

Anti-acne preparations containing tetracycline, azelaic acid and azeloglycine: Optimization of stability and physicochemical properties

Preparaty przeciwtrądzikowe zawierające tetracyklinę, kwas azelainowy i azeloglicynę: optymalizacja stabilności i właściwości fizykochemicznych

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Abstract

Background. Acne vulgaris is a common inflammatory skin condition affecting almost 85% of the adolescent and young adult population. The etiopathogenesis of this dermatosis involves an imbalance in the skin microbiome, leading to inflammation of both the skin and hair follicles.

Objectives. The aim of this study was to develop topical anti-acne formulations with increased therapeutic efficacy and reduced risk of developing antibiotic resistance. Six hydrogel formulations containing azelaic acid or its derivative, azeloglycine, in combination with tetracycline hydrochloride were prepared as part of the study.

Materials and methods. The investigated formulations were prepared using an Eprus U500 pharmaceutical mixer and the pH was determined using an ERH-11S electrode designed for dense substances and a CPC-505 Elmetron pH-meter. The formulations were analyzed for tetracycline stability in the presence of additional active ingredients and varying pH over a period of 35 days using high-performance liquid chromatography (HPLC). In addition, the effects of azeloglycine and azelaic acid on the viscosity of the prepared formulations were evaluated using a Brookfield DV2T rotational viscometer.

Results. Chromatographic analysis showed significant stability of tetracycline in most formulations, with azeloglycine-containing formulations showing less degradation of the antibiotic than azelaic acid-containing preparations. In addition, azeloglycine-containing gels exhibited more favorable rheological properties, which may facilitate better application and be more beneficial to patients.

Conclusions. The results suggest that formulations containing azeloglycine and tetracycline may be a promising strategy for acne therapy, offering increased tetracycline stability and an optimal rheological profile, which may result in prolonged therapeutic effect and more effective drug delivery to the skin.

Key words: stability, azelaic acid, tetracycline, potassium azeloyl diglycinate, azeloglycine

Streszczenie

Wprowadzenie. Trądzik pospolity to powszechna zapalna choroba skóry, dotykająca blisko 85% populacji nastoletniej i młodych dorosłych. Etiopatogeneza tej dermatozy obejmuje zaburzenie równowagi mikrobiomu skóry, prowadzące do stanów zapalnych zarówno w obrębie skóry, jak i mieszków włosowych.

Cel pracy. Celem niniejszego badania było opracowanie miejscowych preparatów przeciwtrądzikowych o udoskonalonej efektywności terapeutycznej oraz zredukowanym ryzyku rozwoju oporności na antybiotyki. W ramach badań przygotowano sześć hydrożelowych formułacji, zawierających kwas azelainowy lub jego pochodną — azeloglicynę, w połączeniu z chlorowodorkiem tetracykliny.

Materiały i metody. Badane preparaty otrzymano przy użyciu miksera farmaceutycznego Eprus U500, wartość pH wyznaczono przy użyciu elektrody ERH-11S przeznaczonej do substancji gęstych oraz pH-metru CPC-505 firmy Elmetron. Formułacje analizowano pod kątem stabilności tetracykliny w obecności dodatkowych składników aktywnych oraz zmiennego pH przez okres 35 dni przy użyciu wysokosprawnej chromatografii cieczowej HPLC. Ponadto, oceniano wpływ azeloglicyny oraz kwasu azelainowego na lepkość przygotowanych preparatów przy użyciu viskozymetru rotacyjnego Brookfield DV2T.

Wyniki. Analiza chromatograficzna wykazała znaczną stabilność tetracykliny w większości formułacji, przy czym preparaty zawierające azeloglicynę cechowały się mniejszą degradacją antybiotyku w porównaniu do formułacji z kwasem azelainowym. Ponadto, żele z azeloglicyną wykazały korzystniejsze właściwości reologiczne, co może sprzyjać lepszej aplikacji i być korzystniejsze w użyciu dla pacjentów.

Wnioski. Uzyskane wyniki sugerują, że preparaty zawierające azeloglicynę i tetracyklinę mogą stanowić obiecującą strategię w terapii trądziku, oferując zwiększoną stabilność tetracykliny oraz optymalny profil reologiczny, co może przekładać się na przedłużone działanie terapeutyczne i efektywniejsze dostarczanie substancji aktywnych do skóry.

