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Opinion paper

Ointment containing spray freeze-dried metronidazole effective against rosacea

Klára Szentmihályi [a,*], Krisztina Móricz [b], Gábor Gigler [b], Zoltán May [a], Eszter Bódis [a], Judit Tóth [a,c], Mónika Bakonyi [b], Szilvia Klébert [a], Tivadar Feczkó [a,c], Zoltán Károly [a]

[a] Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Magyar Tudósok Körútja 2, Budapest, H-1117, Hungary
[b] EGIS Pharmaceuticals PLC, Keresztúri út 30-38, H-1106, Budapest, Hungary
[c] Research Institute of Biomolecular and Chemical Engineering, Faculty of Engineering, University of Pannonia, Egyetem u. 10, Veszprém, H-8200, Hungary

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ABSTRACT

Rosacea is a chronic, inflammatory skin disease that affects about 10% of the population. Metronidazole-containing ointments are typically recommended for the daily treatment of symptoms. For increasing bioavailability and effectiveness, it is needed to reduce the particle size. Therefore, micronized metronidazole was prepared by spray freeze-drying (SFD) method, then its most important features were examined such as morphology, crystallinity and particle size. The anti-inflammatory effect of the as-prepared agent was tested on a mouse model of rosacea for effectiveness against oedema and redness of the ears, and it was compared to a reference cream.

Metronidazole size was reduced successfully by SFD to 2.7 μm from 162.6 μm. The material was non-porous and preserved its crystalline state. The spray freeze-dried metronidazole mixed into ointment was effective against oedema and ear-redness. The ointment reduced oedema in five times lower doses (2 × 0.04 mg metronidazole) and the ear-redness in half dose (2 × 0.2 mg metronidazole) than the cream containing reference metronidazole (2 × 0.2 mg and 2 × 0.4 mg metronidazole, respectively). In conclusion, the SFD technique is an adequate and gentle procedure for reducing the size of metronidazole, which is highly effective in rosacea.

1. Introduction

One of the incurable diseases of the skin is rosacea, which makes life difficult for many people. Rosacea is a chronic skin condition consisting of inflammation of the face, nose, chin, forehead and eyelashes. The most frequent symptoms on the face are acne, blisters, redness, red inflamed nodules, rashes, increased sebum production and enlarged nose (rhinophyma). These symptoms appear mainly in the middle ages and first alternate asymptomatic periods with exacerbation of symptoms and then become chronic [1]. Although there is no permanent cure for rosacea, symptoms can be reduced with timely treatment and daily care. The skin care compositions and their ingredients depend on the type of rosacea such as phymatous-, ocular-, erythematous- and papulopustular rosacea, furthermore, it is recommended to avoid sunlight and apply special combination sun creams.

Medical preparations for papulopustular rosacea contain azelaic acid, ivermectin, doxycycline, isotretinoin or metronidazole as well as Ti-dioxide and Zn-oxide [1,2]. However, out of these drugs metronidazole is the most commonly used and administered as monotherapy or in combination for local treatment of papulopustular rosacea [3].

Metronidazole (MTZ) is the derivative of 5-nitroimidazole (Fig. 1) and one of the most effective drugs against different infections caused by anaerobic protozoa (Trichomonas, Treponema, Histomonas) and most anaerobic bacteria (Bacteroides, Fusobacterium, Campylobacter, Clostridium) [4]. It has also anti-inflammatory and immunosuppressive effects that are probably responsible for the excellent efficacy in rosacea,

Abbreviations: c rMTZ, cream containing reference metronidazole; o SFD-MTZ, ointment containing spray freeze-dried metronidazole; MTZ, metronidazole; rMTZ, reference metronidazole; ROS, reactive oxygen species; SEM, scanning electron microscopic/microscopy; SFD, spray freeze-drying; SFD-MTZ, spray freeze-dried metronidazole; TEM, transmission electron microscopy; XRD, X-ray diffraction.

* Corresponding author.
E-mail addresses: szentmihalyi.klara@ttk.hu (K. Szentmihályi), moricz.krisztina@egis.hu (K. Móricz), gabor.gigler@gmail.com (G. Gigler), may.zoltan@ttk.hu (Z. May), bodis.eszter@ttk.hu (E. Bódis), toth@mukki.richem.hu (J. Tóth), bakonyi.monika@egis.hu (M. Bakonyi), klebert.szilvia@ttk.hu (S. Klébert), feczko@mukki.richem.hu (T. Feczkó), karoly.zoltan@ttk.hu (Z. Károly).

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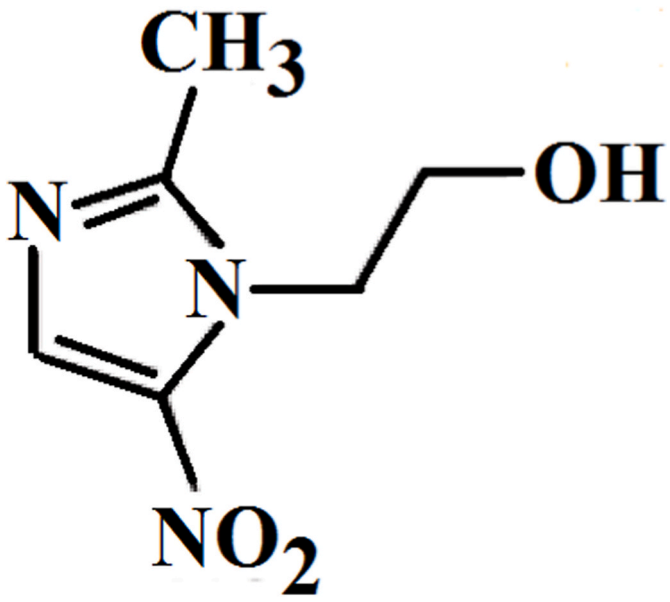

