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磺化锌酞菁对小鼠移植肿瘤的光动力治疗、组织分布和对癌细胞 DNA 的损伤

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Photodynamic therapy of zinc sulfonated phthalocyanine on murine transplanted tumors, its tissue distribution, and damaging effect on DNA of cancer cell

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ABSTRACT Zinc sulfonated phthalocyanine (ZnSPc) 10 mg · kg^{-1} was injected iv into mice bearing S-180 and RA795 lung carcinoma, after 24 h tumor site were irradiated with red light. In mice bearing S-180, tumor regression rate was 31.8–43.5%, tumor growth inhibition rate was 57.4%. The highest concentration was in tumor tissue 24 h after injection of this dye, on d 5 it still retained relatively highest concentration. However, in most other tissues the dye was not detected at this time, disappearance of ZnSPc from plasma was rapid, it showed an open two compartmentmodel, $t_{\frac{1}{2}\alpha}$ 135.8 min, $t_{\frac{1}{2}\beta}$ 70.1 h, V_d 1.92 × 10^{-3} L. In blood, most ZnSPc was bound with plasma protein, the peak light absorption showed blue shift. ZnSPc 2.5 μg · ml^{-1} plus light, percent of DNA double strands greatly decreased, this indicated that DNA was one of target sites for ZnSPc photodynamic action.

KEY WORDS zinc sulfonated phthalocyanine; tissue distribution; sarcoma 180; lung neoplasms; photochemotherapy

提要 S-180 和 RA795 肺癌小鼠尾静脉 iv ZnSPc 10 mg · kg^{-1}，24 h 后肿瘤部位照光，肿瘤抑制率分别是 44.8% 和 57.4%。S-180 小鼠 iv ZnSPc 10 mg · kg^{-1}，24 h 以瘤组织浓度最高，大部分组织于 d 5 药物已测不出，此时瘤组织仍保留较高水平。ZnSPc 从血浆内消除迅速 $t_{\frac{1}{2}\alpha}$ 135.8 min，$t_{\frac{1}{2}\beta}$ 70.1 h，V_d 1.92 × 10^{-3} L。ZnSPc 2.5 μg · ml^{-1} 合并照光，肝癌细胞 DNA 产生显著断链。表明 ZnSPc 对小鼠移植肿瘤有显著的光动力治疗效果。

关键词 磺化锌酞菁；组织分布；肉瘤 180；肺肿瘤；光化学治疗

血卟啉衍生物(HpD)存在一些缺点，酞菁类光敏剂化学性质稳定、易于合成，在红光有强吸收，是一类有发展前途的光敏剂[1]，它对癌细胞的光杀伤[1]、对红细胞膜的光氧化[2]以及对癌细胞的杀伤机制[3]，我们曾予报道。本文介绍合成的磺化锌酞菁(ZnSPc)对小鼠 S-180 和 RA795 肺癌的光动力治疗作用、体内分布和瘤组织摄取以及对肝癌细胞 DNA 的光损伤。

MATERIALS AND METHODS

昆明种小鼠和 739 小鼠(中国医学科学院动物中心供应)，♀，体重 20.5 ± SD 2.0 g。腹水型 S-180 和肝癌(Hep A)每 7 d 传代一次。实验用接种后 d 7 的腹水，用 PBS 稀释至所需浓度。RA795 肺癌按实体瘤接种方法每 7 d 传代一次，实验用 d 6–d 7 肿瘤组织。小鼠左腹股沟 sc 接种 0.2 ml 瘤组织悬液，d 6 肿瘤长到直经约 5 mm，iv ZnSPc，24 h 后照光，光源是 GY S-1 红光治疗仪，波长 600–700 nm，光照度 200 J · cm^{-2}。

磺化锌酞菁(ZnSPc)由中科院感光化学所许慧君教授供给。

ZnSPc 在组织和血浆内的分布 基本按文献[4]小鼠接种 S-180 瘤细胞后在 d 5–d 6，从尾静脉 iv ZnSPc 10 mg · kg^{-1}，不同时间处死，取各器官和瘤组织称重，每 60 mg 湿重

