Experimenteller Teil

1. Material und Methoden

Die Herstellung und die Probenentnahme fanden unter aseptischen Bedingungen in einer LAF-Box statt.
Verwendet wurde Vfend® 200 mg Pulver zur Herstellung einer Infusionslösung (Pfizer) und 0,9% NaCl Macoflex N 100 ml-Beutel von Maco-Pharma.
Im UV/VIS-Spektrometer wurde das Absorptionsmaximum für Voriconazol bei 256 nm ermittelt. Um Messschwankungen weitestgehend auszugleichen, wurden für jeden Arzneistoff drei Konzentrate und drei Infusionsbeutel für die Messung verwendet. Jeder Beutel und jedes Konzentrat wurden wiederum dreimal vermessen. Zur Herstellung von Konzentrat und Infusionsbeuteln wurden sechs Vials auf Raumtemperatur gebracht und jeweils 19 ml Aqua ad injectabilia zugespritzt. Man erhält laut Hersteller ein entnehmbares Gesamtvolumen von 20 ml klarem Konzentrat mit einer Konzentration von 10 mg/ml. Aus drei dieser Vials wurde jeweils der gesamte Inhalt in einen 100 ml NaCl-Beutel gespritzt, aus dem vorher 20 ml entnommen wurden. Man erhält eine Konzentration von 2 mg/ml im Beutel. Beutel und Konzentrat wurden bei 2–8 °C gelagert.

2. Messung

Von Voriconazol wurde eine Eichgerade in Wasser erstellt, da bei einem Vorversuch keine prägnanten Unterschiede in Abhängigkeit vom Lösungsmittel gefunden wurden.
Die sehr guten Regressionskoeffizienten bestätigten unsere Methode ($y = 23{,}037x + 0{,}0023$, $R = 0{,}9999$).
Die Konzentrate und die Beutel wurden auf Raumtemperatur gebracht und die Proben unter aseptischen Bedingungen mit sterilen Eppendorfspitzen bzw. sterilen Spritzen entnommen.
Aus jedem Konzentrat wurden 0,5 ml mit der Eppendorfpipette in einen 100 ml-Kolben überführt und mit Wasser (LiChrosolv®) aufgefüllt. 1 ml dieser Lösung wurden mit 2 ml Wasser (LiChrosolv®) direkt in der Küvette gemischt und bei 256 nm vermessen.
Aus jedem Infusionsbeutel werden 1 ml mit einer 1 ml-Spritze entnommen und in einem 100 ml-Kolben mit 0,9%-NaCl-Lösung verdünnt. Diese Lösung kann direkt bei 256 nm vermessen werden.
Auch hier wurden aus jedem Kolben dreimal die zu vermessenden Verdünnungen erstellt.
Die Proben wurden am Tag 1 hergestellt und an den Tagen 1, 2, 3, 4, 5, 9, 10, 11, 12, 15, 16, 17, 19, 22, 25, 29 und 32 vermessen.
Aus den gemessenen Extinktionen wurde anhand der Daten aus der Eichgerade der Gehalt der einzelnen Proben ermittelt. Die Erstkonzentration im Konzentrat und im Beutel wurde jeweils als 100 % definiert.

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Photodynamic and photo-cross-linking potential of bergamottin

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Bergamottin (5-geranoxypsoralen) is a main component of bergamot and grapefruit oil. In order to investigate the photophysical and photochemical behaviour of bergamottin, absorption and fluorescence properties, production of singlet oxygen and superoxide radical anions and further cross-linking of DNA were studied. Strong photochemical reactions were not observed.

