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Comparison of different ibuprofen-amino acid compounds with respect to emulsifying and cytotoxic properties

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Abstract

Sodium ibuprofen (Ibu-Na) and different ibuprofen-amino acid compounds, lysinate (Ibu-Lys), arginate (Ibu-Arg) and histidinate (Ibu-His), were evaluated for emulsifying, haemolytic and cytotoxic properties.

The highest reduction of surface tension was obtained with Ibu-Lys which shows good emulsifying qualities. At the same time, Ibu-Lys reveals the highest haemolytic activity and affects porcine cornea integrity. However, incorporation of Ibu-Lys into an emulsion system significantly decreases haemolysis.

On the contrary Ibu-Arg, which shows a lower surface tension reduction, allows, unlike Ibu-Na and Ibu-His, for comparably stable emulsions with comparable erythrocyte damage.

Keywords: Ibuprofen; Lysine; Arginine; Histidine; Emulsions; Haemolysis; Histology; Cornea

1. Introduction

Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), can be applied orally and topically as an alternative to a corticosteroidal therapy in inflammation treatment (Tilden et al., 1990; Castell et al., 1987).

For pain relief different ibuprofen salts, especially the lysine salt, are used. In vivo studies have shown a higher bioavailability due to an increased water solubility and dissolution rate (Martin et al., 1990). Ibuprofen lysinate is also found in solutions for intramuscular (Imbun®, i.m.) application or dermal formulations (Dolgit®).

Ocular inflammation therapy with NSAIDs requires, due to low permeation rates (Gupta and Majundar, 1997; Schoenwald and Barfknecht, 1990) and a rapid pre-corneal loss, a frequesnt application and/or highly concentrated eye drop formulations (Pignatello et al., 2001, 2002). Therefore, the elevated water solubility of ibuprofen lysinate is also advantageous.

As ibuprofen itself is surface active its application is connected with local tissue irritation and ocular discomfort (Chakradhara et al., 1992; Han and Roehrs, 1985).

The primary objectives of this study were to evaluate ibuprofen lysinate properties respecting emulsifying and cytotoxic characteristics as compared to sodium ibuprofen. Further ibuprofen-amino acid (Ibu-AA) compounds, ibuprofen arginate and histidinate, were synthesised and examined as well. Solu-

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tions were described regarding reduction of surface tension, haemolysis and influence on porcine cornea tissue integrity. Emulsions were assessed for haemolysis as well as formation and stability properties in contrast with a placebo emulsion.

2. Materials and methods

2.1. Materials

Ibuprofen acid (Ibu-H), sodium ibuprofen (Ibu-Na) and ibuprofen lysinate (Ibu-Lys) were provided by Salutas Pharma GmbH (Barleben, Germany), lysine (Lys), arginine (Arg) and histidine (His) were obtained from Fresenius Kabi (Friedberg, Germany), hydroxypropylmethylcellulose type Metolose 90 SH 400 (HPMC 400), substitution type 2208, USP, from Shin Etsu (Tokyo, Japan), rectified castor oil from Henry Lamotte (Bremen, Germany), Arlacel 83 from Uniqema (Gouda, the Netherlands), sorbitol from Caesar & Loretz (Hilden, Germany), sodium dodecyl sulfate (SDS) from ICN (Eschwege, Germany); sodium chloride, potassium dihydrogen phosphate and disodium hydrogen phosphate (all pro analysi), purchased from Merck (Darmstadt, Germany), were used to prepare isotonic phosphate buffer pH 7.4 (PBS) according to the German Pharmacopoeia (DAB 2001); all substances used were of analytical or pharmacopoeial grade; all formulations were prepared with double-distilled water. All stated concentration data of Ibu compounds refer to ibuprofen acid.

2.2. Methods

2.2.1. Synthesis of Ibu-AA compounds

Ibu-AA compounds were prepared by dissolving equimolar amounts of Ibu-H (5.000 g) and the corresponding amino acid (Arg: 4.222 g, His: 3.761 g) in ethanol 50% (v/v) under short-time heating (maximum 50 °C). The solvent was evaporated under vacuum conditions. The white to off-white crystalline substances formed were dried in a desiccator over silica gel.