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组织加入 0.1 mol · L^{-1} NaOH 3 ml，50℃水浴内放置 2 h 不断振荡，离心，提取液用荧光分光光度计测定，Ex 605 nm，Em 680 nm，Slit 10 nm，另取不给药的正常鼠组织，按上法同样外理，提取液内加入已知浓度 ZnSPc，作标准曲线，从标准曲线计算用药小鼠各组织 ZnSPc 的含量．正常小鼠，尾静脉 iv ZnSPc 10 mg · kg^{-1}，不同时间取血，离心，分离血浆，按上法提取血浆内 ZnSPc 和制备标准曲线，测定条件同上．

ZnSPc 和蛋白质结合试验 基本按文献[5]．取 BSA 1 mg 和 ZnSPc 5 μg 充分混和，终体积 0.105 ml，经 Sephadex G-50 柱洗脱，收集洗脱液，荧光分光光度计测定．Ex 605 nm，Em 680 nm，Slit 10 nm，另取 5 μg ZnSPc，在 BSA 1 mg，BSA 1 mg 加 ZnSPc 5 μg，分别测定荧光强度，正常小鼠尾静脉 iv ZnSPc 10 mg · kg^{-1}，不同时间后取血，分离血浆，按上法提取 ZnSPc 和测定．

癌细胞 DNA 损伤试验 基本按文献[5]．取小鼠腹水肝癌细胞，PBS 稀释成 10^6 / ml 细胞，加入 ZnSPc 37℃温育 20 min，高压钠灯照射 10 min，光照距离 10 cm，光照强度 63 J · cm^{-2}，灯管周围有一密封隔水层，通自来水以散热，按 Birnboim 等法[6]测定细胞的 DNA 解旋．按下式计算 DNA 解旋程度

$$D\%(\text{双链\%数}) = \frac{(P-B)}{(T-B)} \times 100$$

B: 空白管，测定双链 DNA 以外的荧光．T: 测定总的荧光．T−B: 测定提取液内双链 DNA 的量．P: 测定部分解旋后双链 DNA 的量．

RESULTS

ZnSPc 对小鼠 S-180 肿瘤的光动力治疗 接种瘤细胞后 d 6 尾静脉 iv ZnSPc 10 mg · kg^{-1}，24 h 后肿瘤部位皮肤脱毛、照光．照光后 d 14 和 d 20 分别处死动物，称瘤重．有的小鼠经光疗后肿瘤消退，不可触及，处死前未再生长者，为肿瘤消退，计算各组肿瘤消退率和瘤重．应用两种磺化程度不同的 ZnSPc，一为 S / Pc = 2.2，另一为 S / Pc = 2.45．共进行 3 批实验．单照光对肿瘤生长稍有抑制，但统计上无显著差别，而光疗组肿瘤消退率为 31.8% (S / Pc = 2.2)和 43.5% (S / Pc = 2.45)．两种磺化程度的 ZnSPc 光敏效力无明显不同．肿瘤抑制率为 44.8%，和对照组有显著差别($P < 0.01$) (Tab 1)．

ZnSPc 对 739 小鼠 RA795 肺癌的光动力治疗 从表 1 的结果，单照光(200 J · cm^{-2})对肿瘤的生长和对照组差别不显著，对

Tab 1. Photodynamic therapy of ZnSPc on murine sarcoma 180. $n = 3$ expts, ZnSPc 10 mg · kg^{-1} iv, light intensity 200 J · cm^{-2}. Mice were killed on d 14 in expt A and d 20 in expt B after irradiation. $^{*}P > 0.05$, $^{***}P < 0.01$ *vs* control.

Expt	S / Pc	Treatment	Mice with tumor regression / Mice bearing tumors	Tumor regression rate, %	Tumor weight, g ($\bar{x} \pm SD$)	Tumor growth inhibition, %
A	2.45	Control	0 / 23	0	5 ± 0	
		Light	2 / 23	7.0*	4.3 ± 0.6	15.9*
		ZnSPc + Light	10 / 23	43.5***	2.8 ± 0.5	44.8***
B	2.2	Control	0 / 22	0	7.8 ± 0.7	
		Light	1 / 22	4.5*	6.6 ± 0.8	15.1*
		ZnSPc + Light	7 / 22	31.8***	4.3 ± 0.5	44.8***

Tab 2. Photodynamic therapy of ZnSPc on RA795 lung cancer in 739 mice. $n=3$ expts, ZnSPc 10–20 mg · kg^{-1} iv, light intensity 200 J · cm^{-2}. Mice were killed on d 18 in expt A and d 24 in expt B after irradiation. $^{***}P<0.01$.

