Systemic vs local administration of δ-aminolevulinic acid for laparoscopic fluorescence diagnosis of malignant intra-abdominal tumors

Experimental study

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Abstract
Background: Administration of δ-aminolevulinic acid (ALA) either systemically or locally results in tumor-specific accumulation of protoporphyrin IX (PpIX). When excited with light at a defined wavelength and viewed with the appropriate filter cells containing PpIX, have a characteristic red fluorescence. We evaluated both locally (intraperitoneally [IP]) and systemically (intravenously [IV]) administered ALA to compare its effectiveness for laparoscopic fluorescent visualization of intraperitoneal tumors.

Methods: Peritoneal carcinosis was induced in rats using colon carcinoma cells (CC531). Photosensitization was achieved either by intravenous (IV group) or intraperitoneal (IP group) application of ALA solution. Staging laparoscopy was performed in both groups, first using conventional white light and subsequently using blue light (380–449 nm) to excite PpIX-induced fluorescence.

Results: Conventional white light laparoscopy showed 142 visible intraperitoneal tumor foci in the IP group and 116 such foci in the IV group. In the IP group, all tumors (100%) also were fluorescence positive, whereas in the IV group only 32 of the tumors (28%) showed the typical red fluorescence. In the IP group, 30 additional tumors were detected by fluorescence excitation (21%), as compared with eight additional tumors in the IV group (7%).

Conclusions: Fluorescence laparoscopy after local (IP) photosensitization with ALA is a more reliable and effective method than systemic (IV) photosensitization for the detection of small or occult IP tumors.

Key words: ALA — δ-Aminolevulinic acid — FD — Fluorescence diagnosis — Staging laparoscopy

Administration of δ-aminolevulinic acid (ALA), either systematically or locally, overloads the last step in the heme biosynthesis pathway of tumor cells, resulting in an increased accumulation of protoporphyrin IX (PpIX). When stimulated by light of a defined wavelength within its absorption spectrum, PpIX behaves as a fluorescent agent. One main emission wavelength is within the visible light spectrum at 635 nm (red light) [10]. This positive, red fluorescence of PpIX is detectable even in macroscopically invisible tumor foci and can indicate peritoneal carcinosis that would have been missed under conventional white light illumination [4]. Therefore, diagnostic staging laparoscopy for the detection of early intraperitoneal (IP) malignancies may be improved by fluorescence imaging techniques in clinical situations.

One major side effect of systemically applied (intravenous [IV] or oral) ALA results in a general photosensitivity because of PpIX accumulation in skin and other organs [12]. Severe sunburns are likely to occur, and avoidance of sunlight is necessary for an extended period. Forms of ALA for local administration may reduce the phototoxic side effects and improve tumor specific accumulation of PpIX for better fluorescent discrimination of malignant tissue [2].

We evaluated the effectiveness of systemically (intravenously) versus locally (intraperitoneally) applied ALA in fluorescence laparoscopy for disseminated IP tumors in an experimental setting.

Material and methods

Tumor model

A peritoneal carcinosis model was achieved using 1,2-dimethylhydrazin (DMH)-induced colon carcinoma cells (CC531) of male WAG/Rij rats in the same rat species. For tumor cell implantation, the animals underwent IP Ketanest/Valium anesthesia. Under laparoscopic control (4-mm scope,
Karl Storz, Tuttingen Germany), the tumor cell suspension (5 x 10³ 
CCS31 cells in 1.5 ml of RPMI medium, pH 6.5, Sigma, Taufkirchen 
Germany) was injected with a 40-mm 21-gauge needle into the thin peri-
toneal layer at approximately 10 different sites of the peritoneum (Fig. 1).

Photosensitization

After 12 days of tumor growth, the animals weighing 270 to 340 g (mean, 
310 g) were divided randomly into two groups of six each. The IP group 
received ALA via IP lavage with 5 ml of a 3% ALA solution (0.17 mol/l 
NaIICO₃; Medac, Hamburg, Germany). The total ALA dosage was 150 mg 
per animal, corresponding to approximately 440 to 550 mg/kg body
weight. In the IV group, 100 mg/kg body weight of ALA were applied 
intravenously for systemic photosensitization (tail vein injection). Animals 
of both groups were then kept under subdued laboratory conditions with 
free access to water and food in a 12-h light/dark schedule. All experiments 
were performed in accordance with protocols approved by national experi-
mental animal welfare institutions.

