Testing Keratolytic Effect of Carbamide in Various Vehicles

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Background. Urea is a natural protein degradation product widely used in keratolytic preparations due to its property to promote fibrinogen decomposition and to act as a proteolytic agent dissolving and denaturing proteins. Preparations containing 20% or 40% urea in commercial ambiphylic bases are widely used to produce keratolysis. At such high concentration urea recrystallization occurs and is separated over the preparation mass, particularly if the urea content is 40%. This was the initial reason to look for a new preparation formulation that would not have the above-mentioned technological inadequacy. Considering urea’s water solubility and its high content in the preparations, the most convenient form, in which recrystallisation would not occur, was gel. The aim of this work was to experimentally estimate the influence of the vehicle upon the proteolytic effect of urea on keratin in the human hair model.

Methods. Hair geometrical properties, the change in longitudinal mass, and the change of disruptive force and disruptive hair elongation were measured. Results. The effect of 20% and 40% urea was estimated in three various vehicles, of which two were of organic hydrogel type, and one was an ambiphylic base of mixed type - Basiscreme. Conclusion. Keratin proteolysis effects of urea incorporated into various bases, as measured by human hair disruption forces, depended upon the base; preparations containing 20% and 40% urea in Basiscreme (ambiphylic base) had no desired proteolytic effect upon keratin fibers; proteolysis was best expressed by urea preparations in 3.5% NaCMC gel, and proteolytic effect of these preparations was best expressed in the first hour.

Key words: Keratolytic agents; urea; vehicles; excipients; keratin; hair.

Introduction

Urea or carbamide (carbonic acid diamide), is a natural protein degradation product (1, 2). However, urea is an ingredient of the low-molecular fraction of hydrosoluble cutaneous humectants, i.e., natural moisturizing factor (3). As it has high water-binding capacity within its crystal grid, it is added in lower concentrations to skin-care formulations, serving as an effective moisturizing agent (4). It is widely used in keratolytic preparations, because of its property at concentrations of 15% or higher, to promote fibrinogen decomposition and to act as a proteolytic at the concentration of 40%, dissolving and denaturing proteins (5). It is convenient for use as it has no sensitizing or photodynamic effect. It does not stain, and has neither color nor odor itself (1).

Keratins are constituents of protective tissues and are water insoluble. They are high-cystine content proteins. According to their physical properties, especially cystine content, keratins are divided into soft keratins, containing less than 3% sulfur (skin), and hard keratins, containing over 3% sulfur (nails, hair) (6).

Preparations containing 20% or 40% urea in commercial ambiphylic bases are widely used. The purpose of their use is keratolysis (7, 8). As 1 g of urea is dissolved in 1-1.5 g of water (9, 10), in the given preparations (containing 32, and 42% water, respectively), urea recrystallization occurs and is separated over the preparation mass (particularly if urea content is 40%). This prompted us to look for a new preparation formulation that would not have the mentioned technological inadequacy.
Considering urea’s water solubility and its high content in the preparations, the most convenient form, in which re-crystallisation would not occur, is the gel formulation. However, it was necessary to estimate whether the vehicle change increased, decreased or did not affect the keratolytic effect of urea.

The aim of this work was to estimate experimentally the influence of vehicle upon proteolytic effect of urea on keratin in the human hair model, as human hair cortical cells contain keratin in a very high proportion, and cortex makes the most of the human hair total mass (11). In fact, cyto-keratins forming intracellular cytoskeleton within the hair cortex make its largest part responsible for its unique physical properties. About 60% of hair keratins are composed of hard keratins with a low sulfur content and an average molecular weight of 40-60 kDa. Hair matrix serves as a biological glue composed of proteins with a high cystin and sulfur content - high-sulfur proteins (HSP). In the hair extract, HSP make more than 38% of its protein fraction (12). Therefore, we tested the effect of 20% and 40% urea in three various vehicles, two of organic hydrogel type, and one representing an amphiphilic base of mixed type - Basiscreme (13).

**Methods**

**Human hair**

Human hair (12 cm of length), epilated from the scalp as immersed in preparations containing 20% or 40% urea for 1 or 8 hours.

