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·论著·

光动力作用对活体肝组织损伤研究

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摘要

目的 研究光动力作用对活体肝组织的损伤，探讨光动力治疗肝癌的可行性，为临床治疗提供实验依据。

方法 动物实验：将小鼠分成光动力疗法（PDT）组、单血卟啉衍生物（HpD）组、激光组和空白对照组。光敏药物选用血卟啉衍生物，给药量每公斤体重 10 mg，药物用 1 ml 生理盐水稀释，于实验前 48 h 将药物注射入 PDT 组和 HpD 组小鼠腹腔内，避光饲养。将 PDT 组和激光组小鼠固定于实验板上。麻醉后，剖腹暴露右肝前叶，激光直接投照于肝脏表面，光斑直径 5 mm，照射 2 min。激光器为氩离子泵浦染料激光器系统，光波长 630 nm，输出功率 100 mW，每一照射区能量累积约 60 J，照光后关腹，回笼饲养观察。于照光后 1、24、72、120 h 处死各组小鼠，剖腹取肝组织置于 4% 福尔马林液中固定，常规石蜡包埋切片，HE 染色，光镜观察。临床治疗：经病理确诊的肝癌患者，于治疗前 48 h 做皮肤划痕试验，阴性者按每公斤体重 5 mg 静脉给药。治疗时，在 B 超引导下，用 18G 肝穿针经皮穿刺，将石英光纤导入肝肿瘤内。激光波长 630 nm，输出功率 350 mW，每一照射点能量累积 220 J。治疗 1 个月后行二期切除术。标本用 4% 福尔马林固定，常规石蜡包埋切片，HE 染色，光镜观察。

结果 动物实验光镜观察结果显示：PDT 组于照光后 24 h 出现照光区肝细胞大面积坏死，照光区周边肝细胞未见损伤，两者界限清楚，120 h 见坏死区周边纤维组织增生。激光照射组于照光后 1 h 出现肝细胞肿胀，72 h 恢复正常，未见肝细胞损伤。HpD 组和空白对照组未见肝细胞损伤。人肝癌 PDT 后切除标本光镜观察结果显示：肝肿瘤照射区见肝癌细胞大片坏死，肿瘤周边正常肝细胞未见损伤，但见大量淋巴细胞和巨噬细胞聚集。

结论 肝组织内的血卟啉衍生物可被激光激活，引发光动力反应，从而损伤肝细胞。这种损伤的范围有限。通过控制光辐照范围，可以避免肝组织大面积受损。

关键词 肝脏； 血卟啉衍生物； 光动力学疗法

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A Study on Liver Damage Induced by Photodynamic Therapy

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ABSTRCT

Objective To investigate the liver damage induced by photodynamic therapy (PDT) and provide an experimental basis for PDT treatment for liver cancers.

Methods 96 normal mice were divided into 4 groups: PDT group, laser group, HpD group and control group. The photosensitizer used in this study was hematoporphyrin derivative (HpD), diluted in 5% glucose and injected into the peritoneal cavity at a dose of 10 mg/kg body weight 48 h before light irradiation. The mice were kept from sunlight exposure. After anesthesia the abdomen was opened and the right front

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lobe of the liver was exposed. An argon laser pumped dye laser system was used. The liver surface was directly irradiated by the 630 nm laser beam at a power of 100 mW for 2 minutes. The spot size was 5 mm in diameter and the energy density was 60 J/ cm^2. The mice were killed at 1, 24, 72 and 120 hours after laser irradiation, respectively. Samples were embedded in paraffin and HE stained sections were examined under light microscope. Besides, a 46-years old male patient with liver cancer was also included in this study. He received HpD in a dose of 5 mg/ kg body weight, i. v. injected 48 h prior to laser irradiation. Ultrasound-guided liver puncture was performed and optical fibers were inserted into and evenly distributed in the tumor. The 630 nm laser irradiation was carried out at a power of 350 mW, energy density of 250 J/ cm^2 per each spot. The patient was operated one month later and specimens were taken for histopathological examination.

Results Animal experiment: Large necrotic areas were observed in livers of mice 24 hours after PDT. There was a clear demarcation between irradiated and non-irradiated areas observed by both gross and microscopic examination. Fibrous proliferation was seen in the surrounding tissues 120 hours after PDT. Swelling of hepatocytes was observed at 1 h after laser irradiation alone, but returned to normal at 72 h after irradiation. No damage to hepatocytes was observed in livers of both HpD alone and control groups. Clinical case: Wide-spread necrotic areas were present in the PDT irradiated tumor tissue. Normal hepatocytes were observed in the non-irradiated surrounding tissue. There were numerous lymphocytes and macrophages infiltrating in the surrounding areas.

