Coll. Antropol. **32** (2008) Suppl. 2: 67–73
Original scientific paper

Fractionated Illumination Improves the Outcome in the Treatment of Precancerous Lesions with Photodynamic Therapy

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A B S T R A C T

Photodynamic therapy (PDT) is a noninvasive therapy for non-melanoma skin cancer. The aim of this study was comparison of efficacy between fractioned versus single dose illumination in photodynamic therapy (PDT) of actinic keratosis (AK) and Bowen's disease (BD). Fifty-one patients (36 AK and 15 BD) were treated with PDT. They were randomly arranged in two treatment groups. Group one included 26 patients (20 AK and 6 BD) that, after five hours of incubation with 20% 5-ALA, were treated with a single illumination of 100 Jcm^{-2} at fluence rate of 30 $mWcm^{-2}$. Group two included 25 patients (16 AK and 9 BD) that, after 16 hours of incubation with 20% 5-ALA, were treated with two light fractions (50 plus 50 Jcm^{-2}) at same fluence rate with dark interval of two hours between fractions. Twenty-four weeks later, a treated area was incubated for four hours again with 5-ALA in order to detect occult areas of abnormal skin with possible remaining tumor tissue. In case of fluorescence, histological examination was performed. In the group one, fluorescence at the end of the session was absent in 19 (73%) or very weak in 7 (27%). Residual tumor was found in 15 (75%) AK and in 4 (66.6%) BD. In the group two, fluorescence at the end of second session was more intense; in one patient (4%) was absent, very weak in 5 (20%) and weak in 19 (76%) of patients. In this group histology revealed remaining tumor tissue in only 2 (12.5%) AK and 2 (22.2%) BD. Among the patients in the first group, the remaining tumor tissue was significantly bigger ($p=0.005$). The treatment response with clearing of tumor tissue was significantly higher in fractionated illumination than in a single dose illumination group. Fractionated illumination scheme with 16 hours of incubation separated by two hours dark interval significantly improves the therapeutic outcome in tumor eradication.

Key words: *topical photodynamic therapy, 5-ALA, fractionated illumination*

Introduction

Photodynamic therapy (PDT) with topically applied 5–aminolevulinic acid (5-ALA) as the photosensitizer is effective treatment modality for various superficial precancerous and cancerous skin lesions and can be performed on outpatient basis. It is complex photochemical process that shows interaction between the fluence rate, total emitted energy, photosensitizer concentration and oxygen concentration[1]. It was noted that damage of the skin depends upon fluence rate. Low fluence rate causes more tissue damage, as well as fraction of the light. It seems that at high fluences the amount of damage may reach plateau, while oxygen concentration, which is important for porphyrin photobleaching, decreases[1–4].

A conventional protocol uses the topical application of the 20% 5-ALA which serves as precursor drug for 4–16 hours before illumination. According to metabolic process, 5-ALA transforms in the strong photosensitizer protophorphyrin IX (ppIX) which can be activated by 635 nm wavelength red light resulting in generation of reactive oxygen species[1,5]. Several light sources have been used in clinical PDT studies for cutaneous applications,

Received for publication July 30, 2008

including lasers, xenon arch/discharge lamps, incandescent filament lamps, metal halogen lamps, lasers, slide projector illumination assemblies and solid-state light-emitting diodes (LED)[6–9]. Coherent light is not necessary for PDT. The development of energy – efficient LED sources has facilitated the implementation of red-light sources and are likely to become the most frequently used lights in clinical practice[1,10,11]. But, until today no PDT treatment protocol is widely accepted and recommended.

PDT of superficial precancerous and cancerous, but non-melanoma skin tumors using topically applied ALA was introduced by Kennedy and co-workers in 1990[12]. Today, topical PDT has optimal efficacy in non-hyperkeratotic actinic keratosis of the face and scalp, Bowen's disease and superficial histopathologic variant of basal cell carcinoma (less than 2 mm thick). Activated oxygen species within target cells, produce their destruction. The rationale of the therapeutic efficacy of PDT is based on the cytotoxic action of products generated by excited photosensitizer. When a photosensitizer absorbs light of appropriate wavelength it is converted from a stable ground state to a short-living singlet state that may undergo conversion to a longer-living excited triplet state. This photo-active species is responsible for the generation of cytotoxic products. They may either react directly with substrate by hydrogen atom or electron transfer to form radicals (type I reaction), or the triplet state can transfer its energy to oxygen directly to produce singlet oxygen. This singlet oxygen is highly reactive in biological systems (type II reaction), causing photo-oxidation with subsequent cell death. These cytotoxic mechanisms take place in a time scale of microseconds[13]. While 635 nm light may penetrate up to 6 mm (compared with 1–2 mm for light at 400–500 nm), the therapeutically effective maximum depth of PDT will depend on sufficient light dose being delivered to tissue that contains sufficient amount of photosensitizer to achieve a photodynamic reaction. The therapeutically effective depth of PDT in the skin is therefore likely to be lesser, at 1–3 mm at 635 nm depending on the type of tissue[14]. Most clinical application of PDT have used red light around 630–636 nm to achieve adequate penetration[15]. To achieve higher clearance rate of lesions, Morton with co-workers and Haller with co-workers, have treated lesions more than nce, while other authors used single or fractionated illumionation with dark interval lasting for several hours[5,16–18].

