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Saponins can perturb biologic membranes and reduce the surface tension of aqueous solutions: A correlation?

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ABSTRACT

Saponins are secondary plant compounds. They have a triterpenoid or steroidal backbone. Sugars are attached to one or more points of this structure, forming chains that can be branched. This appearance leads to amphiphilic properties giving saponins the ability to interact with both lipophilic and hydrophilic structures. The surfactant behavior lets them lower the surface tension in aqueous solutions and form micelles when reaching the critical micelle concentration (cmc). It also lets them interact with biologic membrane layers that usually consist of phospholipids and cholesterol. This action may perturb the membrane and its function leading to membrane perforation or complete lysis. Thus saponins are also known for their cytotoxicity and membranolytic, respectively hemolytic features. In our studies we wanted to answer the question if there is a correlation between the unspecific detergent behavior when lowering the surface tension and the ability to perforate cell membranes and to act cytotoxic. Do saponins showing a considerable reduction in the surface tension also reveal an evident cytotoxicity or/and a marked cell membrane perforation?

We tested a variety of saponins with distinct structures. The reduction in the surface tension and the cmc were analyzed on a tensiometer using the Wilhelmy plate method. The general cytotoxicity was determined in a cell model by DNA quantification. The cell membrane toxicity or membrane perforation was explored in a cell model by quantification of the leakage of the intracellular enzyme lactate dehydrogenase (LDH).

The experiments revealed a correlation between the membrane toxicity and the reduction in surface tension.

1. Introduction

Saponins are secondary plant compounds and well known for their amphiphilic characteristics generated from lipophilic and hydrophilic sites in their molecular structure. This brings up their ability to interact with both, lipophilic and hydrophilic agents or sites of other molecules. They can be seen as surfactants. Thus saponins lower the surface tension of aqueous solutions.[1] The hydrophilic sugar moiety usually contains glucose (Glc), galactose (Gal), glucuronic acid (GlcA), xylose (Xyl), rhamnose (Rha), but also arabinose (Ara), fucose (Fuc) or quinovose (Qui). The hydrophobic aglycone (sapogenin) may be triterpenoid or steroid in nature. One or more oligosaccharide chains may be attached typically at the C3 position (monodesmosidic) or additionally at the C28 position (bidesmosidic). The great variability of the saponin structures arises from the variability of the aglycone structure, the style of the side chains and the positions of attachment of these moieties to the aglycone.[2] It influences the ability to lower surface.[1]

In general a saponin's surface activity is seen to be linked to its membrane permeabilizing and hemolytic activity, but also to its cytotoxic potential. However, these properties do not show up in the same value for every saponin nor do they obligatory appear concurrently.

A general correlation between the hemolytic activity and the cytotoxic potential could not be shown for steroid saponins, which suggests that the two characteristics are executed by different mechanisms and that the molecular structure plays a decisive role.[3] Another lack of correlation could be shown for the membrane permeabilizing abilities and the hemolytic activity of hopan-type triterpenoid saponins. While all by Gauthier et al. (2009)[4] tested saponins did perforate membranes, the hemolysis activity could only be noted for the oleanane-type, but not for hopan-type saponins. This underlines that a correlation between different surface-related properties does not necessarily exist and that the observed phenomena might be derived from different mechanisms. The cited reports compared the characteristics

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cytotoxicity,[3] membrane permeabilization,[4] and structure[3–5] with the hemolytic activity.

In our work we analyzed the relationship between the general cytotoxicity, the membrane damage or membrane toxicity and the surface activity of monodesmosidic and bidesmosidic saponins, reacting acidic or neutral, steroid and triterpenoid in nature and aglycones. We finally tried to correlate the findings of cytotoxicity tests with the physico-chemical properties of the tested substances. To the best of our knowledge this has not been done so far.

The two types of cytotoxicity, the general cytotoxicity (long term observation) and the membrane toxicity (short term observation) were scrutinized in a cell culture model. The surface activity was researched in a fluid model using a tensiometer.

2. Methods and materials

2.1. Saponins

The listed substances were tested for the general cytotoxicity, the membrane damage or membrane toxicity and the surface activity. Figure 1 shows their chemical structures.

