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Photodynamic therapy of ovarian cancer peritoneal metastasis with hexaminolevulinate: A toxicity study

Laurie Guyon[a,b], Marie-Odile Farine[c], Jean Claude Lesage[b], Anne-Marie Gevaert[c], Sylvie Simonin[d,e], Caroline Schmitt[d,e], Pierre Collinet[a,b], Serge Mordon Ph.D.[b,f,*]

[a] *Department of Gynaecology and Obstetrics, Lille University Hospital, Lille, France*
[b] *INSERM U703, Univ Lille Nord de France, Lille University Hospital, Lille, France*
[c] *Centre de biologie-pathologie, Lille University Hospital, Lille, France*
[d] *Centre Français des Porphyries, Hôpital Louis Mourier, Colombes, France*
[e] *INSERM U773, Paris Diderot University, Paris, France*
[f] *GDR 3049 Médicaments Photoactivables — Photochimiothérapie (PHOTOMED), France*
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Second generation photosensitizer

Summary
Context: While photodynamic therapy (PDT) is a promising treatment for peritoneal carcinomatosis, its use is often limited because of the toxicity of photosensitizers. In this study, safety of PDT with hexaminoevulinate (HAL), a second generation photosensitizer, is assessed.
Methods: PDT of the peritoneal cavity was performed in a rat model of peritoneal carcinomatosis. Rats were treated according to different protocols: with full or half HAL dose, after intraperitoneal or oral administration of HAL, 4 or 8 h after its injection, using red or green light, after protection of the liver or cooling of the abdominal wall. Toxicity was assessed by blood tests quantifying hematocrit, liver and muscular enzymes and by pathological examination of abdominal and intrathoracic organs after treatment. The results were analyzed in the light of quantification of fluorescence and protoporphyrin IX (PPIX) content of the same organs.
Results: PDT with HAL induced rhabdomyolysis, intestinal necrosis and liver function test anomalies, leading to death in 2 out of 34 rats. The liver and the intestine contained high levels of PPIX (3—5 times more than tumor nodules).
Conclusion: HAL PDT lacked specificity. However, the strategy associating diagnosis, treatment and evaluation of the results in one single procedure was effective and should be tested with other photosensitizers.

* Corresponding author at: INSERM U 703, Lille University Hospital, 152 rue du Dr. Yersin, 59120 Loos, France. Tel.: +33 639008635; fax: +33 320446708.
E-mail address: serge.mordon@inserm.fr (S. Mordon).

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Introduction

Photodynamic therapy (PDT) is an investigational treatment with the potential to become an adjuvant therapy to surgery for the management of advanced ovarian or digestive cancer.

The prognosis of these conditions is poor, despite current treatment strategies based on surgery followed by intravenous chemotherapy [1]. PDT offers an attractive alternative to conventional treatments, as it can be performed at the time of the initial surgery and has the capacity to treat microscopic disease. Due to the expected selectivity of photosensitizers for tumor lesions, PDT could be more effective and better tolerated than chemotherapy drugs.

A phase II clinical study about the association of surgery with PDT with Photofrin® was carried out in patients with a peritoneal carcinomatosis from digestive or ovarian origin [2]. Though the strategy was interesting, toxicity was important: bowel perforations, pancreatitis, acute respiratory distress syndrome, cardiac arrhythmia and alteration of blood tests appeared. All patients required intensive peri-operative management [3,4], including prolonged ventilatory support that could last up to several weeks.

The investigators used Photofrin®, one of the oldest photosensitizers, which is known to have little selectivity regarding tumor lesions. For instance, the amount of Photofrin® is as high in the spleen and the small bowel as in tumor implants [5].

However, efficacy and safety of PDT depend on many factors such as light wavelength, illumination protocol (fluence rate, fluence, continuous or fractionated administration of light), the administration conditions of photosensitizers, in particular their dose, and the type of photosensitizer.

5-ALA is the most studied second generation photosensitizer, but offers limited local bioavailability due to its hydrophilic nature. Its lipophilic ester derivatives, such as hexaminolevulinate (HAL) were designed to circumvent this drawback [6]. As a result, the quantity of protoporphyrin IX produced is the same with 30—150 smaller amounts of drug [7]. Two of them are already approved for the treatment or diagnosis of pre-malignant or malignant conditions: methylaminolevulinate (Metvix®) for actinic keratosis and basal cell carcinoma of the skin and hexaminolevulinate (Hexvix®) for the detection of superficial bladder cancer [8].

HAL has already been studied for photodiagnosis and PDT of peritoneal carcinomatosis of ovarian origin. PDT with HAL proved to be effective at inducing necrosis of carcinomatosis lesions [9,10]. Furthermore, photobleaching of tumor lesions was correlated to the extent of their necrosis, allowing for the assessment of treatment results [11].

Nevertheless, these studies about PDT with HAL were carried out on a limited peritoneal surface, thus not allowing to assess the toxicity of the technique. This crucial problem needs an answer before we can suggest using it for patients.

The aim of this study was to determine the toxicity of PDT of the abdominal cavity with HAL in a rat model of peritoneal carcinomatosis.

Methods

Animal model

NuTu-19 is a syngeneic adenocarcinoma used to develop ovarian cancer in an immunocompetent Fischer 344 rat model [12]. Pathogen-free Fischer 344 female rats (Charles River Laboratories, L'Abresle, France) were given intraperitoneal injection with 6×10^6 cells from the NuTu19 cell line using an already descried protocol to induce peritoneal carcinomatosis [11]. The rats were housed throughout the whole experiment in a pathogen free facility with commercial basal diet and water ad libitum and received proper care and maintenance.

They were monitored daily for signs of tumor growth. Animals showing an evident excessive tumor burden (abundant ascites, icterus, discolored eyes) were excluded from the experiments and were sacrificed.

The experiments were started between 6 and 9 weeks after the injection of the cells. The protocol was approved by the animal use and ethics committee of DHURE (Département Hospitalo-Universitaire de Recherche Expérimentale, Lille University, France).

Photosensitizer

Cristalline 5-aminolevulinic acid hexylester hydrochloride (HAL, Photocure ASA, Oslo, Norway) was stored in powder form and kept refrigerated. Samples were prepared immediately prior to use by dissolution of the powder in PBS, so as to obtain a concentration of 100 mg/mL, and were sheltered from light. Each animal received an intraperitoneal injection of 100 mg/kg of HAL, 4 h prior photodynamic therapy.

In some groups of rats, HAL was administered by oral route or at a half dose or 8 h prior PDT.

Photodynamic therapy procedure

Step 1

The rats were anesthetized by IP injection of ketamine (Virbac, Carros, France) 50 mg/kg and Xylazine (Bayer Health Care, Puteau, France) 5 mg/kg. They were placed in the supine position and a blue light mode laparoscopy was performed for later fluorescence analysis (cf ''fluorescence analysis'' paragraph) Fig. 1.

Step 2

The extent of peritoneal carcinomatosis was assessed during white and blue light mode laparoscopy with the score described below.

Two scores were calculated for each rat, one during white light and the other during blue light laparoscopy.

The abdomen was divided into 7 zones: the 2 sides, the midline, the diaphragm, the liver, the bowel and the omentum.

The number of lesions and their fluorescence intensity, if the score was established during blue light mode laparoscopy, were quantified and an intermediate score in white light and in blue light was given to each zone according to these parameters (Table 1).