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The Action of Photosensitizers and Serum in a Bactericidal Process. II. The Effects of Dyes: Hypericin, Eosin Y and Saphranine O

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Abstract

The aim of the present work was to recognize the reasons for differences in the photodynamic action of dyes against various bacterial strains. It is expected that a better understanding of this problem may help in design of new photosensitizers. The sensitivity of 6 various bacterial strains to the photodynamic action of 5 photosensitizers was determined. The hydrophobicity of cell surface and susceptibility of bacteria to the natural defense mechanism of human serum, were estimated. The differences in the photodynamic efficiency of dyes could be contributed to various affinities of cell membrane to dyes, to known details of membrane architecture as well as to different mechanisms of photosensitization.

Key words: photosensitization, hypericin, saphranine O, eosin Y, bactericidal effect of serum

Introduction

The photodynamic action of dyes against microorganisms consists in photosensitization of cells to the action of visible light. The effect is based on oxidative cleavage of cell membrane components initiated by light. Such dyes as porfirines (Doiron and Gomer, 1984; Jori and Preria, 1985; Gabor *et al.*, 2001; Ashkenazi *et al*., 2003), porphycene (Lauro *et al*., 2002) phthalocyanines (Millson *et al*., 1996; Lacey *et al.*, 2001), hypericin and hypocrellin (Chaloupka *et al.*, 1999), saphranine O, eosin Y and others (Friedberg *et al.*, 1991; Castael *et al.*, 2003, Komerick and Wilson, 2002; Wilson, 2004) have been used as photosensitizers in photodynamic therapy of tumors and bacterial infections.

The killing of bacteria by irradiation of photosensitizing dyes within absorption bands is due to production of oxygen containing radicals and/or other reactive oxygen species (ROS)[1]. Two mechanisms (I–II) are supposed to be responsible for this photodynamic action (Halliwell and Gutteridge, 1990). Superoxide anion radical generated in mechanism (I) in the presence of metal ion gives hydroxyl radical $OH^{\bullet}$ that can readily oxidize some components of cell wall due to its extremely high reactivity (Bartosz, 1995). Another basic mechanism (II) of photosensitization consists in generation of singlet oxygen *via* triplet state of a photosensitizer. Hydroxyl radicals ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) formed in mechanisms (I) and (II) respectively, as neutral species soluble in hydrophobic media, can permeate freely through the cell membranes, causing damage of some components of cell envelope.

Since most photosensitizers are efficient only against selected bacterial strains it can be supposed that the photodynamic action of a given dye will depend not only on the dye spectral properties but also on the structure of bacterial cell wall. The reason for variation of sensitivity of bacteria is not completely clear at present. The question arises whether a photosensitizer, to be active, must be bound to the membrane, so that the affinity to the cell may decide of the efficiency. Though ROS species, generated by a dye anchored

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[1] Abbreviations: DMF dimethylformamide, DMSO dimethylsulfoxide.

on a cell, can reach the target place more rapidly than those present in bulk water solution, the binding site may be inaccessible to oxygen and the spectroscopic properties of the photosensitizer may be changed due to binding, leading to a decrease of the efficiency of ROS generation.

The knowledge of the target site damaged by ROS in the membrane and its distance from the binding site is very important. It has been shown in our previous work that chlorophylls as photosensitizers (Jankowski *et al*., 2003) or even ozone (Doroszkiewicz *et al*., 1994) can enhance the bactericidal effect of serum. The bactericidal action of serum is a very important mechanism of resistance against infections of Gram-negative strains (Jankowski, 1995; Doroszkiewicz *et al*., 1994). A correlation between the photosensitization and the action of serum suggest that the place of action of photosensitizers may be close to the site of the membrane attack complex formed by the factors present in serum.