Alpha-Hederin (Carl Roth GmbH + Co. KG, Karlsruhe, Germany).
Beta-Escin (Merck KGaA, Darmstadt, Germany).
Beta-Sitosterol (ARCO-Chemikalien, Berlin, Germany).
Digitonin (VEB Ysat, Wernigerode, Germany).
Ginsenoside RG2 (Phytolab GmbH + Co. KG, Vestenbergsgreuth, Germany).
Glycyrrhetinic acid (Merck KGaA, Darmstadt, Germany).
Glycyrrhizinic acid ammonium salt hydrate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany).
Hederacoside C (Carl Roth GmbH + Co. KG, Karlsruhe, Germany).
Primulic acid 1 (isolation product, purity >95%).

2.2. Cytotoxicity assays

Human urinary bladder epithelial carcinoma cells (ECV-304, ACC 310) were obtained from Deutsche Sammlung von Microorganismen und Zellkulturen GmbH (Leipniz-Institut DSMZ, Braunschweig, Germany). The cells were cultured at 37 °C in saturated water vapor atmosphere containing 5% CO_2. Passages used range from 14 to 33. The cultures were split 1:10 twice a week using trypsin/EDTA (Biochrom AG, Berlin, Germany). The ECV-304 cells were used because they are easy to cultivate, fast growing and the experiments with them generate consistent results. As epithelial cells they are adapted to function on surfaces and boundary layers.

2.2.1. General cytotoxicity

The tolerance of the cells to proliferate in the presence of rising concentrations of the tested substances provides a long term observation of cytotoxicity. Different cytotoxic mechanisms (membrane disintegration due to pore formation[6,7] or complexion of membrane sterols;[8,9] induction of apoptosis;[10] etc.) are probable to lead to a diminished proliferation.

ECV-304 cells were grown in 96-well microplates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a density of 5×10^3 cells per well in 100 μL of Medium 199 Earle with phenol red, stable glutamine, 2.2 g/L $NaHCO_3$, added 2% (v/v) HEPES and 10% (v/v) fetal bovine serum (FBS) (Biochrom AG, Berlin, Germany). After 24 h the medium was changed to 180 μL medium containing different concentrations of the substances to be tested and 20 μL PBS (Biochrom AG, Berlin, Germany) and the cells were incubated for further 72 h.

The general cytotoxic potential of all investigated substances was determined by DNA quantification. Therefore the cell culture medium was discarded and the cells were washed using 100 μL isotonic NaCl solution per well followed by three freeze–thaw-cycles in 100 μL super pure deionized water per well to destroy cellular membranes. To quantify the amount of DNA 100 μL per well of double-concentrated DNA buffer containing 82 mM $Na_2HPO_4{\cdot}2H_20$, 18 mM $NaH_2PO_4{\cdot}H_2O$, 4 mM EDTA and 4 M NaCl were added prior to 10 μL per well of 10 μg/mL Hoechst 33258 dye (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The fluorescence was measured in a microplate-reader, Tecan infinite F200 (Tecan Group Ltd, Mainz, Germany) using an excitation wavelength of 360 nm and an emission wavelength of 465 nm. The fluorescence is proportional to the amount of dsDNA which correlates to the number of surviving cells. The survival ratio was defined as the fluorescence in test wells compared to the fluorescence in control wells.

2.2.2. Membrane toxicity

The membrane toxicity can be rated by the extracellular quantification of a naturally intracellular substance like the enzyme lactate dehydrogenase (LDH). The enzyme may be leaked through pores forming in the cell membrane, membrane rupture or complete membrane destruction caused by the tested substances. Due to the short incubation time, enzyme deliveries by other processes like apoptosis do carry no weight. Hence the LDH assay is a common assay to estimate a substance's toxicity towards cellular membranes in a short term observation, irrespective influences on the cells proliferation.

Cells were grown in 96-well microplates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a density of 2×10^3 cells per well in 100 μL of Dulbecco's minimal essential medium without phenol red, with 3.7 g/L $NaHCO_3$, 4.5 g/L D-glucose, added 1% (v/v) L-glutamine and 10% (v/v) fetal bovine serum (FBS) (Biochrom AG, Berlin, Germany). After 24 h the medium was changed to 100 μL medium containing different concentrations of the substances to be tested and the cells were incubated for further 2 h.

