JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1125–1130

Received January 16, 2007
Accepted May 11, 2007
DOI 10.1211/jpp.59.8.0010
ISSN 0022-3573

Scavenging properties of metronidazole on free oxygen radicals in a skin lipid model system

Sabrina Narayanan, Andreas Hünerbein, Melkamu Getie, Andreas Jäckel and Reinhard H. H. Neubert

Abstract

Reactive oxygen species (ROS) play a vital role in the pathophysiology of the skin disease rosacea, a chronic, genetically-determined and UV-triggered disease, leading to facial redness and blemishes and exhibiting a deep impact on a patient's self-esteem and quality of life. ROS can cause oxidative damage to nucleic acids, sugars, proteins and lipids, thereby contributing to adverse effects on the skin. Metronidazole has been the first-line topical agent therapy for many years; nevertheless the mechanism of action is still not well understood. The therapeutic efficacy of metronidazole has been attributed to its antioxidant effects, which can involve two pathways: decreased generation of ROS within tissues or scavenging and inactivation of existing ROS. Previous investigations have shown that metronidazole reduces ROS by decreasing ROS production in cellular in-vitro systems. The aim of the following study was to demonstrate that metronidazole additionally exhibits antioxidative properties in a cell-free system, by acting as an antioxidant scavenger. A simple skin lipid model (oxidative) system and a complex skin adapted lipid system in conjunction with thiobarbituric acid (TBA) test, a quantitative assay for the detection of malondialdehyde (MDA) and therefore lipid peroxidation, were used to determine the antioxidative properties of metronidazole after UV irradiation. Results clearly show that metronidazole has antioxidative properties in a cell-free environment, acting as a free radical scavenger. Simple skin lipid model: in the presence of 10, 100 and 500 μg mL^{-1} metronidazole the MDA concentration was reduced by 25, 36 and 49%, respectively. Complex skin lipid system: in the presence of 100 and 500 μg mL^{-1} metronidazole the MDA concentration was reduced by 19 and 34%, respectively. The results obtained in this study and from previous publications strongly suggest that metronidazole exhibits antioxidative effects via two mechanisms: decrease in ROS production through modulation of neutrophil activity and decrease in ROS concentration by exhibiting ROS scavenging properties. The remarkable clinical efficacy of metronidazole in the treatment of rosacea is probably due to its ability to decrease ROS via different mechanisms, thereby protecting skin components from induced damage.

Medical Department, Galderma Laboratorium GmbH, Georg-Glock-Str. 8, 40474 Düsseldorf, Germany

Sabrina Narayanan, Andreas Jäckel

Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Wolfgang-Langenbeck-str. 4, D-06120 Halle/Saale, Germany

Andreas Hünerbein, Melkamu Getie, Reinhard H. H. Neubert

Correspondence: R. H. H. Neubert, Institute of Pharmacy, Martin Luther University, Wolfgang-Langenbeck-str. 4, D-06120 Halle/Saale, Germany. E-mail: reinhard.neubert@pharmazie.uni-halle.de

Introduction

Rosacea is a chronic, genetically determined skin disease affecting fair-skinned people of mostly north-western European descent, and has been nicknamed the curse of the Celts (Del Rosso 2004). Primary signs and symptoms include erythema (flushing and redness), papules and pustules and teleangiectasia on the central face and across the cheeks, nose or forehead. Multiple triggers that cause episodes of flushing exist (e.g., UV light, emotional stress, hot drinks, alcohol, spicy foods, exercise, cold or hot weather and hot baths or showers) (Izikson et al 2006). Because the potential manifestations of rosacea are so numerous and varied, the Rosacea Society Expert Committee published a standard grading system for rosacea (Wilkin et al 2004).

Although not a life-threatening condition, rosacea can have a deep impact on a patient's self-esteem and quality of life (Sowinska-Glugiewicz et al 2005; Balkrishnan et al 2006).