The aim of our work is to recognize the interrelation between the structure of bacterial cell membranes and the properties of photosensitizers, their absorption ability on the cell surface and the kind of radicals produced during irradiation. The relation between the action of photosensitizers and of serum is also essential. We have found that some *Shigella* strains differ greatly with respect to the action of some dyes as well as to the bactericidal action of serum. Since the structure of antigen determinants of these strains is known (Simmons and Romanowska, 1987), it seems to be possible to correlate the properties of cell wall and the efficiency of photosensitization.

Experimental

Materials and Methods

Strains and serum. Following strains obtained from Institute of Biotechnology and Environment Protection, University of Zielona Gora were used: *Shigella flexneri* 1a, *S. flexneri* 1b and *S. flexneri* 2a, *Escherichia coli* K12 No. 781 and *Bacillus subtilis* 003. The strain of *E.coli* K1 No. 959 was from Department of Biology and Parasitology, Medical Academy of Wrocław.

Normal human serum (NHS) from 2 healthy donors not treated with any antimicrobial drugs was used. After separation from clot the serum was stored in 0.3 cm^3 portions at –70°C for no longer than 2 months.

Photosensitizers. Solutions in water of following dyes were used, saphranine O (BDH, England), concentration c = 1.00 mM/dm^3, eosine Y c = 1.00 mM/dm^3 (BDH, England), chlorophyll (water soluble) was from C. Erba (Italy) c = 1.00 mM/dm^3. Iron(II) sulfophthalocyanine (FePTS) was a kind gift from dr. Ł. Ostropolska (Chemistry Department, University of Wrocław) c = 5.00 mM/dm^3. Hypericin was obtained by extraction of Deprim (Pharmaceutical and Chemical Company, Ljubljana, Slovenija) by diethylether, evaporation of solvent in darkness and dissolving in DMF (c = 0.12 mM/dm^3). In the cases of hypericin and chlorophyll which are known (Chaloupka *et al*., 1999; Jankowski *et al*., 2003) to be sensitive to visible light, special attention was paid, to protect samples from light until photolysis. Each dye was used in the concentration range where saturation of photodynamic effect (PDE) was observed in a plot of PDE against dye concentration (Fig. 1). In the case of hypericin the results for concentrations of the photosensitizer higher than 0.2 mM/dm^3 could not be obtained because of toxic effect of the dye on most strains investigated.

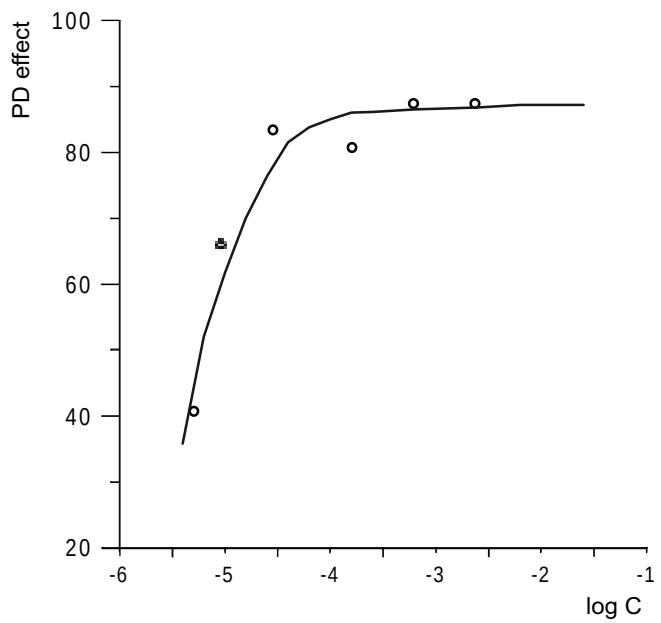

Fig. 1. Dependence of PDE of saphranine against *S. flexneri* 2a on the dye concentration