Material and Methods

In Outpatient department of dermatologic oncology, fifty-one patients have been treated with topical PDT. Prior the therapy informed consent was acquired.

The patients were referred to the PDT with previous histologically confirmed diagnosis of actinic keratosis (AK) (36) and Bowen's disease (BD) (15). Adjunctive therapy to remove thick keratotic crusts with ointments and wet dressing was performed prior the therapy.

Twenty-six patients (20 AK and 6 BD) constituting the group one, were treated with topical PDT after five hours of incubation with 20% 5-ALA. The ALA cream was prepared at the hospital pharmacy just prior the application. It consisted of 20% 5-ALA-HCl (Medac, Hamburg, Germany) in an ointment base (Belobaza, Belupo, Koprivnica, Croatia), 2% of dimethylsulfoxide (DMSO) and 2% of edetic acid disodium salt (EDTA) (Titriplex III, Merck, Germany). After cleaning the area with a saline solution, the cream has been applied to a thickness of approximately 1 mm covering the treated area and 1 cm of the surrounding skin. The area was covered by plastic occlusive dressing (Tegaderm, 3M, UK) and an aluminum foil on the top in order to protect treated skin from exposure to ambiental light. Five hours later the photosensitizing cream was removed, and illumination started. The light source was MediLED 4PDD/PDT system (Division of Laser and Atomic R&D, Ruđer Bošković Institute, Zagreb) with red light wavelength of 635 nm. At the beginning, the distance of light source was determined, and then tumor tissue fluorescence was achieved using violet light of 405 nm. Thereafter, illumination with red light was started. The emitted dose of illumination was 100 Jcm^{-2} in a single dose with fluence intensity of $30mW/cm^2$. To minimize sensation of prickling and pain, spraying with water and cooling with fan was performed. Also, nearby skin was protected with foil. After the treatment, sun-block ointments were recommended for next few days as well as sun protection measures. Our PDT illumination lamp also could detected and register fluorescence. So, the photos of degree of fluorescence have been taken before and after illumination and saved. The control biopsies were performed twenty-four weeks after illumination in patients whom fluorescence after three hours incubation with 5-ALA was detected.

The group two consisted of twenty-five patients (16 AK and 9 BD). They were treated also with PDT after 16 hours of incubation with 5-ALA photosensitizing cream. The procedure of preparing prior illumination was the same as for the group one. The total of 100 Jcm^{-2} (50 Jcm^{-2} plus 50 Jcm^{-2} two hours later) was delivered with fluence intensity of 30 mW/cm^2. After receiving 50 Jcm^{-2} interruption of two hours was done. The photos of degree of fluorescence have been taken before and after illumination, as well as at the end of the first session and at beginning of the second after end of two hours dark interval.

The biopsies from treated areas were performed twenty-four weeks after PDT in case of fluorescence after four hours of incubation with 5-ALA from suspicious lesions. Differences between variables were analyzed by χ^2 test, and $p \geq 0.005$ was considered statistically significant.

Results

In the group one, which consisted of 26 patients, treated with single dose of 100 Jcm^{-2} after five hours of incubation, the fluorescence at the end of PDT was in 7 (26.92%) patients very weak and absent in 19 (73.08%) in comparison of that before PDT (Figures 1 and 2) (Table 1).

Fig. 1. Fluorescence after five hours of incubation with 20% 5-ALA cream.