The pathogenesis of rosacea is still only poorly understood. Many factors have been taken into consideration over the years and the assumption to date is that a multifactorial aetiology is highly likely. In addition to a genetic predisposition, an inter-relationship between cutaneous microvasculature and vascular lability with inflammatory cells, production of reactive oxygen species (ROS), inflammatory mediators and degenerative enzymes

is believed to be involved in the pathogenesis of rosacea (Bamford 2001; Del Rosso 2004; Buechner 2005). ROS are thought to play a vital role in the pathophysiology of this disease, as well as in a number of other dermatological disorders (Jones 2004). ROS, such as superoxide, hydrogen peroxide and hydroxyl radicals, can originate in the environment and in the skin itself (Trouba et al 2002; Briganti & Picardo 2003) and can cause oxidative damage to nucleic acids, sugars, proteins and lipids, leading to protein degradation and possibly apoptosis (Black 2004). For protection against oxidant species, the skin contains a well-organised system of both chemical and enzymatic antioxidants (Briganti & Picardo 2003; Jones 2004). In general, antioxidants attenuate the damaging effects of ROS and can impair many of the events that contribute to epidermal toxicity (Trouba et al 2002). However, overproduction or inadequate removal of ROS can result in oxidative stress, leading to altered metabolism and biomolecular damage, all of which contribute to pathological changes in cell and tissue function (Trouba et al 2002).

Skin is more susceptible than other tissues to the damage caused by ROS. The reasoning behind this is that the skin is exposed to two important factors that are involved in ROS generation, namely oxygen and ultraviolet (UV) light (Öztas et al 2003; Trommer et al 2003a). For example, UV irradiation initiates and activates a complex cascade of biochemical reactions in human skin, causing depletion of cellular antioxidants and antioxidant enzymes, thereby initiating DNA damage, activation of the neuroendocrine system and an increased synthesis and release of pro-inflammatory mediators from a variety of skin cells. The pro-inflammatory mediators in turn increase the permeability of capillaries leading to infiltration and activation of neutrophils and other phagocytic cells into the skin. The consequences are inflammation and free radical generation. The inflammation further activates the transcription of various matrixes degrading metalloproteases, leading to abnormal matrix degradation. In addition, the inflammation and ROS cause oxidative damage to cellular proteins, lipids and carbohydrates, which accumulate in the dermal and epidermal compartments.

Öztas et al (2003) hypothesised that a defective cutaneous antioxidant system may occur in the skin of patients with severe rosacea. This was derived from an ex-vivo study of 19 patients with papulopustular rosacea versus 17 controls. Punch biopsies revealed that patients with severe rosacea had greater levels of malondialdehyde, the major lipid peroxidation product of the fatty acids in skin cells, and less superoxide dismutase activity (oxygen radical-quenching enzyme) (Öztas et al 2003). Additionally, or alternatively, higher levels of ROS can result from the inflammatory process as described above.

Therapeutic options for rosacea depend on the severity of clinical symptoms. As rosacea is a chronic condition with frequent periods of exacerbation, a large percentage of patients relapse after discontinuation of therapy within a relatively short time period (Dahl 2004). Therefore the major criteria for topical therapy, irrespective of effectiveness, is tolerability. The main topical agents used in the treatment of rosacea are metronidazole, azelaic acid, sodium sulfacetamide–sulfur and topical antibiotics (e.g. clindamycin) (Nally & Berson 2006).

Topical metronidazole has been proven worldwide to be an effective and safe treatment option for rosacea and since its release in 1989 has been the predominant first-line agent (e.g. Metrogel, Metrocreme, Metrolotion, Galderma) (Del Rosso 2004; Gupta & Chaudhry 2005). Despite this, the mechanism of action is still not well understood (Del Rosso 2004). Although metronidazole has an antimicrobial effect against anaerobic bacteria and protozoa, topical metronidazole does not cause any significant changes in the skin microflora of rosacea patients (Eriksson & Nord 1987, Gupta & Chaudhry 2002). The reduction of the nitro group of metronidazole leads to the production of short-lived cytotoxic intermediates that interact with deoxyribonucleic acid, thereby inhibiting nucleic acid synthesis. Such pathways are characteristic of susceptible anaerobic protozoa and bacteria and are absent in non-susceptible aerobic cells. Therefore, the efficacy of metronidazole, based on in-vitro and in-vivo studies, has been attributed to its antioxidant and anti-inflammatory effects. Two pathways can lead to the antioxidant effect of a substance: decreased generation of ROS within tissues and scavenging and inactivation of existing ROS (Jones 2004; Zip 2006). Metronidazole has been shown to reduce ROS production through modulation of neutrophil activity. In an in-vitro study by Miyachi et al (1986) involving purified neutrophils, the drug led to significant reduction in ROS. While this study demonstrated that therapeutic doses of metronidazole significantly reduce the ROS concentration in the presence of neutrophils, this effect was not observed in a widely established cell-free oxidative system (xanthine–xanthine-oxidase-system), indicating that the drug may not have the capacity to scavenge ROS. The results of this study were confirmed independently by Akamatsu et al (1990). Interestingly in the study by Akamatsu et al (1990), metronidazole only slightly decreased H_2O_2 and $OH\cdot$ generation by neutrophils in-vitro in the absence of palmitoleic acid, whereas when metronidazole was combined with the free fatty acid, ROS generation by neutrophils was significantly suppressed in a dose-dependent manner (Akamatsu et al 1990). Palmitoleic acid is a free fatty acid found in a large number of tissues. However, this synergistic effect was not found when metronidazole was used together with other free fatty acids (Akamatsu et al 1990).

