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Research paper

The release profiles of intact and enzymatically digested hyaluronic acid from semisolid formulations using multi-layer membrane system

Jamal Alyoussef Alkrad, Yahya Mrestani, Reinhard H.H. Neubert*

Institute of Pharmaceutics and Biopharmaceutics, Martin-Luther-University Halle-Wittenberg, Halle, Germany

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Dedicated to Prof. Dr. Dr.h.c. Bernd W. Müller on the occasion of his 60th birthday.

Abstract

A multi-layer membrane system was used to measure in vitro release of hydrophilic macromolecules such as hyaluronic acid (HA) from semisolid formulations. One enzymatically digested HA-derivative with molecular mass of 22 kDa (HA-D) and 1200 kDa intact HA (HA) were incorporated into three semisolid formulations: water-containing hydrophilic ointment (WHO), amphiphilic cream (AC) and water-containing wool wax alcohol ointment (WWO). Because of the high hydrophilic properties of HA-D and HA, the artificial model membranes consisted of collodion as the matrix and glycerol as the hydrophilic acceptor phase. The area under the concentration–time curve and the mean dissolution time were used as a quantitative parameter to characterise the rate and extent of release in vitro. This study showed that the HA-D and HA release as hydrophilic substances from WHO was higher than both from AC and WWO. It was observed that 83% of HA-D1 was released from WHO after 2 h; in contrast, only 10% was released from 2% HA from the same vehicle during the same time. In conclusion, the in vitro availability of enzymatically digested HA-D was higher for WHO than for the other formulations, AC and WWO. Similarly, the availability of HA-D was higher than that of HA from the same formulations.

Keywords: Hyaluronic acid; Release of hydrophilic macromolecules; Pharmaceutical semisolid formulations; Multi-layer membrane system

1. Introduction

The penetration of a drug into the skin is a function of the nature of the drug, the behaviour of the vehicle and the status of the skin. The major variables accounted for differences in the rate of penetration or flux of different topical drugs or of the same drug in different vehicles are the concentration of drug in the vehicle, the partition coefficient of the drug between the Stratum corneum and vehicle, and diffusion coefficient [1]. Some effective and validated in vitro model systems were described to characterise and optimise drug release and penetration from semisolid vehicle systems [2]. Starting from the system described by Beyer [3,4] in the recent years, multi-layer model systems were used for this purpose. According to Fürst and Neubert, a multi-layer membrane system (MLMS) was developed by using an acceptor which consists of hydrophilic and lipophilic collodion membranes [5–7].

Hyaluronic acid (HA) is a linear polysaccharide formed from disaccharide units containing *N*-acetylglucosamine and glucuronic acid. Its molecular weight is usually in the order of 10^6–10^7 [8]. HA is reported to have a radical scavenger capacity [9]. Therefore, it was incorporated into semisolid formulations in order to give UV-protection. Hence, determination of the release profile of HA from semisolid formulations is important to predict its topical availability. In the literature, there are up to date no publications regarding the biopharmaceutical characterisation of semisolid formulations containing drugs or cosmetics with higher molecular mass such as HA. Therefore, the purpose of this study was to investigate the release of an enzymatically digested (HA-D) from different semisolid formulations and to compare it with the release of intact (HA) by using the MLMS.

* Corresponding author. Institute of Pharmaceutics and Biopharmaceutics, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Strasse 4, 06120 Halle/S., Germany. Tel.: +49-345-5525000; fax: +49-345-5527292.
E-mail address: neubert@pharmazie.uni-halle.de (R.H.H. Neubert).

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Table 1
Compositions of semisolid formulations

Emulsified cetylstearylalcohol	9 P	Glycerolmonostearate 60	4 P	Wool wax alcohol	0.25 P
High-viscosity paraffin	10.5 P	Cetyl alcohol	6 P	Cetylstearylalcohol	3 P
White petrolatum	10.5 P	Medium chain triglycerides	7.5 P	White petrolatum	46.75 P
		White petrolatum	25.5 P		
		Macrogol-1000-glycerolmonostearate	7 P		
		Propylene glycol	10 P		
Water-contained HA	70 P		40 P		50 P
Sum	100 P		100 P		100 P

2. Materials and methods

2.1. Apparatus

2.1.1. Capillary zone electrophoreses (CZE)

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany) ^{3D}CE system. Capillaries with light path (fused silica) obtained from Hewlett Packard (Waldbronn, Germany) with a length to detector (56 cm) and internal diameter (50 μm) with a 150 μm extended light path (bubble cell) were used for the determination of HA-D [10]. The amount of HA-D penetrated into the acceptor membranes was measured by CZE. The detection wavelength was at 195 nm, 40 mM phosphate buffer (pH 6.2), 22 kV, temperature of 25°C and injection time of 30 s at 50 mbar were used for the determination of HA.

