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A new hypothesis for the EPREX case: The rubber leachable Vultac© binds covalently to EPO

Summary

- Poly-tert-butylphenol disulfide (trade name Vultac© TB7) was able to covalently modify EPO.
- This is the first time, at least to our knowledge, that such a modification could be confirmed experimentally.
- The disulfide bonds of EPO are the vulnerable sites for Vultac© attack.
- In the presence of human serum albumin (HSA), Vultac© reacts preferred with HSA, thereby protecting EPO against modification.

Introduction

As described by Sharma [1], leachables consisting of di- and polysulfides of Vultac© 2 (tert.-amylphenol derivatives) were found in EPREX© prefilled syringes. This leachables are suspect to be the cause of an increased frequency of PRCA (pure red cell aplasia). The API in EPREX© is Epoetin alfa (EPO), a glycoprotein consisting of 2 disulfide bridges.

Results*

After tryptic digest (without a reduction step) and deglycosylation by PNGase of Vultac© treated EPO, all peptides containing a cysteine could be detected either modified by addition of Sx (x-times sulfur) or modified by addition of tert-butylphenol Sx (TBPSx). The disulfide bridge in peptide EAENITTGCAEHCSLNENITVPDTK was extended by insertion of 1 or 2 sulfur (tri-and tetra-sulfides). In peptide LYTGEACRLICDSR, the disulfide bridge was cleaved, resulting in 2 modified peptides LYTGEACR+TBPS (or+TBPSS) and LICDSR +TBPS (or+TBPSS).
The sequence of this peptides was confirmed by tandem MS experiments.

The results were presented at the E&L conference Europe, 2 December 2020 and can be accessed via DOI: 10.13140/RG.2.2.16360.90885.

*: Generated in collaboration with A&M Stabtest GmbH, Bergheim, Germany,

Hypothesis

Polysulfides of the Vultac© class can react with proteins by covalent binding to the disulfide bond. In the presence of free thiols like in HSA, polysulfides are trapped. As HSA was replaced by polysorbate, free polysulfides were able to react with EPO. The assumption is, that not the addition of polysorbate, but the removal of HSA is the main cause of EPO related adverse effects.

However, the immunogenicity of Vultac© modified EPO has to be investigated to proof or reject the hypothesis.

[1] Sharma et al, Eur. J. Hosp. Pharm 5, 2004