The latter results encouraged us to re-investigate the potential direct ROS-scavenging properties of metronidazole in a different experimental set-up than previously used. To mimic conditions in human skin, and in rosacea pathogenesis, more closely, as deduced from the results obtained by Akamatsu et al (1990) this might possibly be a vital factor, we used two special skin adapted lipid model systems, recently established to investigate antioxidative properties of substances after UV-exposure (Trommer & Neubert 2005). The lipids in this skin model system were chosen according to the lipid composition of the skin and subsequently prepared as liposomes.

Therefore, in the following study a simple skin lipid model (oxidative) system containing linolenic acid and a complex system containing linolenic acid, dipalmitoylphosphatidylcholine, cholesterol and ceramide III were used to determine whether metronidazole exhibits antioxidative properties in a cell-free system, thereby acting as an antioxidant scavenger.

Materials and Methods

Reagents

α-Linolenic acid (LLA), ferrous sulfate, malondialdehyde-bis-(dimethylacetal), 2-thiobarbituric acid (TBA) and trichloroacetic acid for the TBA assay (all of analytical grade), as well as ferrous sulfate and metronidazole, were obtained from Sigma (Deisenhofen, Germany). Methanol of gradient grade and chloroform (LiChrosolv) were purchased from Merck (Darmstadt, Germany).

Lipid model systems and sample preparation

The samples were obtained after shaking the dispersion for 120 min using the laboratory flask shaker GFL 3006 (Gesellschaft für Labortechnik, Burgwedel, Germany). The concentration of the stock solutions was 1%. The irradiation of the 5-mL samples was started after a 15-min incubation time.

Ferrous sulfate (10 μM) was added to the samples as an electron donor and catalyst of the Haber-Weiss reaction to initiate ROS generation via a Fenton-type reaction. Previous investigations have shown that there is no effect when irradiating lipid model systems without transition metal catalysts (Trommer et al 2001).

The LLA dispersions and test substance solutions were freshly prepared just before use.

Two lipid model systems (as described previously by Trommer and co-workers (Trommer et al 2003a; Trommer & Neubert 2005)) were used. For the simple system (aqueous dispersion of LLA), the following samples were used: sample 1,100 μM α-LLA; sample 2, sample 1 with 5 μM ferrous sulfate; sample 3, sample 2 with 10 μg mL^{-1} (58.4 μM) metronidazole; sample 4, sample 2 with 100 μg mL^{-1} (584 μM) metronidazole; sample 5, sample 2 with 500 μg mL^{-1} (2920 μM) metronidazole; and sample 6, sample 2 with 22.2 μg mL^{-1} (100 μM) bufexamac. For the complex system (liposomal system with ceramide), the following samples were used: sample 7, liposomal system consisting of 100 μM α-LLA, 200 μM dipalmitoyl-phosphatidylcholine, 100 μM cholesterol and 100 μM ceramide III; sample 8, sample 7 with 5 μM ferrous sulfate; sample 9, sample 8 with 10 μg mL^{-1} (58.4 μM) metronidazole; sample 10, sample 8 with 100 μg mL^{-1} (584 μM) metronidazole; sample 11, sample 8 with 500 μg mL^{-1} (2920 μM) metronidazole; and sample 12, sample 8 with 22.2 μg mL^{-1} (100 μM) bufexamac.