2.2.2. Preparation of emulsions

First a w/o emulsion (Ia) was formed and dispersed in an outer HPMC phase (II) to obtain a w/o/w-emulsion in the case of drug-free emulsions (Table 1).

Table 1
Composition of emulsion compounds: w/o-emulsions (Ia and Ib) and outer phase HPMC solution (II)

	I (%)		II (%)
	a	b	
Arlacel 83	2	2	
Castor oil	78	78	
Water	20		
Mixture of ibuprofen compound and water (weighted amounts, see Table 2)		20	
HPMC 400			5
Sorbitol			12.5
Water			82.5

w/o-emulsions, following the composition in Table 1 (I), were prepared by homogenisation using an Ultra-Turrax (Janke & Kunkel, Staufen, Germany). Drug-free (Ia) and emulsions with ibuprofen compounds (Ib) were formulated. Different ibuprofen compounds were incorporated as mixtures with water. The resulting stock emulsions contained amounts equivalent to 10% (w/w) Ibu-H (Table 2).

The outer hydrophilic phase (II) was prepared by dissolving HPMC 400 and sorbitol (Table 1) in water. The solution was autoclaved (121 °C, 2 bar) for 20 min to minimise microbiological contamination of emulsions during storage.

Table 2
Molecular weights of different ibuprofen compounds, corresponding amino acids and concentration of ibuprofen compounds for emulsion preparation and solution for surface tension measurements

	M_r (g/mol)	Concentrations of different ibuprofen preparations equivalent to	
		10% (w/w) Ibu-H	0.1% (w/v) Ibu-H
Ibu-H	206.283	10.000	0.1000
Ibu-Na	228.265	11.066	0.1107
Ibu-Arg	380.486	18.444	0.1844
Ibu-His	361.439	17.522	0.1752
Ibu-Lys	352.472	17.086	0.1709
Arg	174.203		0.0844
His	155.156		0.0752
Lys·H_2O	164.203		0.0796
Lys·HCl	182.650		0.0885

In order to achieve an emulsion containing 2% (w/w) of an ibuprofen compound 20 parts of the w/o-emulsions (I) and 40 parts of the HPMC solution (II) were mixed with 40 parts water and shaken manually. The concentration of emulsions with 0.25, 0.5 and 1% ibuprofen compounds was adjusted by lowering the amount of the inner phase (Ib), which was replaced by placebo emulsion.

Particle size measurements were performed by laser diffraction (Mastersizer MS 20, Malvern, Works, United Kingdom) and calculated by Malvern SB 09 software.

2.2.3. Surface tension

Surface tension measurements were carried out with a thermostatically controlled Processor Tensiometer K100 (Krüss GmbH, Hamburg, Germany) equipped with a Wilhelmi plate (height/width/thickness: 10 mm × 19.9 mm × 0.2 mm) at 20 °C. The apparatus was calibrated with double-distilled water achieving a surface tension of 71.98 mN/m.

The reduction of surface tension caused by different Ibu-AA compounds in contrast to that achieved with pure amino acids was measured. The concentration of the solutions measured was 0.1% (w/v), the amounts weighted are listed in Table 2. Measurements were performed in triplicate every 10 s over 2 min until S.D. was below 0.01 mN/m.

2.2.4. Osmolarity

To guarantee for physiologically tolerable conditions, 5% (w/w) sorbitol was added to all preparations. Osmolarities of all preparations were measured using a freeze point (Semi-micro osmometer, Knauer KG, Berlin, Germany). The apparatus was calibrated with a sodium chloride solution (400 mOsm/kg) and bidistilled water (0 mOsm/kg). Osmolarities of the tested solutions are listed in Table 3 ($n = 3$).

2.2.5. Red blood cell haemolysis

Haemolysis studies were performed as previously described elsewhere (Baydoun and Müller-Goymann, 2003). Red blood cells were gained from human blood (30-year-old male with common blood chemistry).

Ibuprofen compound solutions (0.25, 0.5 and 1% (w/w)) and emulsions (0.25, 0.5 and 1% (w/w)) were tested. The Ibu-Arg emulsion 1% showed a comparably high viscosity. The emulsion/blood mixture had to be centrifuged at much higher rotation rates which could lead to a destruction or higher vulnerability of the erythrocytes. Therefore, the Ibu-Arg emulsion 1% could not be measured.