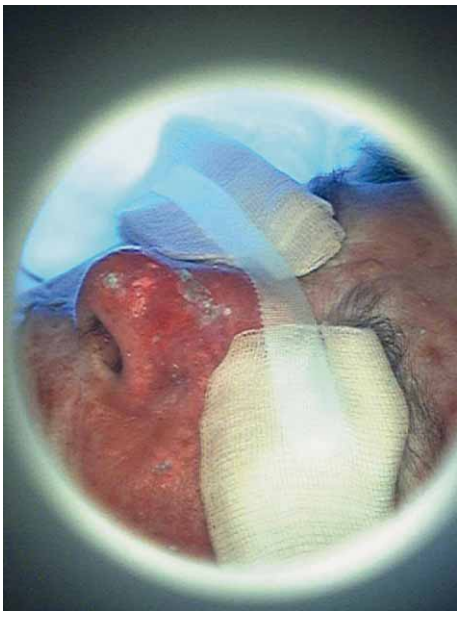

Fig. 2. Fluorescence after 100 Jcm^{-2} received in one session.

The biopsies of suspicious lesions obtained twenty-four weeks after therapy, revealed the presence of AK and BD in tissue in 19 (73%) patients. The residual tumor tissue was found in 15 of 20 AK (75%) and in 4 of 6 BD (66.6%) (Figures 3 and 4). So, the repeating therapy was necessary.

The group two (25 patients), after 16 hours of incubation with 5-ALA, was treated with 100 Jcm^{-2} with an interruption of two hours dark interval after 50 Jcm^{-2} was received, showed much higher degree of fluorescence after finishing the treatment (Figure 5). There was no fluorescence detected in one patient (4%), very weak fluorescence was noticed in 5 (20%) patients, and weak in 19 (76%) (Table 2). Even a higher degree of fluorescence was detected at the beginning of second session after two hours of dark interval. Pathohistological specimens obtained from suspicious lesions after twenty-four weeks after PDT, revealed persistence of tumor tissue in 2 of 16 (12.5%) AK and in 2 of 9 BD (22.2%) (Figures 6 and 7). Statistical analysis showed statistically significance between frequencies of remaining tumor tissue among these two groups according to the first group where in 73% remaining tumor tissue was found (p=0.005).

Discussion and Conclusion

The success of topical ALA-PDT is dependent on several factors. In addition to penetration of 5-ALA into the skin lesion and the formation of therapeutic concentration of ppIX, clearance rate and complete response depend significantly on tumor thickness and duration of 5-ALA application[11]. So, Morton pointed out that curettage before topical 5-ALA application may significantly improve outcome of PDT, particular of thick lesions[16]. In order to enhance effectiveness of 5-ALA-PDT, modified prodrug of 5-ALA – methyl aminolevulate (MAL) and other esters have been used but clinical studies showed a variation in response rate[12,19]. Several groups of authors performed series of pre-clinical studies investigating the effect of fractionating illumination in PDT[12,19,20]. Increased efficacy with fractionated illumination with relatively long dark intervals between two fractions was shown[5,11,18,20]. The mechanism behind the increase in efficacy after the two-fold illumination scheme remains still unclear[20]. The choice of two-fold illumination sche-

TABLE 1
FLUORESCENCE INTENSITY IN THE GROUP ONE AFTER 100 Jcm^{-2} DELIVERED IN A SINGLE DOSE

	Absent (I)	Very weak (II)	Weak (III)	Total
Actinic keratosis	15 (57.70%)	5 (19.23%)		20 (76.93)
Bowen's disease	4 (15.38%)	2 (7.69%)		6 (23.07)
Total	19 (73.08%)	7 (26.92%)		26 (100.00)

TABLE 2
FLUORESCENCE INTENSITY IN THE GROUP TWO AFTER 100 Jcm^{-2} DELIVERED IN THE FRACTIONATED DOSES AFTER TWO HOURS DARK INTERVAL

	Absent (I)	Very weak (II)	Weak (III)	Total
Actinic keratosis	1 (4%)	3 (12%)	12 (48%)	16 (64%)
Bowen's disease		2 (8%)	7 (28%)	9 (36%)
Total	1 (4%)	5 (20%)	19 (76%)	25 (100%)

