Photodynamic therapy for malignant pleural mesothelioma
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NORMAL TISSUE DAMAGE DUE TO INTRATHORACIC MTHPC MEDIATED PHOTODYNAMIC THERAPY: PRECLINICAL STUDIES IN MINIPIGS AND RATS

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To be submitted
Intrathoracic photodynamic therapy (PDT) in combination with surgery is being tested as a means to improve local control in patients with malignant pleural mesothelioma. Clinical studies using this combined modality treatment have, however, been hampered by serious side effects in thoracic organs. In this study we evaluated normal tissue damage to thoracic organs of minipigs and rats by histopathological examination after various PDT schedules using meta-tetrahydroxyphenylchlorin (mTHPC) as photosensitizer. In minipigs tissue injury was assessed at 2 to 4 days after a pleuropneumonectomy combined with PDT with 0.1 mg.kg\(^{-1}\) mTHPC and a total light dose of 10-15 J.cm\(^{-2}\), given at intervals of 48 and 96 hours. The additional effect of a pericardiotomy with boost doses of 10 - 20 J.cm\(^{-2}\) on the myocardium and the chest wall was also investigated. Rat thoracic organ damage was assessed at 3 to 30 days after PDT without pleuropneumonectomy, 0.15 or 0.3 mg.kg\(^{-1}\) mTHPC was given 24 or 96 hours before illumination with total light doses of 5,10 or 20 J.cm\(^{-2}\).

For minipigs no significant differences were observed between the 2 drug-light intervals in any of the thoracic organs. Illumination to > 15 J.cm\(^{-2}\) after pericardiotomy revealed diffuse degeneration of the myocardium and severe damage to coronary arteries. In rats, transmural necrosis of the myocardium was observed after intensified treatment with 0.3 mg.kg\(^{-1}\) mTHPC or 20 J.cm\(^{-2}\) and a drug-light interval of 24 hours. Moderate necrosis of the myocardium healed with formation of fibrosis and calcifications within 30 days. In contrast no necrosis was seen in the esophagus. Only mild edema formation and submucosal bleeding were observed after treatment with 0.3 mg.kg\(^{-1}\) mTHPC.

Severe persistent damage to thoracic organs can be avoided with mTHPC doses < 0.15 mg.kg\(^{-1}\) and a light dose of 10 J.cm\(^{-2}\) after > 48 hours or 5 J.cm\(^{-2}\) after 24 hours. In rats the heart seems to be the most vulnerable organ, which is protected by the pericardium in larger animals.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a disease with a very poor prognosis despite many attempts to improve methods of treatment (1,2). Surgical resection is considered potentially curative for patients with very localized disease, but local control can not be achieved in the vast majority of patients. One of the possible adjuncts to surgery is photodynamic therapy (PDT), which can be added to surgery in the same procedure (3,4). PDT is being successfully used as the sole treatment of small superficial tumors and it may also be a valuable adjunct to improve local tumor control after a debulking procedure by destroying nests of tumor cells remaining after surgical resection. Some studies using the combination of surgery and PDT for MPM showed promising results in patients with stage I or II disease (5,6) but others were less successful and reported a considerable number of patients with serious adverse events (7,8).

In our study of intraoperative PDT for MPM, using the photosensitizer meta-tetrahydroxyphenylchlorin (mTHPC), 3 out of 28 patients died in the perioperative period. Death was due to myocardial infarction, perforation of the esophagus or a bronchial stump fistula (8). Other significant but non-fatal complications were fluid
In the first part of this study minipigs were used because size and shape of the chest in these animals are much alike the geometric characteristics of the human chest. Animals underwent intraoperative mTHPC mediated PDT after a left sided pleuropneumonectomy. Normal tissue damage was compared for two different drug-light intervals of 48 and 96 hours. Surgery and PDT were performed according to our clinical protocol (4). In the second part of this study rats, which are more useful to test a great variety of different PDT parameters, were treated with intrathoracic PDT after lung deflation but without resection. Drug dose, light dose and drug-light interval were varied in order to obtain information about the most favourable combination with respect to damage of the heart and esophagus.

MATERIALS AND METHODS

All experiments were carried out in accordance with protocols approved by the local experimental welfare committees and conformed to national and European regulations.

