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Investigations on topical formulations of clomiphene citrate for treatment of HPV lesions

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Clomiphene citrate (CC), a nonsteroid estrogen analogue with antiestrogenic effects, has been used orally for the treatment of ovulatory failure in women and of human papilloma virus (HPV) – induced venereal warts [1, 2]. The purpose of the use of oral CC administration is to improve ovulation [1]. CC has questionable efficacy in treatment of endometrial hyperplasia. Another route of administration for CC is topical application to the genital area in a dose of 25 mg/g to[1]. Up to 87% of cervical carcinomas contain HPV DNA [3]. When the changes in the DNA of HPV 6, 11, HPV 16, 18, and HPV 31, 35, and 51 were investigated pre- and post-therapeutically using an in situ hybridization technique, it was seen that 100% of condylomata acuminata, 80% of HPV DNA in cervical dysplasia, and 75% of penile genital warts disappeared [1, 3]. All these data indicate that CC has potential efficacy in the treatment of HPV-induced dysplastic lesions [1, 4].

Controlled drug delivery devices applied topically (e.g. bioadhesive drug delivery systems) enable controlled release of the drug and therefore, allow drug remain at the site of application longer [5, 6]. Consequently, dosage frequency is reduced and patient compliance improves. For this reason, we developed new topical formulations of CC for the treatment of HPV dysplastic lesions; bioadhesive gels with controlled release characteristics.

In this study, two bioadhesive gel formulations were prepared using different concentrations of polymer (Noveon AA1-Polycarbophyl and Carbopol 971P: 0.5–1%). The pH values of the gels were measured 5 days apart within 1 month initially. No significant change was observed in pH. This indicated that the bioadhesive gels are stable for 1 month.

The shearing stickness tests were performed for each gel formulation and CC containing ointment, using a custom-made shearing stickness test apparatus at 25 °C. As shown in the Table, the shearing stickness of Formulation FBAG1 (the gel containing 1% w/v bioadhesive polymer) is greater than that of Formulation FBAG2 (the gel containing 0.5% w/v bioadhesive polymer). This is due to the increasing amount of polymer providing better bioadhesion at the ratio of 1 : 1. Formulation FBAG1 will probably remain in the vagina for a longer period than formulation FBAG2.

The viscosity measurements were carried out for both fresh samples (54000 cps and 26000 cps) and 1 month-aged samples. No significant change in viscosity was observed within 1 month for both bioadhesive gel formulations.

After obtaining physically and aesthetically satisfactory gels with strong bioadhesion to vaginal tissue, drug release studies were carried out *in vitro*. Significant differences occurred among formulations with respect to percentage of the released drug. The release of CC from bioadhesive gels was slow in comparison to that from the ointment. During 24 h, 56.7% of drug was released in case of formulation FBAG2 and only 45.7% from formulation FBAG1 (Fig. 1). As predicted period for gel application is once per day from the presented data the most suitable formulation seems to be formulation FBAG2 with a polymer concentration of 0.5%.

In this study, strong bioadhesion to cervical tissue and favorable drug release were achieved. The data obtained in this study suggest that further in vivo investigations of this drug delivery system in treatment of HPV infection are warranted.

Table: Shearing stickiness for both bioadhesive gel formulations. n = 6

	Formulation FBAG1	Formulation FBAG2
Shearing stickiness (g/cm^2)	135 ± 10	100 ± 5

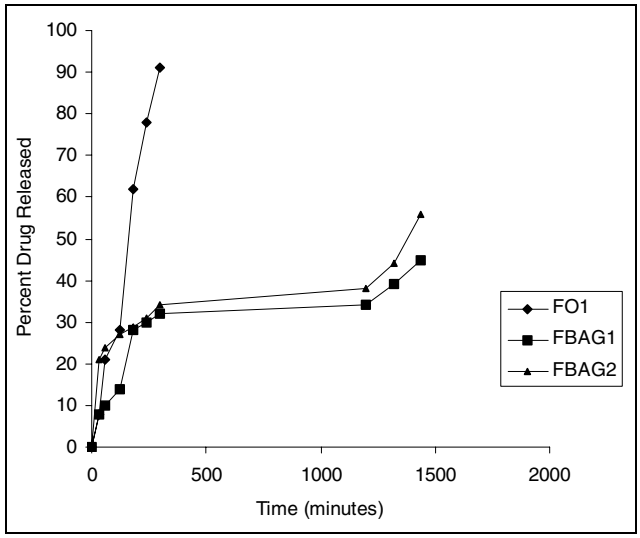

Fig.: Drug release profile for bioadhesive gels and the ointment containing CCs

Experimental

1. Materials

Clomiphene citrate was supplied from Organon, NL. Noveon AA-2 Polycarbophyl and Carbopol 971P were gifts from BF Goodrich, Belgium. All other reagents were of analytical grade.