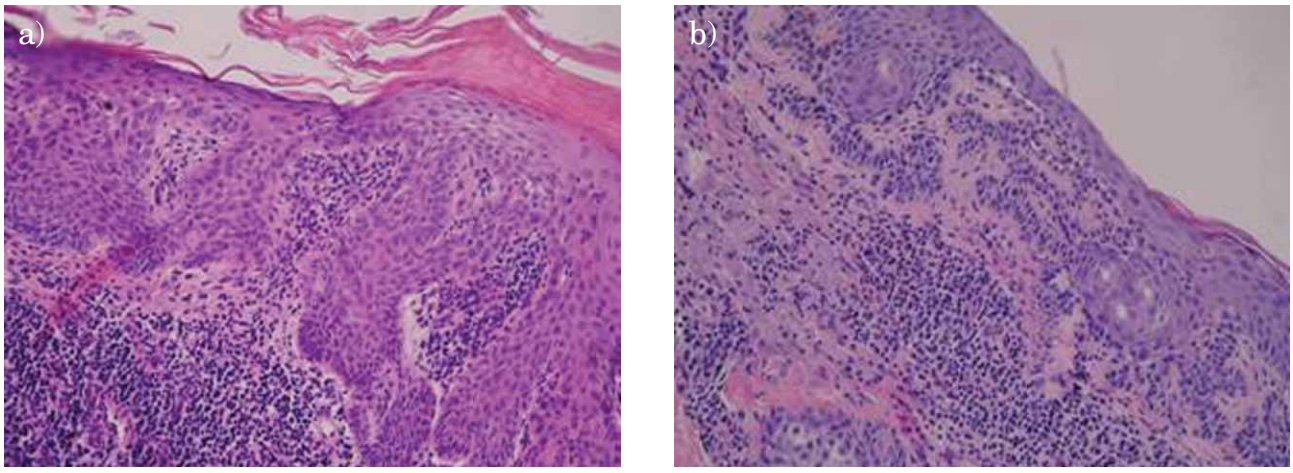

Fig. 3. Pathohistology of the treated actinic keratosis prior (a) and after (b) 100 Jcm^{-2} received (H&E ×100).

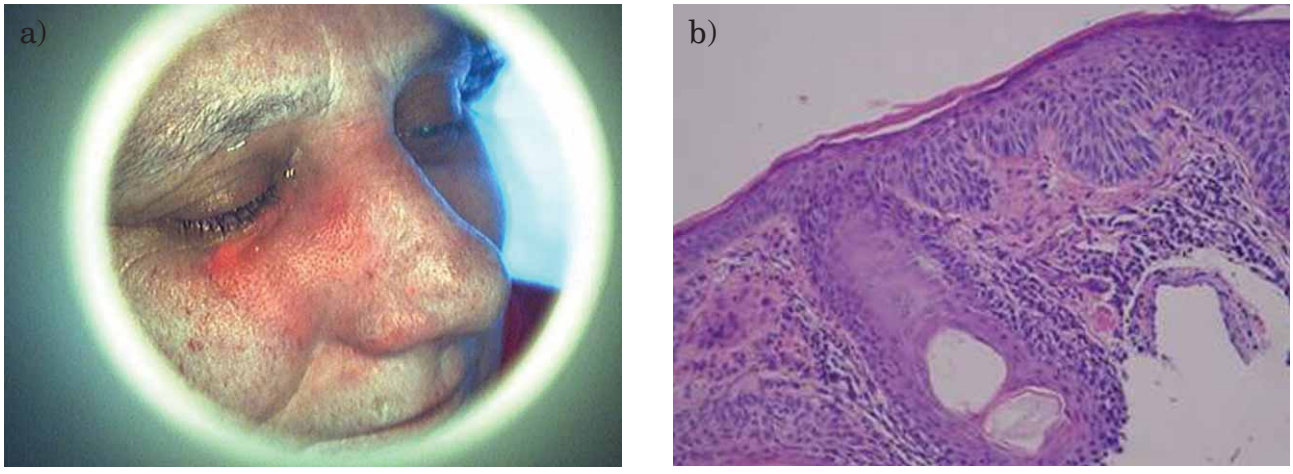

Fig. 4. Pathohistology of the treated Bowen's disease prior (a) and after (b) 100 Jcm^{-2} received in one session (H&E ×100).

me for de Haas and co-workers was based on data from pre-clinical animal studies which showed that following light illumination, tissues continue to synthesize ppIX. The hem synthesis cycle was partly intact and there was still 5-ALA available to be converted into ppIX[12]. Some of pre-clinical studies demonstrated the complexity of tissue response to 5-ALA-PDT. They stress out also importance of duration between two illuminations. A two-hour time interval is necessary to achieve a significant increase in response[12]. That was reason why we choose this scheme in our investigation. The fluence emitted in the first light fraction has great impact on the therapeutic response. Light fraction scheme of 50 + 50 Jcm^{-2} with two-hour dark interval showed greater results than single illumination of 100 Jcm^{-2}. It has been shown that reducing the first light fraction to only 5 Jcm^{-2}, and delivering large fluence in the second session (95 Jcm^{-2}) significantly increases the effectiveness of PDT. Also, very important factors influenced on complete response of tumor were possessing of pigment and thickness of the tumor. It was found in animal model, that the optimal ratio of fluence rates was 20 in the first and 80 Jcm^{-2} in the second fraction[12]. A number of pre-clinical studies have