A. Intrathoracic PDT following pleuropneumonectomy in minipigs

Surgery

Female minipigs with a body weight of 23-29 kg were housed in facilities of the University Animal Hospital (Bern, Switzerland). Animals were cared for by professional veterinary staff and were examined daily after surgery and PDT. The minipigs were fed with standard laboratory food and sterilized water ad libitum. After i.v. injection of mTHPC the minipigs were kept in subdued lighting until they were sacrificed, except during surgery when operation theatre lights were used, although direct illumination at exposed tissue was avoided as much as possible. Before intubation animals were sedated with ketamin, 10 mg.kg\(^{-1}\) i.m, and azaperone, 2 mg.kg\(^{-1}\) i.v. Following endotracheal intubation, anesthesia was maintained with 0.50 + 0.05 % halothane and 70 % nitrous oxide in oxygen whilst the animals were ventilated. Continuous i.v. infusion of fentanyl, 4mg.kg\(^{-1}\).h\(^{-1}\), and pancuronium, 0.5 mg.kg\(^{-1}\).h\(^{-1}\) were administered. The lungs were ventilated with a volume-controlled ventilator with PEEP 3-4 cm H\(_2\)O. Tidal volume was maintained at 10 ml.kg\(^{-1}\) and ventilatory frequency adjusted to maintain PaCO\(_2\) at 4.5-5.5 kPa.

After a lateral thoracotomy, dark surgical drapes were sutured to the skin edges of the incision to avoid skin damage related to light from the operation theatre lights. The right carotid artery and jugular vein were dissected and arterial and venous catheters were inserted for monitoring. The pleura was opened, a left sided pleuropneumonectomy was
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performed and PDT was carried out as described below. After illumination the incision was closed in layers. Animals were extubated after spontaneous breathing resumed. Postoperative pain was controlled using paracetamol i.m.

**PDT procedure**

The minipigs were injected i.v. with 0.1 mg.kg$^{-1}$ mTHPC, dissolved in 25% polyethylene glycol 400, 25% ethanol and 50% water at a final concentration of 0.03 mg.ml$^{-1}$ at 48 (n = 2) or 96 hours (n = 2) before illumination. Distribution of light and total cumulative light dose was monitored with 4 isotropic light detectors, encased in sterile polyethylene extension tubes (Vygom, Ecouen, France) filled with saline. The light detectors were then sutured in the apex of the chest cavity, near the esophagus, on the ventral chest wall and in the dorsal diaphragmal sinus. A sterile, saline filled plastic bag (Steri-drape, 3M) was then placed in the empty chest cavity with the aim to expand diaphragmatic and mediastinal folds. Illumination was performed with a spherical bulb fibre (Cardiofocus, West Yarmouth MA, USA) placed centrally in the saline filled bag and connected to an argon dye laser Spectra Physics 311 (Spectra Physics, USA). Illumination with light of 652 nm at a power setting of approximately 20 J.cm$^{-2}$ was continued until the target dose of 10 to 15 J.cm$^{-2}$ was reached on all four measured positions.

Additional illumination was carried out on three specific locations with a microlens. A light dose of 10 J.cm$^{-2}$ was applied locally on the myocardium after a peri-cardiotomy and the inner chest wall was additionally illuminated with 10 or 20 J.cm$^{-2}$ on two different locations.

**Postoperative period**

During the days following operation and illumination all treated minipigs were somewhat tachypnoic, but not in respiratory distress. Fever, or other signs of infection were not observed. Animals illuminated 96 hours after mTHPC injection were sacrificed 3 days after illumination and those illuminated 48 hours after mTHPC were sacrificed after 2 or 4 days. The minipigs were killed with an overdose of pentobarbital i.v. and rethoracotomy was performed. Biopsies were taken from the left ventricle of the heart (10-15 J.cm$^{-2}$, 20-25 J.cm$^{-2}$), the chest wall (10-15 J.cm$^{-2}$, 20-25 J.cm$^{-2}$ and 30-35 J.cm$^{-2}$), esophagus, bronchial stump, pulmonary artery, aorta, phrenic nerve, vagal nerve and diaphragm (all 10-15 J.cm$^{-2}$). Biopsies were fixed in buffered formalin (10%), paraffin embedded and sectioned at right angles to the surface. Slides were stained with hematoxylin and eosin and were examined by two pathologists (J-Y S and M vd V).