Table 3
Osmolarities of different Ibu compound solutions (mean value ± S.D., $n = 3$)

Ibu solutions (% (w/w))	Osmolarity (mOsm/kg)
Ibu-Na	
1	352 ± 8.1
0.5	314 ± 5.6
0.25	298 ± 2.2
Ibu-Lys	
1	376 ± 9.8
0.5	329 ± 5.8
0.25	306 ± 4.0
Ibu-Arg	
1	381 ± 9.6
0.5	330 ± 6.1
0.25	302 ± 3.9
Ibu-His	
1	372 ± 7.1
0.5	324 ± 4.3
0.25	305 ± 4.2

2.2.6. Light microscopy of histological preparations

For the examination of the influence on corneal structure and integrity cornea was removed from fresh porcine eyes according to the literature (Baydoun and Müller-Goymann, 2003), incubated at 37 °C for 1 h in different Ibu compound solutions and compared to incubated material in PBS and SDS solution in PBS 0.1% (w/w) ($n = 3$). Additionally Ibu-Lys and Ibu-Arg emulsions (0.1, 0.25 and 1% (w/w) of either compound) have been investigated ($n = 3$). pH values of all tested systems in histology and haemolysis studies ranged between 7 and 9.

After incubation corneas were washed with PBS and immediately fixed with a formaline solution 8%(w/w). The material was dehydrated with an alcohol gradient, put in melted paraffin and solidified in block form. Cross sections (<1 μm) were cut, stained with haematoxyline and eosine (H&E), blinded and microscopically observed in co-operation with a pathologist.

Eyes that lost intraocular pressure during transportation or revealed epithelium injury because of improper enucleation were not analysed due to a loss of corneal

barrier function. Further histological material, which gave hints on previous eye inflammation, indicated by a presence of macrophages in the tissue, was not taken into account.

3. Results and discussion

3.1. Surface tension

Fig. 1 reveals that Ibu-Na and Ibu-AA compounds are surface active as compared to the pure corresponding amino acids. The highest reduction of surface tension can be obtained with Ibu-Lys followed by Ibu-Na, Ibu-Arg and Ibu-His, respectively.

3.2. Emulsion formation and stability

Fig. 2a presents the stabilising effect of Ibu-Lys already on the inner w/o-emulsion system according to the composition listed in Table 1 (Ib). None of the other tested ibuprofen compounds is capable of such a w/o-emulsion stabilisation.

w/o/w-emulsions are formed when preparing emulsions following the composition in Table 1 (Ia + II). This placebo emulsion breaks after storage at 20 °C within 7 days.

As soon as ibuprofen compounds are incorporated, fine o/w-emulsions result with mean particle sizes around 1 μm. Nonetheless, the stabilising effect depends on the compound added. Emulsions formed with Ibu-Na (Fig. 2b) and Ibu-His (Fig. 2e) break after 24 h storage. Not so when Ibu-Lys is incorporated. The emulsion remains homogenous showing slight creaming, which is most obvious in the case of 0.25% Ibu-Lys, but no breaking (Fig. 2c). Creaming occurs preferably with lowered Ibu-Lys concentrations. There are no oil droplets detectable on the emulsion surface.

Ibu-Arg stabilised systems, which are less viscous than Ibu-Lys emulsions, tend to creaming but no breaking (Fig. 2d). The effect becomes less pronounced with rising Ibu-Arg concentrations.