Fig. 1. Structure of metronidazole.

although its mechanism of action has not been proven [5,6]. It can be used in several forms such as solution, gel, cream or ointment (treatment of the skin, mucous membranes, oral cavity and vagina), tablet or suspension for oral treatment, and also infusion preparation [7]. However, serious side effects such as gastrointestinal problems (e.g. gastrointestinal disturbances, nausea, abdominal pain, and diarrhoea), neurotoxicity may occur applying it in high doses or for a prolonged period in the case of oral application [4]. It is used against rosacea topically in cream, ointment, gel or lotion with 1% or 0.75% of concentrations. MTZ is absorbed through the skin only to the stratum corneum in significant amounts from cream or gel. It is practically undetectable in the blood, so no side effects are expected systemically [8,9]. However, MTZ can reach the blood if there is an open wound through which it can enter the bloodstream or if a high dose of the active substance is used [10,11]. Side effects may also occur with topical treatment, such as skin irritation, dry skin, abnormal redness and inflammation of the skin (erythema), itching, rash, burning sensation, skin pain and hip sensation. In order to avoid or reduce the mentioned side effects, the dose of MTZ needs to be reduced as low as possible or a smaller size of the active ingredient needs to be used, without lowering the overall efficiency. As the size decreases, the apparent solubility of the drug and in turn the absorption increases although the most important aspect is that a smaller amount of drug is required to achieve the same level of effect [12].

For topical formulations, efficacy, release, dermal absorption, and utilization depend on many parameters, for example, pharmaceutical form, composition, carriers, physicochemical properties (e.g. size, viscosity, pH) of the preparation. Depending on the formulation, no significant difference was found between the commercial preparation and the other formulation containing MTZ in terms of bioavailability based on in vivo studies [13]. However, in vitro literature data on the formulation-dependent release of MTZ and penetration into the skin are conflicting sometimes. MTZ releasing in 60 min was almost 100% from a gel, almost 60% from cream, and just over 40% from ointment [14]. However, according to Dua and coworkers the release from ointment is even slower and lesser extent, about 20% in 1 h and about 40% in 6 h [15]. However, for different cream formulations containing MTZ, the extent of in vitro absorption and profiles were found to be similar regardless of composition [16]. According to Elewski, MTZ penetrates into the skin in the order of cream, lotion and gel, while there is no penetration from ointment [17].

Several methods are available to increase efficiency, e.g. Waszczykowska and co-authors prepared a highly soluble Ag-complex with metronidazole for the treatment of ocular rosacea [18]. Nevertheless, there is another way to increase the effectiveness of metronidazole: producing micron- or nanosized materials makes it much more soluble without chemical transformation [19]. Micronization techniques are one of the novel concepts to ensure advanced drug delivery. The micronization term means to prepare particles with a size smaller than 10 μm [20].

In pharmaceutical science and cosmetics, the size of an active ingredient affects bioavailability, toxicity, shelf life, and efficacy. In drug delivery systems of nanotechnology, the bioavailability of the drug is improved and its biodistribution is better [21,22]. Nanoparticles can penetrate the surface layers of the stratum corneum and reach the epidermis, but do not penetrate the epidermal barrier of intact skin [23–26]. Only particles smaller than 36 nm pass through the intracellular pathway [27]. They can deposit on the follicles, but they do not penetrate the skin through the follicles [28,29]. Transcellular transit depends mainly on charge and lipophilicity, but penetrates deeper layers as size decreases and the effect of nanomaterials grow exponentially [23,30]. Nanoscale use of poorly water-soluble active ingredients (e.g. rutin, hesperedine) is effective in anti-aging and skin protection cosmetics [31]. The use of nanosized titanium dioxide (Tayca MT-100TV) in a sunscreen product increases the sunscreen effect eightfold [32] while the pharmacokinetic efficacy of the nanocomposition containing 307 nm of the active substance apremilast and poly (D, L-lactide-glycoglycolide) is 2.25-fold higher [33]. Nanomaterials in cosmetic products is also useful because such small-scale active ingredients become transparent, e.g. white titanium dioxide, zinc oxide, or originally black fullerene, which also allows their use in moisturizing creams [34].

Small-sized active ingredients can be prepared by several methods such as changing the solvent, milling, melting, shifting the pH or applying supercritical fluid, spray-drying (SD), freeze-drying (FD) or spray freeze-drying (SFD) methods [20,35–41]. The great advantage of the spray freeze-drying (SFD) method is that it can be used for sparingly water-soluble and heat-sensitive materials where the obtained product has good efficiency and quality [42]. Currently, in the pharmaceutical industry, emphasized research focuses on the development of new drug delivery routes and methods applying SFD techniques (Supplementary data, Fig. 1), since using sustained-release injections or the delivery of a needleless epidermal drug (as a non-invasive vaccine) are more time- and energy-efficient procedures compared with the previously used SD and FD techniques [41]. Furthermore, the SFD technique is often used to increase the apparent solubility of a drug when pulmonary, nasal or intradermal delivery is needed [43,44].