2.1.2. High performance liquid chromatography (HPLC)

A chromatograph, LC-10 AD SCHIMADZU (Schimadzu, Kyoto, Japan) coupled with detector: SPD-10 A SCHIMADZU (UV Spectrophotometric Detector, Schimadzu, Kyoto, Japan) was employed. The substances were separated with column, Nucleosil-100 C 18 (5 μm) (Knauer, Berlin, Germany). HA was determined using HPLC. The resulting solution after digestion and neutralisation of HA was injected directly into the column. The mobile phase (water/methanol, 96:4) was run at a flow rate of 0.8 ml/min. Detection was done at 280 nm [10]

2.1.3. Multi-layer membrane system

The model apparatus consists of polyacrylate (Piacryl, Piesteritz, Germany) cells. It was described previously [5–7]. The cells were fitted together and placed in a chamber maintained at 32 ± 0.2°C during the experimental period. Each acceptor system contained three glycerol–collodion membranes (Fig. 1).

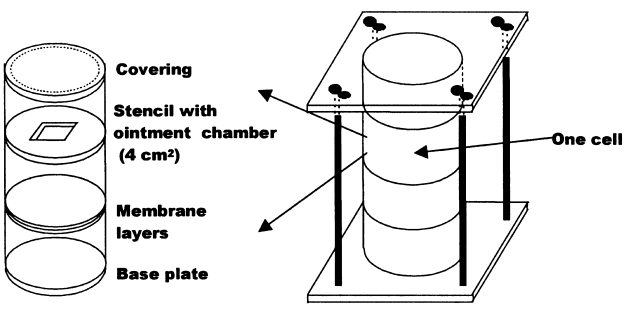

Fig. 1. Multilayer membranes system (MMS).

2.1.4. pH-meter

HI 9321 microprocessor, Hana-Instrument (Karlsruhe, Germany).

2.2. Materials

HA derivative (22 kDa) and HA (1200 kDa) were obtained from Hans-Knöll-Institut (Jena, Germany). Sulphuric acid was obtained from Fluka (Buchs, Switzerland); methanol was obtained from J-T-Baker (Deventer, Netherlands); Sodium hydroxide (NaOH) from Roth (Karlsruhe, Germany); dipotassium phosphate, monopotassium phosphate from Merck (Darmstadt, Germany); collodion and glycerol were purchased from Caesar and Loretz (GmbH Hilden, Germany); absolute ethanol and ether obtained from Merck (Darmstadt, Germany); white petrolatum, high-viscosity paraffin, propylene glycol, glycerolmonostearate 60, cetyl alcohol, cetylstearylalcohol and emulsified cetylstearylalcohol were obtained from Hansen and Rosenthal (Wasserfuhr, Bonn, Germany); medium chain triglycerides were obtained from Pharma GmbH & Co.(Eschwege, Germany); wool wax alcohol from Croda (Synopharm, Barsbüttel, Germany) and Macrogol 1000 from BASF (Ludwigshafen, Germany).

2.3. Methods

2.3.1. Semisolid formulations preparation

The HA was dissolved in the hydrophilic phase before mixing the hydrophobic and hydrophilic phases in order to produce three systems, namely, water-containing hydrophilic ointment, water-containing wool wax alcohol ointment (DAB) [11] and amphiphilic cream (DAC) [12] (Table 1). Formulations were prepared according to DAB and DAC. HA was incorporated with different concentrations (1, 2 and 5%) in different formulations, where HA-D was incorporated in concentration of 2%.