Therefore the light absorption of the processed kit's dye was measured at 450 nm and 620 nm in a microplate-reader, Tecan SPECTRA Fluor (Tecan Group Ltd, Mainz, Germany). The light absorption is proportional to the amount of processed dye which correlates to the amount of released LDH being a surrogate parameter for the amount of destroyed cells. The survival ratio was defined as the light absorption in test wells compared to that in positive (complete cell destruction) and negative (spontaneous cell destruction) control wells.

2.3. Experiments on surface tension

2.3.1. Reduction in surface tension

Liquids molecules underlie cohesive forces. In the liquid these forces are equal into each direction. For those molecules on the surface, the lack of neighboring molecules above lets them execute stronger forces on proximate molecules. This circumstance results in an enhancement of attractive forces, forming the surface tension. The surface tension also depends on the type of liquid and has a value of 72 Nm/m at 25 °C for pure water.[11] Surfactants can perturb the development of cohesive forces between liquid molecules and thereby lower the surface tension.

The influence of the examined substances on the reduction in surface tension of liquids was performed using a Krüss K10 tensiometer (Krüss GmbH, Hamburg, Germany). The Wilhelmy plate method was considered to be the optimal measurement method for this purpose. Therefore a defatted and thoroughly cleaned roughed platinum-plate was dipped into the liquid. The tensiometer measures the force that is required to pull the plate out. This force reaches a maximum just before the plate leaves the liquid and is proportional to the surface tension.

All substances were dissolved in 0.1 M PBS. The solutions were put into a sample vessel, tempered to 20 °C and given a resting