UV irradiation

UV-B irradiation was carried out using a UV irradiation chamber (Dr Gröbel UV-Elektronik, Ettlingen, Germany) enabling a selective exposure to UV-B because of the special lamp F15/T8 15 W with a main emission range of 290–320 nm (Sankyo Co., Tokyo, Japan). Before irradiation, 5.0-mL samples (polysaccharide concentrations as given in the figures) were transferred to 55-mm open glass dishes. The optical pathlength was 2.1 mm and a homogeneous exposure was assumed in spite of light scattering.

The samples were treated with an UV-B dose of 0.25 J cm^{-2}, which corresponded approximately to 10-fold the minimal erythema dose (MED) of normal-pigmented (type II in the skin type classification) people (Kindl & Raab 1998). This high dosage was required to provoke stress conditions.

Thiobarbituric acid assay

The thiobarbituric acid (TBA) test is a quantitative assay for the detection of malondialdehyde (MDA), and is the most widely used technique to determine lipid peroxidation products (Schaffazick et al 2005; Trommer et al 2003b). In this study, the Buege–Aust method of the TBA assay was applied (Buege & Aust 1978). Briefly, 2.0 mL of a stock TBA reagent containing 15% (w/v) trichloroacetic acid in 0.25 M HCl and 0.37% (w/v) TBA in 0.25 M HCl was added to 1.0 mL of the UV-B treated sample. After heating at 90°C for 15 min and cooling down, the red TBA–MDA complex (2:1) appears allowing fluorescence measurement. An HPLC system (Merck-Hitachi, Darmstadt, Germany), equipped with an auto sampler AS-4000A, interface D-6000A, pump L-6200A, UV-VIS-Detector L-4250 and fluorescence detector F-1080, was used to quantify the pigment. A reversed-phase column (LiChrospher 100; RP 8, particle size 5 μm) was used with a mobile phase of methanol–water 30:70 for HPLC procedure. The excitation wavelength was 515 nm and the emission measurement was performed at 555 nm.

A calibration curve was generated using MDA, which was formed from malondialdehyde-bis-(dimethylacetal) under acidic conditions.

Statistical analysis

All data shown represent the mean values ± s.d. of triplicate measurements (n = 3). Statistical analysis of the effects of the different polysaccharides on the TBA-RP concentration after UV-B irradiation was performed using a one-way analysis of variance. In all cases, post-hoc comparisons of the means of individual groups were performed using Dunnett's multiple comparison test. $P < 0.05$ was accepted as being statistically significant. All calculations were performed using GraphPad Prism 2.0 (GraphPad Software Inc., San Diego, CA).

Results

Simple skin lipid system

Exposure of the simple system to ferrous ions resulted in the production of MDA whether or not the sample was treated with irradiation (Figure 1), indicating that ROS formed as the result of the Fenton-type reaction facilitated the oxidation of α-LLA. The amount of MDA formed when the sample was irradiated with UV was significantly higher than with the non-irradiated sample. This observation makes the role of UV radiation on potentiating ROS production by the Fenton-type reaction much clearer. Metronidazole at concentrations of 10 (sample 3), 100 (sample 4) and 500 (sample 5) μg mL^{-1} reduced the concentration of MDA by 25, 36, and 49%, respectively. The effect of metronidazole at the highest

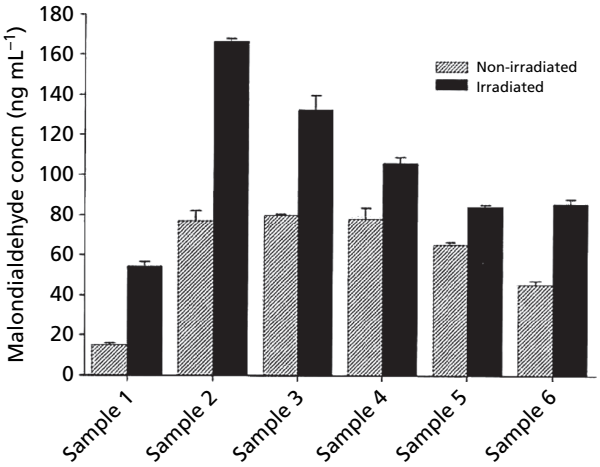

Figure 1 The protective effect of metronidazole against oxidation of linoleic acid upon exposure to UV radiation using the simple lipid model. Data are means ± s.d., measurements were done in triplicate. For definition of the samples see Materials and Methods (Lipid model systems and sample preparation).

concentration—500 μg mL^{-1} (sample 5)—on reducing MDA formation was comparable with the effect of the positive control, bufexamac, at a concentration of 22.2 μg mL^{-1} (sample 6).