Photolyses. The influence of photosensitizers on the survival of bacteria was tested by a modified procedure described by Wilson and Pratten (1994). To 0.1 cm^3 of bacterial suspension (10^9 cells/cm^3) placed in a well of pH plate 0.1 cm^3 dye solution and 0.3 cm^3 physiological salt solution (PS) were added. The concentration of dyes in the photolysed samples was: saphranine, eosin and chlorophyll $c = 2 \times 10^{-4}$ M/dm^3, FePTS 10^{-3}M/dm^3 and hypericin 2.4×10^{-5}M/dm^3. Mean absorbance of the photolysed samples at 500–600 nm was similar for all dyes investigated. The samples were covered by glass plate to prevent evaporation of solvent and illuminated (by stirring) during 90 min by white non mutagenic light (5 W/cm^2) in a special compartment enabling optimal illumination conditions and maintaining temperature near to 30°C (26–35°C). Another plate containing analogous samples was incubated in darkness (the pH plate was covered by black glass plate).

The samples were incubated parallelly in light and in darkness in order to determine the PDE. The difference between the survival percent of bacteria in the illuminated and not illuminated sample was treated as the measure of photodynamic action.

The compartment for photolyses, plates and other furniture were sterilized by UV bactericidal lamp overnight before the experiment.

The survival of bacteria was determined by visible count method immediately after photolysis. For each sample at least 3 separate experiments and in each experiment 3 separate counts were performed. Bacterial strains showing survival percent higher than 50 were treated as resistant.

The bactericidal effect of serum. To some samples of bacterial culture, before photolysis, 0.2 cm^3 of PS instead of 0.3 of PS were added. After the photolysis, 0.1 cm^3 of serum diluted 1:1 by PS was added and the samples were incubated during 60 min (37ºC) without illumination. Then the survival percent of cells was determined as described above. This protocol was aimed to avoid deleterious effect of photosensitizers and radicals on serum.

Determination of $OH^\bullet$ radicals. The amount of hydroxyl radicals, produced in the solutions of photosensitizers, was determined at the same conditions as in the experiments with bacteria, but instead bacterial suspension 0.1 cm^3 of 4((9-acridinecarbonyl)amino)-2,2,6,6 tetramethyl piperidin-1 oxyl, free radical (TEMPO, Molecular Probes Inc, USA, solution 1.7 mg/10 cm^3 DMSO) was added to the samples. This nonfluorescent compound, by reaction with $OH^\bullet$ is converted to strongly fluorescent 9-acridine derivative which can be detected by its fluorescence at 440 nm (excited at 300 nm, see Haugland, 1996). This system was standarized by measuring of acridine fluorescence (40 ng/dm^3 –1 mg/dm^3) and the fluorescence of TEMPO, at the same conditions in the presence of $OH^\bullet$ generating system (Fe^{2+}/ascorbate; see Yokoyama *et al*. 2002). We determined also lipid peroxidation products, as described previously (Jankowski *et al*., 2003).

Cell surface hydrophobicity. It was determined by salting out test (Jankowski *et al*., 1997; Ljungh *et al*., 1985). This method is considered as a comparative test of cell surface affinity to hydrophobic compounds. The various amounts of $(NH_4)_2SO_4$ (0–3.2 M/dm^3) were added to bacterial suspension. Aggregation was observed 3 and 5 min after addition and the salt concentration needed to induce the aggregation was assumed to be inversely proportional to the hydrophobicity of cell surface.

Results

Photodynamic effect of dyes. The influence of saphranine O and eosin Y on the survival of bacteria is shown in Tables I and II. The number of cells (N) in the sample with the dye, related to N in the control compartment (without dye), gives the survival percent (S%). S % in the presence of dyes but without illumination, is treated as the reference for determination of the photodynamic effect (PDE).

Saphranine O is the most efficient photosensitizer tested at the given optimal experimental conditions. *S. flexneri* 1b, 2a, and *E. coli* K12 strains are sensitive to the photodynamic action of saphranine O but the effect is practically absent in the cases of *S. flexneri* 1a (Table I). The PDE of saphranine O on the strain *S. flexneri* 1b is lower than that of this photosensitizer on *S. flexneri* 2a or *E.coli* K12. *B. subtilis* 003 strain appears to be not liable to the PDE of investigated dyes though some weak susceptibility to saphranine O cannot be excluded.