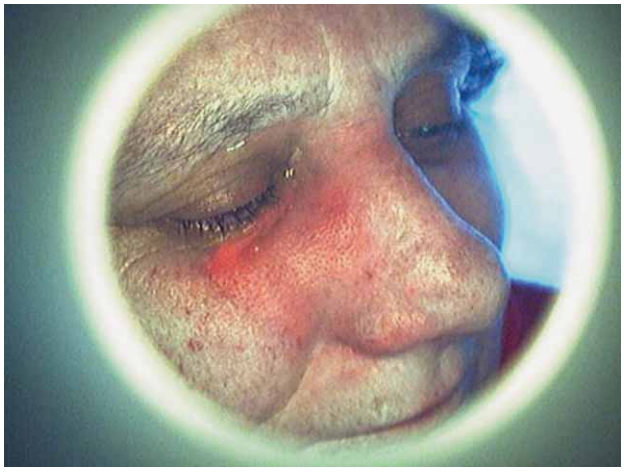

Fig. 5. Fluorescence after 100 Jcm^{-2} received in fractionated PDT with two hours dark interval.

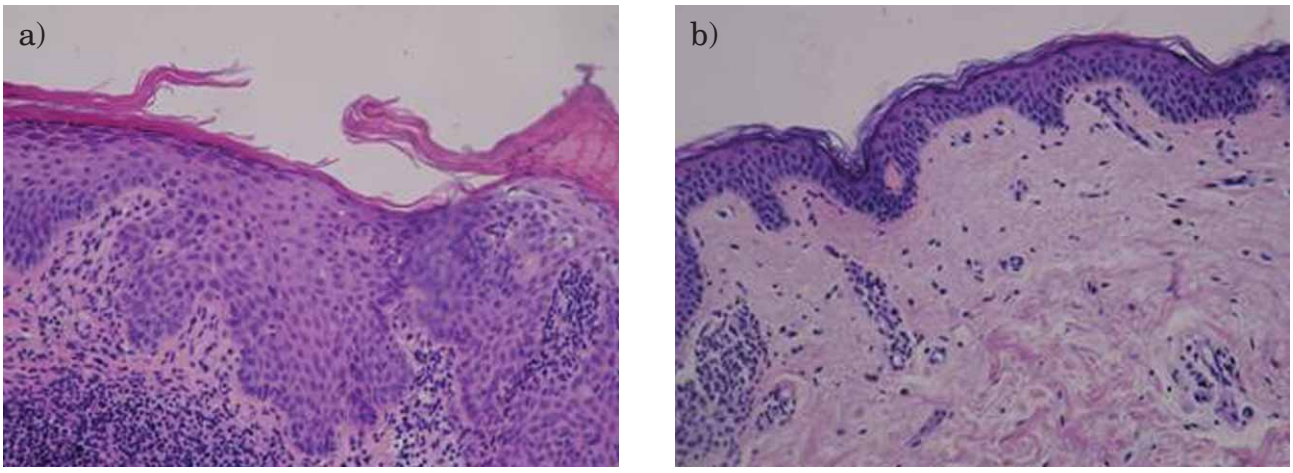

Fig. 6. Pathohistology of the treated actinic keratosis prior (a) and after (b) 100 Jcm^{-2} received in fractionated PDT with two hours dark interval (H&E ×100).