Complex skin lipid system

The concentration of MDA was much lower after radiation of the complex (liposomal) system than using the simple lipid model system consisting of an aqueous dispersion of LLA (Figure 2). Metronidazole at a concentration of 10 μg mL^{-1} (sample 9) had no influence on the concentration of MDA. On the other hand, metronidazole at a concentration of 100 (sample 10) and 500 (sample 11) μg mL^{-1} reduced the concentration of MDA by 19 and 34%, respectively. In contrast, using the complex (liposomal) lipid system application of bufexamac at a concentration of 22.2 μg mL^{-1} (sample 12) reduced the concentration of MDA much more than all used concentrations of metronidazole.

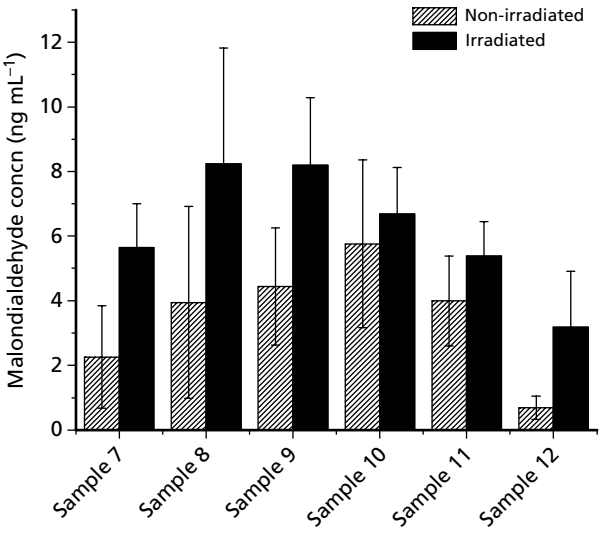

Figure 2 The protective effect of metronidazole against oxidation of lipids upon exposure to UV radiation using the complex lipid model. Data are means ± s.d., measurements were done in triplicate. For definition of the samples see Materials and Methods (Lipid model systems and sample preparation).

Discussion

Lipid peroxidation refers to the oxidative degradation of lipids. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds, including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition. Measurement of MDA has been used as an indicator of lipid peroxidation (Esterbauer et al 1991). The most widely used method is the TBA reaction with MDA. This method is of particular interest because of its procedural simplicity and nanomolar sensitivity.

Using this method, we showed that in contrast to previous reports (Miyachi et al 1986; Akamatsu et al 1990), metronidazole had antioxidative properties in a cell-free environment, acting as a free radical scavenger. This can be concluded from the fact that metronidazole was able to effectively protect linolenic acid from UV-induced oxidation in both a simple and complex lipid oxidative system. These systems have been previously used to screen for new antioxidative compounds for topical administration (Trommer et al 2003b; Trommer & Neubert 2005). The results impressively showed the protective effect of a lipid, such as LLA, being incorporated into vesicles (liposomes), as the concentration of MDA after radiation of this complex system was lower compared with the results obtained after radiation of the simple lipid system consisting of an aqueous dispersion of LLA.

As previously postulated, ROS play a central role in the pathophysiology of rosacea. Metronidazole exhibits antioxidative effects via two mechanisms. Previous studies have shown that metronidazole decreases ROS production through modulation of neutrophil activity (Miyachi et al 1986; Akamatsu et al 1990). In addition, our study shows evidence that metronidazole also has ROS scavenging properties. Due to the contradictory results obtained in this study, and along with the synergistic influence of palmitoleic acid on decreasing of ROS generation by metronidazole observed by Akamatsu et al (1990), we hypothesise that the nature of the cell-free system, and also the presence or absence and type of fatty acids incorporated play a vital role in the experimental setup in-vitro and maybe in the clinical efficacy in the skin, respectively. Therefore, it can be concluded that the remarkable clinical effectiveness of metronidazole in treating rosacea is due to its ability to decrease ROS. For example, metronidazole would thereby prevent the activation of metalloproteinases in-vivo, which

can result in the breakdown of dermal collagen (Miyachi 2001; Rijken et al 2006).

