blood cell lysis) following test agent exposure.

Protocol

Test System
Anticoagulant treated whole blood

Species
Human and rat (other species available)

Incubation Time
45 min at 37ºC

Test Article Concentration
8 concentrations up to 250 µM or the highest concentration at which the compound is soluble; two-fold dilutions

Number of Replicates
N=3 per concentration

Quality Controls
Vehicle (negative control)
Triton X-100 (positive control)

Test Article Requirements
50 µL of 20 mM solution

Analysis Method
Spectophotometry at 540 nm

Data Delivery
Degree of lysis at each concentration
IC_{50}

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Hemoglobin released into plasma can be assessed as a measure of red blood cell lysis following drug/chemical exposure.

Table 1

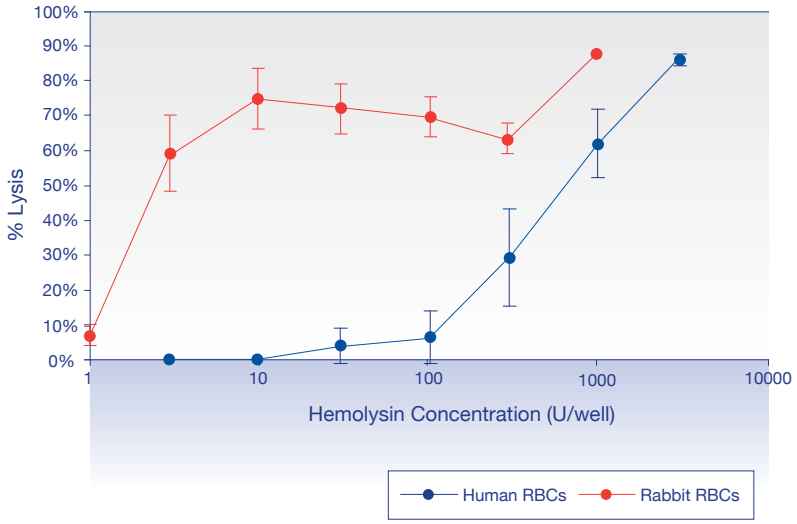
Hemolysis by Control Compounds

Compound	Concentration (%, v/v)	% Lysis
Triton X-100	1%	70%
Triton X-100	0.1%	14%
Triton X-100	0.01%	2%
SDS	1%	82%
SDS	0.1%	18%
SDS	0.01%	7%
Saponin	1%	57%
Saponin	0.1%	19%

Human erythrocytes were treated with agents known to cause hemolysis. The percent lysis increased with increasing concentrations of the agents.

Figure 1

Hemolysis by Hemolysin in Human and Rabbit Erythrocytes



Rabbit erythrocytes are much more susceptible to hemolysis by the bacterial exotoxin, hemolysin, than human erythrocytes, demonstrating the need to use human erythrocytes in hemolysis experiments.

References

[1] Dausset J and Contu L (1967) *Ann Rev Med* **18**; 55-70
[2] FDA Guidance for Industry - Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients (May 2005)
[3] Lubran MM (1989) *Ann Lab Clin Sci* **19**; 114-21
[4] Deibler GE (1959) *J Appl Physiol* **14**; 133-6