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Missing evidence for toxicity of high PFAT5 levels in mixtures of lipids

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SUMMARY

Background: The compliance of lipid admixtures to physical emulsion stability parameters is extremely important to ensure the safety of patients. For example, admixtures containing a percentage of fat globules larger than 5 μm in diameter (concept known as PFAT5) of more than 0.05% might produce toxic effects in lung and liver. This concern is mainly based on a limited number of animal studies investigating admixtures with high PFAT5 levels resulting from 48 h of admixture storage. However, all effects observed in these studies might as well be attributed to chemical instability like lipid oxidation, which was not analysed and therefore could not be excluded.

Aims: This study aims at investigating the correlation of high levels of PFAT5 in lipid emulsion admixtures with lipid oxidation parameters under different storage conditions.

Methods: We studied the physical (PFAT5 value) as well as the chemical (pH, primary and secondary oxidation parameters) stability of an admixture of a lipid emulsion and an amino acid solution after up to 48 h following different storage conditions (exposure to oxygen, exposure to artificial light).

Abbreviations: ALT, alanine amino transferase; Anv, p-anisidine value; AST, aspartate amino transferase; GST, glutathione-S-transferase; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; PN, parenteral nutrition; USP, US pharmacopeia.

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Results: High levels of PFAT5 were only observed after exposure of the admixture to oxygen. Additional exposure to artificial light led to a parallel increase in the primary and secondary oxidation parameters, while the pH was unchanged.
Conclusions: The admixtures investigated in the former animal studies were obviously both physically and chemically unstable and all effects observed in the studies could just as well be caused by chemical instability, namely the administration of lipid peroxides with the admixture.

1. Introduction

The stability of lipid emulsions and their admixtures during the infusion period is paramount to the safety of the patients. Thus, the intravenous infusion of lipid emulsions over a certain period of time should comply with pharmacopeia specifications to achieve the potential benefits of the lipid emulsions without the adverse effects consequent to physical or chemical instability.

Physical instability occurs when the net negative charge upon the lipid droplets is reduced by the presence of oppositely charged cations, which are introduced from admixture components such as electrolytes, minerals or amino acids. The domination of the positive attractive forces over the negative repulsive forces favours the coalescence of oil droplets. Infusion of fat globules exceeding the internal diameter of the microvasculature (5 μm) are assumed to induce fat embolism in the lungs, therefore in lipid emulsions the volume-weighted percentage of fat globules greater than 5 μm, the "PFAT5 level", has to be below 0.05% [1]. The US pharmacopeia (USP), however, does not regulate the PFAT5 limit of admixtures.

The potential toxicity of lipid admixtures with high PFAT5 level has been investigated in several animal studies [2–5]. One of these studies addressed the toxic effects of a high PFAT5 level on the lung. In this study, two admixtures of a lipid emulsion with an amino acid solution were used: One prepared shortly before infusion and one prepared 48 h in advance, leading to the infusion of an "aged", unstable solution with a high PFAT5 level. Surprisingly, no microvascular thrombosis was reported in the lungs after a 24 h infusion of the unstable admixture to guinea pigs [5]. In contrast, these animals showed an increased cellularity in the lung [5]. This raised the question whether in the respective study a factor other than PFAT5 was responsible for the unexpected type of lung pathology. Interestingly, malondialdehyde (MDA) was also increased in the lungs of the guinea pigs treated with the unstable admixture [5]. In general, MDA results from lipid peroxidation of polyunsaturated fatty acids [6]. It can either arise endogenously during oxidative stress or exogenously from lipid admixtures containing high levels of polyunsaturated fatty acids in the presence of oxygen and/or ambient light during long-term storage [7]. Of note, lipid peroxidation end products are not limited to MDA and also include other aldehydes like 4-hydroxynonenal (4-HNE). Therefore, if MDA was administered to the guinea pigs, the even more toxic 4-HNE was administered as well [8]. Administration of 4-HNE induces mitochondrial dysfunction in human airway epithelial cells *in vitro* [9] and leads to the accumulation of inflammatory cells in the lung *in vivo* [10], similar to the observed changes in the guinea pigs [5]. Chemical instability of the admixture infused to the guinea pig therefore is another potential explanation for the observed changes, especially as neither protection from light nor protection from oxygen has been reported in the guinea pig study [5].

We hypothesized that the admixtures infused in the respective animals studies were chemically unstable despite limited time (48 h) of potential exposure to oxygen and artificial light. Therefore we have studied the physical (PFAT5 level) and chemical (pH, primary and secondary oxidation