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Do surface active parenteral formulations cause inflammation?

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ABSTRACT

Local irritation and inflammation at the site of administration are a common side effect following administration of parenteral formulations. Biological effects of surface (interfacial) activity in solutions are less well investigated than effects caused by other physico-chemical parameters such as pH and osmolality. The interfacial activity in different systems, including human plasma, typical amphiphilic substances with fundamental biological relevance such as free fatty acids, anesthetic depot formulations and six different antibiotics was measured. The relative interfacial pressure, and/or concentration of active substance, required to obtain 50% of the maximal attainable effect in terms of interfacial pressure were calculated. The aim was to test the hypothesis that these parameters would allow comparison to biological effects reported in in vivo studies on the investigated substances. The highest interfacial activity was found in a triglyceride/plasma system. Among the antibiotic tested, the highest interfacial activities were found in erythromycin and dicloxacillin, which is in accordance with previous clinical findings of a high tendency of infusion phlebitis and cell toxicity. Independently of investigated system, biological effects were minimal below a 15% relative increase of interfacial activity. Above 35–45% the effects were severe. Interfacial activity in parenteral formulations may well cause damages to tissues followed by inflammation.

1. Introduction

European and US pharmacopoeias recommend that physico-chemical parameters such as osmolality and pH are adjusted in parenteral preparations to avoid toxicity or local irritation. There is no similar recommendation regarding interfacial (surface) activity, despite the fact that it is known that interfacial active agents may cause toxic effects.

It is evident from animal experiments that intravenous administration of solutions or emulsions containing typical amphiphilic agents such as free fatty acids (FFAs), causes marked and acute toxicity (Orö and Wretlind, 1961; Connor et al., 1963). At levels above normal physiological concentrations, FFAs can induce morphological changes in erythrocytes both in vitro and in vivo (Kamada et al., 1987; Söderberg et al., 2009), and several diseases are associated with elevated concentrations of FFAs (Dhainaut et al., 1987; Lefevre et al., 1988; Roden et al., 1996; Egan et al., 1999; Kurien and Oliver, 1966; Oliver, 1972; Mozaffarian et al., 2006).

In a previous study, injectable anesthetic depot formulations (lidocaine–prilocaine 1:1) were tested on rats to induce ultra-long nerve blockades by administration directly next to the sciatic nerve (Söderberg et al., 2006). Below a concentration of 20% active

Abbreviations: C, concentration; $EC50_{in\ vitro}$, concentration required to obtain 50% of the maximal attainable effect in terms of measured interfacial pressure in vitro; n_H, Hill coefficient; Π, interfacial pressure; $Π_{max}$, maximal attainable interfacial pressure; $Π_{rel}$, relative interfacial pressure; $γ_0$, interfacial tension of the pure fluid/solution (reference system); $γ$, interfacial tension of the pure fluid/solution including the active substance; $C_{2:0}$, acetic acid; $C_{6:0}$, caproic acid; $C_{8:0}$, caprylic acid; $C_{10:0}$, capric acid; $C_{12:0}$, lauric acid; $C_{16:0}$, palmitic acid; AP, ampicillin; BP, benzylpenicillin; CE, cefuroxime; CX, cloxacillin; DC, dicloxacillin; ER, erythromycin; EDTA, edetate dipotassium; FFAs, free fatty acids; HSA, human albumin; PBS, phosphate-buffered saline solution; EaHy926, endothelial hybrid cell line; HUVEC, human umbilical vein endothelial cell; ICAM, intercellular adhesion molecule.

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substance in the formulation, the duration was short, and histopathological findings (such as inflammatory responses) were only minimal and occasional. Above a concentration of 60%, the duration of nerve block was prolonged, but inflammatory responses were marked and frequent. The mechanisms responsible for these observations are not fully understood, but both lidocaine and prilocaine express interfacial activity at physiological pH.

The high incidence of infusion phlebitis in the clinical setting indicates a significant problem associated with the administration of parenteral formulations. Based on studies from the 1980s, it was estimated that 1.3 million patients in the United States alone, suffer from infusion phlebitis annually (Stonehouse and Butcher, 1996). A review of the research on the topic reveals that many factors may be involved, such as infusion/flow rate, duration, catheter diameter and material, other drugs administered, insertion site, gender etc. Although several studies have been carried out to quantify specific risk factors, the vast numbers of factors involved makes it difficult to pinpoint the key factors affecting safety. Parenteral administration of antibiotics is associated with particularly high risks (Lanbeck and Paulsen, 1995, 2001; Lanbeck et al., 2002, 2004). The risk varies depending on the antibiotic, and here dicloxacillin and erythromycin causes the highest vessel irritation and are the most toxic to endothelial cells in vitro (Lanbeck et al., 2002, 2004).