2.3.2. Preparation of hydrophilic membranes

Solution A contained 100 g 4% collodion. Solution B

Table 2
The area under concentration–time curve (AUC) and the mean dissolution time (MDT) of HA-D (22 kDa) and HA (1200 kDa) release from semisolid formulations

Formulations	HA		HA	
	AUC (% min)	MDT (min)	AUC (% min)	MDT (min)
Water-containing hydrophilic ointment 5%	1020 ± 56.4	4	–	
Water-containing hydrophilic ointment 2%	1040 ± 52.2	3	30767.5 ± 1636.7	31.1
Amphiphilic cream 5%	670 ± 55.5	1.7	–	
Amphiphilic cream 2%	–		15810.0 ± 1310.2	34.6
Water-containing wool wax alcohol ointment 5%	540 ± 61.4	1.8	–	
Water-containing wool wax alcohol ointment 2%	–		10277.5 ± 1168.9	20.6

was prepared in such a way that 4 g of mixture was removed from 100 g mixture of ether and ethanol (85:15) and then 4 g glycerol was added to the remaining mixture. Finally, A and B were mixed and then the resulting mixture was placed on a glass surface of a film-forming device [5–7]. The membrane was dried for 4 h at room temperature and cut into discs of 4 cm diameter. The content of glycerol in the membrane was 2.5 mg/4 cm^2.

2.3.3. Solutions preparation

A 40 mM phosphate buffer solution (pH 6.2) for CZE was prepared by dissolving 4.599 g potassium hydrogen phosphate and 1.079 g potassium dihydrogen phosphate in water, filling up to a volume of 1000 ml. The pH of the buffer was measured at 25°C using a pH-meter (Hana-Instrument). The mobile phase for HPLC was freshly prepared by adding 40 ml of HPLC-grade methanol to 960 ml of bidistilled water (water/methanol 96:4 (v/v)). This solution was degassed by ultrasonic bath for 30 min and kept in tightly closed bottle.

2.3.4. Release studies

An accurately weighed quantity of the topical formulation (10 mg) was applied to the acceptor system, which was fixed in a penetration cell with an exposed application area of 4 cm^2. The release cells were fixed in the model constructed and placed in a chamber maintained at 32 ± 0.2°C during the experimental period. The model apparatus was removed from thermostat chamber at selected time intervals. The release cells were separated and the amount of applied formulation remaining on the first acceptor layer was removed. The membranes were extracted with 3 ml distilled water: the lipid phase was removed with 5 ml ether, while the aqueous phase containing HA-D extracts were filtered with 4 μm filter before measuring in CZE [10]. HA extracts were dried in an autoclave at 100°C. After an hour, each sample was dissolved with 0.25 ml of concentrated H_2SO_4 and kept for 5 min and then adjusted to pH 5 with 1 N NaOH and measured using HPLC [10].

3. Results and discussion

HA as well as HA-D (after the enzymatic digestion) are radical scavengers. It is therefore important to incorporate either HA or HA-D in semisolid formulations to protect the skin from damage associated with radicals. Characterisation of the release of HA and HA-D from semisolid formulations is essential to have optimal therapeutic effect. In this study, HA (1200 kDa) and HA-D (22 kDa) were used to evaluate their in vitro releases from semisolid formulations. The in vitro release of HA was monitored only over a period of 120 min because of its low release rate; on the other hand, HA-D release was measured over a period of 360 min. TOPFIT 2.0 PC program [13] was used to determine the area under concentration–time curve (AUC) and mean dissolution time as parameter to characterise in vitro rate and extent of HA and HA-D release. The results were expressed as plot of HA and HA-D concentration (%), respectively, in the acceptor membrane versus time (Figs. 2 and 3).

As shown in Fig. 2, the amounts of HA released from both 2 and 5% WHO after 20 min incubation time were more than 5% (only 10 and 25 μg, respectively, of the initial amounts of HA (200, 500 μg) present in 10 mg of the

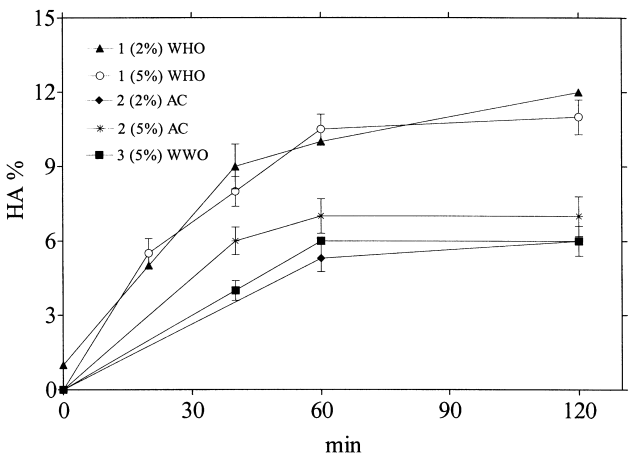

Fig. 2. In vitro release profile of HA (1200 kDa) from three different semisolid formulations with different concentrations: 1, 2 and 5% (1: water-containing hydrophilic ointment (O/W cream), 2: amphiphilic cream, 3: water-containing wool wax alcohol ointment (W/O cream).