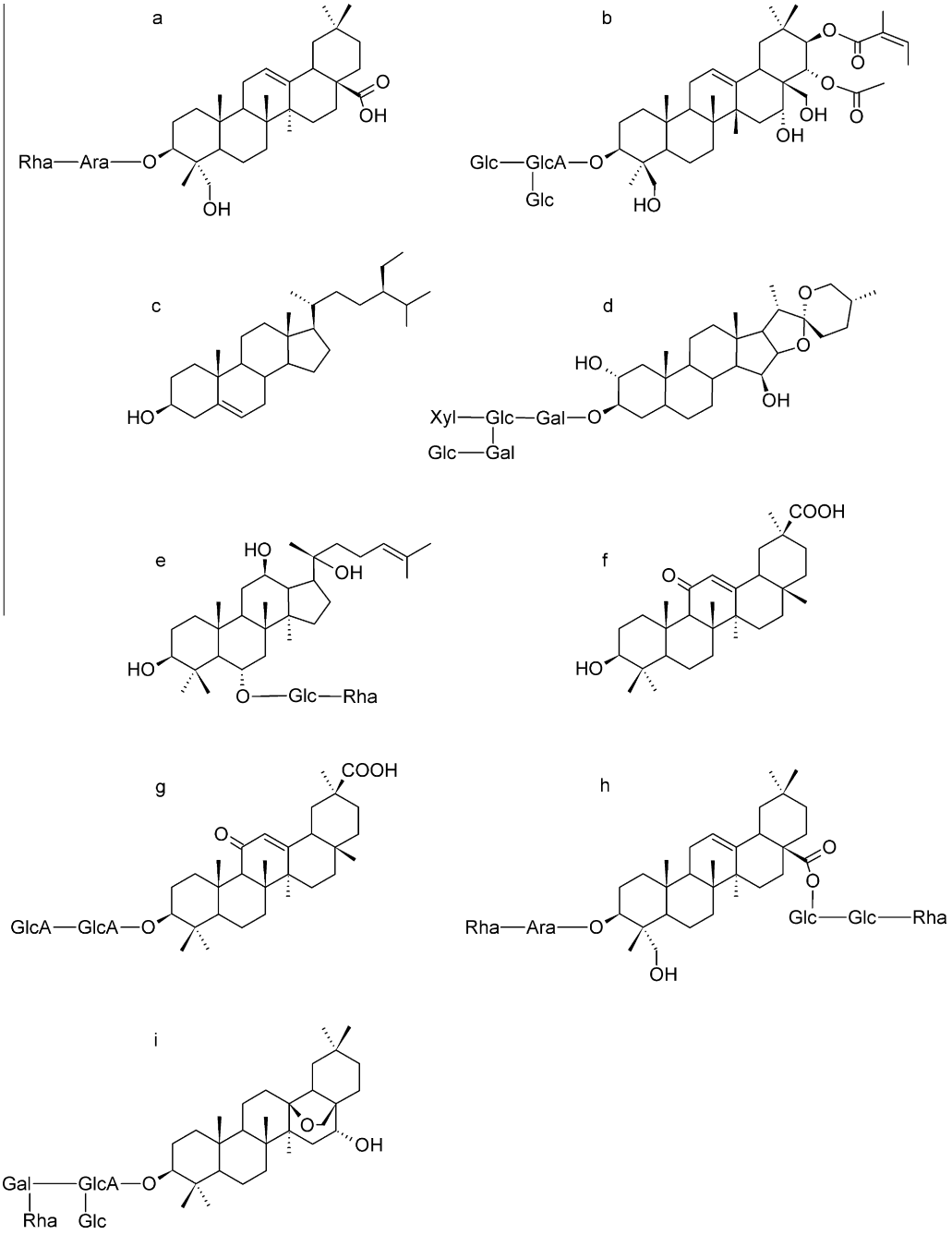

Figure 1. Chemical structures of the tested substances: (a) alpha-hederin, (b) beta-escin, (c) beta-sitosterol, (d) digitonin, (e) ginsenoside RG2, (f) glycyrrhetinic acid, (g) glycyrrhizinic acid ammonium salt hydrate, (h) hederacoside C, (i) primulic acid 1.

period of 10 min to build up equal surfaces. Afterwards the measurements were performed in triplicate for each substance and concentration. After every measurement the plate was cleaned and the resting period was given before the new measurement started.

2.3.2. Critical micelle concentration (cmc)

At the critical micelle concentration a liquid's surface is completely saturated with surfactant molecules and the surface tension reaches its minimum. The amount of surfactant molecules at the surface stays in a steady state at further addition of surfactant and dissolved molecules begin to associate forming micelles. The amount of dissolved free surfactant molecules remains constant too. Micelles may appear as spheres, cylinders or bilayers while shape and size depend on the surfactant's molecular geometry. The cmc is an important characteristic of a surfactant.

The cmc was also assessed on a Krüss K10 tensiometer (Krüss GmbH, Hamburg, Germany) using the Wilhelmy plate method. All measurements were carried out as described under *Reduction in surface tension*. The cmc indicated an inflection point when the surface tension was plotted as a function of the logarithm of the concentrations.[12] If tangents are applied to the rapidly declining part of the curve and to nearly constant section, the cmc-value is displayed as an intersection point in the chart.

3. Results

Table 1 displays the results of all performed experiments. Results of the cytotoxicity assays are presented as IC_{50}-values, results from the experiments on the reduction in surface tension

Table 1
IC_{50}- and EC_{50}-values of the tested substances and critical micelle concentrations (cmc), '>' indicates IC_{50}- and EC_{50}-values could not be observed and the listed concentrations are the highest tested

	$IC_{50}/\mu M$		$EC_{50}/\mu M$	cmc/μM
	General cytotoxicity (DNA assay)	Membrane toxicity (LDH assay)	Reduction in surface tension	
Alpha-hederin	35	29	4	13
Beta-escin	52	34	7	88
Beta-sitosterol	61	472	19	241
Digitonin	13	15	8	163
Ginsenoside RG2	>127	783	25	>127
Glycyrrhetinic acid	101	292	16	106
Glycyrrhizinic acid	>119	>238	128	>1429
Hederacoside C	>164	>164	10	82
Primulic acid 1	13	58	8	90

are presented as EC_{50}-values. Results from the critical micelle concentration experiments are given as absolute concentrations.

3.1. Cytotoxicity assays

The values are expressed as the concentrations that decreased the number of cells by 50% (general cytotoxicity) or that caused a delivery of 50% LDH into the supernatant (membrane toxicity) compared to the control (=IC_{50}). The authors define that a moderate to strong general cytotoxicity and/or potential to perforate cell membranes is expressed in IC_{50}-values around 50 μM and below. This threshold value was set since to the authors' experience other non-toxic natural compounds habitually express by far higher IC_{50}-values.