Słowa kluczowe: kwas azelainowy, stabilność, azeloglicyna, diglicynian azeloilu potasu, tetracyklina

Background

Acne vulgaris, a common inflammatory skin condition, affects nearly 85% of the adolescent and young adult population.^{1,2} This dermatosis is associated with an overgrowth of the skin microbiome, including *Cutibacterium acnes*, as well as increased skin sebum secretion, increased keratinization of the sebaceous gland orifices and the development of local inflammation.^{3–5} Topical therapy is used for mild to moderate forms of acne, with satisfactory results in more than half of patients.^{6,7} In the present study, an attempt was made to develop anti-acne formulations with multidirectional topical activity. The key formulation ingredients to inhibit pathogenic microorganisms are tetracycline hydrochloride and azelaic acid or potassium azeloyl diglycinate, called azeloglycine. This combination aims to increase the efficacy of topical therapy while reducing the risk of developing antibiotic resistance.^{8–11}

Materials and methods

Tetracycline hydrochloride (Merck Life Science Sp. z o.o., Poznań, Poland), 2-amino-2-methyl-1,3-propanediol (AMPD; Merck Life Science Sp. z o.o.), Carbopol 980 NF – polyacrylic acid crosslinked with allyl pentaerythritol (Lubrizol, Wickliffe, USA), ethanol (Stanlab, Lublin, Poland), potassium azeloyl diglycinate (azeloglycine), sol (Zrób Sobie Krem, Prochowice, Poland), azelaic acid (Pol-Aura, Morąg, Poland), and demineralized, bi-distilled water were used to prepare the formulations. Acetonitrile (Merck Life Science Sp. z o.o.), formic acid (Merck Life

Science Sp. z o.o.) and demineralized, bi-distilled water were used in the high-performance liquid chromatography (HPLC) analysis.

Six hydrogels were prepared, differing in the type of active ingredient. Three hydrogels (designated 1KA, 2KA and 3KA) contained azelaic acid at concentrations of 1%, 2% and 3%, while the other 3 (1A, 2A and 3A) contained azeloglycine at analogous concentrations. All hydrogels contained the same tetracycline content of 0.2%. The Carbopol content of the formulations was constant and homogeneous for all gels. The concentration of AMPD was uniform in all groups of formulations, but was higher in gels containing azelaic acid than in gels containing azeloglycine due to the acidic nature of this ingredient. All formulations also contained the same amount of ethanol, which acted as a co-solvent. The detailed composition is summarized in Table 1.

The formulations were obtained by homogenization for 16 min using an Eprus U500 pharmaceutical mixer (Eprus, Bielsko-Biała, Poland) at 630 rpm to obtain optimal dispersion of the active ingredient. The developed gels were stored at 3°C in opaque containers protected from light throughout the study.

The pH of all hydrogels was measured using an ERH-11S pH electrode (Elmetron, Zabrze, Poland), designed for the analysis of highly viscous materials, and a CPC-505 pH meter (Elmetron). Five pH measurements were made for each of the hydrogels tested.

Viscosity measurements of all formulations were performed using a Brookfield DV2T rotational viscometer (Ametek, Middleborough, USA) with spindle number 6. The viscosity of each formulation was measured

Table 1. Compositions of the analyzed formulations

Formulation	TC [g]	Azelaic acid [g]	Azeloglycine [g]	Ethanol [g]	AMPD [g]	Carbopol 980NF [g]	Water [g]
1KA	0.2	1.0	0.0	14.0	3.4	1.5	79.9
2KA	0.2	2.0	0.0	14.0	3.4	1.5	78.9
3KA	0.2	3.0	0.0	14.0	3.4	1.5	77.9
1A	0.2	0.0	1.0	14.0	2.1	1.5	81.2
2A	0.2	0.0	2.0	14.0	2.1	1.5	80.2
3A	0.2	0.0	3.0	14.0	2.1	1.5	79.2

at a constant spindle speed of 120 rpm for 1 min. To ensure repeatability of results, identical measurement conditions were maintained for all samples. Six measurements were performed for each hydrogel.

Each hydrogel tested was subjected to HPLC analysis at equal intervals every 7 days for a period of 35 days. For sample preparation, 0.5 g of hydrogel was taken from each formulation and dissolved in 99.5 g of water. The dissolution process was carried out on an Arex Digital Pro magnetic stirrer (Velp Scientifica, Usmate, Italy) at a constant speed of 900 rpm for 20 min. Six 1 mL samples were taken from each preparation.