According to our knowledge, there is no data in the literature for the effectiveness of a bioactive agent prepared by the SFD method for the treatment of rosacea. In this study, our goal was to produce small-sized and more efficient metronidazole by SFD method and test the anti-inflammatory effect for its applicability in rosacea in the form of ointment.

2. Materials and methods

2.1. Materials

Metronidazole was obtained with European Pharmacopoeia (EuP) specification from Aarti Drugs Limited, Mumbai, India. Liquid nitrogen was provided by Linde Gas Magyarország Zrt. with a purity of 99.999%. High purity water (18.3 MΩ/cm) was made by Millipore equipment (Merck Ltd.).

Medical-grade white vaseline was obtained from Dilube, S.A. (Barcelona, Spain), while white paraffin was from MOL-LUB Kft. (Budapest, Hungary).

The mice were from Toxi-Coop Zrt., Budapest, Hungary. The reference cream was Rozamet (10 mg metronidazole/g cream) obtained from

Jadran Galenski Laboratory, Croatia. The composition of reference preparation was glycerol, liquid paraffin, water, emulsifier, preservatives, and 1% metronidazole.

2.2. Preparation of spray freeze-dried metronidazole

The freeze granulator (LS2-PowderProAB freeze granulator, Gothenburg, www.powderpro.se) has two separate units, the freeze granulator and freeze dryer (Supplementary data, Fig. 2). Freeze granulation consists of two main steps, atomization and spraying by spray freezing into vapour over liquid (SFV/L) technique [45]. The freeze granulator includes a metering pump (peristaltic pump, Watson Marlow 323 pump) and a mixer, as well as a spray head. The pump speed was set at 20 l/min, while the atomizing gas pressure was 0.4 bar. The stirrer is an IKA RCT basic magnetic stirrer with a power of 600 Watts and a speed set at 300 l/min. The dissolved active ingredient (1 wt% aqueous MTZ solution) was sprayed into a liquid nitrogen bath (−196 °C), and the frozen material was subsequently freeze-dried in a vacuum (Supplementary data, Fig. 3). The process conditions and settings were preliminary optimized in terms of particle size. The lyophilizer was a Scanvac Coolsafe 55-9 Pro Control type 5-tray freeze dryer. The product was signed as SFD-MTZ.

2.3. Scanning and transmission electron microscopy (SEM and TEM) measurement

The initial and spray freeze-dried metronidazole were characterized by SEM (Zeiss EVO40) using tungsten hairpin filament operated at 20 kV. The images were acquired at different magnifications by a secondary electron detector (SED).

For transmission electron microscopic (TEM) measurement and to reach higher magnification of the studied material, we used a FEI Morgagni 268D transmission electron microscope (TEM). The tungsten filament was operated at 100 kV, and we observed the morphology and the grain size of the samples at different magnifications.

2.4. Measurement of apparent surface

The nitrogen adsorption isotherms were measured at −196 °C by an Autosorb 1C (Quantachrome, Boynton Beach, FL, USA) static volumetric computer-controlled instrument. The apparent surface area was calculated using the Brunauer–Emmett–Teller (BET) model [46]. All the samples were outgassed for 24 h in a vacuum at 60 °C before the gas adsorption measurements.

2.5. Measurement of particle size

The particle size and distribution were determined by the laser diffraction method. For the calculation, the material refractive index 1.6 and the absorption coefficient 0 and 0.1 were used for reference MTZ (rMTZ) and SFD-MTZ, respectively. The average particle size was reported as a volume equivalent sphere diameter and the width of the size distribution as span (span = (d90 – d10)/d50). The measurements were carried out in wet dispersion in cyclohexane containing 0.1% soy lecithin by a Malvern Mastersizer 2000 instruments (Malvern Instruments, Malvern, UK) using the SM dispersion unit and a stirring rate of 2500 rpm. 10 mg of SFD sample was weighed in 1 mL of cyclohexane solutions containing 0.1% soy lecithin and sonicated for 40 s at 30% of the power with 6 mm probe by Sonics VCX 130 instrument, then the obtained suspension was poured into the dispersion unit for measurement. The rMTZ was added directly to the solutions to reach an obscuration around 10.

2.6. X-ray diffraction measurement

The X-ray powder diffraction (XRD) measurements were performed to analyse the crystallinity of SFD metronidazole using a Philips PW-1050 diffractometer. It is equipped with a Bragg-Brentano parafocusing goniometer and operated by a Cu tube at 40 kV and 35 mA, using a secondary beam graphite monochromator and a proportional counter. The scans were recorded in step mode with 0.04° step size for 1 s between 10° and 70° 2θ.

2.7. Preparation of ointment containing SFD-MTZ

SFD-MTZ (1 g) was suspended in liquid paraffin (9 g) by sonication and dispersed in white vaseline (90 g).