Table I
The photodynamic effect (PDE) of saphranine O (1 mM/dm^3) on the investigated strains

Strain	Incubation type						
	Not illuminated sample Number of cells × 10^6			Illuminated sample Number of cells × 10^6			PDE
	Control	dye	S%	Control	dye	S%	
S. flexneri 1a	70.6	67.7	95.9	77.3	77.3	100.0	0
S. flexneri 1b	71.1	70.3	98.8	63.7	28.3	44.4	55.6 (p^1<0.05)
S. flexneri 2a	60.3	57.5	95.3	75.6	9.6	12.7	87.3 (p^1<0.05)
E. coli K12	73.0	65.1	89.1	57.3	14.1	24.6	75.4 (p^1<0.05)
B. subtilis. 003	50.7	47.2	93.1	33.9	28.8	83.5	16.5

[1)] probability that the samples illuminated and not illuminated belong to the same population.

Table II
The photodynamic effect (PDE) of eosin Y (1 mM/dm^3) on the investigated strains

Strain	Incubation type						
	Not illuminated sample Number of cells x10^6			Illuminated sample Number of cells x10^6			PDE
	Control	dye	S%	Control	dye	S%	
S. flexneri 1a	39.5	37.1	93.9	27.7	20.5	74.1	25.9
S. flexneri 1b	43.9	39.9	90.9	38.7	15.1	39.1	60.9 ($p^1<0.05$)
S. flexneri 2a	73.8	68.3	92.5	53.2	21.8	40.7	59.2 ($p^1<0.05$)
E. coli K12	30.5	26.6	87.2	31.3	14.5	46.3	53.6 ($p^1<0.05$)
B. subtilis. 003	53.2	52.2	98.7	40.7	39.1	96.1	3.9

[1] see legend to Table I.

Eosin Y (Table II) exerts noticeable photodynamic action against *S. flexneri* 1b, *S. flexneri* 2a and *E. coli* K12 though the efficiency against these strains, seems to be lower than that of saphranine O except *S. flexneri* 1b appearing slightly more sensitive to eosin (the difference seems to be within experimental error).

Fe(II) sulfophthalocyanine (anionic dye of strongly polar, hydrophilic character) and chlorophyll (water soluble preparation) are photodynamically inefficient against the strains investigated (results not shown).

It is shown that the PDE greatly depends on the bacterial strain and for a given strain it varies with the type of photosensitizer. The observed differences in PDE of a given dye, between bacterial strains of the same species, must be connected with some special properties of cell membranes.

The *S. flexneri* 1a strain undergoes aggregation by ammonium sulphate 0.2 M after 5 min while the strain 1b shows analogous behavior at 0.8 M and *S. flexneri* 2a strain at 3.2 M salt solution. These results indicate (Ljungh *et al.*, 1985; Jankowski *et al.*, 1997) that the cell envelopes of *S. flexneri* 1a strain are the most hydrophobic and *S. flexneri* 2a the most hydrophilic of the strains tested by us, while *S. flexneri* 1b strain shows intermediate hydrophobicity. This feature as well as the chemical nature of antigenic determinants (Simmons and Romanowska, 1987) rendering the cell surface of *S. flexneri* 1a strain more compact implies probably lower affinity of *S. flexneri* 1a cells to ionic dyes such as saphranine O and eosine Y than that of *S. flexneri* 2a and 1b. The difference between these serotypes resides in the structure of pentasaccharide repeating unit: *S. flexneri 1a* membrane has a sequence of 3 unsubstituted deoxymannose (rhamnose) residues and in 1b species rhamnose III is acetylated and in 2a serotype it is glycosylated. This structural elements may be responsible for the lower affinity to water soluble dyes and much lower PDE of hydrophilic dyes against *S. flexneri* 1a. The efficiency of water soluble dyes: saphranine O and eosine Y against *S. flexneri* 2a, *S. flexneri* 1b and *S. flexneri* 1a strains follows the order of affinity to water of the cells. This suggests that the efficiency of the photosensitizers against bacteria is proportional to the absorption ability of a dye to cell envelopes.