**B. Intrathoracic PDT in rats**

**Surgery**

Male Sprague Dawley (SD) rats, weighing between 200 and 300 grams, were bred and maintained in autoclaved filter top micro isolators in our animal house facility, and fed standard laboratory pellets and acidified water ad libitum. Prior to treatment animals were brought under anesthesia by breathing 6% halothane. After subcutaneous (s.c.) injection of 0.025 mg atropin. Rats were then intubated and ventilated with an infant
ventilator Dräger Babylog N (Dräger, Lubeck, Germany). Anesthesia was maintained with 2% halothane.

The left chest cavity was opened by a lateral incision in the fourth intercostal space and the deflated left lung was gently pushed aside. After the PDT procedure, the deflated lung was reexpanded by briefly increasing the inspiratory pressure and the chest wall was closed, stitching it in two layers. Animals were detubated when awake and received 0.0025 mg atropin and 0.0025 mg buprenorfine s.c. at the end of the surgical procedure.

**PDT procedure**

Rats were injected with mTHPC (0.15 or 0.3 mg.kg\(^{-1}\)) at 24 or 96 hours before illumination (Table 1). Illumination was performed using a spherical bulb fibre, placed between the heart and the esophagus and connected to a diode laser. (Applied Optronics, South Plainfield, U.S.A.), emitting light at 652 nm, at a power setting of 20 mW.cm\(^{-2}\). The cumulative light dose on the heart and esophagus was measured with an isotropic light detector placed on each organ. In the experimental groups 3 to 8 the effect of variation in drug light interval from 24 to 96 hours was investigated for a constant drug and light dose. In experimental groups 9 to 12 the influence of increasing drug and light dose was investigated for a constant drug-light interval of 24 hrs.

**Postoperative period**

At intervals of 3 to 30 days after operation biopsies were taken from the heart, esophagus, lung, chest wall, aorta and diaphragm. The specimens were fixed in buffered formalin (4 %) and sectioned at right angles to the surface. Slides were sectioned with hematoxylin and eosin and were examined by two pathologists (J-Y. S. and M. vd V.).

**RESULTS**

**A. MINIPIGS**

**Heart**

The combination of surgery and PDT (10-15 J.cm\(^{-2}\)) resulted in severe degenerative damage to the epicardium in all treated animals. The myocardium exhibited focal necrosis, but most of the muscle tissue showed no evidence of detrimental changes. Damage to the endothelium of the coronary arteries varied from mild to severe, but remained focal. There was no difference in PDT induced myocardial injury for illumination at 24 versus 96 hours after mTHPC administration. Boost illumination up to a total light dose of 20-25 J.cm\(^{-2}\) resulted in diffuse degeneration of the myocardium (Fig 1B) and damage to coronary artery endothelium with visible denudation (Fig 1C). Again no striking differences in effect were observed between the 2 drug-light intervals.
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<th>Table 1. Experimental protocol normal tissue damage in SD rats</th>
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N = 4 to 6 rats per group, fluence rate was 20 mW.cm⁻². All treatment groups underwent the surgical procedure.
Figure 1

Histological assessment 3 days after treatment (H&E x 200). 

A: myocardium with normal myocardiun; normal myocardiun is only visible in the central upper part (white arrow).

B: myocardium with diffuse degeneration, normal myocardiun is still visible in the lower left part, and C: a coronary artery with myocardium.

PDT in minipigs using MTHPC 0.1 mg/kg, 5-7.5 J/cm^2, drug-light interval 48 hours, light dose 20-25 J/cm^2.
Figure 2

PDT in rats using mTHPC 0.1 mg.kg⁻¹, drug-light interval 24 hours, light dose 20 J.cm⁻². A: normal myocardium (H&E x 100), B: extensive myocardial bleeding (black arrow) and necrosis (white arrow, H&E x 200) and C: submucosal (left side) and subserosal bleeding (right side) (both indicated with white arrows) and submucosal edema (black arrow) in the esophageal wall (H&E x 200). Histological assessment 3 days after treatment.
Esophagus

Biopsies of the esophagus showed edematous changes in the serosal layers of minipigs treated after drug-light intervals of 48 and 96 hours. Muscularis mucosa swelling was found sporadically in animals treated 48 hours after drug administration. The mucosal lining was not injured in any treatment condition used in these experiments.

Other organs

Striated muscles of the chest wall and the parietal pleura showed varying degrees of degeneration and necrosis. Significant damage was seen with illumination to total light doses of 10-15 J.cm$^2$ at 48 or 96 hours after drug administration. Additional illumination with 10 J.cm$^2$ resulted in endothelial detachment from the submucosal layers, and some perineurial edema. A boost of 20 J.cm$^2$ resulted in focal chest wall necrosis.