3.1.1. General cytotoxicity

A moderate to strong general cytotoxicity is expressed in IC_{50}-values around 50 μM and below. This applies to alpha-hederin (35 μM), beta-escin (52 μM), primulic acid 1 (13 μM) and digitonin (13 μM). A lower cytotoxicity expressed in clearly higher IC_{50}-values can be observed for ginsenoside RG2 (>127 μM), glycyrrhetic (101 μM) and glycyrrhizinic acid (>119 μM) and for hederacoside C (>162 μM). Beta-sitosterol (61 μM) revealed, compared to the results from the membrane toxicity experiments, a markedly lower IC_{50}.

3.1.2. Membrane toxicity

A moderate to strong potential to perforate cell membranes is expressed in IC_{50}-values around 50 μM and below. That applies to alpha-hederin (29 μM), beta-escin (34 μM), primulic acid 1 (58 μM) and digitonin (15 μM). Little membrane damage expressed in clearly higher IC_{50}-values can be observed for beta-sitosterol (472 μM), ginsenoside RG2 (783 μM by approximation), glycyrrhetic (292 μM) and glycyrrhizinic acid (>238 μM) and hederacoside C (>164 μM).

3.2. Experiments on surface tension

3.2.1. Reduction in surface tension

The values in Table 1 are presented as the concentration of the semi-maximal effect, which was the reduction in the surface tension in our case. It is indicated as EC_{50} in μM.

Since all substances possess a more or less amphiphilic chemical structure, the EC_{50}-values of all nearly all substances show up below 50 μM. Only glycyrrhizinic acid showed an EC_{50} of 128 μM.

Looking closer to the EC_{50}-values a certain pattern is perceptible: Substances that revealed a moderate to high general cytotoxic potential show up with EC_{50}-values in the single-digit range. That fits to alpha-hederin (4 μM), beta-escin (7 μM), digitonin (8 μM) and primulic acid 1 (8 μM). In contrast substances that revealed a lower cytotoxicity reach double to triple digits EC_{50}-values, such as beta-sitosterol (19 μM), ginsenoside RG2 (25 μM), glycyrrhetinic (16 μM) and glycyrrhizinic (128 μM) acid and hederacoside C (10 μM).

3.2.2. Critical micelle concentration (cmc)

The lowest cmc by far was recorded for alpha-hederin (13 μM). Beta-escin (88 μM), glycyrrhetinic acid (106 μM), hederacoside C (82 μM) and primulic acid 1 (90 μM) gave cmc-values in the same range. No cmc could be assessed for ginsenoside RG2 (>127 μM) and glycyrrhizinic acid (>1429 μM). The noted concentrations for these substances are the highest tested.

4. Discussion

Our results show that a prediction of the reduction in surface tension by the tested substances is only possible by a limited extent. The ratio of lipophilic and hydrophilic sites in the molecular structure should play a decisive role. It is a bit surprising that glycyrrhizinic acid bearing only two sugars reduces the surface tension by far by a lower value than its aglycone glycyrrhetinic acid. Another discrepancy is seen for the bidesmoside hederacoside C and its monodesmoside alpha-hederin which lowers the surface tension by a much bigger extent than the bidesmoside. Since only little is known about definite physico-chemical mechanisms of saponins on surfaces of aqueous solutions we tried to evaluate our results from the experiments on the surface tension by comparison with results from bio-chemical experiments like experiments on cytotoxicity and membrane toxicity.

What are the bio-chemical mechanisms that induce the general cytotoxicity and the membrane toxicity? And may these mechanisms be divided into processes leading to membrane toxicity and processes leading to general cytotoxicity? If so, three scenarios are possible: (1) processes leading to a high membrane toxicity resulting in a general cytotoxicity, (2) processes leading to a high membrane toxicity resulting in no general cytotoxicity and (3) processes leading to no membrane toxicity but resulting in a general cytotoxicity. Scenario 1 should fit for most substances perturbing biologic membranes through specific processes or not beyond a certain degree and could be observed for nearly all of the herein tested substances, except beta-sitosterol. This degree of perturbation should be mainly dependent on the substance itself, the concentration and the membrane and is a relative value. Beyond this degree of damage to the cellular membrane cells are no longer capable of proliferation or underlie a complete lysis. Lesser membrane toxicity also results in lesser general cytotoxicity. Scenario 2 does not occur to the authors' experience. Scenario 3 only fits for substances that perturb biologic membranes in a little extent and cause cytotoxicity through a specific intracellular mechanism. This could be observed for beta-sitosterol.