Conclusions Selective and sharply demarcated photodamage to liver tissue can be induced by selective laser irradiation after HpD administration. It is suggested that photodamage to surrounding normal tissues can be avoided by carefully controlled laser irradiation during photodynamic therapy of liver cancers.

Key words Liver; Hematoporphyrin derivative; Photodynamic therapy

光动力疗法已成功地应用于人体多个系统肿瘤的治疗[1,2]，并显示出独特的优点。但将光动力疗法用于肝癌的治疗，人们普遍持谨慎态度[3]，肝组织中存留的血卟啉衍生物含量很高[4]是其原因之一。为了探讨光动力治疗肝癌的可能性，为临床治疗提供实验依据，作者采用小鼠在体实验，光镜观察小鼠肝脏受光动力作用后的病理改变及转归，在此基础上，我们还对 1 例肝癌患者光动力治疗后二期切除的肝脏标本进行观察，了解肝癌治疗后肝组织的形态学变化。现将实验结果报道如下：

材料与方法

一、实验研究

1. 实验动物　普通级昆明种小鼠(福建省卫生防疫站提供，合格证号：92001)体重 20～22 g，雌雄兼用。

2. 药物　血卟啉衍生物(北京制药工业研究所)，小鼠给药量每公斤体重 10 mg，药物用 1 ml 生理盐水稀释，于照光前 72 h 腹腔内注射，给药后避光饲养。

3. 激光器　采用氩离子激光泵浦染料激光器系统，波长选用 630 nm，激光束由芯径 400 μm 的石英光纤传输，光纤末端输出光功率 100 mW。

4. 实验动物分组　将小鼠随机分为光动力疗法组、激光组、单血卟啉衍生物组和空白组，每组 24 只，并分别于实验后 1、24、72 和 120 h 观察结果。

5. 照光方法　小鼠固定于实验板上，硫贲妥钠麻醉，腹部常规脱毛消毒，切开腹部，暴露右肝前叶，将激光投照于肝脏表面，光斑直径为 5 mm，照光时间 2 min，累积能量 60 J。照光后关腹，回笼饲养观察。

6. 标本处理　每组小鼠分别于照光后 1、24、72 和 120 h 断颈处死。开腹取右肝组织置于 4%福尔马林液中固定，常规石蜡包埋切片，HE 染色，光镜观察。

二、临床治疗

1. 临床资料　患者男，46 岁。CT 检查示右肝叶占位性病变，大小 12cm× 11. 5cm，AFP＞ 400 ng/ml；肝肿瘤病理检查：肝细胞癌。

2. 药物　血卟啉衍生物(北京制药工业研究所生产)，常规皮肤划痕试验，阴性者按每公斤体重 5 mg 静脉给药，用药后 48～72 h 行光辐照治疗，需避光一个月。

3. 激光器系统　氩离子激光泵浦染料激光器系统，光波长 630 nm，石英光纤芯径 400 μm，末端为 1 cm 长的柱状弥散头，输出功率 350 mW。

4. 激光治疗　患者取平卧位，常规消毒铺无菌巾，1%利多卡因局部麻醉。B 超引导，经皮穿刺，将 18G 肝穿针插入肝肿瘤内，退出针芯，将芯径 400 μm 的石英光纤导入瘤内照射。光纤末端输出功率

350 mW, 每点照射 12 min, 累积能量约 220 J。

5. 手术标本处理　患者于 PDT 后一个月行肝肿瘤不规则切除术, 术后标本用 4%福尔马林固定, 取肿瘤组织及周围肝组织进行常规石蜡包埋, 切片, HE 染色, 光镜观察。

结　果

一、HpD 组

于注射 HpD 后 1、24、72 和 120 h 解剖小鼠, 肝脏表面照射区无肉眼变化。光镜下见肝小叶结构正常, 肝细胞索排列规则, 未见炎症细胞浸润, 未见肝细胞变性坏死(图 1)。

二、激光照射组

于激光照射后 1 h 和 24 h 解剖小鼠, 见两组小鼠肝脏表面照射区均出现轻度泛白改变。光镜下见肝细胞肿胀, 肝窦间隙变小, 胞浆轻度嗜伊红性, 胞核染色性无变化。24 h 组的部分小鼠镜下见光纤通道内少量肝细胞坏死(图 2), 照射区肝细胞未见坏死。72 h 和 120 h 见小鼠肝脏表面照射区恢复正常。光镜下见肝细胞正常, 未见坏死灶。