Human hair swelling in water is a reversible process, provided there are no additional influences such as alkaline or acid pH, high temperature, etc. Wet hair properties are essentially different from those of the dry hair; therefore, after the treatment, human hair samples were conditioned over a drying medium (silica gel) for 24 hours in order to avoid the hydrolytic effect of residual moisture in hair (6), which was not the subject of the present study. Then the treated and control (untreated) hairs were used to measure their vector properties, as described below.

**Preparations**

Urea formulations used in the study were the following: 20% urea in Basiscreme (CB20), 40% urea in Basiscreme (CB40), 20% urea in 0.5% Carbopol 940 gel (C20), 40% urea in 0.5% Carbopol 940 gel (C40), 20% urea in 3.5% sodium carboxymethylcellulose gel (7pp) (CM20), and 40% urea in 3.5% sodium carboxymethylcellulose gel (7pp)(CM40). Measurement of pH in the preparations was performed on the Iskra’s MA 5740 pH-metre.

**Measurement of hair vector properties**

Alterations of treated hairs vector properties compared to non-treated hairs ("0") were analyzed. Geometrical properties, the longitudinal mass, and two straining static parameters, disruptive force and disruptive hair elongation were measured. One hundred measurements were performed per a hair specimen, and the mean value was calculated.

Longitudinal mass measurement: In order to define the disruptive force, as the force by the unit area, it was necessary to determine the hair thickness, i.e., longitudinal mass. As hair has the elliptical rather than the ideal circular cross-section, the longitudinal mass was measured by determining the degree of ellipticity (the ratio of longer transsectional radius to wards the shorter transsectional radius) and this value was expressed in decites (dtx). The longitudinal mass measurement was performed by a vibroscpical method based upon the determination of resonant frequency of a single hair strained by a certain pre-load (6), in our case 500 mg. Measurements were performed on the Vibroscope device.

Measurement of disruptive elongation: Hair strength is defined as the resistance of hair to the mechanical force. The hair resistance to the elongation force was measured by Frank’s hydraulic dynamometer in a static test, i.e. one end of the hair was fixed while the other was strained by force. The disruptive elongation was expressed as percentage (6).

**Results**

Table 1 shows the pH of freshly tested preparations which were the same after 12 hours.

**Table 1**

| Preparation | CB20 | CB40 | C20 | C40 | CM20 | CM40 |
|-------------|------|------|-----|-----|------|------|
| pH          | 6.90 | 7.00 | 6.80 | 7.10 | 7.20 | 7.00 |

*CB20 and CB40 = 20 g or 40 g urea in Basiscreme; C20 and C40 = 20 g or 40 g urea in 0.5% Carbopol 940 gel; CM20 and CM40 = 20 g or 40 g urea in 3.5% sodium carboxymethylcellulose gel.*

*pH was measured in freshly made preparations, as well as after 12 hours. There was no measurable difference in any of the samples.

Tables 2. and 3. show the values required for calculating the disruptive force, which was the basic indicator of keratolysis.

Having analyzed the changes of longitudinal mass and disruptive elongation in all the groups after 1 and 8 h of exposure, it was noticed that in hairs treated with an amphiphyl base as urea carrier for 1h longitudinal mass and disruptive elongation were increased; only a longer exposure of hair to the...
The action of these preparations (8h) caused a slight decrease in longitudinal mass value, and elasticity still remained higher than initially.

Table 4 shows the influence of the time on the disruptive force exerted by tested preparations. If the value obtained for the disruptive force of untreated hair is taken as zero value, then Table 4 represents the percentage of disruptive force decrease in hairs treated with the analyzed preparations. As keratolysis was expected, and therefore a decrease in disruptive force, the minus signified an increase in disruptive force, i.e. a "keratoplastic" effect.