Whether the increased ROS concentration in the skin of rosacea patients results from overproduction or inadequate removal of ROS (or both) remains to be determined. Öztas et al (2003) demonstrated, in an ex-vivo study, that patients with mild rosacea had superoxide dismutase (SOD) activity that was increased to maintain the antioxidant capacity, thereby resulting in unchanged MDA levels. However, patients with severe rosacea showed a significant decrease in SOD activity, resulting in increased MDA levels due to ROS. These results strongly support the hypothesis that ROS play a vital role in the pathogenesis of rosacea. Increased levels of ROS can cause oxidative damage to different proteins and a wide range of other biomolecules, inducing protein degradation and possibly apoptosis (Black 2004). Skin is more susceptible than other tissues to the damage by free oxygen radicals because of its direct exposure to oxygen and ultraviolet (UV) light (Öztas et al 2003; Trommer et al 2003b). Metronidazole exhibits an antioxidative effect via two pathways: decreased generation of ROS within tissues and scavenging and inactivation of existing ROS. This is likely to be the primary reason for the outstanding clinical efficacy of metronidazole in the treatment of rosacea, especially in regard to papules and pustules.

Conclusion

Metronidazole significantly decreases the major contributing factor (reactive oxygen species) for skin damage in rosacea leading to the breakdown of collagen and elastic fibres, dilation and increased permeability of blood vessels, release of pro-inflammatory mediators and apoptosis. The therapeutic efficacy of metronidazole has been attributed to its antioxidant effects, which can involve two pathways: decreased generation of ROS within tissues and scavenging and inactivation of existing ROS. Previous investigations have shown that metronidazole reduces ROS by decreasing ROS production in cellular in-vitro systems. The aim of this study was to demonstrate, for the first time, that metronidazole exhibits antioxidative properties in a cell-free system by acting as an antioxidant scavenger. A simple skin lipid model (oxidative) system containing linolenic acid and a complex skin system containing linolenic acid, dipalmitoylphosphatidylcholine, cholesterol and ceramide III were used in conjunction with thiobarbituric acid (TBA) test, a quantitative assay for the detection of malondialdehyde (MDA). Results clearly show that metronidazole has antioxidative properties in a cell-free environment, acting as a free radical scavenger.

References

Akamatsu, H., Oguchi, M., Nishijima, S., Asada, Y., Takahashi, M., Ushijima, T., Niwa, Y. (1990) The inhibition of free radical generation by human neutrophils through the synergistic effects of metronidazole with palmitoleic acid: a possible mechanism of action of metronidazole in rosacea and acne. *Arch. Dermatol. Res*. **282**: 449–454

Balkrishnan, R., McMichael, A. J., Hu, J. Y., Camacho, F. T., Shew, K. R., Bouloc, A., Rapp, S. R., Feldman, S. R. (2006) Correlates of health-related quality of life in women with severe facial blemishes. *Int. J. Dermatol*. **45**: 111–115

Bamford, J. T. (2001) Rosacea: current thoughts on origin. *Semin. Cutan. Med. Surg*. **20**: 199–206

Black, H. S. (2004) ROS: a step closer to elucidating their role in the etiology of light-induced skin disorders. *J. Invest. Dermatol*. **122**: xiii–xxiv

Briganti, S., Picardo, M. (2003) Antioxidant activity, lipid peroxidation and skin diseases. What's new. *J. Eur. Acad. Dermatol. Venereol* **17**: 663–669

Buechner, S. A. (2005) Rosacea: an update. *Dermatology* **210**: 100–108

Buege, J. A., Aust, S. D. (1978) Microsomal lipid peroxidation. *Methods Enzymol*. **52**: 302–310

Dahl, M. V. (2004) Rosacea subtypes: a treatment algorithm. *Cutis* **74**: 21–24

Del Rosso, J. Q. (2004) Medical treatment of rosacea with emphasis on topical therapies. *Expert Opin. Pharmacother*. **5**: 5–13