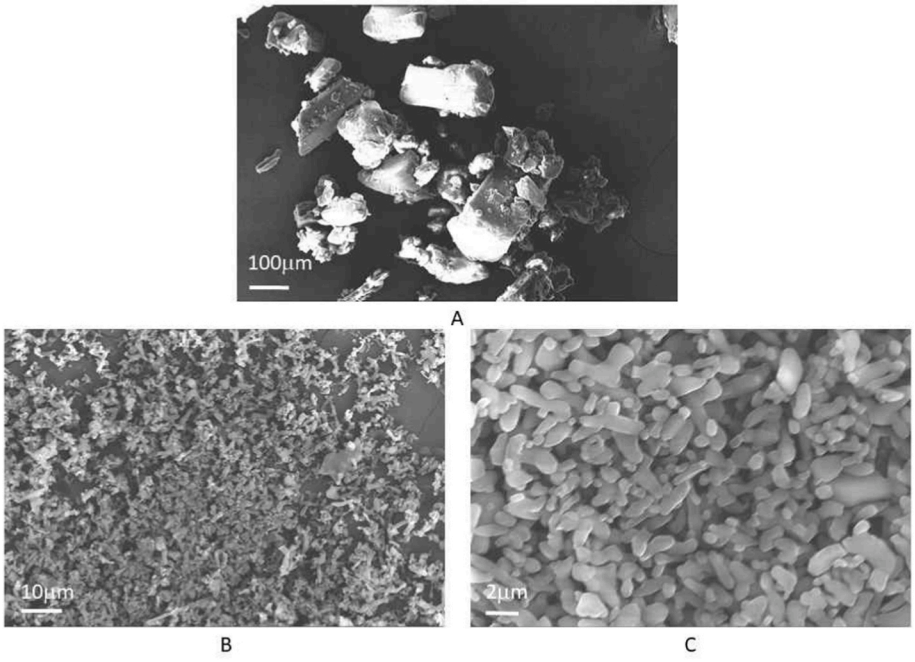

Fig. 2. Scanning electron microscopy of the reference metronidazole (rMTZ: A) and the spray freeze-dried metronidazole (SFD-MTZ: B and C).

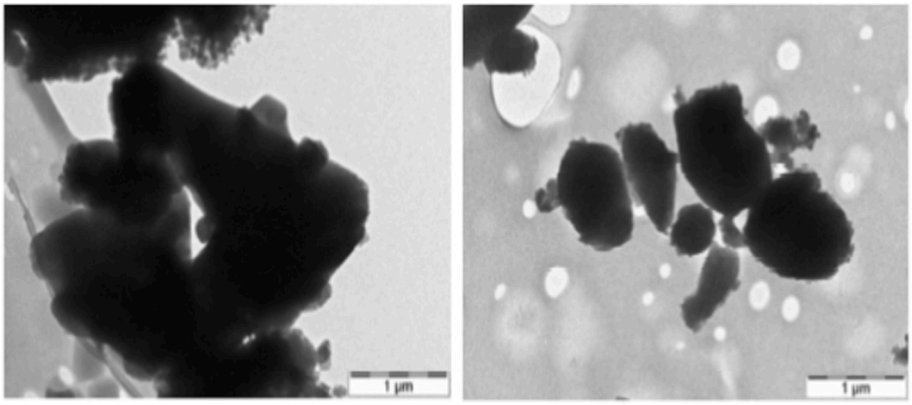

Fig. 3. Transmission electron microscopy of the reference metronidazole (rMTZ: A) and the spray freeze-dried metronidazole (SFD-MTZ: B).

2.8. Animal study

6 weeks old male NMRI mice (30–37 g) were divided into three groups with 10 animals in each. Vasodilatation and oedema were induced in each group with croton oil on both sides of the right ear [47]. The first group was a control treated with croton oil, the second group was treated with croton oil and reference cream while the third group was tested with croton oil and ointment containing SFD-MTZ (10 mg metronidazole/g ointment). The base thickness of ears of mice was measured by calliper (Mitutoyo, Japan), then the second and third groups were pre-treated topically with different doses of metronidazole preparations (20, 10, 5 or 2 µl cream or ointment on inner and outer surfaces of the right ear, the cumulative doses were 0.4, 0.2, 0.1 and 0.04 mg metronidazole/right ear). Thirty minutes later inflammation was induced in the right ear of each mouse by topical application of 10 µl of a 2% croton oil solution (solved in acetone), both on the inner and outer surfaces of the animal's ears. The second treatment with the tested preparations was done 50 min after the croton oil treatment. Six hours later the thicknesses of ears were measured again. The redness of each ear was determined by visual observation.

All animals were housed in plastic cages, under standard laboratory conditions (24 ± 2 °C, 40–60% relative humidity) with allowed free access to standard laboratory pellet for mice and tab water. The study conformed to the Declaration of Helsinki guidelines, the EU Directive 2010/63/EU for animal experiments, and was approved by the local animal ethical committee. All animal procedures were under the Protection and Careful Treatment of Animals (40/2013. II. 14., Hungarian Government Decree) and were approved by the Institutional Animal Care and Use Committee.

2.9. Evaluation of the in vivo effectiveness

The degree of oedema was characterized by the difference of thickness of the right ear before and 6 h after the croton oil treatment and calculated as follows:

Oedema thickness (mm) = right ear diameter at 6 h after the croton oil treatment (mm) - right ear diameter before the treatments (mm).

The score of redness was determined as the sum of outer surface points and inner surface points of the ear. On the outer surface of the ear, 1 point was counted for the red edge or red base of the ear.

On the inner surface of the ear, 1 or 2 points were calculated for the red or red and swollen base of the ear, partially red or red auricle, while 1 point for visible telangiectasia. Zero-point means no visible symptom, while 7 points can be the maximum total score for visible symptoms.