Expt	ZnSPc, mg · kg^{-1}	Treatment	Mice	Tumor weight, g ($\bar{x}\pm$ SD)	Tumor growth inhibition, %
A	10	Light	20	5.9 ± 0.65	
		ZnSPc + Light	20	3.23 ± 0.11	45.8***
B	20	Light	24	5.4 ± 0.86	
		ZnSPc + Light	21	3.19 ± 0.30	57.4***

Tab 3. ZnSPc content (μg / g tissue) in 4 mice bearing S-180. $n=4$ mice, $\bar{x}\pm$ SD.

Tissue	ZnSPc content (μg / g tissue)			
	1 d	2 d	3 d	5 d
Tumor	19.04 ± 16.82	16.04 ± 7.35	11.31 ± 4.53	11.88 ± 9.39
Liver	17.65 ± 17.91	30.81 ± 16.28	21.35 ± 10.47	0
Bladder	16.27 ± 15.92	11.08 ± 4.81	1.73 ± 3.98	0
Intestine	13.27 ± 8.67	8.88 ± 6.53	9.58 ± 7.44	0.69 ± 5.08
Heart	3.92 ± 2.79	7.04 ± 7.35	0	0
Kidney	3.81 ± 2.50	2.31 ± 1.38	0	0
Muscle	3.69 ± 5.76	3.23 ± 3.22	1.04 ± 2.65	0
Spleen	3.46 ± 4.40	1.96 ± 8.96	1.04 ± 6.47	0.92 ± 8.22
Skin	2.54 ± 10.79	6.69 ± 11.49	3.92 ± 11.41	0
Lung	1.15 ± 2.29	0	0	0
Stomach	1.62 ± 4.99	3.00 ± 0.79	2.88 ± 2.53	0
Brain	0	0	0	0

RA795 肺癌的光疗，只采取照光组与光疗组比较.（光疗后 d 18 和 d 24 分别处死动物，称瘤重）. ZnSPc (S / Pc = 2.2) 10 mg · kg^{-1} 和 20 mg · kg^{-1} 抑瘤率分别是 45.8% 和 57.4% (Tab 2).

ZnSPc 在带瘤小鼠组织内的分布 S-180 小鼠尾静脉 iv ZnSPc 10 mg · kg^{-1}，不同时间测各组织内药物含量，结果见 Tab 3. 在 S-180 小鼠，给药后 24 h 以瘤组织含量最高，大部分组织于 d 5 已测不出药物，仅肠和脾含微量. 肿瘤组织 d 5 仍保留较高水平.

ZnSPc 在小鼠血浆内的分布 昆明种小鼠尾静脉 iv ZnSPc 10 mg · kg^{-1}，不同时间取血，测定血浆内药物浓度. 给药后 5 min ZnSPc 浓度是 95.9 ± 9.60 μg · ml^{-1}，6 h 降至 23.9 ± 9.36 μg · ml^{-1}，24 h 降至 6.8 ± 7.17 μg · ml^{-1}，从药-时曲线，ZnSPc 在小鼠体内代谢呈开放性二室模型，$t_{\frac{1}{2}\alpha}$ 135.8 min，$t_{\frac{1}{2}\beta}$ 70.1 h，V_d 1.92×10^{-3} L.

