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Stability of anthralin in liposomal phospholipids

G. Mahrle[1], B. Bonnekoh[1], M. Ghyczy[2], and W. Wiegrebe[3]

[1] Universitäts-Hautklinik, Joseph-Stelzmann-Strasse 9, W-5000 Köln 41, Federal Republic of Germany
[2] Nattermann & Cie GmbH, Köln, Federal Republic of Germany
[3] Pharmakologisches Institut, Universität Regensburg, Regensburg, Federal Republic of Germany

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Autooxidation of anthralin by air can be enhanced by irradiation with UV or visible radiation, by increase in temperature or pH and by the presence of zinc ions [14]. At the biological level anthralin readily binds to serum albumin and epidermal protein and is then oxidized [10]. Oxidation intermediates, such as anthralin or anthralin-free radicals, may be responsible for the biological effect. Therefore, anthralin should be stable in therapeutical prescriptions, but oxidation in vivo should not be prevented. Anthralin has dual solubility characteristics due to the hydrophilic hydroxyl and carbonyl groups and the lipophilic 10-methylene group [1]. It is, however, relatively unstable in aqueous or alcoholic solutions [7, 11], but relatively stable in white soft paraffin [8].

Liposomes, consisting of phospholipids and forming vesicles in aqueous solutions, have been shown to be ideal carriers and penetration enhancers for some topical dermatological drugs, e.g. glucocorticoids and antimycotics [3, 4]. We therefore tested anthralin stability in phospholipid liposomes. Two different phospholipid mixtures were used for the formulation of liposomes (Table 1). Formulations 1, 2 and 3 contained mainly phosphatidylcholine and phosphatidylethanolamine, which meant that these liposomes did not carry electrical charges and were mainly lipophilic. Formulation 4 contained a large amount of negatively charged phospholipids resulting in electrically charged liposomes at physiological pH with hydrophilic characteristics. There was virtually no difference in particle size and lamellarity between the four formulations.

Formulation 3 had a higher content of electrolytes than the other formulations, in which liposomes were fixed in a gel matrix. Liposomes in formulation 3 were mobile in a dispersion, since electrolytes prevent the formation of a gel matrix. The main fatty acids in the phospholipids were linoleic acid (75%) and linolenic acid (7%). The ratio of unsaturated to saturated fatty acids was 9:1 in all formulations.

Formulation 2 was enriched with 10% primrose oil which contained 71% linoleic acid, 7% oleic acid, 10% γ-linolenic acid, 9% palmitic acid, 1% stearic acid, and 2% other fatty acids [2]. The ratio of unsaturated to saturated fatty acids was 9:1, the same as in the original liposomal phospholipids. In addition to the fatty acid composition of formulations 1, 3 and 4, with 7% α-linolenic acid, formulation 2 contained 1% γ-linolenic acid.

Fresh 1% anthralin phospholipid was prepared by incorporation of 50 mg purified anthralin in 5 g of the vehicle. Purification was by column chromatography (SiO_2, dichloromethane). During the time of investigation the preparation was stored at 5°C under light protection. Samples were taken at different intervals and analysed by thin layer chromatography [15]. C4-lactone, a hydrophilic derivative of anthralin with a lacton ring at position 10, was synthesized as described elsewhere [16]. C4-lactone (derivative X) has been shown to inhibit cell growth in vitro more effectively and to be less toxic than anthralin [5]. It was therefore compared with anthralin, using a 1% concentration in phospholipids.

We found that anthralin rapidly degraded in formulation 2, less rapidly in formulations 1 and 3, and relatively slowly in formulation 4 (Table 2). This meant that there was virtually no difference between the degradation time in the gel or the dispersion formulations 1 and 3, even though anthralin was less soluble in the dispersion than in the gel. The addition of primrose oil reduced the degradation time. The lowest anthralin degradation rate was obtained with the liposome preparation containing a higher content of negatively charged phospholipids. C4-lactone was more stable than anthralin, at least in formulations 2 and 3.

The results demonstrate that anthralin is oxidized within days in liposomal phospholipids. The oxidation process is retarded by using liposomes consisting mostly

Offprint requests to: G. Mahrle

Table 1. Liposomal phospholipid formulations

	Formulation			
	1: NAT 8257	2: NAT 8308	3: NAT 8417	4: NAT 8418
Phospholipid (wt%)	20[a]	18[a]	19[a]	20[b]
Ethanol (wt%)	16	16	16	16
Other ingredients (wt%)	–	10% primrose oil	4% choline chloride	–
Water (wt%)	to 100	to 100	to 100	to 100
Particle size (nm)	200 ± 20	200 ± 20	160 ± 20	190 ± 20
Lamellarity	4–6	4–6	3–5	2–4
pH	6.5	6.1	5.7	7.1
Physical form	Transparent gel	Non-transparent gel	Dispersion	Non-transparent gel

[a] 85% PC, 10% PE, 5% acidic PL; [b] 28% PC, 2% PE, 43% acidic PL, 6% sterine and derivatives, 21% soya oil.
Abbreviations: PL, phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine. Acidic PL are phosphatidic acid, phosphatidylinositol and N-acylphosphatidylethanolamine

Table 2. Stability of anthralin (C4-lactone) in liposomal phospholipid formulations. +, unaffected or minimally oxidized; ±, highly degraded; –, completely oxidized; n.d., not done

Day	Formulation			
	1	2	3	4
0	+ (+)	+ (+)	+ (+)	+ (+)
1	+ (+)	± (+)	+ (+)	+ (+)
2	+ (n.d.)	± (n.d.)	+ (n.d.)	+ (n.d.)
5	+ (+)	± (+)	+ (+)	+ (+)
9	+ (±)	± (±)	± (+)	+ (+)
23	± (±)	– (±)	± (±)	+ (+)
36	– (±)	– (±)	– (±)	± (±)

of negatively charged phospholipids. Compared with other vehicles (Table 3) anthralin in phospholipids was less stable than in white soft paraffin, but more stable than in aqueous or acetone solutions.

Table 3. Stability of anthralin in different vehicles. HLT, half-life time, i.e. the time after preparation at which at least 50% of anthralin is degraded; LCD, liquor carbonis detergens; DMEM, Dulbecco's modified Eagle's medium

Vehicle	HLT	Reference
White soft paraffin	> 1 year	6, 8–10, 12
+ coal tar (room temp.)	< 3 weeks	
+ coal tar (4° C)	> 3 weeks	
+ LCD	about 10 weeks	
Pasta zinci oleosa	< 1 month	13
+ salicylic acid	about 6 months	
Aqueous buffer (pH 7.5)	about 1 h	7
Acetone solution	< 1 h	
0.1% acetone in DMEM (under cell culture conditions)	10 min	5
Phospholipids	< 1 day to < 5 weeks	Present paper

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