The photodynamic effect of hypericin against the strains investigated is given in Table III. The most striking feature of the results presented is a great difference in the photodynamic action of hypericin on

Table III
The photodynamic effect of hypericin on the strains investigated

Strain	Incubation type								
	Not illuminated			Illuminated			Photodynamic effect		
	Number of cells x10^6		S%[1]	Number of cells x10^6		S%[1]	PDE %	t_d[2]	p[3]
	Control	Hypericin		Control	Hypericin		Hypericin		
S. flexneri 1a	70.0	29.0	41.4	57.8	23.2	40.1	1.3	0.1	>0.05
S. flexneri 1b	35.8	35.2	98.3	39.4	7.9	20.0	78.3	6.3	<0.002
S. flexneri 2a	3.9	3.9	100	3.5	2.2	62.8	37.2	2.6	<0.02
B. subtilis. 003	40.0	28.1	70.2	61.5	42.5	69.1	1.2	0.1	>0.05
E. coli K12	58.8	39.6	67.3	93.2	18.4	19.7	47.6	3.4	<0.01
E. coli K1	90.5	40.7	44.9	94.4	19.5	20.6	24.3	3.4	<0.01

[1] S% is survival percent of cells surviving the treatment, with respect to control; [2] Student's test; [3] see legend to Table I.

S. flexneri strains 1a, 1b and 2a. In the case of the strain 1b greater part of cells are killed in the presence of hypericin during illumination, while the strain 1a shows practically no photodynamic effect. Since hypericin is the most hydrophobic of the dyes tested by us, low PDE against this strain, showing high surface hydrophobicity, is unexpected. This apparent discrepancy between strength of PDE and affinity of a dye to cell surface of *S. flexneri* 1a is probably connected to the type of radicals produced and the explanation of this result will be presented below.

S. flexneri 2a strain and both *E. coli* strains tested show lower sensitivity to the action of hypericin than the cells of *S. flexneri* 1b serotype in accord with the ranking of membrane surface hydrophobicity. *B. subtilis* 003 is resistant to the PD effect though some toxic action cannot be excluded.

In most cases (Tables I, II and III) the number of cells (N) in the not illuminated samples, surviving the treatment, is close to the reference sample (without dye addition), so the survival percent (S%) in such samples is close to 100. These data indicate that the dyes are in most cases not toxic to the strains tested in our experimental conditions. However for chlorophyll (results not shown) and for hypericin (Table III), in the case of *S. flexneri* 1a and *E. coli* K1 and K12, the value of N in the not illuminated samples was essentially lower than in the control (without dye). This difference can be explained by some toxic effect of the dye on bacteria, independent on the presence of light.

Hypericin, a substance insoluble in water, was used in our experiments in a form of a solution in dimethylformamide (DMF). It was shown in a separate experiment that DMF is not toxic for the strains investigated at our experimental conditions.

Combined effect of serum and photosensitizers against bacterial strains. The impact of the photodynamic action of hypericin and the bactericidal effect of serum on survival of *S. flexneri* 1b and *E. coli* K12 seems to be comparable to the sum of the activities of these separate antibacterial factors (Table IV). It must be noticed however that an addition of serum leads to enhancement of antibacterial action against *S. flexneri* 1b and *E. coli* K12 that cannot be attained by increasing the concentration of the photosensitizer because of a saturation effect (Fig. 1).

