Research progress on photodynamic detection of peritoneal metastases using 5–aminolevulinic acid (ALA)

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Abstract Peritoneal metastasis (PM) has long been regarded as the terminal stage or cancer spreading. Since the 1990s, an integrated treatment strategy combining cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) has been gradually developed by the surgical oncology community and has definitely improved the survival in selected patients with PM. In such comprehensive treatment strategy, completeness of cytoreduction (CCR) is the most important independent prognostic factor for survival benefit. The current CRS technique, however, is inadequate to identify the minute tumor nodules hidden at less accessible sites in the abdomen and pelvis. Thus PM still accounts for the most frequent form of cancer recurrence after CRS + HIPEC. There is an urgent need to develop sharper techniques to identify the minute PM nodules. Among the emerging technologies, photodynamic diagnosis (PDD) attracts the increasing attention for PM diagnosis. This review summarizes the application of 5-aminolevulinic acid (5–ALA)-based PDD for the diagnosis and treatment of PM.

Key words 5-aminolevulinic acid; photodynamic diagnosis; peritoneal surface malignancy; PEPT1; ABCG2; ferrochelatase

http://kns.cnki.net/kcms/detail/11.3662.R.20180606.1751.044.html
(peritoneal cancer index, PCI) and completeness of cytoreduction (CCR) are the most important independent survival predictors.

However, current surgical techniques are prone to overlook occult or microscopic deposits that may recur postoperatively even after a thorough CRS, with recurrence rates as high as 70%.

It is urgently needed to develop new methods to detect PM or micrometastases.

In recent years, ALA-PDD has been developed and utilized for the detection of gastrointestinal malignancies, ovarian cancer, and malignant mesothelioma.

The purpose of this review is to summarize the basic principle, basis, and clinical research status of ALA-PDD.

### 1 ALA-PDD

#### 1.1 Protoporphyrin IX (PpIX)

ALA is the natural precursor of PpIX and heme. In the human body, ALA is synthesized from neighboring CoA and glycine under the catalysis of ALA synthase and transferred to the cytoplasm, where it is catalyzed by ALA dehydratase leading to protoporphyrinogen. In the presence of ferrochelatase, PpIX is converted to heme. ALA can be transported into cancer cells through the PEPT1 transporter, thus increasing PpIX synthesis and accumulation.

#### 1.2 PpIX Selective Accumulation

Selective accumulation of PpIX in cancer cells and tissues is the theoretical basis of ALA-PDD. The mechanism of selective accumulation of PpIX in cancer cells includes enzyme-related and transport-related mechanisms.

1.2.1 Enzyme-Mediated Mechanism

Kaneko et al. observed that in glial tumors, the activity of ferrochelatase was lower than in normal brain tissues. After administering ALA, ALA-PDD and PpIX fluorescence were observed in the normal brain cells, whereas no fluorescence was observed in the tumor cells. Kaneko et al. also observed a similar phenomenon in bladder cancer.

1.2.2 Transport-Mediated Mechanism

The expression and metabolism of ALA transporters can promote selective accumulation of PpIX.

Fig. 1 Biolosynthesis pathway of protoporphyrin IX and heme

ALA: aminolevulinic acid; PpIX: protoporphyrin IX.
ABCG2 mRNA in the cytoplasm of tumor cells may be involved in the regulation of cell PpIX levels.

PEPT1, which is predominantly expressed on the surface of cancer cells, plays a critical role in the selective accumulation of PpIX in tumor cells.

In a study by Collin et al., the expression levels of PEPT1 and ABCG2 were measured in 20 gastric cancer cell lines and 13 primary gastric tumors. PEPT1 expression was found to be significantly correlated with the selective accumulation of PpIX in tumor cells.

In another study by Guyon et al., the expression levels of PEPT1 and ABCG2 were measured in 20 colorectal cancer cell lines and 13 primary colorectal tumors. PEPT1 expression was found to be significantly correlated with the selective accumulation of PpIX in tumor cells.

In a study by Onemura et al., the expression levels of PEPT1 and ABCG2 were measured in 20 head and neck squamous cell carcinoma cell lines and 13 primary head and neck squamous cell carcinoma tumors. PEPT1 expression was found to be significantly correlated with the selective accumulation of PpIX in tumor cells.

In a study by Kishi et al., the expression levels of PEPT1 and ABCG2 were measured in 20 breast cancer cell lines and 13 primary breast cancer tumors. PEPT1 expression was found to be significantly correlated with the selective accumulation of PpIX in tumor cells.

**Author Animal Model Number of case Drug Administration Dose**

Hornung [18] Fischer344 rat EOC PM 24 ALA IV 100 mg/kg
Cani [10] BD IX rat EOC PM 36 ALA IV 100 mg/kg
Chao [10] Fischer344 rat EOC PM 9 ALA IV 100 mg/kg
Ludwig [10] Fischer344 rat EOC PM 11 HAL IP 4 – 12 mmol/L
Collin [10] Fischer344 rat EOC PM 21 ALA/HAL IP 100 mg/kg
Guyon [10] Fischer344 rat EOC PM 42 HAL IP/PO IP: 100 mg/kg PO: 50 mg/kg
Gahlerer [10] WAG Rij rat CC PM 12 ALA IV/IV 440 – 550 mg/kg
Kishi [10] BALB/c nude mice CC PM 8 ALA IP 250 mg/kg