The side effects of injectable preparations, including hemolysis, muscle contractions and peripheral nerve injuries, have been well known for a long time (Svendsen, 1983; Svendsen et al., 1985; Comereski et al., 1986; Sutton et al., 1996). Oshida et al. investigated approximately 300 different preparations used in Japan in the late 1970s, by measuring physico-chemical parameters such as the pH and the osmotic properties (Oshida et al., 1979). They found wide ranges in relative osmotic pressure from 0.2 to 36 (relative to that of physiological saline solution) and pH 1.4–12.8. The hemolytic potential of the preparations tested was closely related to the severity of muscle lesions in their animal experiments, but provoking erythrocytes using sodium chloride solutions of serial concentrations with increasing osmotic ratio (0.5–8) caused no corresponding hemolysis. Unfortunately, no results from interfacial activity measurements on these formulations were reported.

Although several risk factors are reported in the literature, surprisingly little attention has been paid to the possible correlation between interfacial activity and toxic or inflammatory responses caused by the administrations of parenteral formulations or for that matter, a possible correlation between interfacial activity in biological systems and the occurrence of sterile inflammation.

The aim of this study was to elucidate the role of interfacial activity as a risk factor in relation to tissue damage and inflammation at the site of administration. To do this, the interfacial tension of solutions containing either biologically relevant interfacial active agents such as FFAs, lidocaine:prilocane anesthetic depot formulations and ordinary (water-based) antibiotic parenteral formulations associated with high risk in the clinical setting, was measured. The results were compared to findings from previous studies on cell cultures (Söderberg et al., 2009; Lanbeck et al., 2004), a rat model (Söderberg et al., 2006) and observations in the clinic (Lanbeck et al., 2002).

2. Materials and methods

2.1. Materials

Ampicillin sodium (Doktacillin®, Meda), bensylpenicillin sodium (Bensylpenicillin®, Meda), cefuroxime sodium (Zinacef®, GlaxoSmithKline), cloxacillin sodium (Cloxacillin Stragen, Stragen Nordic), dicloxacillin sodium (Dikloxacillin Meda, Meda), erythromycin lactobionate (Abboticin®, Amdipharm) and human albumin (Albumin Behring CLS GmbH) 200 g/l (HSA), were supplied by the Malmö University hospital pharmacy. Solutions were diluted to their final concentrations using distilled water. Saturated fatty acids (purity >99%) were purchased from Sigma–Aldrich. Medium chain triglyceride oil (triglyceride) and lidocaine base were of European Pharmacopoeia quality, and were supplied by Apoteket AB (Sweden). Prilocaine base was produced by Synthelec AB. Saturated fatty acids and lidocaine:prilocaine were diluted in medium chain triglyceride oil to final concentrations as previously described (Söderberg et al., 2009, 2006). Chemicals for the preparation of phosphate-buffered saline solution (pH 7.4) (PBS) were of analytical grade. Human plasma was obtained by collecting venous blood in a Vacutainer (Becton Dickinson AB). This procedure yields a concentration of 5 mM of edetate dipotassium (EDTA), a standard method described previously (Söderberg et al., 2009).

2.2. Interfacial tension measurements and calculations

The interfacial tension measurements were performed with a Tracker Drop Tensiometer (Teclis, Longessaigne, France). Each formulation was tested in duplicate at 37 °C. Mean values were calculated from duplicate measurements made over 5 min for interfaces to air and 10 min for systems including lipids. The reason for using a shorter time interval for the interfacial tension measurements of water solutions was that it was assumed that during intravenous infusion or injection in the clinical setting, the formulation is immediately washed away from the site of administration by the blood stream. Immediate response can thus be expected. The time interval for measurements on systems including free fatty acids was chosen to correspond to the exposure time used in a previous study on the effects of fatty acids on red blood cells (Söderberg et al., 2009). In addition, the adsorption kinetics are expected to be rapid due to the small size of the molecules tested.

The interfacial pressure (Π) is obtained from Eq. (1), where γ_0 is the interfacial tension of the pure fluid (reference system) and γ is the interfacial tension of the solution including the active substance.

$$\Pi = \gamma_0 - \gamma \quad (1)$$

The concentration required to obtain 50% of the maximal attainable effect in terms of measured interfacial pressure in vitro ($EC50_{\text{in vitro}}$), and the "Hill coefficient" (n_H), describing the shape of the curve and degree of cooperativity were estimated by curve fitting to Hill's equation (Eq. (2)). In this expression, Π is the interfacial pressure measured at concentration C, and Π_{max} the maximal attainable interfacial pressure.

$$\Pi = \frac{\Pi_{max} \times C^{n_H}}{C^{n_H} + EC50_{\text{in vitro}}} \quad (2)$$

The relative interfacial pressure (Π_{rel}) is calculated using Eq. (3).

$$\Pi_{rel} = \frac{\Pi}{\gamma_0} \quad (3)$$

Non-linear least square regression analysis was performed using SCIENTISTS software (Micromath Scientific Software, Salt Lake City, UT, USA).

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