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Short communication

Increased urinary coproporphyrin excretion observed in patients with differently staged Hodgkin's disease treated with chemotherapy

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Abstract

It has been reported that patients with Hodgkin's disease (HD) show altered porphyrin metabolism, and suggested that the cause is the neoplastic process itself. If this is true, disease progression should be associated with higher levels of porphyrin excretion.

The aim of this study was to evaluate urinary coproporphyrin levels in patients with Hodgkin's disease at different stages. As many of the patients received chemotherapy, another aim was to verify experimentally whether chemotherapeutic agents might increase porphyrin levels in rabbits.

All of the patients had above-normal urinary coproporphyrin levels.

On the other hand, rabbits receiving the porphyrin precursor 5-aminolevulinic acid (5-ALA), and also treated with doxorubicin, showed very high plasma porphyrin levels.

The increased levels of urinary coproporphyrins seem to be due to the disease itself, since the patients in stages III and IV had higher excretion values, presumably due to biochemical heme synthesis lesions that lead to the availability of the porphyrin precursor, as well as coproporphyrin accumulation and excretion.

The altered porphyrin synthesis may be attributable to the cytotoxic oxygen species generated in the presence of NADH and iron.

As the patients also received extensive chemotherapy regimes, the altered porphyrin metabolism may be affected by antineoplastic treatment generating oxygen reactive radicals. The alterations in porphyrin metabolism induced by chemotherapeutic agents appear to be demonstrated in rabbits in which doxorubicin increases porphyrin synthesis after porphyrin precursor treatment.

Keywords: Hodgkin's disease (HD); Stages; Urinary coproporphyrins; Iron; Anthracycline; Toxic oxygen species; Rabbits; 5-Aminolevulinic acid (5-ALA); Doxorubicin; Plasma porphyrins

1. Introduction

Much interest has been aroused in the possible association between malignancy and altered porphyrin metabolism [1].

The levels of hemoprotein enzymes, such as catalase and cytochrome c-oxydase are much reduced in tumour tissue, and some authors have concluded that malignant cells are impaired from synthesising heme, a characteristic that may account for the decreased respiratory activity of tumours. All of these data have been extensively reviewed by Greenstein [2]. The fall in heme levels (the natural repressor of ALA-synthase) increases the activity of this enzyme, which plays a key role in the biosynthesis and excretion of porphyrins [3].

In particular, alterations in the metabolism of porphyrins and their precursors have been described in a number of hematological malignancies [4]. The lymphocytes of patients with active malignant lymphoproliferative diseases show increased porphobilinogen deaminase activity [5,6]; Palma-Carlos and Palma-Carlos have found that subjects with

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Hodgkin's disease (HD) have increased levels of free erythrocyte porphyrins [7]; and Sharabasy et al. have analysed heme biosynthesis and the enzyme activities associated with the porphyrin metabolic pathway in various neoplastic blood diseases, including Hodgkin's lymphoma [8]. It has been suggested that the cause of the altered porphyrin metabolism is the neoplastic process itself [4].

The above reports did not consider the possible interrelationship between porphyrin excretion and the stages of Hodgkin's disease. If the alterations in porphyrin metabolism are due to the neoplastic disease, they should become more pronounced as the disease progresses.

The main aim of this study was to verify this hypothesis, by selecting patients with Hodgkin's lymphoma at different stages and evaluating the differences in their urinary coproporphyrin excretion levels.

As patients affected by Hodgkin's disease at different stages and with high coproporphyrin excretion values are exposed to extensive chemotherapeutic treatment, it is presumable that the administration of antineoplastic drugs, such as doxorubicin, may affect the induction of porphyrin biosynthesis.

It seems of interest to evaluate the contribution of chemotherapeutic treatment to porphyrin biogenesis in animals. As the antineoplastic agents used in Hodgkin's disease, such as anthyracycline compounds, may affect heme metabolism [9], stimulate the induction of 5-ALA synthase and leave more 5-ALA available for porphyrin biogenesis [3], another aim of this study was to see whether porphyrin synthesis may be affected by the presence or absence of chemotherapeutic treatment with doxorubicin in rabbits receiving 5-ALA.

2. Materials and methods

2.1. Patients

The study involved 14 patients (8 males and 6 females) with a mean age of 35 years (range 11–71 years), and 6 healthy controls.

All of the patients and controls gave their informed consent to participate in the study, which was conducted in accordance with the recommendations of the Declaration of Helsinki and the local Ethics Committee.

2.1.1. Diagnosis

Hodgkin's disease was pathologically diagnosed on the basis of excision lymph node biopsy [10].

2.1.2. Staging

The patients underwent a physical examination, chest radiography, bone marrow biopsy and cytology [10], and computed tomography of the chest, abdomen and pelvis. The presence of systemic symptoms (B symptoms) (low grade fever, night sweats, weight loss exceeding 10% of body weight) was reported in eight patients. The patients were classified as being in stages I, II, III and IV according to the "Report of the Committee on Hodgkin's Disease Staging Classification" [11].

Furthermore, their histological profiles were studied according to the Nomenclature Committee [12], and the nodular sclerosis, mixed cellularity, lymphocyte depletion, and lymphocyte predominance subtypes were identified and reported.

2.1.3. Therapy

All 14 patients received radiotherapy. Patients in stages I and II in the case of the presence of unfavourable clinical features and bulky involvement of one or more lymph nodes received short courses of chemotherapy with adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) followed by local radiotherapy [13]. Six patients in stages III received ABVD cycles combined with radiotherapy according to the regimen of Milan's National Cancer Institute [13]. Two patients in stage IV were treated with mecloretamine, vincristine, procarbazine and prednisone (MOPP) alternated with ABVD, followed by radiotherapy [14].

2.1.4. Porphyrin analysis

Twenty-four-hour urine samples were collected from all of the subjects, and urinary coproporphyrins were detected as methyl esters using the method described by Pinelli and Gaspari [15].

2.2. Animal treatment

The investigation was carried out in conformity with the guidelines for the care of laboratory animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was performed as shown in Fig. 1.

Male New Zealand white rabbits weighing 3000 ± 100 g were obtained from Harlan (Correzzana, Milan, Italy) and divided into groups of five animals each. The experiments were carried out using four groups of animals treated with saline (controls), doxorubicin, 5-aminolevulinic acid (5-ALA) or a combination of both chemicals. Doxorubicin was administered subcutaneously at a daily dose of 7 mg/kg (divided into three injections at 4 h intervals) for 3 days. 5-Aminolevulinic acid was given, subcutaneously, at a dose of 50 mg/kg on the third and fourth day. In the group receiving the combined treatment, 5-aminolevulinic acid was given 1 h (third day) and 15 h (fourth day) after the last doxorubicin injection.

The animals were sacrificed on the fourth day, 19 h after the last doxorubicin injection and 4 h after the last 5-aminolevulinic acid administration.

Blood was collected using citrate, and the plasma was prepared and stored at $-20\,^{\circ}C$ until assay. Plasma porphyrins were measured according to the method described by Colombi et al. [16].