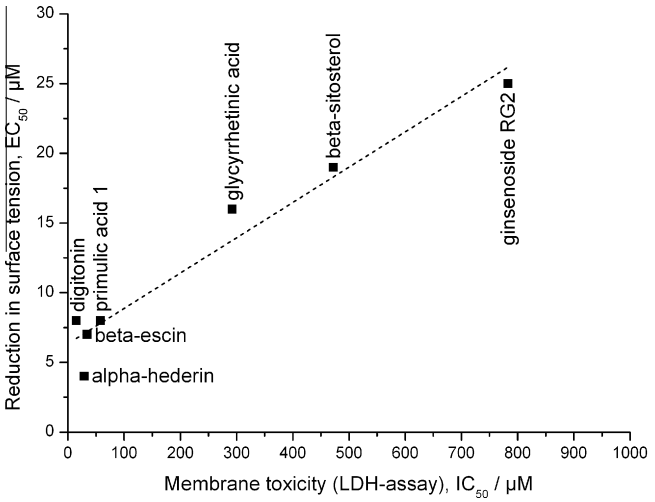

Figure 2. Plot of the IC_{50}-values of the tested substances from membrane toxicity analyzed by lactate dehydrogenase-assay (LDH) versus the EC_{50}-values from the reduction in surface tension, glycyrrhizinic acid and hederacoside are not displayed due to missing IC_{50}-/EC_{50}-values [linear regression: $y = 0.02535x + 6.33327$; $r^2 = 0.9384$].

Do the results of possibly specific processes leading to strong membrane toxicity correlate with the unspecific reduction in surface tension of aqueous solutions? As Figure 2 shows, this question, which was already asked in the title can be answered with yes: in our tests a correlation between the membrane toxicity and the reduction in surface tension could be established for the tested substances.

The selection of the substances to be investigated in our tests was orientated to cover various structural properties that are common for amphiphilic substances like saponins: monodesmosidic and bidesmosidic saponins reacting acidic or neutral, steroid and triterpenoid in nature and aglycones were tested.