Biopsies from the bronchial stump and muscular layers of the pulmonary artery showed focal edema after illumination at 48 and 96 hours after mTHPC. Muscle degeneration in the pulmonary artery only occurred for the 48 hours drug-light interval. Fatty tissue surrounding the aorta, exhibited edema with scattered bleeding after PDT. The aorta itself was not affected.

The phrenical and vagal nerve showed some focal edema after both drug-light intervals. Edema of the diaphragm was seen in all animals, but significant muscular damage was only seen for the 96 hours drug-light interval.

B. RATS

Heart

After standard PDT (0.15 mg.kg$^{-1}$ and 5 J.cm$^2$) a moderate to severe cardiac necrosis was seen at 3 days after treatment in 60% of rats. The incidence of this early necrosis increased to 100% with increasing drug dose (Fig 2B and 3A) or light dose (Fig 3C). Transmural necrosis was even seen in 25% and 60% of rats treated with dose intensified schedules of 0.3 mg.kg$^{-1}$ / 5 J.cm$^2$ or 0.15 mg.kg$^{-1}$ / 20 J.cm$^2$, respectively. The incidence of cardiac necrosis after standard PDT (0.15 mg.kg$^{-1}$ / 5 J.cm$^2$) reduced with increasing follow up time, but this was accompanied by an increase in fibrosis and calcification (Fig 3D). At 30 days after treatment the incidence of calcifications was higher in rats given an increased total light dose but there was no significant increase in late fibrosis or necrosis for 10 J.cm$^2$ compared with 5 J.cm$^2$ (Fig 3C). For the longer drug-light interval of 4 days, no significant damage was observed (Fig 3B).

Esophagus

Mild inflammation and edema were seen in 20-40% of rats at 3 days after operation in all treatment groups, including controls. This had resolved by 30 days post treatment. Significant damage to the esophagus was only seen in animals treated with the intensified PDT schedules. In these groups, 30-70% had moderate to severe edema at 3 days after treatment, although this had resolved by 30 days (Figure 4). In 50% of the animals treated with the increased drug dose, submucosal bleeding was also seen (Fig 2C).
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Figure 3
Incidence of pathologic changes in the myocardium of PDT treated rats for each experimental group (see Table 1). Effect of differences in effect of drug dose (A), drug-light interval (B), total light dose (C) and follow up time (D) are shown.

Other organs

The combined procedure of surgery and PDT included temporary compression of the left lung which always resulted in some damage to this lung. Control animals treated with light or drug only, all showed some degree of interstitial pneumonia and mesothelial proliferation of the pleura. The pathological changes were more pronounced after PDT with 0.15 mg kg⁻¹ mTHPC and a total light dose of 5 J cm⁻². This was seen after both drug-light intervals of 24 and 96 hours. Most indications of active inflammation were replaced by fibrotic changes 7 or 30 days after treatment. Intensified PDT resulted in moderate to severe necrosis and bleeding.

The ribs showed some osteoclastic activity with periostal reaction throughout all experimental groups. After standard PDT treatment there was little evidence of muscle damage 7 days after treatment and complete recovery after 30 days. After intensified PDT schedules the initial muscular damage persisted to 30 days and coagulation necrosis in striated muscles was seen in 2 animals treated with 20 J cm⁻² at 24 hours after mTHPC.
Pathologic changes were not seen in the aorta, with the exception of 1 rat illuminated with 10 J/cm². This rat developed calcifications of the aortic wall which was evident at 30 days after treatment.

Transient mesothelial damage was observed on the diaphragm of animals illuminated 24 hours after drug administration, with a significant trend for repair by 30 days after treatment. Escalating the drug dose increased diaphragmal damage. Necrosis, bleeding and calcifications were also seen in muscles of the dose intensification groups with only little recovery after 30 days.

**DISCUSSION**

Intrathoracic Photodynamic Therapy has been used as an adjuvant to surgery in treatment protocols for malignant pleural mesothelioma (5,6,7,8). The rationale for this approach is that surgery may achieve macroscopically radical resections, but the majority of patients experience local recurrence within a few months of operation. This indicates that microscopic residual disease was still present after surgery. Thoracic PDT can destroy minimal residual disease and should, theoretically, lead to improved local control. However, clinical experience with intraoperative PDT for MM can be associated with severe complications. It is therefore important to gain insight into how PDT parameters (drug dose, drug-light interval and total light dose) are associated with toxicity.