Recently, more information about the photocytotoxic and photogenotoxic potential of bergamottin (5-geranoxypsoralen, 5-GOP) was demanded (Anon. 2003). Furthermore, the increasing world-wide usage of dietary supplements containing bergamottin has to be considered.
Although *in vitro* photoactivity was found in mammalian cells (Ashwood-Smith et al. 1992), *in vivo* studies in humans and albino guinea pigs showed no phototoxicity of bergamottin (Zaynoun et al. 1977; Naganuma et al. 1985). In both *in vivo* studies bergamottin was directly applied to the skin. Thus, gastro-intestinal or hepatic metabolism could not be relevant for photoinactivity in these cases, since bergamottin is known as a partly irreversible inhibitor of cytochrome P450 3A4 (Bailey et al. 2003). Furthermore, hardly any phototoxic effect and no morphologic alterations of epidermal Langerhans cells were observed after applying bergamottin to the skin of mice (Aubin et al. 1994). Some *in vitro* studies did not notice phototoxicity, either (Ashwood-Smith et al. 1992).
In order to gain more information about the photoactivity of bergamottin, we examined some photophysical and photochemical properties of bergamottin. Absorption and fluorescence spectra (Table) do not differ from those of bergapten, suggesting similar photophysical properties.
For the appearance of phototoxicity and photogenotoxicity, the following photoreactions are important (Potapenko 1991): cross-linking of DNA, production of singlet oxygen ($^1O_2$) and oxygen radicals ($O_2^{\cdot -}$, e. g.). Strong cross-linking by bergamottin and UV irradiation did not appear, as there were only small differences (ΔT_m) in melting points of irradiated and non-irradiated DNA treated with bergamottin (Table). Therefore, bergamottin can be regarded as a monofunctional furocoumarin, probably as a result of the inability to intercalate due to the bulky geranoxy-substituent. In 1995 Laquerbe et al. showed that cross-links possess a higher photocytotoxic potential than monoadducts in human cells. This might explain the marginal phototoxicity of bergamottin. In contrast to Morlière et al. 1990, we found only slight production of singlet oxygen

Table: Properties of bergamottin (in comparison data of psoralen and bergapten)

Compd.	R	Absorption maxima	Fluorescence maxima	Relative $^1O_2$-photoproduction	Relative $O_2^{\bullet -}$-photoproduction	ΔT_m (°C)
Bergamottin		λ_{max} 310 nm log ε 4.22	$\lambda_{max, ex}$ 338 nm $\lambda_{max, em}$ 474 nm	0.06 ± 0.02	0.03 ± 0.00	0.5 ± 0.4
Psoralen	−H	λ_{max} 328 nm log ε 3.79	$\lambda_{max, ex}$ 343 nm $\lambda_{max, em}$ 428 nm	1.00 ± 0.03	0.08 ± 0.00	7.9 ± 0.4
Bergapten	$-OCH_3$	λ_{max} 310 nm log ε 4.10	$\lambda_{max, ex}$ 338 nm $\lambda_{max, em}$ 476 nm	0.10 ± 0.04	0.01 ± 0.00	11.9 ± 0.9

Riboflavin as a positive control with a relative $O_2^{\bullet}$-photoproduction of 1.00 ± 0.03; results partly from Bode and Hänsel 2005

by bergamottin and UV irradiation (Table). It must be noticed, however, that the authors did not use pure bergamottin but depleted bergamot oil with reduced contents of other furocoumarins. Therefore, the reported production of singlet oxygen may be explained by the presence of other compounds than bergamottin. In addition, we did not detect large amounts of superoxide radical anion by bergamottin and UV irradiation (Table). In conclusion, we assume that bergamottin exhibits only poor photodynamic properties.

Apparently, the described weak photoactivities and other possible photoreactions (formation of DNA-monoadducts and of adducts with lipids and proteins) suffice for photoactivity *in vitro*, but not for *in vivo* phototoxicity as reported by Naganuma et al. (1985) and Zaynoun et al. (1977).

It may be assumed that bergamottin, reacting as a monofunctional furocoumarin, is less photogenotoxic as other bifunctional furocoumarins since this relation is reported for other monofunctional furocoumarins (Kinley et al. 1994), too. This fact has to be ensured by experiments with different cell systems because the relative efficiency of cross-links and monoadducts differs due to varying repair mechanisms (Laquerbe et al. 1995). Preliminary experiments revealed no photomutagenicity in the bacterial reverse mutation test using different *Salmonella typhimurium* strains (Anon. 2003). Besides further photomutagenicity tests (e. g. with eukaryotic cells), the photoclastogenicity of bergamottin should also be examined. Suitable *in vitro* test systems are the photo-micronucleus test and the photo-comet assay (Brendler-Schwaab et al. 2004). Possible positive results should be verified *in vivo* for photocarcinogenicity (Müller et al. 2001).

Experimental

Bergamottin was purchased from Fluka. Production of singlet oxygen (bleaching of *N*,*N*-dimethyl-4-nitrosoaniline) and superoxide radical anions (reduction of nitro blue tetrazolium), cross-linking (melting of Poly(dA-dT)-Poly(dA-dT)-DNA) and absorption spectra were determined according to Bode and Hänsel (2005). Fluorescence spectra were obtained on a Perkin Elmer LS 50B luminescence spectrometer with monochromator slits of 15 nm at the excitation and emission maxima using solutions containing 100 μM of the furocoumarin in EtOH Uvasol® (Merck).

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