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Hyaluronic acid as a modulator of the cytotoxic effects of cationic surfactants

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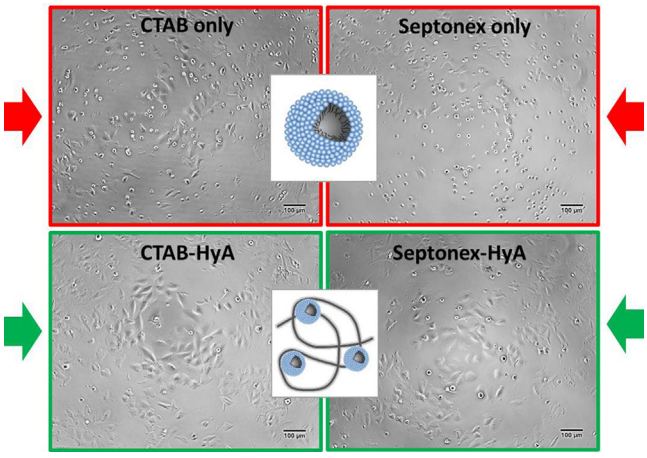
HIGHLIGHTS

- Surfactants (CTAB and Septonex) are toxic for osteoblasts.
- When complexed with hyaluronan their cytotoxicity is suppressed.
- Fetal bovine serum plays a positive role in the cytotoxicity suppression.

GRAPHICAL ABSTRACT



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ABSTRACT

CTAB (cetyltrimethylammonium bromide) and Septonex (carbethoxypendecinium bromide) are cationic surfactants known for harmful effects on different cell types (bacteria, fungi, mammal cells, etc.). Colloidal complexes of CTAB or Septonex with oppositely charged hyaluronic acid (HyA), based primarily on electrostatic interactions, were prepared with the aim to test potential modulation of surfactants cytotoxic effects. Complexes were tested for their cytotoxicity on human osteoblasts—the cell metabolic activity was determined after 24 h of treatment. Our data show that CTAB–HyA or Septonex–HyA complexes reduce (in different rate according to the used surfactant and HyA concentrations) cytotoxicity of both surfactants in all tested concentrations. In addition, a significant role of fetal bovine serum (important supplement of cell culture medium) in cell recovery under the stress conditions like CTAB or Septonex presence was observed. Taken together, HyA could be a useful modulator of CTAB or Septonex effects on cells at diverse levels. Drug or nucleic acid delivery system, diagnostic dye carriers or cosmetic industry are the possible applications of prepared complexes.

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1. Introduction

Cationic surfactants are known for their cytotoxic properties [1–4] but due to their interactions with negatively charged substances – some kinds of drugs, nucleic acids, cellular surfaces, etc. – they can serve as an interesting tool in drug or gene cell delivery, for the study of cell trafficking processes, or in other cell structure visualisation techniques. The ability to form micelles is another positive property of surfactants and a benefit which is used in drug carriers [5]. Cetyltrimethylammonium bromide (CTAB), in particular, is commonly used as a compound in drug delivery systems; for example, it is an ideal "shape-inducing" agent [6]. In general, it is known that cationic surfactants exhibit the highest cytotoxicity in comparison to anionic and non-ionic ones [7]. In spite of this, several studies have shown the anticancer effect of CTAB or other molecules containing the quaternary ammonium group [8–11]. Additionally, it was showed that these surfactants can behave as cytotoxic agents in dependence on the target cell type – surfactants were substantially cytotoxic to non-polarized cells in contrast to polarized cells [12]. Interestingly, it was shown that CTAB cytotoxicity can be depressed by polymers: Alkilany et al. reduced the CTAB-induced cytotoxicity of a CTAB-capped nanorods solution by PAA (polyacrylic acid) polymer over-coating [13].

The ideal polymer for our study, aimed at surfactant cytotoxicity modulation by forming complexes with oppositely charged biopolymer, appeared to be hyaluronan (HyA), because in our previous study, the reduction of the cytotoxic effects of CTAB on specific cell types in the presence of free sodium hyaluronate (HyA) was described [14]. Hyaluronan is a naturally occurring glycosaminoglycan composed of repeating β-1,4-D-glucuronic acid and β-1,3-*N*-acetyl-D-glucosamine disaccharide subunits [15]. HyA exhibits a wide spectrum of functions at various organism levels [16] and due to its favourable properties – biocompatibility, biodegradability, unique biomechanical features, and modifiability (functional groups) – HyA is called a biomaterial of the near future. Many HyA functions are conditioned by interactions with HyA-binding proteins, which are specific to the place of concrete HyA action [17–20].