三、PDT 组

光动力治疗后 1 h 解剖小鼠, 见肝脏表面照射区泛白, 光镜下见肝小叶、肝索结构正常, 肝细胞肿胀, 肝窦和肝静脉充血, 未见细胞坏死。24、72 和 120 h 解剖小鼠, 见三组小鼠肝脏表面照射区均呈黄白色或灰白色改变, 与周围非照射区活组织颜色形成鲜明反差, 界限清楚。光镜观察见照射区肝组织结构破坏, 细胞溶解, 炎症细胞浸润(图 3), 但照射区边缘肝细胞正常, 两者间有明显界限(图 4)。这些结果, 与以往周传农等[5] 的报道一致。120 h 组坏死区未见扩大, 光镜下可见纤维组织增生(图 5)。

四、各实验组和对照组肝组织病理变化

见表 1。

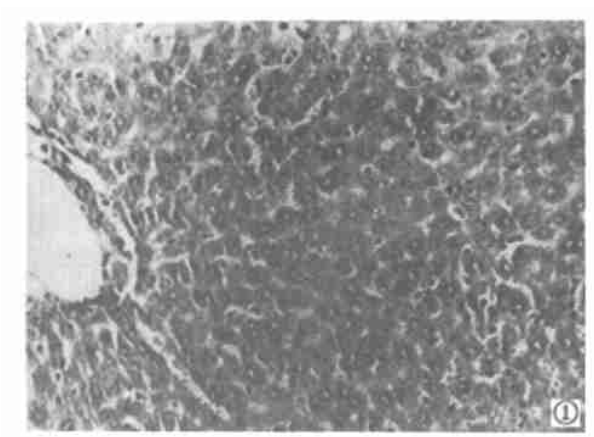
表 1　PDT、HpD 和激光对小鼠肝细胞的损伤

Tab. 1　Histological changes of hepatocytes in different groups of mice

组别 Group	肝细胞光镜表现 Histological changes of hepatocytes			
	1 h	24 h	72 h	120 h
PDT	肿胀 Swelling	坏死 Necrosis	坏死 Necrosis	坏死及纤维组织增生 Necrosis &fibrous proliferation
HpD	正常 Normal	正常 Normal	正常 Normal	正常 Normal
激光 Laser	肿胀 Swelling	肿胀 Swelling	正常 Normal	正常 Normal
空白 Control	正常 Normal	正常 Normal	正常 Normal	正常 Normal

五、人肝癌 PDT 后一个月手术切除标本

肉眼见肿瘤照射区表面及切面呈黄白色豆腐渣样改变, 肿瘤周围的肝组织表现为正常深红色, 组织弹性好, 血供丰富, 未见异常改变。光镜下见肿瘤细胞大片坏死(图 6), 肿瘤周边肝组织小叶结构正常, 肝细胞索排列规则(图 7), 两者之间见大量淋巴细胞和部分嗜酸细胞, 巨噬细胞聚集(图 8)。



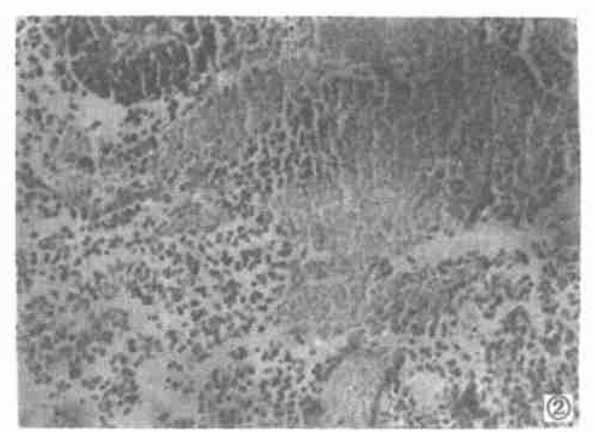


图 1　注射 HpD 后 24 h, 肝小叶结构正常, 肝细胞索排列规则, 未见肝细胞变性坏死　HE × 40　**图 2**　PDT 后 24 h, 肝组织结构破坏, 细胞溶解, 有的仅存裸核　HE × 40

Fig. 1　Mouse liver. 24 h after HpD administration. Normal appearance of hepatocytes. HE stained. × 40.　**Fig. 2**　Mouse livers. 24 h after PDT. Necrosis of hepatocytes and destruction of hepatic tissue. Some naked nuclei were also seen. HE stained. × 40.