3.3. In vitro haemolysis studies

The haemolytic activity depends on the concentration of the irritant and incubation time. Fig. 3 shows

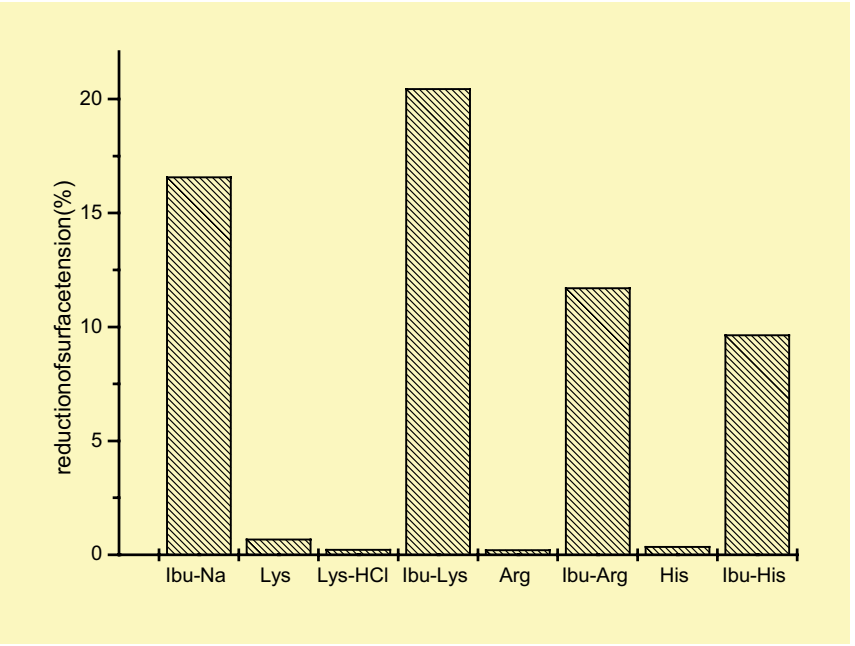

Fig. 1. Reduction of surface tension caused by different Ibu compounds in aqueous solutions 0.1% (w/v) in comparison to pure amino acids, mean value ($n = 3$).