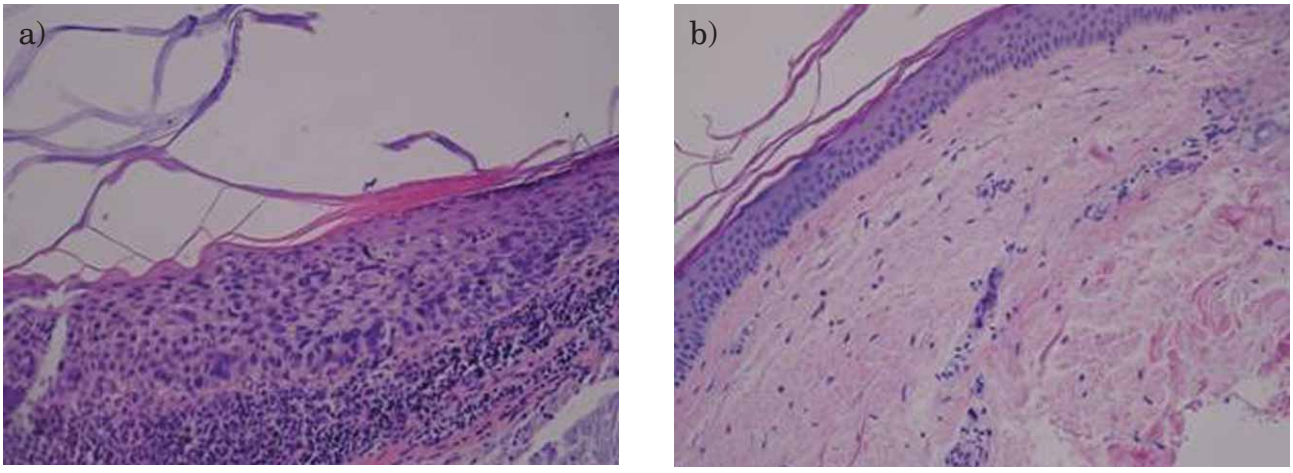

Fig. 7. Pathohistology of the treated Bowen's disease prior (a) and after (b) 100 Jcm^{-2} received in fractionated PDT with two hours dark interval (H&E ×100).

shown that photobleaching of the ppIX limits the PDT dose that can be delivered in a single light fraction at fixed fluence rate. So, in normal mouse skin 100 Jcm^{-2} does not result in significantly more damage than 50 Jcm^{-2}. The relationship between response to 5-ALA-PDT and fluence has not been investigated in the clinical practice[12]. Oberdanner with co-workers stated that fractionated delivery in PDT may enhance the photodynamic effect in tumor areas with insufficient blood supply by enabling the re-oxygenation of the treated area *in vitro* system. Fractionated irradiation with light/dark intervals of 45/60 s decreases reactive oxygen species (ROS) production and cytotoxicity of PDT. This effect can be reversed by addition of an inhibitor of the glutathione reductase which can be regenerated during dark intervals, thereby rendering cells less susceptible to ROS produced by PDT compared with continuous irradiation[18]. They noted a discrepancy in the ROS level and cytotoxicity for these two modalities of treatment and try to explain this effect by two hypotheses: 1. singlet oxygen or other ROS is decomposed during the dark intervals of fractionated irradiation, thus leading to less cytotoxicity; and 2. cellular repair mechanisms restore protein and lipid elements during dark intervals causing less cytotoxicity[18]. These results could be of interest for PDT of any well vascularized tissue or tumor in which oxygen deprivation is not to be expected. So, in clinical implementation, it has to be considered that strong antioxidant glutathione recycling may antagonize the therapeutic efficiency[18]. It is evident that low fluence rate causes more damage than high rate for the same total fluence[2]. Fractions during light delivery seem to increase the amount of damage done by given fluence. The oxygen concentration in illuminated tissue decreases with illumination[3]. This can be seen to recover at lower fluence rates below 30 mWcm^{-2} [1]. Also characteristic photobleaching rate may decrease with increasing fluence rate. It is less pronounced or absent at fluence rates above 30 mWcm^{-2} [21]. The mechanism of the cell death after 5--ALA-PDT is complex and heterogeneous. Early after the

5-ALA administration, ppIX is localized in the mitochondria and it is well known that cell death can occur by apoptosis and or necrosis[22]. Grebenova with co-workers have shown that during and after illumination the mitochondria are damaged resulting in cytochrome-c release and caspase 3 activation followed by necrosis. Simultaneously, a second route to apoptosisis is activated[23]. The study of de Bruijn and co-workers shows in the study in mice that the timing of the second illumination is important[11]. Some studies have shown that fluence delivered in the first fraction is important, while a single illumination with same fluence resulted in minimal skin damage at day 1 to 7 after treatment, despite the fact that almost 60% of the total amount of ppIX is photobleached[24,25]. Gederaas has shown a similar process *in vitro*[26]. Fractionated PDT prefer some Japanese authors as Togashi and Uehara who showed that tumor cells surviving from PDT have proliferative potential, and that oxygenation in tumors may be recovered after 24 hours. They concluded that re-growth of tumor was significantly suppressed by fractionated PDT because re-accumulation of ppIX in tumor tissue, what occurs during dark time of pause between two illuminations, enables better efficacy in selective tissue damage of illuminated field[27]. Group of authors from Netherland stressed out that two hours dark interval is necessary to achieve a significant increase in response[12]. Also, it is shown that choice of flueunce for the first light fraction is critical, and a high fluence for the second illumination is necessary for better tissue response[12]. So, Hass with co-workers found that treatment scheme for PDT with two-fold illumination using 20 Jcm^{-2} in the first and 80 Jcm^{-2} in the second fraction with two hours dark interval is optimal for obtaining maximal clearance rate of treated lesion[12]. Very similar experience as previously mentioned authors we showed during our practice. Fractionated PDT seems to be much more effective than single illumination because of biochemical events which recover during pause and enhance final effect of PDT. The argument for this result confirm also relative high grade of fluorescence at the end of two-fold illumination that implies higher concentration of available ppIX than after single illumination mode. In our opinion, investigating of histopathologic specimens in serial sections of tumor and precancerous lesions after twenty-four weeks revealed that remaining tumor tissues was found in lesions where alterations were found around follicular structures and alteration affected deeper parts of follicular epithelia. We compared specimens with their previous biopsy prior the PDT treatment. In the serial sections it was obvious that lesions were thicker than lesions which were completely resolved. Also, atypical keratinocytes infiltrated deeper part of acrotrychia. Probably hydrophylic ALA couldn't perform strong reactions within keratinocytes which were probably especially protected with rich lipid content of follicular lumina. In the second group of patients, longer incubation of 16 hours with 5-ALA probably enhanced penetration and contributed favourable therapeutic outcome. So it could be possible reason for an unsatisfactory therapeutic response, particularly in the single illumination scheme.