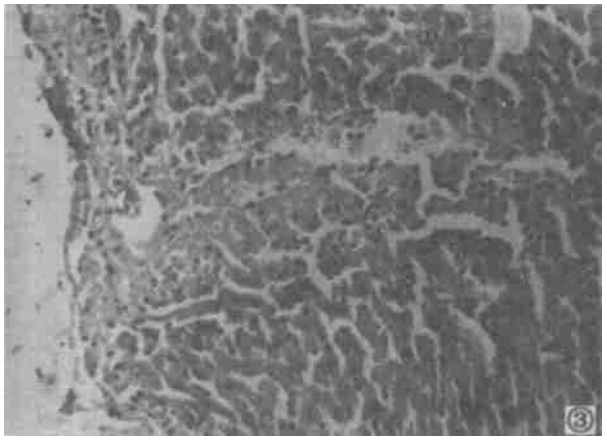
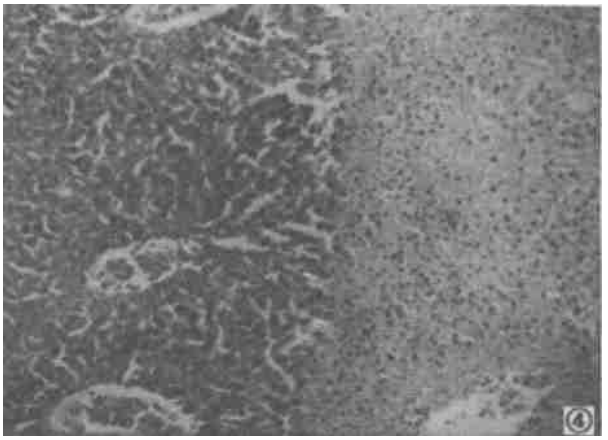
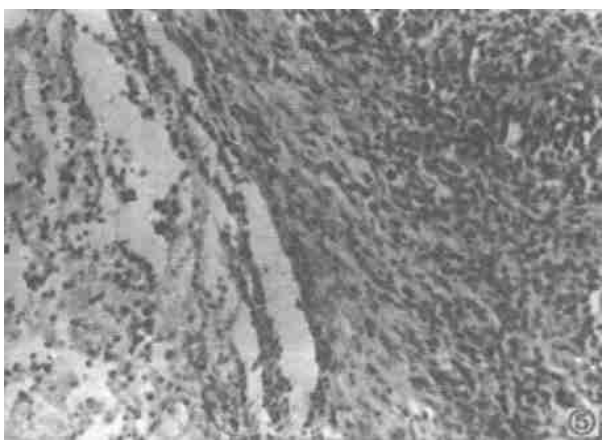
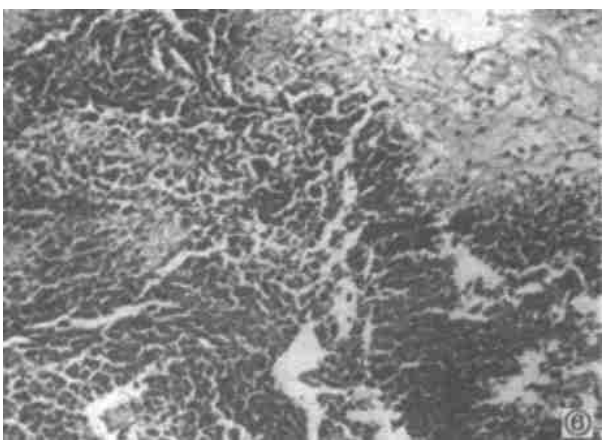
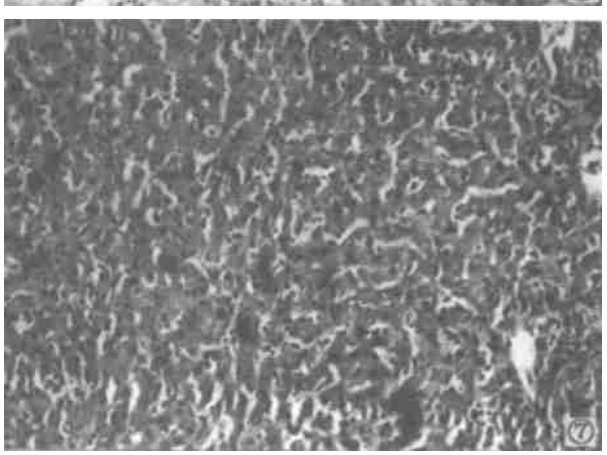
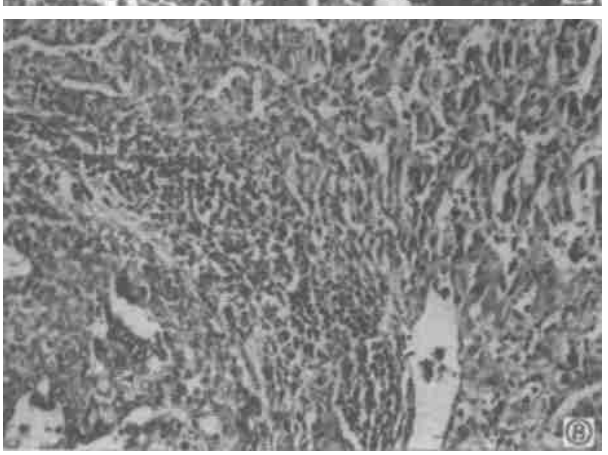