### Table 2

| Preparation | Longitudinal mass (dtex) | Disruptive force (cN/dtex) | Disruptive elongation (%) |
|-------------|--------------------------|---------------------------|--------------------------|
| "0" (control)† | 57.8                     | 1.84                      | 54.9                     |
| CB20        | 58.8                     | 2.00                      | 58.8                     |
| CB40        | 58.3                     | 1.84                      | 59.8                     |
| C20         | 56.3                     | 1.76                      | 50.2                     |
| C40         | 52.8                     | 1.72                      | 53.6                     |
| CM20        | 54.0                     | 1.72                      | 53.7                     |
| CM40        | 50.7                     | 1.61                      | 51.8                     |

† See legend under Table 1.
†† dtex = decitex [longer transsectional radius (TR)/shorter TR]
‡‡ cN = centi Newton
† urea untreated hairs

### Table 3

| Preparation | Longitudinal mass (dtex) | Disruptive force (cN/dtex) | Disruptive elongation (%) |
|-------------|--------------------------|---------------------------|--------------------------|
| "0" (control)† | 59.60                   | 1.94                      | 60.0                     |
| CB20        | 59.39                    | 1.91                      | 64.1                     |
| CB40        | 57.83                    | 1.89                      | 64.6                     |
| C20         | 56.54                    | 1.93                      | 64.7                     |
| C40         | 56.83                    | 1.91                      | 64.7                     |
| CM20        | 56.15                    | 1.82                      | 62.1                     |
| CM40        | 55.71                    | 1.70                      | 62.0                     |

† See legend under Table 1.
†† dtex = decitex [longer transsectional radius (TR)/shorter TR]
‡‡ cN = centi Newton
† urea untreated hairs

### Discussion

The essential parameter of this work was the pH value of the tested preparations, since urea is stable only in a neutral pH and at concentrations over 2 mol/l (i.e., 12%) (14).

### Table 4

| Preparation | Duration of treatment | 1 hour | 8 hours |
|-------------|-----------------------|--------|---------|
| CB20        | - 8.7                 | 1.55   |         |
| CB40        | 0                     | 1.58   |         |
| C20         | 3.26                  | 1.04   |         |
| C40         | 6.52                  | 1.55   |         |
| CM20        | 6.52                  | 6.20   |         |
| CM40        | 12.50                 | 12.32  |         |

† See legend under Table 1.
as an amphoteric gel, having both acid and alkaline groups with approximately equal inversion capabilities (15). They are most stable at a neutral pH in the preparations. Excluding the influence of other factors, one could directly study the influence of the alkaline media (inhibiting or potentiating) on the extent of proteolysis of the human hair keratin induced by urea.

Such an effect could be attributed to the effect of the vehicle itself upon keratin, or to the base inhibitory effect on keratolytic properties of urea, especially in the preparation containing 20% of urea, as urea at lower concentration causes loosening of cell membrane and corneocyte intercellular spaces (3). As bases of this type contain surface active compounds (in this case nonionogenic), the effect of an amphoteric base upon the hair properties could be a subject of special studies. For the time being we can only certify that the bases of this type (i.e., particularly Basiscreme) are not suitable for manufacturing preparations with high urea content, in which its keratolytic effect is expected.

It was interesting to analyze the changes in hair elasticity in gel bases. After the initial stability or a slight decrease, the longer hair treatment produced elasticity increase, i.e. the disruptive elongation, which was more evident in Carbopol base than in the cellulose base. A possible explanation could have found in the kinetics of bonds’ formation and decomposition. While urea could disrupt intramolecular hydrogen bonds in protein chains (2) in the initial phase, later a cross-linkage of the lysed bonds occurred and new chemical bonds were created at the lateral protein functional groups. These were more flexible than the primary bonds (3).

The subject of our analysis was testing the hair’s strength alterations by measuring the disruptive force before and after the treatment with urea-containing preparations. The higher degree of hair lysis, the less force was necessary to apply per unit area to disrupt the hair. Therefore, the percentage of disruptive force decrease of the treated hair relative to that of untreated hair indicated the proteolysis achieved with urea.