2. Methods

2.1. Preparation of the bioadhesive gels and the ointment

Formulations FBAG1 and FBAG2

	FBAG1 (g)	FBAG2 (g)
Clomiphene citrate 2.50 g	2.50	2.50
Noveon AA-1 Polycarbophyl 1–0.5 g	1	0.5
Carbopol 971P 1–0.5 g	1	0.5
Glycerol 30–18 g	30	18
Triethanolamine 1.74–3.07 g	1.74	3.07
Distilled water q.s. 100.00 g	100.00	100.0

Polycarbophyl and CP 971P were mixed in distilled water with the aid of a mini shaker at 1000 rpm. CC was mixed with glycerol using a vertical mixer and added to the homogenous polymer solution. The mixture was mixed using a mini shaker. Triethanolamine was added to the resultant homogenous mixture for gelation.

Formulation B (FO1)

An ointment containing 2.50 g of CC was prepared using white petrolatum.

2.2. Determination of pH

The pH of each formulation (the gels and the ointment) was measured at 0., 5., 10., 15., 20., 25., and 30. day.

2.3. In vitro evaluation of polymer adhesion

The shearing stickness at 25 °C was determined to evaluate polymer adhesion in vitro for each gel formulation (FBAG1 and FBAG2), using a cus-

tom-made test apparatus similar to that described in the literature [7, 8]. The apparatus consists of a pulley, a motor and a balance. The gel with the thickness of 0.3–0.4 mm was placed between two glass-plates. A piece of string was wound by means of a motor working at a constant speed of 140 cm/min. The shearing stickness values were represented by the reading on the spring balance attached to the set up when two glass-plates were separated with the applied force of motor.

2.4. Determination of release rate of CC from the bioadhesive gels and the ointment

Drug release studies were carried out using the apparatus for disintegration of vaginal tablets described in BP with some modifications [9, 10]. 2 g sample was applied on top of the perforated plate assembly. The cage was immersed into buffered medium held at 37 ± 0.5 °C and rotated at 50 rpm. The amounts of released CC were determined spectrophotometrically at 232 nm after 30 min, 1, 2, 3, 4, 5, 20, 22, and 24 h.

2.5. Determination of viscosity of the formulations containing CC

Viscosity of bioadhesive gels and the ointment containing CC were measured using a Brookfield viscometer (Mode RVT, Stoughton, MA, 02072, USA). The viscosity measurements were carried out just after preparation and one month later. Each measurement was repeated three times.

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Neuropogonines A, B and C, new depsidon-type metabolites from *Neuropogon* sp., an Antarctic lichen

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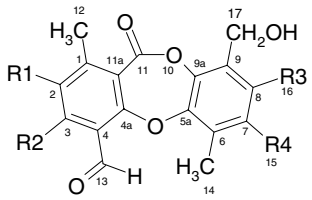
Lichens from special regions of the globe have been suggested as a source of chemically diverse bioactive metabolites [1]. Antarctica is a particularly interesting biosphere due to the special climatic conditions and the distance to other continents. Amongst the aromatic structures from lichens the depsidon-type metabolites form a major group [2–5]. They were identified as constituents of various genera and species of lichens. However, the determined substitution pattern of the hitherto known tricyclic depsidone do not reflect the chemical diversity of these compounds. Here we report structures and biological activities of new depsidones, neuropogonines A, B and C (**1**, **2**, **3**), which were isolated from the Antarctic lichen *Neuropogon* sp.

A 150 g sample of dried *Neuropogon* sp. collected on the Antarctic Livingstone Island in the course of the Bulgarian Antarctic Expedition in 1995, was extracted twice by 500 ml MeOH/$CHCl_3$ (3 : 1) for 48 h. The combined extracts were evaporated and the residue (1.6 g) was subjected to CC on silica gel 60 (Merck, column 5 cm × 40 cm, elution by a) $CHCl_3$, b) $CHCl_3$/MeOH (9 : 1). Thereby five components were eluted which stained blueish on TLC with 1% vanillin/conc. H_2SO_4. Final purification was achieved by preparative HPLC (Lichrospher 100, RP-18, 7 µm, 10 × 250 mm; gradient 95% water/0.1% TFA to 95% acetonitrile, 28 min, 4 ml/min, detection at 210 nm) affording **1**, **2** and **3** in addition to protocetraric (**4**) and usnic acids (**5**) [6].



	R1	R2	R3	R4
1	OH	H	H	CH_2OH
2	H	OH	OCH_3	COOH
3	H	OH	H	COOH
4	H	OH	OH	COOH

4 and **5** were readily identified as known metabolites [6] on the basis of their mass spectrometric data and NMR measurements. Compounds **1**, **2** and **3** were shown as new structures (EI-MS: **1**: MW 344, m/z 344.08981 (M^+, calcd. 344.08979 for $C_{18}H_{16}O_7$); **2**: MW 388, m/z 388.08099 (M^+, calcd. 388.08251 for $C_{19}H_{16}O_9$); **3**: MW 358, m/z 271.06408 ([$M-CO_2$, $-CO$, $-CH_3$], calcd. 271.06708 for $C_{15}H_{11}O_5$).

The presence of aromatic carboxylic groups in **1**, **2** and **3** was confirmed by λ_{max} 1725 cm^{-1} in the IR spectra and λ_{max} 314–320 nm in the UV-VIS spectra.