ZnSPc 和蛋白质结合试验 ZnSPc 5 μg 和 BSA 1 mg 充分混匀，终体积 0.105 ml，经过 Sephadex G-50 柱洗脱，收集洗脱液，荧光测定 ZnSPc 浓度，Ex 605 nm，Em 680 nm，在过柱时，酞菁蓝色带内蛋白含量最高，可知 ZnSPc 绝大部分是和蛋白结合. 固定激发波长于 605 nm，进行荧光扫描，与蛋白结合的 ZnSPc 最大光吸收峰在 684 nm，与自由 ZnSPc 的最大光吸收一致(Fig 1)，说明与 BSA 结合后 ZnSPc 的光化学性质未发生改变. 小鼠尾静脉 iv ZnSPc 10 mg · kg^{-1}，24 h

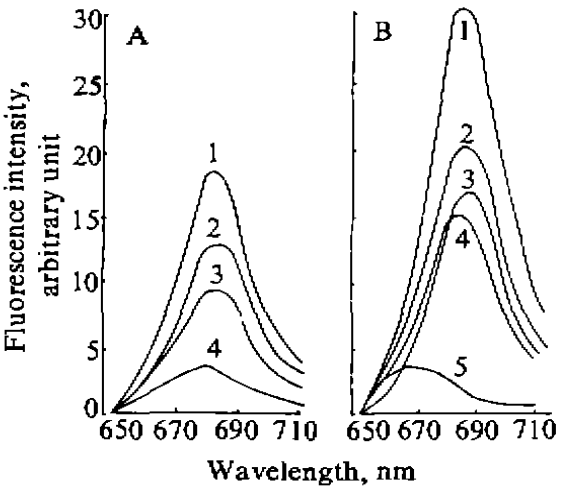

Fig 1. Emission spectra (excitation 605 nm) of ZnSPc in PBS and in bovine serum albumin (BSA) (A) and in mouse plasma after iv dye 10 mg · kg^{-1} (B). (A) 1: BSA 1 mg + ZnSPc 5 μg, 2: ZnSPc 5 μg, 3: BSA 1 mg + ZnSPc 5 μg followed by Sephadex G-50 elution, 4: BSA 1 mg. (B) 1: 5 min after ZnSPc, 2: 20 min after ZnSPc, 3: ZnSPc 1 μg in PBS, 4: 60 min after ZnSPc, 5: Plasma of control mouse.

后取血，分离血浆，提取血浆中 ZnSPc，荧光扫描测定，条件同上. 给药后 60 min 血浆内 ZnSPc 最大光吸收峰稍稍兰移(Fig 1).

ZnSPc 对小鼠肝癌细胞 DNA 的光损伤

取小鼠腹水肝癌细胞 4× 10^6 / ml，加入 ZnSPc，37℃温育 20 min，照光 10 min，光强 63 J · cm^{-2}. Birnboim 法[6]测定 DNA 双链解旋. 以双链%数(D%)表示解旋程度，对照肝癌细胞 D%在 73-79%，ZnSPc 1.25 μg · ml^{-1} 合并照光使 D%减少 15%左右，2.5 μg · ml^{-1} 使 D%值比对照减少 40%左右，加大 ZnSPc 剂量，D%值未进一步减少(Tab 4).

DISCUSSION

本文结果表示，ZnSPc 对小鼠 S-180 和 RA795 肺癌有显著的光动力治疗作用，在瘤组织内有较高浓度且贮留时间较长，说明此光敏剂和瘤组织有较大亲和力. 脑组织测不出药物，表示此药不能透过血脑屏障. 在体内主要和血浆蛋白结合，结合后最大光吸收峰稍兰移，四磺化酞菁和金属螯合(Al, Zn, Cu)在体外不显示光氧化效应[2]. 体内也没有光动力治疗作用. 本文应用不同磺化程度的 ZnSPc (S / Pc = 2.2-2.45)体内显示明显的光敏效力，说明药物的疏水性与光敏作用有密切关系. 为了使酞菁能够注射应用而又不丧失光敏效力，有人应用脂质体包埋 AlPc[7]. 我们曾试用磷脂和胆固醇制备脂质体包埋 ZnPc，对小鼠肿瘤有明显的光动力治疗作用[8]，效果和 ZnSPc 相似.

这些结果说明，控制酞菁的磺化程度可以得到和脂质体包埋同样的效力. 酞菁类光敏剂对细胞膜[2]和亚细胞器膜[3]有明显的光敏效力. 本文观察到对癌细胞 DNA 有显著的光损伤，表示 DNA 是酞菁类化合物光敏作用的靶

Tab 4. Photodynamic damage of ZnSPc on DNA double strand of mouse hepatoma cells. Hepatoma cells 4 × 10^6 cells / ml, incubated with ZnSPc at 37℃ for 20 min, irradiated with high pressure sodium lamp for 10 min, irradiation distance 10 cm, light intensity 63 J · cm^{-2}, n = 3 mice, $\bar{x} \pm$ SD. $^*P < 0.05$ *vs* control, $^{**}P < 0.001$ *vs* control.

