Diamide Does Not Improve The Analysis Of IL1β And TNFα In Dithiothreitol-treated Sputum Supernatants

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Introduction
There is one report (Milone et. al. ERS 2012) that diamide can be used to oxidize disulfide bonds that were separated by dithiothreitol (DTT) treatment during the homogenization of sputum samples, a process that can affect the analysis of mediators in sputum supernatants. It was the aim of this study to test this method for two selected cytokines in sputum samples of healthy subjects induced before and after inhalation of LPS.

Methods
Three sets of experiments were performed:

1. IL1B and TNFα ELISA standards (R&D DuoSet) were treated with DTT (final 0.05%) or PBS and frozen. After thawing different concentrations of diamide were added (3.75-60 mM), incubated for 30 min and then analysed by ELISA.

2. DTT treated sputum supernatants from 8 healthy subjects at baseline and following LPS challenge (Holz et al. BMC Pul Med 2012) were thawed and incubated with diamide (7.5 or 60 mM) to test if the addition of diamide had any effect.

3. Pooled DTT treated sputum supernatants were spiked with IL1B and diamide (2-120 mM) was added before freezing or after thawing.

Results
Treatment of assay standards with DTT resulted in a significant decline in the slope of the standard curves, which was reversed to the level of PBS treated standards by the addition of diamide. The effect was smaller for TNFα, which was already very sensitive to PBS treatment (>60% reduction).

The median concentration of IL1B after LPS treatment was 56.3, 61.6 and 56.5 pg/ml (without, 7.5, 60mM diamide) and only 2/8, 4/8 and 2/8 samples had detectable IL1B levels at baseline. IL1B levels in sputum supernatants were clearly detectable without addition of diamide and did not further increase with diamide. No effect of diamide was observed for TNFα and most baseline and LPS sputum supernatants were below the detection limit.

The median recovery of IL1B was 49 and 66 % (125 and 15.6 pg/ml spike) when diamide was added before freezing and 47.6 and 61.7 % when diamide was added after thawing. The recovery did not differ from samples that were not treated with diamide.

Conclusion
In our hands the addition of diamide was not successful in reversing the negative effects that DTT treatment has on the analysis of IL1B and TNFα in sputum supernatants. As DTT treatment remains to be the most simple and economic method to homogenise sputum, its effects on novel assays and markers need to be checked in advance.

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