4.1. Saponins with higher toxic potential and reduction in surface tension

4.1.1. Alpha-hederin, beta-escin, digitonin, primulic acid 1

In 1962 the formation of pits and pores in viral membrane coats as a consequence of saponin treatment was discovered by Dourmashkin et al. using electron microscopy.[7,13] In the same year two other groups reported the occurrence of cholesterol in the target membrane to be essential to induce the pore formation.[6,7] Since then, different research groups confirmed this observation whereas others disproved it. Certainly, processes leading to pits and pores, involving membrane cholesterol or not, could explain the undoubtedly noticeable membranolytic effects for a wide range of saponins. Schlösser (1969) described the formation of complexes between cholesterol and a number of saponins including some used in our tests, namely alpha-hederin, beta-escin and primulic acid 1. By binding the added saponins with free cholesterol, he could inhibit membranolysis (hemolysis) of bovine erythrocytes.[14] Several groups approved the discovery since that time, but revealed a strong dependency between the ability to bind cholesterol and the saponin's structure.[9,15] These findings might explain the results of our cytotoxicity tests: The above named substances and digitonin showed up with strong general cytotoxicity as well as a high membrane leakage. The interaction of digitonin and some analogs with the membrane cholesterol was depicted by Nishikawa et al. (1984). Digitonin could bind to the cholesterol in erythrocytes, granulocytes and in a liposomal membrane model. The binding was strongly influenced by the complexity of the sugar moieties of the analogs in the manner digitonin > desglucodigitonin > glucosy-galactosyl-digitonin > galactosyl-digitonin.[8] Since alpha-hederin, beta-escin, digitonin and primulic acid 1 are capable of binding membrane cholesterol, leading to the loss of the cholesterol's cell membrane-stabilizing function, a strong membrane perturbation is likely and a strong general cytotoxicity presumable.[8,14,16,17] The influence of the sugar chains was also analyzed by Wang (2007), Hu (1996) and Gauthier (2009) and their colleagues.[3,4,15] According to their findings, sugars attached to the C3 hydroxyl-group of the triterpenoid or steroid backbone like glucuronic acid (see beta-escin, primulic acid 1), arabinose (see alpha-hederin), rhamnose or galactose (see digitonin) support cytotoxicity and membrane permeabilization. Moreover it seems the hydrophobic strength of this moiety is beneficial for membrane binding activities of such saponins, since the more hydrophobic arabinose linked to glucose or glucuronic acid at the C3 hydroxyl-group amplifies the membrane perturbation more than galactose. The same observation can be made for a more hydrophobic butyl residue esterified to the glucose or glucuronic acid instead of a methyl residue.[15] This restores the proposal of Glauert et al. (1962), who predicted saponin-cholesterol complexes with the saponins hydrophilic sugar chains centrally orientated in micellar-like structures. These were responsible for the formation of aqueous pores increasing the membrane permeability.[6,7] The formation of saponin-cholesterol complexes definitely is a membrane perforation and cytotoxicity enhancing process but not the essential mechanism. There are indications that saponins bearing oleanolic acid as aglyconic component are capable of permeabilizing membranes devoid of cholesterol by applying their rhamnose bearing sugar residues on so called rhamnose receptors. This initiates a chain of events leading to cytotoxicity due to cytosolic leakage or the internalization of cytotoxic substances like lectins.[4,15,18] Another enhancement in cytotoxicity and membrane permeabilization can be found for substances glycosylated at two sides (bidesmosidic), the C3 hydroxyl-group and the C28 carboxyl-group of oleanolic acid.[15,19,20]

Saponins showing up with strong cytotoxicities and high membrane permeabilizing potentials stand out with a high reduction in the surface tension and lower cmc-values. EC_{50}-values of saponins that executed lower cytotoxicities and fewer membrane perturbations are twice as high in the mean. This suggests a correlation between cytotoxicity, in many times carried out through membranolytic effects and the ability to react as detergent. This correlation should be influenced by the saponins structures. The monodesmosidic alpha-hederin, digitonin and primulic acid 1 with their (at least the latter two) complexly branched sugar residues and beta-escin featuring a hydrophilic sugar moiety as well as other hydrophilic centers in its chemical structure clearly demonstrate that surface activity is linked to the structure.

4.2. Saponins with weak toxic potential and reduction in surface tension

4.2.1. Glycyrrhetinic acid, glycyrrhizinic acid, hederacoside C

Glycyrrhizinic acid, applied as ammonium salt and its aglycone glycyrrhetinic acid, although being oleanane-type triterpens, appear with weak general cytotoxic and membranolytic potential. Especially glycyrrhizinic acid executed very low general cytotoxicity, very low membrane perforating effects, the lowest reduction in surface tension and showed the highest cmc-value. Taking into mind that some of the values were only noted by approximation due to dissolving problems at high concentrations, it is possible to state that this structure has very poor detergent properties. The observed behavior might find its origin in the aglycone, whose triterpenoid structure differs considerably from oleanolic acid. Accordant with various other authors researching structure–activity relationships of saponins (Bang et al., Gauthier et al.,

Voutquenne et al., Wang et al.) the aglycone is a decisive factor for the cytotoxicity and membrane perforating features of a saponin. Saponins derived from oleanolic acid have a high potential in both cytotoxicity and membrane perforation.[3–5,21,22] Slight changes to this structure leading to aglycones like gypsogenin, quillaic acid or hederagenin hardly have effect on these properties [unpublished results]. That becomes obvious as most saponins with these aglycones strongly lower the surface tension of aqueous liquids and exhibit low cmc-values. Further changes to this structural backbone leading to glycyrrhetinic acid result in an evident decrease in the named effects irrespective of the sugar chains.[4,21] The weak general cytotoxicity and membrane permeabilization of hederacoside C stays in contradiction to the reported structural requirements and is not convincingly to explain. In regard to the strong general cyto- and membrane toxicity of alpha-hederin a at least equivalent toxicity was expectable for the bidesmoside. This observation coincides with those made in other publications: in comparison to alpha-hederin hederacoside C exhibits a much weaker cytotoxicity and membrane perforation and lower or lesser effects on receptors and tissues.[23–27]