2.10. Statistical analysis

GraphPad Prism 6.0 (GraphPad Software, San Diego, USA) software was used for statistical calculations. Mean and standard deviation was calculated from the observed data. The statistical evaluation was performed by one-way analysis of variance followed by Dunnett's multiple comparison tests (control vs. treated groups) for oedema thickness and Kruskal-Wallis ANOVA followed by Dunn-test was used for the visual score of redness. The significance was determined as $p < 0.05$.

3. Results and discussion

3.1. Characterization of SFD-MTZ

The scanning electron microscopy (SEM) analysis shows that reduced MTZ size could be obtained by the SFD method compared with the reference sample (Fig. 2 and Supplementary data, Fig. 4). The SFD synthesised particles showed a slightly elongated shape (Fig. 2C) with size varying within a relatively wide range, comprising of smaller and larger particles (Fig. 2 B). This finding is also supported by the transmission electron microscopy (TEM) measurement (Fig. 3) and the light microscopic picture (Supplementary data, Fig. 5).

In general, SFD prepared materials are spherical and highly porous, however, using low concentration solutions, the particles tend to acquire non-spherical fragments [48]. We applied a 1 wt% solution, which was a rather low concentration, although close to the saturation point since the solubility of MTZ is 10.5 mg ml^{-1} at 25 °C [35, 49]. The concentration, however, accounts for the non-spherical particles only in part and the particles are not fragmented. According to Wanning and co-authors [41], the explanation can be that the colliding droplets dissipate their energy and freeze immediately at the moment of the impact. The morphology of the as-prepared material is basically influenced by the applied experimental conditions. In our case, the rapid cooling of the liquid sprayed into the vapour over liquid nitrogen (−196 °C) determined the morphology of our material. This SFD technique allows the droplets to become super-cooled already in the gas phase over the cryogenic liquid [27]. Furthermore, SFD-MTZ material lacks porosity, as can be seen in Fig. 2C. The measurement of the apparent surface area showed only 5 $m^2 g^{-1}$ and the Type II shape of the isotherm supports a non-porous feature. Till now there is no exact information on the relationship between shape (sphericity) and porosity [50]. Although SFD technique alone is suitable for the preparation of micro- and/or nano-sized drugs without excipients, in most cases the drug is prepared together with a carrier (e.g. liposomes, polymer particles, dendrimers, miscellaneous nanoparticles). The resulting materials are larger than 5 µm in size, sometimes reaching 16 mm [51,52]. Using

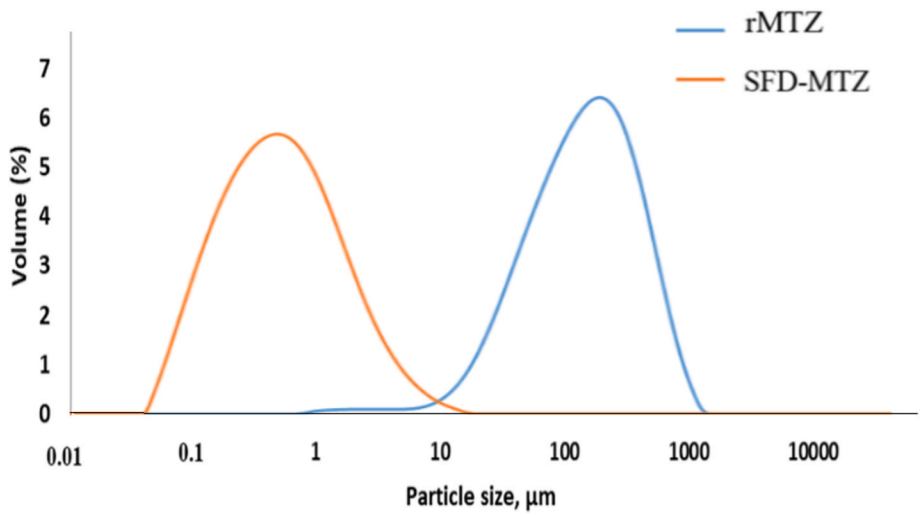

Fig. 4. The particle size distribution of the SFD-MTZ sample and the reference metronidazole (rMTZ).

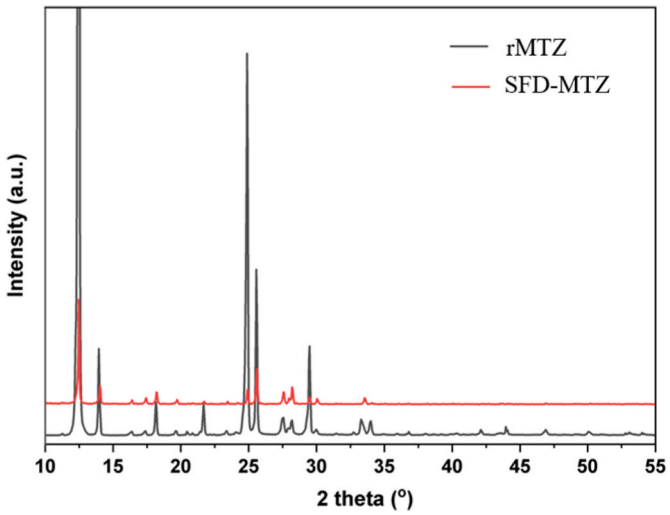

Fig. 5. X-ray diffraction image of the spray freeze-dried (SFD-MTZ) sample and the reference metronidazole (rMTZ).

this SFD technique, the atomized droplets of the mixture solution freeze rapidly in the cryogenic medium and ice crystals can form in the frozen droplets. Upon lyophilization, the resulting mixture becomes a porous material due to the removal of sublimated ice crystals. However, this property does not characterize the drug, but the drug and carrier together. In the case of the active substances produced alone by SDF technique, that was characterized as a porous material, a material larger than 20 μm was produced in each case and no adsorption studies were performed, only the SEM images showed that a porous material was obtained [53]. For active substances below 1 μm, a drug was prepared from a solution concentration of less than 1% [54]. Although our material does not appear to be porous in SEM recordings, we confirmed this by an adsorption test. The morphology of SFD powders depends on several factors including spraying, freezing and drying technique, concentration of the solute, potential excipients, etc. Due to the very low solute concentration and the lack of excipients, high porosity was not expected anyway.