In this study we investigated normal tissue toxicity in relation to mTHPC mediated intrathoracic PDT in minipigs and rats. Particular attention was paid to damage of the heart and the esophagus, which are important factors determining recovery after

![Figure 4](image)

Figure 4

Incidence of edema and submucosal bleeding of the esophagus in rats of experimental groups treated with intensified PDT (see Table 1)
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resection and PDT in clinical protocols (5,8). Organ damage, assessed by postmortem histological examination, was generally greatest for short drug-light intervals and increased light and drug doses. Results of the minipig experiments suggested less influence of the drug-light interval than total light dose, but this was not confirmed in the more extensive rat studies.

In minipigs myocardial injury was mild and focal, without evidence that the drug-light interval influenced the extent of injury after illumination with 10-15 J.cm². This is consistent with a previous study in dogs using Photofrin mediated PDT (9).

Pericardiectomy and illumination with higher light doses resulted in severe myocardial necrosis and coronary artery injury. Unfortunately it is impossible to differentiate from these experiments between the effect of the pericardiectomy and the additional illumination. The pericardium may have offered considerable protection due to its thickness (± 1 mm), as indicated in other studies (10). The relatively thin pericardium in rats is accompanied with more light exposure to the myocardium than is seen in larger animals. At least some myocardial necrosis was observed in all PDT treated rats. After the intensified PDT protocols, transmural necrosis was seen. Animals with transmural necrosis did not recover fully from their treatment, and the intensified PDT schedules were discontinued, without including 30 day follow-up.

Thoracic PDT in the minipig or rat resulted in only mild edema in the mucosal lining of the esophagus if the total light dose did not exceed 10 J.cm², although significantly more damage was observed for higher light doses. (Fig 2C and 4). It seems reasonable to conclude that for a drug dose < 0.15 mg.kg⁻¹, esophageal injury is not a serious threat for light doses up to 10 J.cm⁻². This corresponds well with findings from a dog study with Photofrin mediated PDT, showing no significant esophageal damage (9). However, esophageal perforation has been reported from several clinical studies (3,8). Factors other than the photochemical reaction could possibly increase the risk of perforation during combined surgery and PDT. Tumor excision on the adventitial side may have contributed to injury of the esophageal wall. Thermal injury, which alters the submucosal collagen architecture (11), may also have occurred during intrathoracic illumination if the light source was positioned in close proximity to the esophagus (7,8). Some authors have advocated lower wavelength illumination, for instance 514 nm for mTHPC, to reduce the risk of transmural necrosis for direct PDT of the esophagus (12,13). But this is more imminent for intraluminal PDT of the esophagus, because light dosimetry is notoriously difficult during these treatment procedures (14). Such adjustments may not be necessary for intrathoracic PDT, provided that the total light dose to the esophagus is kept below 10 J.cm².

The influence of the drug-light interval on the extent of damage was much more marked in the rat than in the minipig, although only 2 minipigs per group were investigated and the shortest drug-light interval was 2 days.

Although proper light dosimetry was only performed for the heart and esophagus in the rat studies, some interesting information could be obtained from histological assessment of other thoracic organs. Transient interstitial lung injury was seen in most animals, including control groups, but this was possibly caused by temporal compression of the left lung during illumination. Necrosis and bleeding were not seen in the control groups and seemed to be more related to the PDT effect. Hemorrhagic necrosis of lung tissue after intrathoracic PDT has also been observed in two other studies after intrathoracic PDT (15,16). Although microscopically impressive, the clinical relevance of this type of damage may be limited. It has been shown in pigs that if damage to lung
tissue was confined to a limited area, healing capacity was preserved in all animals treated with mTHPC mediated PDT (15).

In conclusion, thoracic PDT with mTHPC doses < 0.15 mg.kg\(^{-1}\) and light doses of 10 J.cm\(^{-2}\) after 48 to 96 hours or 5 J.cm\(^{-2}\), after 24 hours is associated with mild transient normal tissue damage. Escalation of drug or light dose was associated with more severe persistent damage. In rodents the heart seems to be the most vulnerable organ. In larger animals, including humans, the pericardium has an important protective effect. In addition to the photochemical damage, factors like surgical and thermal injury may contribute to damage in thin walled structures like the esophagus.

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