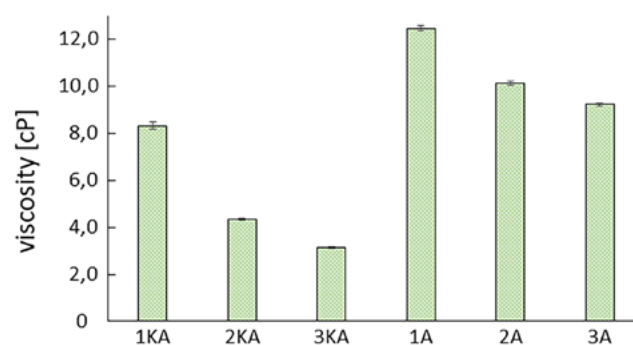
The HPLC analysis was performed on a Thermo Scientific Dionex UltiMate 3000 liquid chromatograph (Dionex Corporation, Sunnyvale, USA) using an TCC-3000SD column oven, LPG-3400SD pump, WPS-3000TSL autosampler and DAD-3000 detector. A RP-18 LiChroCART column (125 mm × 4 mm, 5 µm) was used for the chromatographic separation. Separation was performed at 40°C using a mixture of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B) as mobile phase. The phase flow rate was 1.0 mL/min. The elution gradient started at 7% of phase B for 0.5 min, reached 50% at 4th min and increased to 95% at 5th min, which was maintained for 2 min. From the 7th to the 9th min, the gradient returned to 7% phase B. The retention time was 4.14 min and detection was performed at 280 nm. A series of market dilutions of tetracycline were prepared and a standard curve was obtained with a correlation coefficient (R) of 0.9993 ($y = 0.2517x - 0.0428$).

Results

The highest pH value, with an average of 7.89, was recorded for hydrogel 1KA, while the lowest pH value for formulation 3KA was 5.38. Hydrogels containing azelogyline had similar pH values of around 7.78. The results of all pH measurements are summarized in Table 2.

Hydrogels containing azelaic acid had significantly lower viscosity values compared to azelogyline formulations. There were also more significant differences in viscosity between the azelaic acid gels. Hydrogel 1KA had the highest viscosity, with an average value of approx. 8,314 cP. The viscosity of formulation 2KA was around 4,336 cP and gel 3KA had the lowest viscosity of around 3,133 cP. The azelogyline hydrogels showed significantly higher viscosity values, with smaller differences between the values. Hydrogel 1A had the highest viscosity close to 12,460 cP. The viscosity value for gel 2A was around 10 127 cP and gel 3A was 9,222 cP. The viscosity values of all formulations are shown in Fig. 1.

Chromatographic analysis showed tetracycline stability for most formulations over a period of 35 days. The greatest decrease in antibiotic concentration was observed in the hydrogel, 1KA, in which tetracycline concentration decreased from 10.86 µg/mL on the 1st day to 5.45 µg/mL on the last day of measurements. Tetracycline in the 2KA and 3KA hydrogels remained more stable. In the 2KA formulation, it decreased from 11.77 µg/mL on 1st day to 10.5 µg/mL on 35th day. In the 3KA hydrogel, the initial concentration was 10.2 µg/mL and reached 9.27 µg/mL on the last day. In the formulations with azelogyline, small decreases in drug concentration were observed over the 35 days of analysis. In hydrogel 1A, the concentration

**Fig. 1.** The viscosity values of formulations containing azelaic acid – 1KA, 2KA and 3KA, and azelogyline – 1A, 2A and 3A**Table 2.** The pH values of evaluated formulations containing azelaic acid – 1KA, 2KA, 3KA and azelogyline – 1A, 2A and 3A

Formulation	1KA	2KA	3KA	1A	2A	3A
pH value	7.89 ±0.01	5.90 ±0.01	5.38 ±0.01	7.82 ±0.01	7.81 ±0.01	7.71 ±0.01

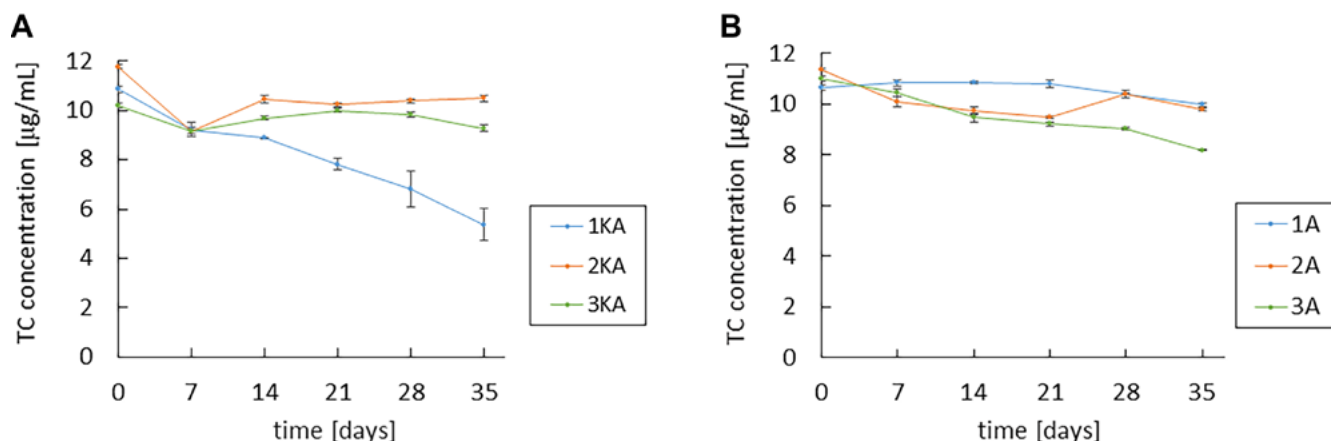


Fig. 2. Changes in tetracycline concentration values in formulations containing azelaic acid – 1KA, 2KA and 3KA (A), and azeloglycine – 1A, 2A and 3A (B) during 35-day observation