Results

There was no morbidity or mortality related to either tumor growth or laparoscopy in either group (IP or IV). Dissemi-
nated tumor growth was observed exclusively in the parietal peritoneum. The entire abdominal cavity was assessable for 
laparoscopic inspection.

IP group (ALA administered by peritoneal lavage)

In the IP group, 142 tumors were visible under conventional white light. The size of the tumors ranged in diameter from 
1 to 10 mm. In the PDD mode, all 142 tumors showed the 
PPIX typical red fluorescence. But there also were 30 (21%) 
additional tumors visible that had not been detected in the 
WL mode (Table 1).

IV group (ALA administered by tail vein injection)

In the IV group, the WL mode showed 116 tumors, of which 
only 32 (28%) were fluorescence positive in the PDD mode. 
Under blue light, eight additional, macroscopically occult 
tumors (7%) were found (Table 1).

All the tumors, whether detected by conventional white light or blue light (PDD) were histologically examined. The 
tumors all were found to be malignant tumors derived from 
the CCS31 cell line. There were no false-positive findings in 
either the IP or the IV group.

Discussion

Endoscopic detection of fluorescence using locally admin-
istered ALA was described already for the detection of early 
bladder cancer several years ago. The results of these stud-
ies indicate that porphyrin-induced fluorescence increases 
sensitivity to detect plane urothelial lesions such as dyspla-
sias, carcinoma in situ, and papillary tumors, as compared 
with conventional white light cystoscopy alone [7]. How-
ever, ALA-induced PPIX fluorescence also has been used as 
a diagnostic aid for gastrointestinal malignancies in several 
experimental and clinical studies [1, 3, 5, 8, 9]. Most of 
these studies administered ALA systemically, either orally 
or intravenously.

Staging laparoscopy

A 5.5-mm laparoscopic sheath with an insufflation side port was inserted 
via the linea alba of the lower midabdominal wall into the abdominal

cavity. A carbon dioxide (CO₂) pneumoperitoneum was created with 
amaximum pressure of 5 mmHg (Laparoflator, Karl Storz, Tuttingen,
Germany). For laparoscopy of the entire abdominal cavity, a 4-mm endoscope 
(Hopkins II, 12°, Karl Storz Endoscopes, Tuttingen, Germany) connected 
to a modified CCD (Change Couple Device)-camera (Telecom, Karl Storz
Endoscopes Tuttingen, Germany) was introduced through the trocar.

For conventional white light and fluorescence laparoscopy, the D-light 
system (Karl Storz, Tuttingen, Germany) was used. This system is based 
on a 300-W xenon short-arc lamp with special optical properties for fo-
cusing high intensities of light into a light guide. The system is capable of 
two different illumination modes: (a) conventional white light mode (WL 
mode) and (b) blue light mode for PPIX fluorescence excitation (PDD 
mode, 380-440 nm). These two modes can be switched easily at any time 
by a footswitch or directly by a button on the CCD camera head. To 
enhance the emitted fluorescence of the tissue, special spectral filters ("cut-
coff" or "longpass" filters) in the endoscope’s eyepiece block the blue 
excitation light and allow only a defined part of the blue light to reach the 
endoscopist’s eye and the CCD camera.

First, a complete examination of the entire abdominal cavity was per-
formed using white light. All tumors detected were counted, and the exact 
location of the tumors was mapped. The same procedure followed in the 
PDD mode. Previously detected tumors were evaluated according to their 
fluorescence, and the peritoneum was examined for additional fluorescence 
positive tumors.

Laparotomy

All the animals were killed, and the laparoscopic findings were confirmed 
by open inspection and palpation of the entire peritoneum. No additional 
tumor foci were found after laparotomy. All tumors, identified in either white light or fluorescence, were measured and removed for histologic 
examination.

PDI Mode

Fig. 1. Laparoscopically controlled tumor cell (CCS31) implantation into 
the peritoneal layer.