	Percent of DNA double strand (D%) ZnSPc, μg · ml^{-1}			
	1.25	2.5	5	10
Control	74.8 ± 5.0	79.0 ± 1.4	79.5 ± 1.1	73.2 ± 2.3
ZnSPc + Light	64.1 ± 2.9*	49.1 ± 3.8**	54.2 ± 4.4**	50.7 ± 2.4*

部位之一. 酞菁类化合物性质稳定，对红光有强吸收，而 HpD 最大光吸收在 400 nm 左右，临床治疗是红光照射，所以酞菁类能够发挥其最大光敏效力. 从我们和他人对酞菁类光敏剂的研究结果，提示它是一类较好的光敏剂，有希望成为临床有用的光敏药物.

REFERENCES

1 Fu NW, Huang L, Xu HJ, Chou CF. Investigation on photodynamic action of phthalocyanines. *Chin J Oncol* 1988; 10 : 239

2 Fu NW, Huang L, Xu HJ, Chou CF. Photodynamic action of phthalocyanines on red cell membrane. *Tumor* 1988; 8 : 319

3 Fu NW, Huang L, Xu HJ. Photodynamic action of phthalocyanine tetrasulfonate on mitochondria and microsome of hepatoma cell. *Prog Biochem Biophys* 1989; 16 : 287

4 Chan WS, Marshall JF, Lam GYF, Hart IR. Tissue uptake, distribution, and potency of the photoactivatable dye chloroaluminum sulfonated phthalocyanine in mice bearing transplantable tumors. *Cancer Res* 1988; 48 : 3040

5 Fu NW, Chu YX, Huang L, Yan LX, Quan LP, An JY. Photodynamic action of hypocrellin A on tumor cell and its distribution in tumor bearing mice. *Chin J Cancer* 1989; 8 : 450

6 Birnboim HC, Jevcak JJ. Fluorometric method for rapid detection of DNA strand breaks in human white blood cells produced by low doses of radiation. *Cancer Res* 1981; 41 : 1889

7 Valduga G, Reddi E, Jori G. Spectroscopic studies on Zn(II)-phthalocyanine in homogeneous and microheterogeneous systems. *J Inorg Biochem* 1987; 29 : 59

8 Fu NW, Yan LX, Xu HJ, Jiang L, Chang JY. Photodynamic therapy of sulfonated zinc phthalocyanine encapsulated in liposomes on murine hepatoma. *Chin J Oncol* 1990; 12 : 399

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苯环利定对离体兔脑基底动脉和整体脑血流的作用

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Effects of phencyclidine on rabbit basilar artery *in vitro* and rabbit cerebral blood flow *in vivo*

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ABSTRACT The effect of phencyclidine [1-(1-phenylcyclohexyl)piperidine, PCP] on rabbit basilar arteries was studied with an *in vitro* model of ring segment arteries. PCP 0.05-500 $\mu mol \cdot L^{-1}$ caused vasoconstriction of basilar arteries in a concentration-dependent manner. Its maximal effect (E_{max}) was 94 ± 21 mg and the concentration causing half maximal effect (EC_{50}) was 25 ± 18 $\mu mol \cdot L^{-1}$. PCP 0.01-10 $\mu mol \cdot L^{-1}$ also concentration-dependently augmented the vasoconstriction induced by electric stimulation in rabbit basilar arteries. Its E_{max} was 91 ± 18 mg and EC_{50} was 0.27 ± 0.17 $\mu mol \cdot L^{-1}$.

The effects of PCP on mean arterial blood pressure (MABP) and heart rate (HR) of rabbits were observed. PCP iv 4 $mg \cdot kg^{-1}$ reduced MABP from 14.3 ± 0.8 to 12.2 ± 1.0 kPa and HR from 300 ± 0 to 278 ± 5 bpm in 5 min.

Using the technique of radionuclide imaging in rabbit brain *in vivo*, we studied the effect of PCP on

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