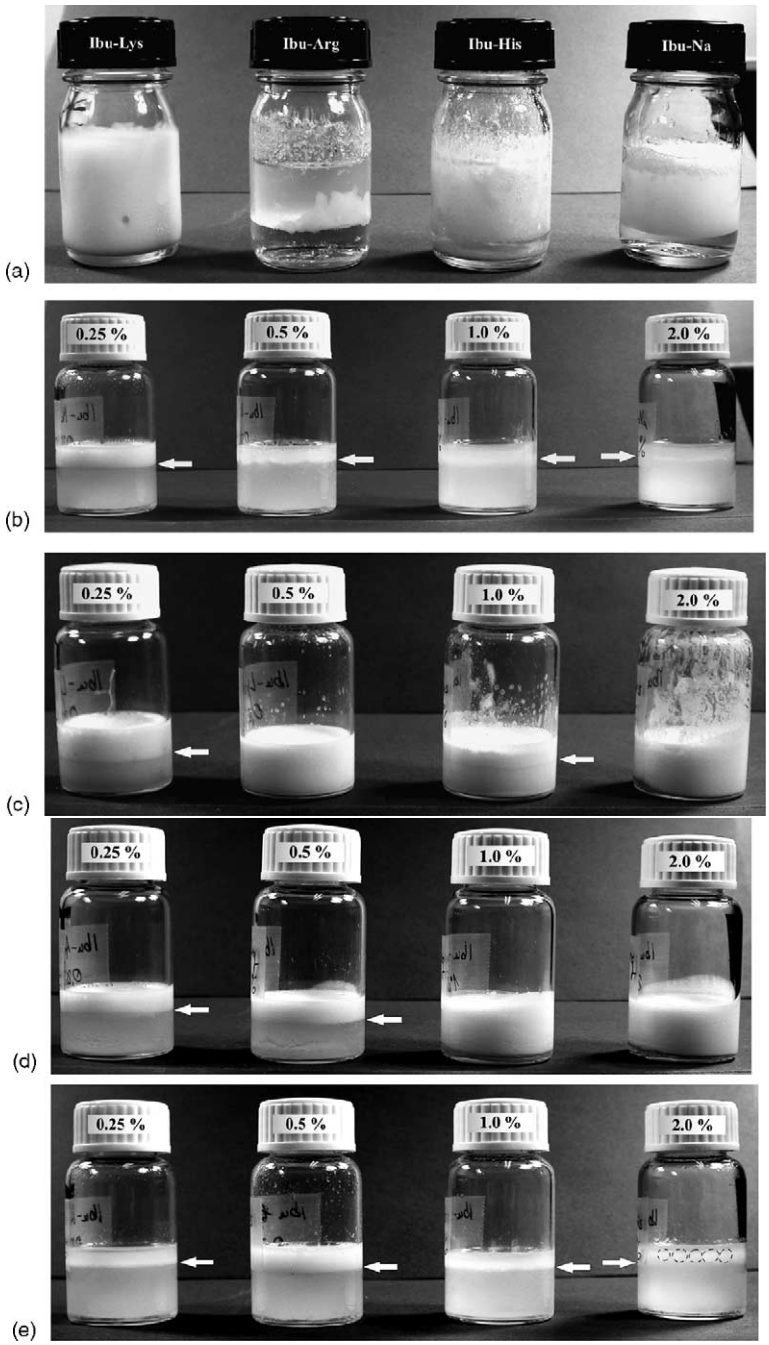

Fig. 2. Stability of different emulsions (arrow indicates phase interface): (a) w/o-emulsions following composition Ib (Table 1), ibuprofen compound equivalent to 5% Ibu-H. (b–e) o/w-emulsions containing 0.25, 0.5, 1 and 2% (w/w) of Ibu compound after 1 week of storage. (b) Ibu-Na: arrows indicate line of phase separation. (c) Ibu-Lys: arrows indicate line of phase separation. (d) Ibu-Arg: arrows indicate line of phase separation (e) Ibu-His: arrows indicate line of phase separation, oil droplets are marked in 2% preparation.

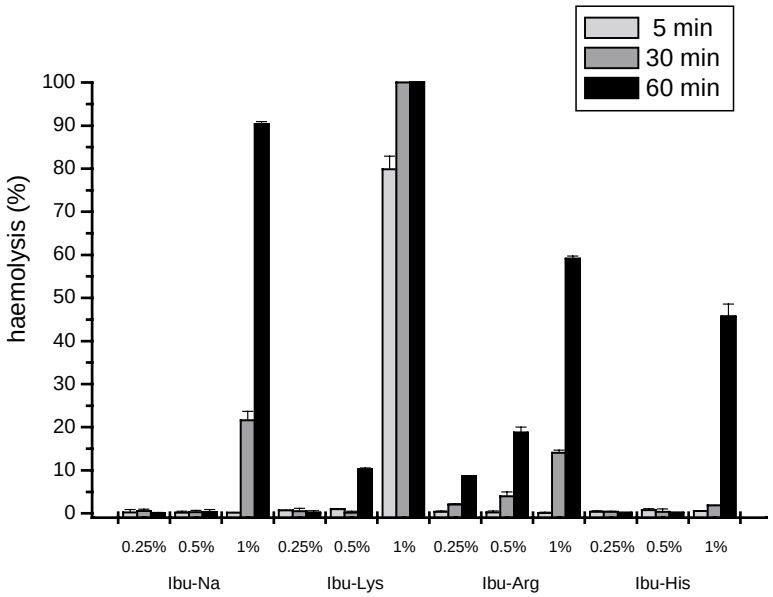

Fig. 3. Red blood cell haemolysis caused by different Ibu compound solutions ($n = 3$), each value represents mean ± S.D.

the haemolytic behaviour of different Ibu-Na and Ibu-AA solutions isotonised with 5% sorbitol and incubated for 5, 30 and 60 min. Ibu compound solutions at 0.25% (w/w) show no significant cytotoxicity independent of incubation time. Ibu-Arg reveals the highest haemolysis of around 8%, which is still an acceptable value. When raising the concentration to 0.5% (w/w) only Ibu-Lys and Ibu-Arg, the two compounds with the best emulsifying qualities, reveal at an incubation time of 60 min obvious erythrocyte membrane damage resulting in activities over 10%. At a concentration of 1% Ibu-Na and Ibu-Arg reveal respective data even within 30 min. Ibu-Lys is the most damaging agent showing a lysis of 83% after