Results presented in Table 4 show that the greatest percentage of disruptive force was achieved by the preparation containing 40% urea in 3.5% NaCMC gel, after 1h of treatment. Results achieved with the same preparation after 8 h were almost identical to the results achieved after 1h; certain decrease was even noted (as well as in the preparations containing 20% urea in the same basis). One of the possible explanations of the obtained results could be based upon the already described cross-linkage of the previously cleft bonds in the first hour. But because urea acted as a stabilizing agent for non-keratin proteins (which amount to 17% of the human hair medulla), this property obviously manifested itself only after the disruption of the keratin layer, and this also led to a decrease in the urea’s proteolytic properties in the function of time (6).

It was observed that the preparations with the amphoteric bases produced almost identical results after 8 hours, regardless of urea content, while 20% urea in the initial hours caused an increase in disruptive force by 8.7%, i.e. paradoxically stabilized the hair. In the preparation containing 40% urea, in the first hour, disruptive force in the treated hair was identical to the one in the untreated hair. Such results for an amphoteric base as urea vehicle were completely in accordance with the previously described results for the disruptive elongation and the longitudinal mass.

In the case of Carbopol base, doubling urea content caused a two-fold increase in the disruptive force percentage in the first hour, while after 8 h a sudden efficiency decrease occurred, especially in the preparation containing 40% urea. As Carbopol base is synthesized by neutralization of acrylic acid with an organic alkali (as in our case), it would be necessary to analyze the relationship between the base and keratin.

It has to be pointed out that only in the preparations made in NaCMC gel one could find out that the double urea content produced two-fold decrease of disruptive force percentage both at the first and the eighth hour, whereas this regularity in preparations made with 0.5% Carbopol gel was only found in the first hour, however with the efficacy almost 50% lower than in the NaCMC gel.

**Conclusion**

1. Keratin proteolysis effects of urea incorporated into various bases, measured by human hair disruption forces, depended upon the type of the base.
2. Preparations containing 20% and 40% urea in Basiscreme (amphoteric bas) had no desired proteolytic effect upon keratin fibers.
3. Proteolysis was best expressed by urea preparations in 3.5% NaCMC gel and proteolytic effect of these preparations was best expressed in the first hour.

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Urea je prirodni produkt degradacije proteina koji se naširoko koristi u keratolitičkim preparatima zbog svoje osobine da povećava razgradnju dekopoziciju fibrinogena i da se ponaša kao proteolitičko sredstvo za rastvaranje i denaturaciju proteina. U širokoj upotrebi, kao keratolitici, su preparati koji sadrže 20% i 40% uree u komercijalnim ambifilnim podlogama. Pri tako visokoj koncentraciji uree u preparatima dolazi do njene rekristalizacije u masi preparata, naročito ako je sadržaj uree 40%. Ovo je bio osnovni razlog da se traže nove formulacije preparata koje ne bi imale gore navedene tehnološke nedostatke. Razmatrajući mogućnost rastvaranja uree u vodi i njen visok sadržaj u preparatima, najpodesniji oblik, u kome ne bi došlo do rekristalizacije, je gel. Cilj ovog rada bio je da se na modelu humane dlake objektivno proceni uticaj podloge na proteolitički efekat uree na keratinska vlakna. Procenili smo efekat 20%-tne i 40%-tne uree u tri različite podloge od kojih su dve tipa organskih hidrogela, a jedna je ambifilna podloga mešovitog tipa - Basiscreme. Merene su geometrijske osobine dlake: promena u longitudinaloj masi, promena sile kidanja i promena sile istezanja. Iz te studije izvučeni su sledeći zaključci: efekti proteolize kera-
tina ureom inkorporisanim u različite podloge, mereni promenom sile kidanja humane dlake, zavise od podloge; preparati koji sadrže 20% i 40% uree u Basiscre-
me (ambifilna podloga) nemaju željeni proteolitički efekat na keratinska vlakna; pro-
teoliza je najefikasnija sa preparatima uree u 3,5%-nom gelu NaCMC, kao podlozi i najbolje proteolitičke efekte ovi preparati uree pokazuju u prvom satu.

**Ključne reči:** Keratolitici; urea; vehikulumi; ekscipijensi; keratin; dlaka.