Based on the particle size analysis, the average size of the SFD-MTZ sample (2.7 μm) was two orders of magnitude smaller as compared to the original reference material (162.6 μm). The width of the size distribution given as span was 2.75. The particle size distribution can be seen in Fig. 4.

For the determination of the crystalline form of the SFD-MTZ sample, XRD measurements were applied. XRD patterns clearly show that the crystallinity of the sample was preserved during SFD (Fig. 5). The intensity of the peaks of the SFD-MTZ sample decreased compared to the rMTZ material, implying a decreased size of metronidazole.

3.2. *In vivo mouse experiment*

Mouse model of rosacea by croton oil-induced ear inflammation [47] was applied, where a strong inflammatory response was elicited with croton oil in the ears of mice, leading to oedema and redness of the ear. Both oedema and flushing are well inhibited by metronidazole containing preparations. The effectiveness of ointment containing SFD-MTZ (o SFD-MTZ) was tested compared to the cream available in the market (since ointment containing MTZ is not available), which contains larger crystalline metronidazole, however, both preparations are of 1 wt% metronidazole.

The effect of the dose amount and the type of the used drug is illustrated in Fig. 6, while the data are found in the Supplement data Table 1. The administration of 2 wt% croton oil caused ear swelling for control mice. The reference cream in the dose of 2 × 10 μl and 2 × 20 μl inhibited significantly ($p < 0.05$) the croton oil-induced ear swelling. These effective cream volumes are equal to 0.2 mg and 0.4 mg metronidazole/ear. The SFD-MTZ containing ointment reduced the ear thickness by dose-proportional in the dose of 2 × 2 μl, 2 × 5 μl and 2 × 10 μl, which corresponds to 0.04, 0.1 and 0.2 mg MTZ. Although no significant differences could be observed between the two products at the same and different doses because of the overlapping range of standard deviations, yet the invariably lower mean values of the developed ointment (oSFD-MTZ) as compared to the reference cream (rMTZ) suggests better results for the former substance. For reducing the induced ear oedema significantly, the lowest effective dose was 0.04 mg in the case of micro sized SFD-MTZ containing ointment, meanwhile to attain the same oedema thickness, 2 mg rMTZ containing cream was needed. This means that 5 times less micro sized SFD-MTZ containing ointment is enough comparing with reference cream to achieve the right effect.

The preparations were tested for croton oil-induced skin redness on mice ears at the same doses as in the previous experiment. The redness of the inflamed area is visible due to the dilation of the blood vessels and the abundant blood supply. In human rosacea, redness of the face is also optically confusing for patients, so its alleviation is an important consideration in the treatment [55]. This can only be quantified by scoring, assessing the change in the color of the affected area. Score

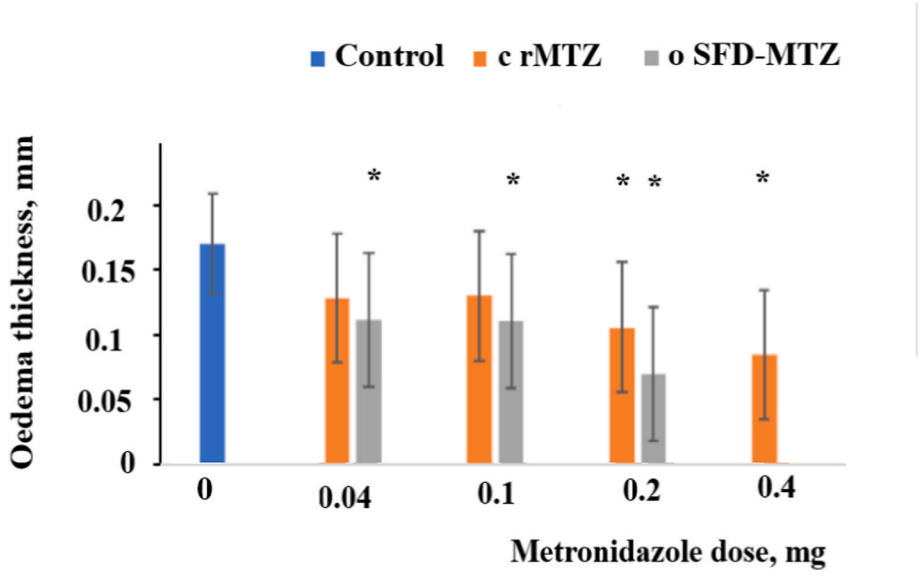

Fig. 6. Oedema thickness (mm) of right ears of mice after treatment with croton oil (Control), cream containing reference metronidazole (c rMTZ) and ointment containing spray freeze-dried metronidazole (o SFD-MTZ. Data are expressed as means ± SD, n = 10, * significant difference at $p < 0.05$ to the control value.