图 3 激光照射后 24 h, 光纤通道内部分肝细胞坏死, 周围肝细胞结构正常 HE × 40 **图 4** PDT 后 24 h, 照射区(右)肝组织结构破坏, 细胞溶解。照射区周边(左)肝细胞结构正常, 肝窦与肝静脉充血, 两者界限清楚 HE × 40 **图 5** PDT 后 120 h, 坏死区周边纤维组织增生 HE × 40 **图 6** PDT 后 1 个月, 肿瘤细胞结构破坏, 呈大片坏死 HE × 40 **图 7** 人肝癌 PDT 后 1 个月, 肿瘤周围肝组织结构正常, 未见肝细胞变性坏死 HE × 40。**图 8** 人肝癌 PDT 后 1 个月, 肿瘤照光区与邻近肝组织间大量淋巴细胞, 巨噬细胞集聚 HE × 40

Fig. 3 Mouse liver. 24 h after laser irradiation alone. Only a few hepatocytes around the needle path were necrotized. The surrounding hepatocytes preserved their normal sturcture. HE stained. × 40. **Fig. 4** Mouse liver. 24 h after PDT. A clear demarcation is seen between irradiated and necrotic zone (right) and surrounding non-irradiated, normal tissue(left). HE stained. × 40. **Fig. 5** 120 h after PDT. Fibrous proliferation can be seen at the edge of necrotic, irradiated area. HE stained. × 40. Fig. 6 Surgical specimen of human liver cancer, 1 month after PDT, showing large areas of necrosis. HE stained. × 40. **Fig. 7** Normal structure of liver tissue was preserved near the irradiated, necrotic area of liver cancer in the surgical specimen 1 month after PDT. HE stained. × 40. **Fig. 8** Infiltration of numerous lymphocytes and macrophages was present in surrounding liver tissue near irradiated cancer area. 1 month after PDT. HE stained. × 40.

讨 论

光动力治疗肝癌的安全性问题是人们关注的重点之一。光动力作用究竟对肝组织产生什么样的损伤,这种损伤能否控制,能否防范,这是临床治疗前必须探讨的。我们知道光动力杀伤范围与靶组织内光敏药物的贮留量、靶组织的光学特性、靶组织的光生物反应密切相关。本组实验动态观察了治疗量激光对小鼠活体肝组织的影响、治疗量血卟啉衍生物的影响以及血卟啉衍生物加激光对活体肝组织的影响。结果显示:单纯激光照射的肝细胞只出现局限性的一过性肿胀,72 h 即恢复正常,不导致细胞死亡。单用血卟啉衍生物后肝细胞没有出现任何镜下损伤。血卟啉衍生物加激光照射则肝细胞出现不可逆转的严重损伤,于实验第 1 h 即出现细胞水肿,24 h 见大面积坏死,120 h 见纤维组织增生,但这种损伤表现仅见于照光区内。我们在进行光辐照时,对光斑区外的组织未给予任何保护,观察结果时发现照射区的黄白色坏死范围与照射光斑大小一致,且与周边正常深红色肝组织形成明显对比,好似描画出来一般。镜下观察见照射区坏死细胞与邻近非照射区活细胞之间界限也十分清楚, 。

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PDT 后 1 个月行二期手术切除,

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