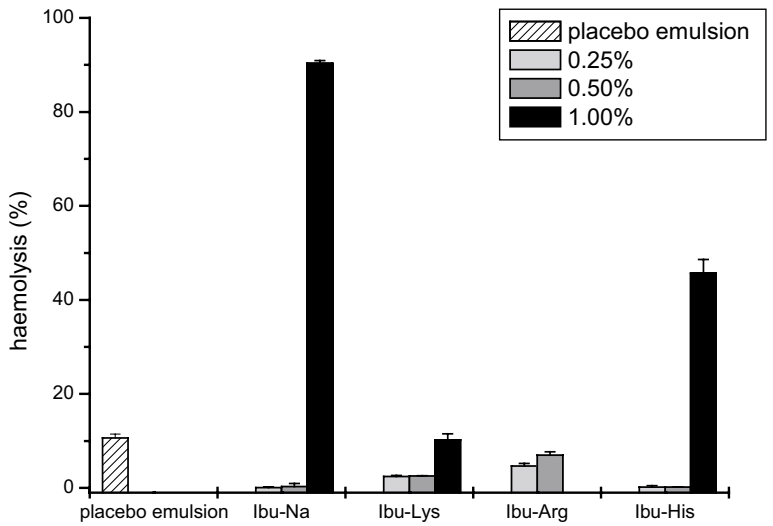

Fig. 4. Red blood cell haemolysis caused by different Ibu compound emulsions ($n = 3$), each value represents mean ± S.D.

5 min and total lysis after 30 min. Ibu-His and Ibu-Arg produce a distinct lysis only after 60 min at a concentration of 1%. When now the haemolytic activities of the Ibu compound solutions under the same conditions (60 min, 1%) are taken into consideration, the results correlate with the rank order of surface activity reduction measurements (Ibu-Lys > Ibu-Na > Ibu-Arg > Ibu-His).

The haemolytic behaviour of freshly prepared emulsions containing Ibu-Na and Ibu-His are comparable to their corresponding solutions (Fig. 4). On the other hand, the harmful effect caused by