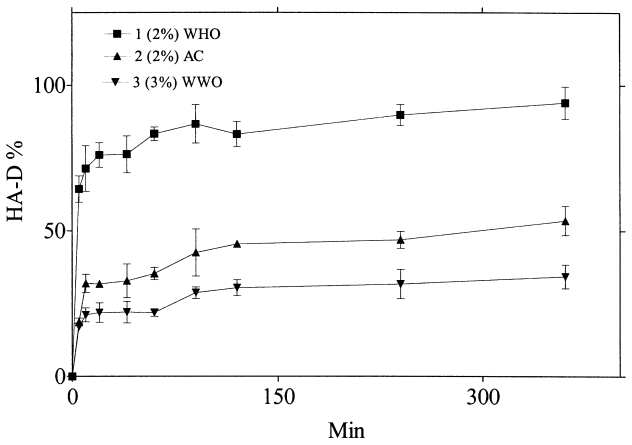

Fig. 3. In vitro release profile of HA-D (22 kDa) from three different semisolid formulations with concentration of 2% (1: water-containing hydrophilic ointment, 2: amphiphilic cream, 3: water-containing wool wax alcohol ointment).

respective ointments). The maximum released amount of HA was 5% from 5% WHO after 60 min and 12% from 2% WHO after 120 min. Except from 2% WHO (after 20 min) and 2% AC (after 60 min), the amount of HA released from 1 and 2% ointments in all the three formulations were below the detection limits. Similarly, the amounts of HA released from 5% WWO and AC were undetectable with the incubation time of less than 40 min. The maximum released amounts of HA from 5 and 2% AC were 7 and 5.5%, respectively, and were obtained after 60 min. Also, 5.5% was the maximum amount of HA released from 5% WWO.

The AUC of HA released from semisolid formulations were calculated and presented in Table 2. The AUC obtained from the release profile of HA from WHO was higher than that obtained from both AC and WWO.

HA-D of 64% was released from 2% WHO after 5 min and 94% after 360 min (Fig. 3). Only 18% of HA-D was released from 2% AC after 5 min and 53% after 360 min. This indicated that HA-D showed a higher affinity to AC than to WHO. The lowest release of HA-D was observed from 2% WWO; 17 and 34%, after 5 and 360 min, respectively.

Generally, it was found that the highest quantity of HA-D was released from WHO followed by AC and then WWO. The AUC of HA-D released from the three vehicle systems are presented in Table 2. The semisolid formulations were arranged according to their AUC as follows: WHO > AC > WWO.

The differences in the release profiles of equal molecular mass HA from different vehicles, HAs with different molecular mass from the same vehicle, could be due to differences in the physicochemical properties of HA such as its solubility and partition coefficient. Therefore, the release behaviour, which is thermodynamically controlled, can be predicted by physicochemical parameters such as solubility in the different phases of the reams and partition coefficients [14]. It is in general evident that it easier to get a higher release of a hydrophilic macromolecule such as HA from an O/W cream (WHO) than from a W/O cream (WWO) or AC. These results indicate that the HA affinity to the hydrophilic cream (O/W cream) vehicle is less than both to amphiphilic cream and to the lipophilic cream (W/O cream) because of release of a hydrophilic macromolecule such as HA from the W/O cream is limited and can be controlled by its solubility in the outer phase of the cream.

The MLMS measurement of the in vitro release of HA from the same semisolid formulations exhibited very low release as compared to HA-D. Hence, it is possible to assume that the size of the HA molecules could also exert an influence on their release from different vehicle systems.

4. Conclusion

The topical availability of the HA varied from different vehicle systems. So does the availability of HA and HA-D with different molecular mass from the same vehicle system. The affinity of HA to the O/W cream is lower than both to the AC and to the W/O cream. In addition, the topical availability of HA-D (22 kDa) was found to be higher than the availability of HA (1200 kDa). It is, therefore, more useful to use enzymatically digested HA-D instead of intact HA and hydrophilic creams (O/W creams) as vehicle systems.

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