Since this investigation is a part of extensive study, comparison between modalities of single illumination with 100 Jcm^{-2} after 16 hours of incubation and two fold illumination with two hours dark period (50 plus 50 Jcm^{-2}) after five hours incubation with 5-ALA, is going to be performed. Unfortunately, in this moment, we can not surely estimate and evaluate contribution of incubation time (5 or 16 hours) on definitive therapeutic outcome, but in our opinion it seems to be negligible.

As it was pointed out, until today, no treatment protocol was accepted. So we tried to achieve more effective mode of treatment with excellent therapeutic and cosmetic results. It was shown that treatment modality with 16 hours of incubation in two session after two hours dark interval achieved superior therapeutic outcome than after five hours incubation and single illumination scheme with the same fluence rate.

Acknowledgements

The study was supported by Ministry of Science, Education, and Sports of the Republic of Croatia, grant No.141-2180056-481, »Photodynamic therapy in dermatological oncology«.

REFERENCES

1. LANGMARCK K, MEHTA R, TWYMAN P, NORRIS P, J Photochem Photobiol, 60 (2001) 37. — 2. ROBINSON DJ, DE BRUJIN HS, VAN DER VEEN N, STRINGER MR, BROWN SB, STAR WM, Photochem Photobiol, 67 (1998) 140. — 3. TROMBERG BJ, ORENSTEIN A, KIMEL S, BARKER SJ, HYATT J, NELSON JS, BERNS MW, Photochem Photobiol, 52 (1990) 375. — 4. MOAN J, SOMMER S, Cancer Res, 45 (1985) 1608. — 5. BABILAS P, SCHACHT V, LIEBSCH G, WOLFBREIS OS, LANDTHALER M, SZEIMIES RM, ABELS C, Br J Cancer, 88 (2003) 1462. — 6. MORTON CA, WHITEHURST C, MOSELEY H, MOORE JV, MACKIE RM, Laser Med Sci, 10 (1995) 165 — 7. SZEIMIES RM, KARRER S, SAUERWALD A, LANDTHALER M, Dermatology, 192 (1996) 246. — 8. KARRER S, BAUMLER W, ABELS C, HOHENLEUTNER U, LANDTHALER M, SZEIMIES RM, Lasers Surg Med, 25 (1999) 51. — 9. LAZIĆ R, GABRIĆ N, DEKARIS I, GAVRIĆ M, BOSNAR D, Coll Antropol, 31 (2007) 71. — 10. MORTON CA, WHITEHURST C, MOORE JV, MACKIE RM, Br J Derm, 143 (2000) 767. — 11. DE BRUJIN HS, VAN DER PLOEG – VAN DER HEUVEL A, STERENBORG HJCM, ROBINSON DJ, J Photochem Photobiol, 85 (2006) 184. — 12. DE HAAS ERM, KRUIJT B, STERENBORG HJCM, NEUMANN M, ROBINSON DJ, J Invest Dermatol, 126 (2006) 2679. — 13. RIGEL DS, FRIEDMAN RJ, DZUBOW LM, REINTGEN DS, BYSTRYN JC, MARKS R, Cancer of the skin (Elsevier Saunders, Philadelphia, 2005). — 14. HENDERSON BW, DOUGLAS TJ, Photochem Photobiol, 55 (1992) 145. — 15. POTTIER RH, CHOW YFA, LAPLANTE JP, ET AL, Photochem Photobiol, 44 (1986) 679. — 16. MORTON CA, MACKIE RM, WHITEHURST C, MOORE JV, McCOLL JH, Arch Dermatol, 134 (1998) 248. — 17. HALLER JC, CAIMDUFF F, SLACK G, SCHOFILED J, WHITEHURST C, TURNSTALL E, ET AL, Br J Dermatol, 143 (2000) 1270. — 18. OBERDANNER CB, PLAETZER C, KIESSLICH T, KRAMMER B, Photochem Photobiol, 81 (2005) 609. — 19. VAN DER AKKER JT, BOOT K, VERNON DI, BROWN SB, GROENENDIJK L, VAN RHOON GC, ET AL, Photochem Photobiol Sci, 3 (2004) 263. — 20. VAN DER VEN H, HEBEDA KM, DE BRUJIN HS, STAR