Table IV
Combining of the bactericidal effect of hypericin and of serum against the investigated strains[a]

Strain	Number of cells [x10^{-6}]		Survival % relative to control							
Incubation conditions	Control		Hypericin (H)			Serum (Se)		H + Se		
	illum.	dark	illum.	dark	PDE%	illum.	dark	illum.	dark	PDE%
*S. flex*neri 1b	25.6	28.9	18.4	94.1	75.7	31.2	38.1	14.4	35.6	23.7
S. flexneri 1a	22.0	23.0	45.9	47.4	1.5	60.4	52.2	24.1	33.5	9.4
E. coli K12	70.0	67.7	26.3	58.5	32.2	34.3	29.1	12.4	19.6	7.2
B. subtilis 003	50.0	59.7	81.0	81.0	0.0	88.6	93.8	81.2	83.1	1.9

[a] In all cases for the PDE in *S. flex*neri 1b and *E. coli* K12 $p<0.05$ was found by means of Student's test.

In the case of *Shigella flexneri* 1a which can be treated as resistant to the bactericidal action of complement (Survival >50 %), photodynamic action of hypericin leads to a marked strengthening of the antibacterial effect, manifested by the fact that the number of cells in the illuminated sample with hypericin and after addition of serum (S = 24.1%) is much lower than in the sample with serum (S = 60.4%) or in the illuminated sample containing the photosensitizer (S = 45.9%). *B. subtilis* 003 strain seems to be resistant to the photosensitizer and serum and their combined action.

The concentration of hydroxyl radicals. In order to get insight into the molecular mechanism of the effects observed it is necessary to asses the nature of radicals and reactive oxygen species generated during our experiments.

The amount of hydroxyl radicals produced during 90 min illumination of photosensitizers solutions is given in Table V.

A considerable amount of $OH^{\bullet}$ radicals, in our experimental conditions is produced only in the cases of saphranine O and eosine Y. For the same dyes largest photodynamic effects against most strains tested, are observed (Tables I and II) so that the survival percent of bacteria is inversely proportional to the amount of hydroxyl radicals produced.

Table V
Production of $OH^{\cdot}$ radicals during photolysis of the dye solutions (90 min)

Photosensitizer	$\Delta F^{1)}$	C_A [nM/dm]$^{2)}$
Safranine O	25	294.0
Hypericin	0	0
Eosine Y	11	129.4
Chlorophyll (soluble)	3	35.3
Fe(II) sulphophthalo-cyanine	0	0

[1)] Difference of the fluorescence intensity (at 440 nm) between illuminated and not illuminated sample; [2)] The concentration of acridine derivative produced by reaction of TEMPO with $OH^{\bullet}$

Our experiments suggest that $OH^{\bullet}$ are efficient ROS species damaging cell membranes. The determination of the peroxidation products (results not shown) gives roughly the same result. Hypericin however appears to be inactive with respect to $OH^{\bullet}$ production (Table V) though it gives strong antibacterial photodynamic effect against some strains (Table III). This apparent contradiction can be explained by another mechanism of the photosensitization: in the case of hypericin is operating probably the mechanism (II) mentioned in the introduction. This reaction sequence leads to production of singlet oxygen ($^1O_2$) rather than $OH^{\bullet}$ (Mirossay *et al*., 1999; Weiner and Mazur, 1992). Another type of photosensitization might be resposnible for the fact that hypericin shows practically no PDE against *S. flexneri* 1a in spite of high affinity to cell surface while for other dyes tested usually there is a correlation between absorption on cell membrane and PDE.

Discussion

It is commonly known that the cells of various types may differ essentially in their sensitivity to the PD effect of dyes (Wilson, 2004; Weiner and Mazur, 1992; Halliwell and Gutteridge, 1990). In the present work high PD effect of hypericin against *S. flexneri* 1b and practically no photodynamic action of the same dye against *S. flexneri* 1a was found. Analogously in the case of 2 strains of *E. coli* (K12 and K1) various effect of chlorophyll (extract from leaves) has been reported (Jankowski *et al*., 2003).