**ALA**: aminolevulinic acid; **PDD**: photodynamic diagnosis; **PM**: peritoneal metastasis; **EOC**: epithelial ovarian carcinoma; **CC**: colon cancer; **GC**: gastric cancer; **HAL**: hexaminolaevulinate; **IV**: intravenous; **IP**: intraperitoneal; **PO**: per os; **NR**: not reported.
Hornung 等报道，24 只 Fischer 344 小鼠经腹腔注射卵巢癌细胞制作 PM 模型，接种 4 周后，静脉给予ALA 100 mg /kg，之后1、3、6、9...0%)标本的转移结节检出率。此外，在 60% 的结肠癌 PM 中，可检测到转移结节。在胃癌 PM 和阑尾黏液肿瘤中，PDD 检测率较低，分别为25.7%和16.4%，详见表3。

2 ALA-PDD 用于肿瘤检测

3 ALA-PDD 用于肿瘤检测

3.1 ALA PDD

采用不同 ALA 的 PDD 的 PM 试验结果

| 组别 | ALA | 剂量 | 给药方式 | 显像时间 | 检出量 | 检出率 |
|------|-----|------|----------|----------|--------|--------|
| 1    | ALA | 100 mg /kg | 静脉 | 1 h | 62.5% | |
| 2    | ALA | 50 mg /kg | 口服 | 2 h | 37.5% | |
| 3    | ALA | 25 mg /kg | 口服 | 3 h | 25%  | |

3.2 ALA PDD

采用不同 ALA 的 PDD 的 PM 试验结果

| 组别 | ALA | 剂量 | 给药方式 | 显像时间 | 检出量 | 检出率 |
|------|-----|------|----------|----------|--------|--------|
| 1    | ALA | 20 mg /kg | 静脉 | 1 h | 62.5% | |
| 2    | ALA | 10 mg /kg | 口服 | 2 h | 37.5% | |
| 3    | ALA | 5 mg /kg | 口服 | 3 h | 25%  | |

ALA-PDD 用于肿瘤检测的临床应用

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Table 2: ALA-PDD for detecting PM in clinical trials

| Author            | Disease       | Number of case | Administration | Dose (/mg kg⁻¹) | Incubation time/h | Sensitivity/\% | False positive/\% | Specificity/\% |
|-------------------|---------------|----------------|----------------|-----------------|------------------|----------------|------------------|----------------|
| Lonin[14]         | EOC           | 29             | IP             | 30              | 5                | 92             | 2                | NR             |
| Liu[16]           | EOC           | 20             | PO             | 20              | 2                | 95             | 0                | 100            |
| Yonemura[16]      | PM            | 138            | PO             | 20              | 2                | 46             | 0                | 100            |
| Murayama[10]      | GC            | 13             | PO             | 10 - 15         | 3                | 100            | 0                | 100            |
| Hillemanns[16]    | EOC           | 26             | PO             | 10              | 9 - 16           | 75             | 0                | 100            |

ALA: aminolevulinic acid; PDD: photodynamic diagnosis; EOC: epithelial ovarian carcinoma; PM: peritoneal metastasis; GC: gastric cancer; IP: intraperitoneal; PO: per os; NR: not reported.

Table 3: Positive rate of ALA-PDD for detecting PM of different origins

| Primary site                        | Number of case | Positive rate/\% (\%) |
|-------------------------------------|----------------|----------------------|
| Ovarian cancer                      | 26             | 22 (84.6)            |
| Mesothelioma                        | 8              | 5 (62.5)             |
| Pancreas cancer                     | 4              | 3 (75.0)             |
| Colorectal cancer                   | 29             | 27 (96.5)            |
| Cholangiocarcinoma                  | 3              | 2 (66.7)             |
| Small intestine cancer              | 8              | 4 (50.0)             |
| Gastric cancer                      | 10             | 9 (90.0)             |
| Appendiceal mucinous carcinoma      | 55             | 9 (16.4)             |
| Total                               | 143            | 81 (56.6)            |

PM: peritoneal metastasis; ALA: aminolevulinic acid; PDD: photodynamic diagnosis.

Table 4: PpIX contents in PM of different origins

| Primary sites                        | Number of case | PpIX content (/nm mg⁻¹) |
|-------------------------------------|----------------|------------------------|
| Ovarian cancer                      | 10             | 0.018 ± 0.007          |
| Mesothelioma                        | 5              | 0.015 ± 0.010          |
| Pancreas cancer                     | 5              | 0.010 ± 0.010          |
| Colorectal cancer                   | 29             | 0.010 ± 0.000          |
| Gastric cancer                      | 10             | 0.010 ± 0.001          |
| Appendiceal mucinous carcinoma      | 15             | 0.002 ± 0.001          |

PM: peritoneal metastasis; PpIX: protoporphyrin IX.

5 ALA-PDD PM

Kamp[12] 84 ALA 44 PDD 56 ALA-PDD 13 PM 115 ALA-PDD 84 ALA 44 PDD 56 ALA-PDD 13 PM 115 ALA-PDD

4 ALA PDD

ALA ALA ALA ALA ALA ALA ALA.
6

ALA-PDD PM PEPT1 ABCG2 PpIX ALA-PDD PM PEPT1 ABCG2

ALA-PDD PM PEPT1 ABCG2 PpIX ALA-PDD PM PEPT1 ABCG2

ALA-PDD PM PEPT1 ABCG2 PpIX ALA-PDD PM PEPT1 ABCG2

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