4.2.2. Beta-sitosterol, ginsenoside RG2

The monodesmosidic triterpenoid saponin of dammarane-type ginsenoside RG2 revealed a weak cytotoxicity, a very low membrane perforation, low reduction in surface tension and high cmc-values. Only little is known about the cyto- and membrane toxicity of dammarane-type saponins. One study pointed out the number of attached glycosyls to be decisive for the cytotoxicity.[21,28] In that case dammarane-type saponins bearing only one sugar molecule were most toxic, those with more sugars, possibly even subdivided to different junctions of the aglycone showed only weak cytotoxicity. Another study on dammarane-type saponins concluded the number of free hydroxyl-groups in the side chain to be crucial for the cytotoxicity.[21,29] Two hydroxyl-groups bring up a stronger cytotoxicity than one or three. According to these information ginsenoside RG2 lacks of the mentioned structural requirements and is indeed weakly cytotoxic. Moreover the sugar chain of ginsenoside RG2 is linked to C6 of the ring B. This is a rather uncommon position. Assuming a glycosylation at C3 of the ring A also enhances cytotoxicity for dammarane-type saponins as it is observed for oleanane-type saponins, and that the lack of a sugar residue at this position leads to a drop of cytotoxicity, our observation for ginsenoside RG2 becomes conceivable.

Steroid saponins that are known to act cytotoxic usually consist of diosgenin or pennogenin.[3,21,30] Beta-sitosterol has a strong structural similarity to cholesterol which is a natural component of all mammalian membranes. An exchange between membrane-cholesterol and beta-sitosterol was reported and beta-sitosterol might be capable of adopting the membrane-stabilizing function of the exchanged cholesterol.[31,32] Under these circumstances very weak membrane toxicity and therefore very weak general cytotoxicity were predictable. The influence of beta-sitosterol on the membrane does not lead to the formation of pores, pits or tubes. Our results show very low membrane perforation, but also an unexpected strong general cytotoxicity. Possibly the general cytotoxicity of beta-sitosterol finds its origin in another mechanism this substance is known for: numerous studies reported apoptosis-inducing properties for beta-sitosterol on an array of cancer cell lines.[10,31,33] Beta-sitosterol causes cellular death by perturbation of mitochondrial membranes leading to the loss of mitochondrial membrane potential. Moreover the substance targets different caspase-dependent pathways of cellular death and the production of reactive oxygen species (ROS).[33]

The reduction in surface tension by beta-sitosterol and ginsenoside RG2 is in the same range like that of other cytotoxic and/or membranolytic inactive substances. This is markedly since beta-sitosterol has no sugars attached to its rather lipophilic steroidal backbone that might provide amphiphilic features. The high cmc-values compared to the other inactive tested substances might be a consequence of the small molecular size, but other conditions also influence the cmc like the shape of the molecule, proportion of hydrophobic and hydrophilic groups, nature of these groups and polarity.[12]

5. Conclusion

Over all and as Figure 2 indicates a general correlation between a saponin's activity on biologic surfaces and the ability to lower surface tension in liquids is given. Biological surfaces are natural cell membranes, commonly from mammalian cancer cell lines or artificial membrane models, mostly liposomes emulating natural membranes with compositions of phospholipids and cholesterol. We were able to show that biologic highly active amphiphilic substances bring up a higher surface activity in a liquid model than those with a lower or no biologic activity. Although different specific mechanisms to enhance a saponin's general cytotoxicity and membrane perforation are discussed and even if plenty of structural requirements seem to be necessary to work with these mechanisms a non-specific detergent activity is notable for nearly all of the tested amphiphilic substances. This non-specific activity should originate from the yet unknown structural requirements underlying the principles of physical chemistry and applies to any surface. Hence, parts of the influence of amphiphilic substances like saponins on cell membranes as well as on liquids surfaces results from the same cause and a correlation is in all likelihood.

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