Influence of humic substances on the cytotocixity of surfactants

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Summary

Aiming to reduce the toxic behaviour of surfactants the interaction of humic substances with four different types of surfactants was investigated. Using the human promonocytic cell line U937 the cytotoxicity of the individual surfactants and their combinations with isolated naturally occurring and synthetic humic acids were measured by means of the XTT tetrazolium reduction assay. Due to their negative charges humic acids are able to reduce the cytotoxicity of cationic surfactants, but differ in their effect on amphoteric agents. No influence of humic substances was found on the cytotoxicity of non-ionic and anionic surfactants.

Key index words: naturally occurring humic acids; synthetic humic acid like polymers; anionic, cationic, non-ionic and amphoteric surfactants; cytotoxicity

Introduction

Surface-active agents are components of many drug formulations, cosmetics, and body care products. Because of their physico-chemical properties they are used as emulsifiers, detergents, conserving, cleansing or wetting agents. Surfactants develop more or less cytotoxic and membrane cytotoxic effects (Jelinek and Klöcking, 1998, Jelinek *et al.*, 2002). Aiming to reduce the toxic behaviour of surfactants the interaction of humic substances with four different types of surfactants was investigated.

Materials and methods

Humic substances

Naturally occurring humic acids (HA) isolated as sodium humate (Na-humate) from the water of a rain-moor peat situated near Dierhagen-Neuhaus (Mecklenburg-Vorpommern, Germany) according to Klöcking *et al.* (1977), HA from the peat of the Altteich peatland near Bad Muskau/Weisswasser (Saxony, Germany) and the sodium salt of the synthetic HA-like polymer caffeic acid oxidation product (Na-KOP) prepared according to Helbig *et al.* (1994) were studied.

Surfactants

The amphoteric surfactant TEGO® betaine CKD (Th. Goldschmidt AG, Essen, Germany), the non-ionic surfactant Plantacare® 2000 (Henkel KGaA, Düsseldorf, Germany), the anionic surfactants sodium dodecyl sulfate (Ferak, Berlin, Germany) and sodium cetylstearyl sulfate (Vaseline Fabrik E. Wasserfuhr GmbH, Bonn, Germany), the cationic surfactants benzalkonium chloride (Caesar & Loretz GmbH, Hilden, Germany) and didecyldimethylammonium chloride (Merck Schuchardt OHG, Hohenbrunn, Germany) were used.

Cells

Human promonocytic U937 cells (ATCC CRL 1593) were cultivated in RPMI 1640 medium containing 10% foetal bovine serum and 5% CO_2. They were split every two days at a ratio of 1:3 and used for the experiments two days after passaging.

Cytotoxicity

Using the XTT tetrazolium reduction assay EZ4U (Biozol Diagnostica Vertrieb GmbH, Eching, Germany) the surfactants were tested for cytotoxicity singly and in combination with either Na-humate or Na-KOP, 1 h after addition to the cells. The principle of this test corresponds to the method described by Klöcking *et al.* (1995, 1998). To compare the cytotoxicity-reducing effect of humic substances, half-maximum cytotoxic concentrations (CC_{50}) of surfactants both in the presence and in the absence of the humic substances containing test materials were calculated.

Results

The cytotoxicity of the amphoteric surfactant TEGO® Betaine CKD is significantly reduced by Na-humate isolated from the Altteich peat deposit, but not by the synthetic HA-like polymer Na-KOP. Neither Na-humate nor Na-KOP did exert an influence on the cytotoxicity of the non-ionic surfactant Plantacare® 2000. Furthermore, Na-humate from the water of the Dierhagen rain-moor peat and the synthetic Na-KOP decreased the cytotoxicity of the cationic surfactants benzalkonium chloride and didecyldimethylammonium chloride. Neither Na-humate nor Na-KOP exert an influence on the toxicity of the anionic surfactants sodium dodecyl sulfate and sodium cetylstearyl sulfate, respectively (Table 1).

The half-maximum cytotoxic concentration (CC_{50}) after 1-h exposure amounts to 334 (295-377) µg/ml for Na-humate and 421 (340-521) µg/ml for Na-KOP, respectively.

The cytotoxicity-reducing effect of HA on cationic surfactants can be explained by the interaction of both components resulting in a less toxic product. Koopal *et al.* (2004) were able to show that the two cationic surfactants dodecyl- and cetylpyridinium chloride bind strongly to purified Aldrich HA due both to electrostatic and hydrophobic attraction. In contrast, no significant binding was observed between Aldrich HA and the anionic surfactant sodium dodecyl sulfate.

Conclusion

Based on these results we conclude that naturally occurring as well as synthetic humic substances due to their negative charges are able to reduce the cytotoxicity of cationic surfactants, but differ in their effect on amphoteric agents. No influence of humic substances was found on the cytotoxicity of non-ionic and anionic surfactants. In no case an increase of the surfactants' cytotoxicity due to the combination with humic substances was observed.

For future research it would be interesting to explore whether the desired physico-chemical properties of surfactants such as conserving, foaming and cleansing effects are also affected by humic substances.

Table 1. Half-maximum cytotoxic concentrations (CC50) of surfactants in the presence and in the absence of humic substances 1h after exposure
(n = number of experiments).

Type	Surfactant	Humic substance	CC_{50} (µg/ml)	Confidence interval	n
Amphoteric	TEGO® Betain CKD	without	34.1	30.4 - 38.2	3
	TEGO® Betain CKD	Na-humate [1]	42.5	39.8 - 45.3	3
	TEGO® Betain CKD	Na-KOP [2]	33.9	32.0 - 35.9	3
Non-ionic	Plantacare® 2000	without	127.0	119 - 136	3
	Plantacare® 2000	Na-humate [1]	136.0	114 - 162	3
	Plantacare® 2000	Na-KOP [2]	132.0	120 - 145	3
Anionic	Sodium dodecyl sulfate	without	45.6	44.7 - 46.5	7
	Sodium dodecyl sulfate	Na-KOP [2]	51.4	48.9 - 54.1	3
	Sodium cetylstearyl sulfate	without	21.8	18.9 - 25.0	3
	Sodium cetylstearyl sulfate	Na-KOP [2]	22.7	18.2 - 28.2	3
Cationic	Benzalkonium chloride	without	5.0	4.8 - 5.2	10
	Benzalkonium chloride	Na-humate [3]	8.5	7.2 - 10.0	3
	Benzalkonium chloride	Na-KOP [2]	7.0	6.6 - 7.3	3
	Didecyldimethylammonium chloride	without	3.3	3.2 - 3.4	6
	Didecyldimethylammonium chloride	Na-humate [3]	5.4	5.1 - 5.6	3
	Didecyldimethylammonium chloride	Na-KOP [2]	5.5	5.0 - 6.1	3

1 extracted from the Altteich peatland near Bad Muskau / Weisswasser (Saxony, Germany)
2 synthetic HA-like polymer caffeic acid oxidation product
3 extracted from the water of rain moor peat situated near Dierhagen- Neuhaus (Mecklenburg-Vorpommern, Germany)

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