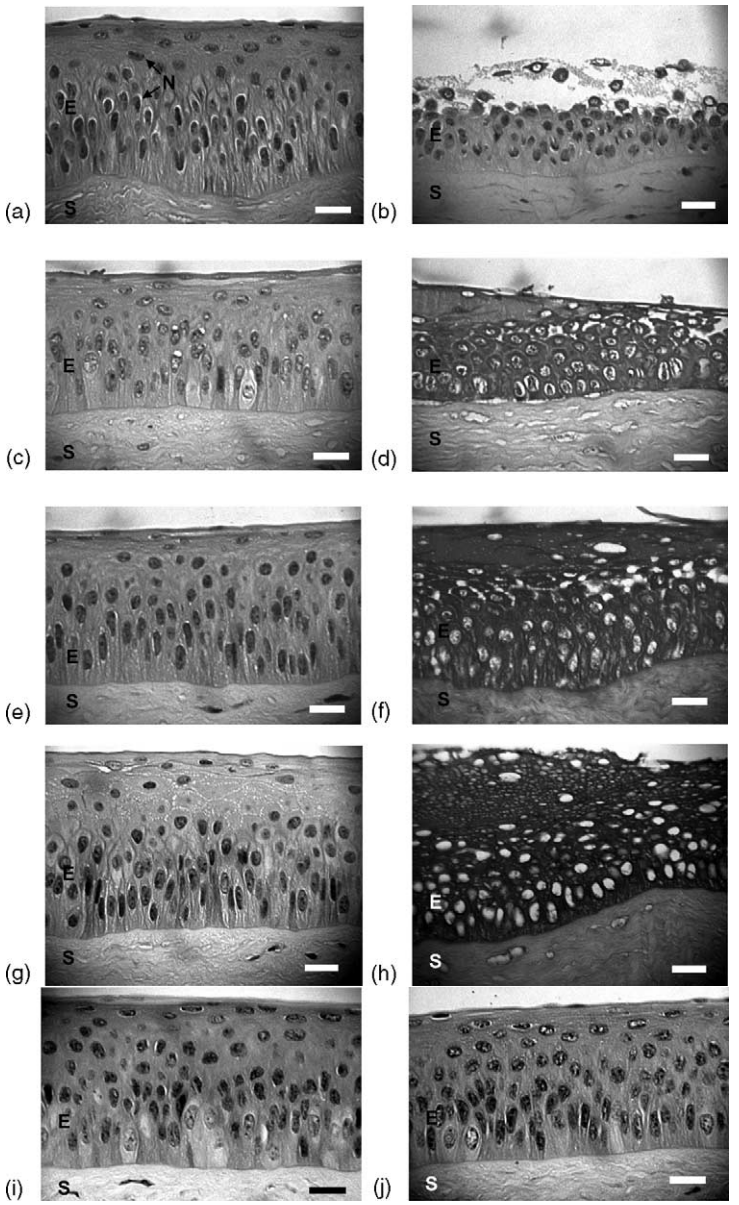

Fig. 5. Cross sections of excised porcine cornea (H&E, scale bar 20 μm), incubated for 1 h at 37 °C with (a) PBS (2 h), (b) SDS 0.1% (0.5 h), (c) Ibu-Na solution 0.5%, (d) Ibu-Na solution 1%, (e) Ibu-Lys solution 0.5%, (f) Ibu-Lys solution 1%, (g) Ibu-Arg solution 0.5%, (h) Ibu-Arg solution 1%, (i) Ibu-His solution 0.5%, (k) Ibu-His solution 1%. E, epithelium; S, stroma; N, nuclei.

Ibu-Lys is obviously significantly diminished when Ibu-Lys is applied as an emulsion (Fig. 4). This observation is in accordance with previously obtained results already described in the literature (Jumaa and Müller, 2000). The haemolytic activity of a highly lytic agent could be greatly decreased in solutions/dispersions with other surfactants that can build a micellar or liposomal structure or by incorporation into an emulsion formulation. It was proposed that the lytic agent was either merged into the lipophilic phase or intercalates between the emulsifier molecules at the interface. The good emulsifying properties of Ibu-Lys lead to a restriction of the molecule in the interface which decreases the direct contact of Ibu-Lys with the erythrocyte membrane. In solutions, the concentration of the free lytic agent is rather high leading to an attack on erythrocytes and a great haemolytic effect (Jumaa and Müller, 2000). Haemolysis caused by Ibu-Arg seems to be similar to Ibu-Lys. The haemolysis caused by the placebo emulsion (10.7%), which is comparable to results obtained with a Ibu-Lys emulsion 1%, can be explained with the poor stability leading to high regional emulsifier concentrations during the experiment.

3.4. Histology

Fig. 5a–k present corneal cross sections after incubation of freshly excised pig corneas with various solutions to investigate their influence on corneal cell structure and tissue integrity. After incubation in an isotonic phosphate buffer solution pH 7.4 (Fig. 5a), epithelium (E) and stroma (S) structure is maintained. Typical stratified epithelial layer can be recognised by the basal columnar cells and the squamous surface cells appearing with a bulge at the nuclei (N). When corneal epithelium is exposed to an irritant, like SDS (Fig. 5b), previously narrow intercellular spaces are widened, cells and nuclei are deformed and superficial epithelial cells are detached from tissue assembly. Treating corneas with the tested ibuprofen solutions has a lytic effect on the nuclei which is already notable at lower and obvious at higher concentrations. In agreement with the lowest surface activity, Ibu-His shows the smallest degree of tissue destruction. Ibu-Lys and Ibu-Arg emulsion systems do not affect corneal structure.

4. Conclusions

Ibu-Na, which reveals haemolysis only at higher concentrations but notable cornea tissue damage, is no appropriate emulsion stabiliser. Ibu-His which has the less pronounced effect on erythrocyte membranes and cornea does not stabilise emulsions efficiently either.

Ibu-Lys, which reveals the comparably highest reduction of surface activity, is a good emulsifying agent leading to fine o/w-emulsions with mean particle sizes around 1 μm. However, Ibu-Lys affects porcine cornea integrity and is highly haemolytic. The latter effect can be clearly decreased by incorporating Ibu-Lys into an emulsion system.

Despite of a lower surface activity reduction Ibu-Arg allows for comparably stable emulsions at comparable erythrocyte damage. However, Ibu-Arg solutions as well have a destructive effect on corneal tissue elements.

On the contrary both, Ibu-Lys and Ibu-Arg, applied as an emulsion do not affect corneal integrity.

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