Eriksson, G., Nord, C. E. (1987) Impact of topical metronidazole on the skin and colon microflora in patients with rosacea. *Infection* **15**: 8–10

Esterbauer, H., Schaur, R. J., Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med*. **11**: 81–128

Gupta, A. K., Chaudhry, M. (2002) Topical metronidazole for rosacea. *Skin Ther. Lett*. **7**: 1–3, 6

Gupta, A. K., Chaudhry, M. M. (2005) Rosacea and its management: an overview. *J. Eur. Acad. Dermatol. Venereol*. **19**: 273–285

Izikson, L., English, J. C., Zirwas, M. J. (2006) The flushing patient: differential diagnosis, workup, and treatment. *J. Am. Acad. Dermatol*. **55**: 193–208

Jones, D. (2004) Reactive oxygen species and rosacea. *Cutis* **74**: 17–20

Kindl, G., Raab, W. (1998) *Licht und Haut. Bräunung, Lichtschutz, Pflege: ein Leitfaden für die Beratung in der Praxis*. Govi Verlag, Eschborn

Miyachi, Y. (2001) Potential antioxidant mechanism of action for metronidazole: implications for rosacea management. *Adv. Ther*. **18**: 237–243

Miyachi, Y., Imamura, S., Niwa, Y. (1986) Anti-oxidant action of metronidazole: a possible mechanism of action in rosacea. *Br. J. Dermatol*. **114**: 231–234

Nally, J. B., Berson, D. S. (2006) Topical therapies for rosacea. *J. Drugs Dermatol*. **5**: 23–26

Öztas, M. O., Balk, M., Ogus, E., Bozkurt, M., Ogus, I. H., Ozer, N. (2003) The role of free oxygen radicals in the aetiopathogenesis of rosacea. *Clin. Exp. Dermatol*. **28**: 188–192

Rijken, F., Kiekens, R. C., van den Worm, E., Lee, P. L., van Weelden, H., Bruijnzeel, P. L. (2006) Pathophysiology of photoaging of human skin: focus on neutrophils. *Photochem. Photobiol. Sci*. **5**: 184–189

Schaffazick, S. R., Pohlmann, A. R., de Cordova, C. A., Creczynski-Pasa, T. B., Guterres, S. S. (2005) Protective properties of melatonin-loaded nanoparticles against lipid peroxidation. *Int. J. Pharm*. **289**: 209–213

Sowinska-Glugiewicz, I., Ratajczak-Stefanska, V., Maleszka, R. (2005) Role of psychological factors in course of the rosacea. *Rocz. Akad. Med. Bialymst*. **50** (Suppl. 1): 49–53

Trommer, H., Neubert, R. H. (2005) Screening for new antioxidative compounds for topical administration using skin lipid model systems. *J. Pharm. Pharmacol. Sci*. **8**: 494–506

Trommer, H., Wagner, J., Graener, H., Neubert, R. H. (2001) The examination of skin lipid model systems stressed by ultraviolet irradiation in the presence of transition metal ions. *Eur. J. Pharm. Biopharm*. **51**: 207–214

Trommer, H., Wartewig, S., Bottcher, R., Poppl, A., Hoentsch, J., Ozegowski, J. H., Neubert, R. H. (2003a) The effects of hyaluronan and its fragments on lipid models exposed to UV irradiation. *Int. J. Pharm*. **254**: 223–234

Trommer, H., Platzer, M., Raith, K., Wohlrab, W., Podhaisky, H. P., Neubert, R. H. (2003b) Examinations of the antioxidative properties of the topically administered drug bufexamac reveal new insights into its mechanism of action. *J. Pharm. Pharmacol*. **55**: 1379–1388

Trouba, K. J., Hamadeh, H. K., Amin, R. P., Germolec, D. R. (2002) Oxidative stress and its role in skin disease. *Antioxid. Redox Signal*. **4**: 665–673

Wilkin, J., Dahl, M., Detmar, M., Drake, L., Liang, M. H., Odom, R., Powell, F. (2004) Standard grading system for rosacea: report of the National Rosacea Society Expert Committee on the classification and staging of rosacea. *J. Am. Acad. Dermatol*. **50**: 907–912

Zip, C. (2006) An update on the role of topical metronidazole in rosacea. *Skin Ther. Lett*. **11**: 1–4