The *Shigella* strains 1a and 1b show also several sensitivity to the bactericidal action of serum (Table III) though the differences between strains is less marked than that of PD effect (Tables I and II). Combining of PDE and the bactericidal action of serum is relatively more efficient in the case of the *S. flexneri* 1a strain, less sensitive to both antibacterial actions while separate. The enhancement of the bactericidal efficiency of serum by addition of photosensitizers, observed in the present work, suggests that the membrane structure damaged by photosensitization is localized near to the site of complement (C) binding. The complex formed by C is probably bound to lipid A (Joiner, 1988; Jankowski, 1995) structure connected directly to the membrane outer layer containing the antigenic determinants in Gram-negative bacteria.

The diversity of the PDE of hypericin within *Shigella* species can be explained taking into account high surface hydrophobicity of *S. flexneri* 1a strain shown in our test – a feature connected probably to the nature of repeating sugar sequences occurring in the membrane surface (Simmons and Romanowska, 1987) and responsible for antigenic properties. We suppose that hypericin, hydrophobic compound insoluble in water and soluble in DMF, is readily absorbed on the surface of 1a strain cell membranes, more hydrophobic than those of *S. flexneri* 1b and 2a species. Strong binding of hypericin to cell membrane of *S. flexneri* 1a cells leads to the toxic effect independent on light (see Table II). On the other hand such a strong binding on the cell surface hinders penetration of excited dye to the target site situated in an inner layer in the membrane which may be responsible for a lower PDE.

In the case of 1b serotype, that probably has a lower affinity to hypericin than that of 1a strain, because of its lower hydrophobicity, the binding of the dye to cell membrane may be weaker but the excited dye can penetrate deeper into the membrane to the target site (perhaps lipid A) and therefore well marked PDE is observed (Table I).

The fact that hypericin shows lower PDE against *S. flexneri* 2a strain that that in 1b serotype may be explained by more hydrophilic cell surface properties of species 2a.

Such type of behavior – unexpected lack of PDE of hypericin against *S. flexneri* 1a that should have highest affinity of cell surface toward this dye, is noticeable in the case of hypericin, the dye giving probably singlet oxygen ($^1O_2$) in type II photosensitizing mechanism. On the other hand saphranine O and eosin, dyes of more hydrophilic character, and producing $OH^\bullet$ radicals are more efficient against *S. flexneri* 2a and 1b than to *S. flexneri* 1a cells, in accord with expected order of affinities to cell membranes. If it is assumed that the strong binding of hypericin to *S. flexneri* 1a cells results in lack of PDE the question arises why strong binding does not prevent PDE in the case of hydrophilic dyes.

It must be taken into account that the process of the dye binding to bacterial cell membrane proceeds by another way in the case of hypericin added in DMF solution than for photosensitizers soluble in water. It is possible that hypericin molecules aggregate in water suspension of microorganisms before binding disabling penetration into membrane.

We suppose that hydrophilic dyes (eosin, saphranine) are bound closer to the target site (tentatively identified above as lipid A) while hypericin, to be effective photodynamically, must be transported to some distance through the membrane. Therefore strong binding on the outer membrane layer prevents PDE in the case of hypericin.

$^1O_2$ produced in the photodynamic action of hypericin is characterized by a lower oxidative potential, higher lifetime in tissues and longer mean distance of penetration in the medium than that of $OH^\bullet$ (Halliwell and Gutteridge, 1989; Weiner and Mazur, 1992). Its is also supposed that $^1O_2$ formation in low polar media is inhibited Reddi *et al.*, 1984). Specific properties of radicals generated can be the cause of the differences in effectivity between hepericin and other dyes tested.

Conclusions: 1) The photodynamic efficiency of dyes depends essentially on the absorption site of photosensitizers on the target cell wall and on the kind of ROS produced. 2) Combined the action of photosensitizers and serum can essentially enhance the bactericidal effect especially in the case of less sensitive strains.

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