evaluation is partly represented in Supplementary data Fig. 6. The ear-redness was also decreased in a dose-proportional manner for both preparations, but the reduction was significant only at the highest doses (2 × 20 μl for reference cream, and 2 × 10 μl for SFD-metronidazole containing ointment) (Fig. 7 and Supplement data Table 2). The mean results at each dose obtained for the two formulations were rather similar, however, there is significant difference in respect of the standard deviations of the two substances. Due to the smaller standard deviations of the results of the ointment containing SFD-MTZ, it can be declared to yield significant reduction in ear redness at a dose of 0.2 mg, while in the case of the reference cream a dose of 0.4 mg rMTZ was required. The results showed that the ointment containing SFD-metronidazole is already effective in half the dose of the reference cream.

In our experiment, a micro-sized ointment containing SFD-MTZ was used and compared to a commercially available cream containing a non-micro-sized active ingredient. The two preparations differed in the formulation and size of the active agent. The smaller size gives the drug many beneficial properties such as overcoming physiological barriers [56]. The small active ingredients are excellent for the topical treatment of skin diseases, some of which interact with the pathogen in the skin tissues at the subatomic level [57]. Due to the smaller size, the higher surface area to volume ratio of the active ingredient allows a higher exposure on the skin surface, so that the contact surface, biological and chemical interaction with the pathogen may be higher [58,59]. In inflammatory skin diseases, small substances penetrate deeper into the inflamed skin due to impaired epidermal barrier function without systemic absorption and side effects [60]. Dermal active ingredients, for which the achievement of a systemic effect may also be important, e.g. in the case of diclofenac-diethylamine, the nanometer particles provide greater penetration, thus increasing the beneficial systemic effect and reaching the deeper layers of the skin. If this higher permeation rate is unfavorable and there is a side effect, it is still advisable to use a smaller but micro-sized active ingredient. Skin penetration of the micro sized active agent is minimal and systemic toxicity can be significantly reduced or avoided beside achieving a long-lasting local effect [61]. The

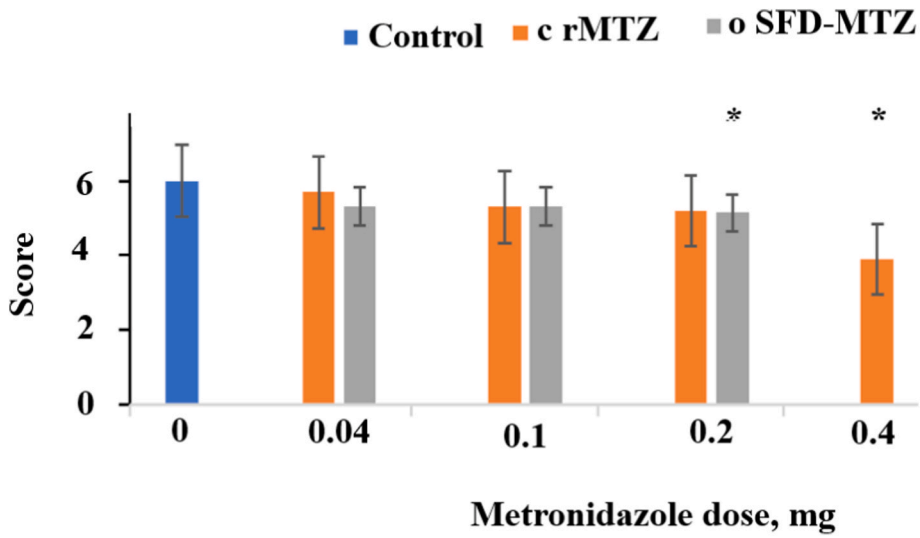

Fig. 7. Ear-redness of right ears of mice after treatment with croton oil (Control), cream containing reference metronidazole (c rMTZ) and ointment containing spray freeze-dried metronidazole (o SFD-MTZ). Data are expressed as means ± SD, n = 10, * significant difference at $p < 0.05$ to the control value.

average particle size of the active ingredient in our composition is 2.7 μm, which is just above the nanoscale range. However, it can be clearly seen in Fig. 4 that a significant part of the material is still in the nanoscale range, while the other part is already in micrometer size. Based on this, it can be said that the SFD-MTZ-containing composition contains both nano- and micrometer sized active ingredients, thus combining its beneficial properties such as penetration into the deeper layers of the skin, while achieving a long-lasting local effect with higher exposure due to the higher surface area to volume ratio of the particles.

According to the literature, the release of the active ingredient from the cream is much better as compared to the ointment [17] although previous comparative studies with metronidazole containing preparations have shown that there were no significant differences in the efficacy regardless of formulation, formulation composition, metronidazole concentration, and frequency of use. [[62–65]]. Nevertheless, taking into account the results in the literature, we can assume that higher efficiency can be ascribed obviously to the modified morphology instead of formulation. The active ingredient does not get into the skin in the case of an ointment but remains on the surface, so one would expect the cream to be more effective [9]. In our experiment, however, the ointment was effective in 5 times lower dose against oedema and in a half dose in ear-redness, which is clearly due to the smaller size of the active ingredient.