of tetracycline decreased from 10.65 $\mu\text{g/mL}$ to 9.98 $\mu\text{g/mL}$ and in hydrogel 2A from 11.35 $\mu\text{g/mL}$ to 9.8 $\mu\text{g/mL}$. Only formulation 3A showed a greater decrease, from 11.0 $\mu\text{g/mL}$ to 8.18 $\mu\text{g/mL}$. The course of drug concentration changes is shown in Fig. 2.

Discussion

The formulations presented in this paper constitute a new proposal for the treatment of acne vulgaris. The choice of tetracycline allows the use of its anti-pathogenic and anti-inflammatory properties, which are crucial in the context of anti-acne therapies.^{11,12} Additional ingredients incorporated into the formulation, such as azelaic acid or azeloglycine, have a proven use in the topical treatment of acne lesions. They have comedolytic and keratolytic properties and lighten acne hyperpigmentation Fig. 3.^{8–10}

The pH of the formulations is critical to the stability of tetracycline, which is most stable at pH conditions ranging from weakly acidic to neutral. Either an excessively acidic or an excessively alkaline environment will result in rapid degradation of the antibiotic. At a pH above 8, maroon quinones can be formed in the presence of oxygen and light.^{13,14} At a pH close to 2, the tetracycline molecule is dehydrated and nephrotoxic compounds such as 4-epianhydrotetracycline are formed.^{15–17} In the pH range of 2–6, a reversible epimerization process can also take place, leading to the formation of 4-epitetracycline, which has no antimicrobial activity.^{18,19} Therefore, all the preparations tested were characterized by a weakly acidic or neutral pH, which favors their stability.²⁰

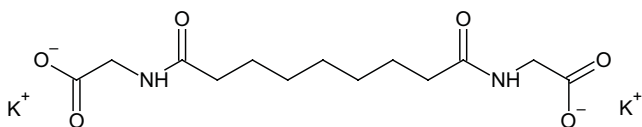


Fig. 3. The chemical structure of azeloglycine

Formulations containing azeloglycine presented higher viscosity values compared to hydrogels containing azelaic acid. Azelaic acid was found to cause a significant reduction in hydrogel viscosity, which should be taken into account when designing formulations containing this substance.

The HPLC analysis showed high stability of the antibiotic in most of the formulations analyzed. In formulations 2KA, 3KA, 1A, and 2A the degradation process was very slight. A modestly higher loss of drug concentration was observed in hydrogel 3A, but the process was also found to be slow. The greatest degradation was observed in hydrogel 1KA, where disintegration was faster than in the other formulations and the tetracycline concentration at day 35 was only 50% of the initial value.

Azeloglycine has more effective therapeutic activity at lower concentrations than azelaic acid. It is also more widely used than azelaic acid in skin care formulations due to its near-neutral pH, low number of interactions and high compatibility with various ingredients in cosmetic preparations.



The proposed formulations can have multidirectional efficacy based on the antibacterial activity of the antibiotic used. In addition, azeloglycine is an excellent ingredient for anti-acne formulations due to its ability to lighten hyperpigmentation, reduce redness and regulate excess sebum. The alcoholamine AMPD contained in the preparations can support the cleansing process of the hair follicles from sebum deposits thanks to its activity against free fatty acids.²⁰

Conclusions

The present study focuses on new anti-acne preparations that combine the bacteriostatic and anti-inflammatory properties of tetracycline with the anti-acne effects of azelaic acid and azeloglycine. The use of these substances aims to treat acne vulgaris more effectively while reducing the risk of antibiotic resistance.

A comparison of the rheological properties showed that azelogylicine provided a formulation viscosity averaging approx. 10,500 cP, which may contribute to more favorable skin application compared to azelaic acid gels with lower viscosity values averaging approx. 5,200 cP. Chromatographic results showed high tetracycline stability in most of the formulations tested, and formulations with azelogylicine showed less loss of drug. The developed azelogylicine-containing formulations could be an interesting and effective proposal for a topical anti-acne preparation due to the high persistence of the antibiotic and the favorable rheological parameters.

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