WM, Photochem Photobiol, 70 (1999) 921. — 21. IINUMA S, SHOEMACKER KT, VAGNIERES G, RAJADHYAKSHA M, BAMBERG M, MOMMA T, HASAN T, Cancer Res, 53 (1993) 1249. — 22. WILSON BC, OLIVIO M, SINGH G, Photochem Photobiol, 65 (1997) 166. — 23. GREBENOVA D, KUZELOVA K, SMETANAN K, PLUSKALOVA M, CAJTHAMLOVA H, ET AL, J Photochem Photobiol, 69 (2003) 71. — 24. ROBINSON DJ, DE BRUJIN HS, STAR WM, STERENBORG HJCM, Photochem Photobiol, 77 (2003) 319. — 25. ROBINSON DJ, DE BRUJIN HS, WOLF J, STERENBORG HJCM, STAR WM, Photochem Photobiol, 72 (2000) 794. — 26. GEDERAAS OA, THORSTENSEN K, ROMSLO I, Scand J Clin Lab Inves, 56 (1996) 583. — 27. TOGASHI H, UEHARA M, IKEDA H, INOKUCHI T, Oral Oncology, (2006) in press.

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FRAKCIONIRANO OSVJETLJAVANJE POBOLJŠAVA TERAPIJSKI UČINAK FOTODINAMSKE TERAPIJE PREKANCEROZA

S A Ž E T A K

Fotodinamska terapija je neinvazivna metoda liječenja ne-melanomskih tumora kože. Cilj studije je usporediti učinkovitost frakcioniranog nasuprot jednokratnome obasjavanju tijekom fotodinamske terapije (PDT) aktiničkih keratoza i Bowenove bolesti. Fotodinamskom terapijom liječen je 51 bolesnik (36 AK i 15 BD). Slučajnim odabirom podijeljeni su u dvije skupine. Prva skupina sastojala se od 26 bolesnika (20 AK i 6 BD) koji su nakon pet sati inkubacije sa 20% 5-ALA kremom obasjavani jednokratno sa 100 Jcm^{-2} uz intenzitet od 30 $mWcm^{-2}$. Druga skupina bolesnika uključivala je 25 bolesnika (16 AK i 9 BD) koji su obasjani nakon 16 sati inkubacije sa 20% 5-ALA kremom i to dvokratno uz dva sata pauze (50 plus 50 Jcm^{-2}) pri istom intenzitetu. Poslije 24 tjedna, tretirana područja su ponovno inkubirana sa 20% 5-ALA kremom tijekom četiri sata s ciljem otkrivanja mogućeg zaostalog tumorskog tkiva. U slučaju fluorescencije učinila bi se probatorna ekscizija uz patohistološku verifikaciju. U prvoj skupini bolesnika fluorescencija na kraju tretmana nije bila viđena u 19 (73%) bolesnika dok je u 7 (27%) bila vrlo slaba. Zaostalo tumorsko tkivo nađeno je u 15 (75%) AK i u 4 (66.6%) BD. U drugoj skupini bolesnika, fluorescencija je pri kraju druge seanse bila nešto intenzivnija; u jednog bolesnika (4%) je bila odsutna, u pet (20%) je bila vrlo slaba dok je u 19 (76%) bila slaba. U ovoj se skupini zaostalo tumorsko tkivo našlo u samo dva (12,5%) AK i dva (22,2%) bolesnika sa BD. Statističkom obradom potvrđena je veća učestalost zaostajanja tumorskog tkiva u prvoj skupini bolesnika ($p=0,005$). Terapijski odgovor se pokazao statistički značajno boljim u skupini sa frakcioniranim obasjavanjem nego u skupini sa jednokratnim obasjavanjem. Frakcionirano obasjavanje sa 16 sati inkubacije uz dva sata pauze među seansama značajno poboljšava terapijski rezultat i iskorjenjivanje tumora.