By reducing the particle size the efficacy increases because of the enhanced absorption [12,66]. Normal-sized MTZ is said to enter the cell of an anaerobic organism by passive diffusion, where its reduction occurs by forming cytotoxic intermediates and free radicals which damage the cell, its DNA, and its proteins [67]. The greater antibacterial effect of nanoscale drugs is also related to the fact that nanomaterials already interact with the membrane and enter the bacterial cell by damaging it [68], which is schematically illustrated in Fig. 8. In our two in vivo studies, the increase in efficacy is clearly shown that a smaller amount was required from the ointment containing SFD-MTZ than the cream containing the non-micro-sized drug. This can be explained by the fact that the smaller active ingredient results in more particles in the same amount of weight, can be mixed more homogeneously, evenly smeared and gives a larger total surface area [1]. Comparative efficacy or release studies depending on the size of the active substance or carrier of MTZ are scarce in the literature. Furthermore, no mention is made of a similar experiment where the efficacy could have been increased by reducing the size of the drug. However, by reducing the carriers to a smaller size, the application of a nanolipid career increased the uptake of MTZ into the skin [69] getting similar and favourable results that support our experiment. Based on an in vitro study without comparison with an agent of other size or other product, MTZ was released in 60–80% during 6 h from microspheres filled with polyvinyl alcohol, and during 10 h from the gel containing MTZ [70].

4. Conclusion

One way to increase the effectiveness of MTZ-containing products used in the daily treatment of rosacea is to reduce the size of active ingredients. Spray freeze-drying is a very well-suited method for downsizing heat-sensitive materials as MTZ. In this work, a crystalline drug of two orders of magnitude smaller size (2.7 μm) was prepared successfully from the original crystalline MTZ (162.6 μm) by the SFD method. The SFD-MTZ particles lack porosity and showed a slightly elongated shape with a span of 2.75.

The anti-inflammatory efficiency of the prepared SFD-MTZ was tested in a rosacea mouse model. The two characteristic symptoms of rosacea inflammatory skin are oedema and redness of the inflamed area, so these were evaluated in the experiment. The ointment containing SFD-MTZ showed to be highly effective against both oedema and ear-redness since inhibited oedema at five times lower concentrations and the ear-redness in a half concentration than the reference cream.

In summary, the smaller crystalline MTZ produced by the gentle SFD technique is suitable and highly effective for cosmetic and medical use in rosacea.

Credit author statement/contributions

Klára Szentmihályi: conceptualization, investigation, data curation, writing – original draft. Krisztina Móricz: methodology, formal analysis, investigation, data curation. Gábor Gigler: resources, supervision. Zoltán May: investigation. Eszter Bódis: investigation, data curation. Judit Tóth: investigation, data curation. Mónika Bakonyi: investigation, Tivadar Feczkó: conceptualization, project administration, supervision, editing. Szilvia Klébert: investigation, visualization. Zoltán Károly: conceptualization, project administration, supervision, editing.

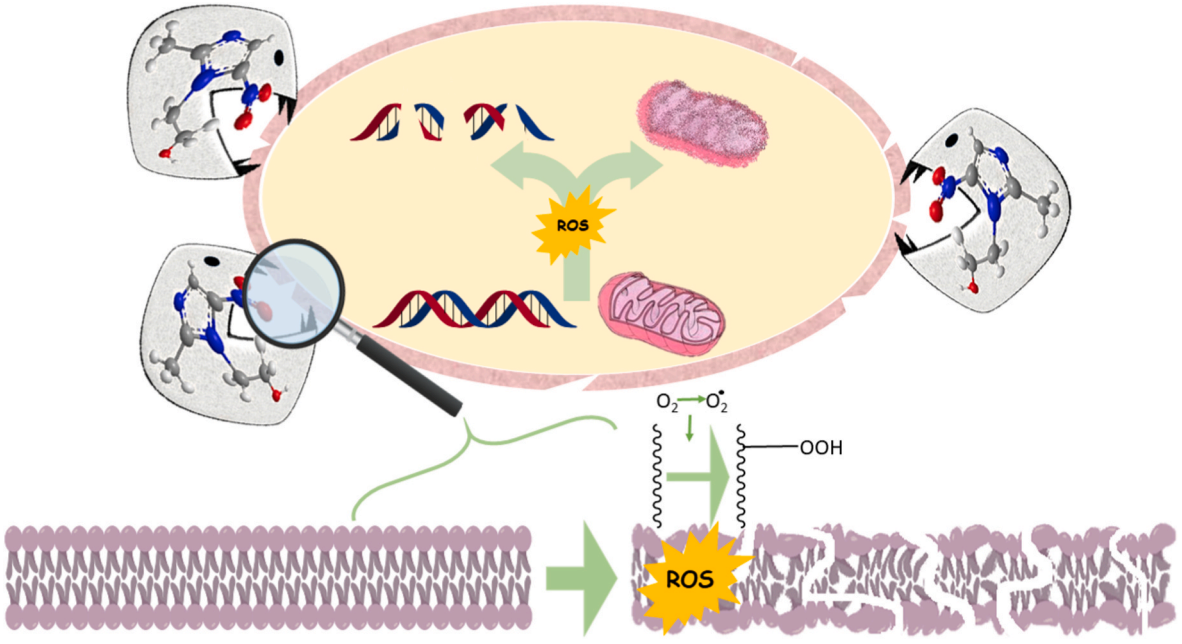

Fig. 8. Schematic characterization of mechanism action of nanosized metronidazole on a bacteria, ROS reactive oxygen species.

Declaration of competing interest

The authors declare that they have no competing interest in the content of the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2022.103559.

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