As mentioned above, surfactants cytotoxicity could be regulated when complexed with hyaluronan. However, HyA in these complexes can play more roles, not only cell protecting but also it can also help the complex to bind onto the cell surface (via its receptors) and subsequently to move within the cell. Thus, the surfactant-HyA complex can serve as a carrier of non-polar drugs solubilized within the cores of surfactant micelles. HyA is degraded by hyaluronidases (Hyals), especially Hyal1 and Hyal2. Extracellular HyA is attached to Hyal2 anchored in the cell membrane and then cleaved [21]. It seems that this process is in cooperation with the HyA receptor CD44. HyA is then transferred into the cell by endocytosis. In lysosomal vesicles, HyA is cleaved again, but by HyAl1 and then by exoglycosidases into monomers [16,22,23]. This pathway alone could be a way for the delivery of complexes to cells. The effects of HyA on cells have been well described, mostly thanks to its wide medical applications and the needs of regenerative medicine [24–31].

In this work, CTAB–HyA and Septonex–HyA complexes were prepared and their cytotoxicity was determined in comparison to native surfactants. In contrast to the previous study [14], in which the surfactant and hyaluronan were added to cells separately one at a time, the pre-prepared surfactant–HyA complexes were applied on cells. Further, Septonex, a structural analogue of CTAB, was also investigated. In addition, we were interested in the role of fetal bovine serum (FBS) in the ability of cells to overcome stress conditions (i.e. the presence of native surfactants or surfactants–HyA complexes). FBS (the blood fraction after clotting, free of blood cell elements) is a crucial component of the cell growth medium because it provides supplements important for cell cultivation in vitro (for adhesion, division, survival etc.). However, the major compound of FBS, bovine serum albumin, is known to interact with various molecules (it provides a variety of binding sites for both hydrophobic and negatively charged hydrophilic moieties), and the behaviour of surfactants in complexes could be affected by this protein [33,34]. Moreover, it has already been demonstrated that cell behaviour and morphology can be substantially influenced by the presence or absence of FBS in general [14,35].

Surfactants with regulated cytotoxicity might play a role in drug, gene, or diagnostic dye carriers (thanks to the use of the natural HyA-transport system) and could exhibit only a moderate and controllable antiseptic activity (thanks to HyA's protective activity). The results could help to raise the profile of surfactant–HyA complexes with respect to their use in practical cell biology and clinical applications.

Table 1
Composition of hyaluronan–surfactant complexes and their zeta potential (values in parentheses represent the standard deviation).

CTAB (mM)	HyA (mg/l)	Zeta potential (mV)
0.04	5	−14(2)
0.05	5	−8(2)
0.05	30	−15(2)
0.05	50	−34(5)
0.08	30	−29(1)
0.08	50	−19(5)
0.10	30	−26(1)
0.10	50	−28(4)
Septonex (mM)	**HyA (g/l)**	**Zeta potential (mV)**
0.03	1	−70(1)
0.06	1	−72(2)
0.08	1	−68(3)

2. Materials and methods

2.1. Surfactants and hyaluronan

Cetyltrimethylammonium bromide (CTAB) was purchased from Sigma–Aldrich (Czech Republic) and carbethoxypendecinium bromide (Septonex) from GNB chem (Czech Republic), both used as received. Hyaluronan was purchased from Contipro Biotech (Czech Republic); two batches were acquired—one with a weight-average molecular weight of 1000 kDa was used in the preparation of complexes with CTAB, while the other with a weight-average molecular weight of 936 kDa was used in complexes with Septonex.

Solutions of complexes were prepared by mixing hyaluronan and surfactant stock solutions, prepared in deionized water, to obtain the desired final concentration. The surfactant solution was always added dropwise to the hyaluronan solution. In the case of CTAB–hyaluronan complexes, the hyaluronan concentration had to be sufficiently low in order to prevent precipitation and prepare homogeneous solutions of complexes. The concentrations of CTAB in complex solutions were 40, 50, 80 and 100 μM; three hyaluronan concentrations were tested: 5, 30 and 50 mg/l. In the case of Septonex–hyaluronan complexes, only one hyaluronan concentration was used (1 g/l) at three different surfactant concentrations: 30, 60 and 80 μM. Eleven different samples of complexes were thus prepared and used in experiments; their exact composition is given in Table 1.

The prepared complexes were characterized by their particle size distribution (measured by dynamic light scattering) and their zeta potential (measured by laser Doppler micro-electrophoresis) using a Zetasizer Nano ZS (Malvern Instruments, UK).