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Quilamine HQ1-44, an iron chelator vectorized toward tumor cells by the polyamine transport system, inhibits HCT116 tumor growth without adverse effect

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ABSTRACT

Tumor cell growth requires large iron quantities and the deprivation of this metal induced by synthetic metal chelators is therefore an attractive method for limiting the cancer cell proliferation. The antiproliferative effect of the Quilamine HQ1-44, a new iron chelator vectorized toward tumor cells by a polyamine chain, is related to its high selectivity for the Polyamine Transport System (PTS), allowing its preferential uptake by tumoral cells. The difference in PTS activation between healthy cells and tumor cells enables tumor cells to be targeted, whereas the strong dependence of these cells on iron ensures a secondary targeting. Here, we demonstrated *in vitro* that HQ1-44 inhibits DNA synthesis and cell proliferation of HCT116 cells by modulating the intracellular metabolism of both iron and polyamines. Moreover, *in vivo*, in xenografted athymic nude mice, we found that HQ1-44 was as effective as *cis*-platin in reducing HCT116 tumor growth, without its side effects. Furthermore, as suggested by *in vitro* data, the depletion in exogenous or endogenous polyamines, known to activate the PTS, dramatically enhanced the antitumor efficiency of HQ1-44. These data support the need for further studies to assess the value of HQ1-44 as an adjuvant treatment in cancer.

1. Introduction

1.1. Iron metabolism, a new target for anticancer therapy

Iron is essential in many biological functions and a potential target for anticancer therapy [1]. Initially developed for the treatment of iron overload, iron chelators have shown an antiproliferative action on tumor cells, thus demonstrating their potential value in the treatment of some cancers [2]. Thus, the high efficiency and selectivity of new thiosemicarbazone chelators against various tumors have been reported *in vitro* and *in vivo* [3]. In fact, growing tumor cells require large quantities of iron, leading to an increase in the iron importing proteins and a decrease in the expression of iron exporting proteins [4–6]. In addition, iron exposure stimulates the proliferation of tumor cells *in vitro* and *in vivo* [7]. Therefore, decreasing the exchangeable intracellular iron level with synthetic iron chelators appears an attractive method to reduce the proliferation of cancer cells [8]. Our previous results dealing with deferasirox (ICL670), an iron chelator currently used in the treatment of secondary iron overload disease, suggested that its antiproliferative activity results from its inhibitory effects on both iron and polyamine metabolisms [9]. However, these chelators were designed to treat patients exhibiting iron excess. Therefore, their use in patients without iron excess may lead to major side effects, thus limiting their use as antitumoral agents in patients not affected by iron overload. Thus, for potential iron chelators to be used in cancer therapy, they must be vectorized toward tumoral cells.

1.2. Polyamine, a vector for anticancer agents

In tumor cells, polyamine metabolism, including biosynthesis and uptake, is particularly amplified [10,11]. Targeting polyamine

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metabolism by using polyamine synthesis inhibitors or polyamine catabolism activators was demonstrated to prevent and reduce risk factors associated with the tumor development [12]. The efficiency of the polyamine transport system (PTS) is also greatly increased in tumoral cells. This property is of potential interest for targeting antitumor agents [13–28].

Based on these observations, we have developed a new generation of iron chelators, the Quilamines, in which an 8-hydroxyquinoline chelating subunit is grafted onto polyamine vectors, in order to vectorize the iron chelator inside the cancerous cell with an overactive PTS [29]. The difference in PTS activation between healthy and tumor cells enables tumor cells to be targeted whereas the stronger dependence of these latter cells on iron ensures a secondary targeting.

We previously reported that the Quilamine HQ1-44 was highly taken up by the PTS and displayed an efficient antiproliferative activity [29]. As expected during the design of Quilamines, the polyamine chain, involved in the metal coordination, reinforced the affinity for iron (III) and the ferric complex was shown to adopt preferentially a 1:2 [Fe/HQ1-44] stoichiometry at pH = 7.4.

We extended the screening of the antiproliferative activity of HQ1-44 to various tumor cell lines and compared it to two non-tumoral cell lines [30]. The relationship between PTS activity and antiproliferative efficiency of HQ1-44 in these various cancer cell lines clearly demonstrated that the Quilamine HQ1-44 was the most efficiently recognized by the active PTS of human colon adenocarcinoma HCT116 cell lines. Consequently, this Quilamine exhibited the higher antiproliferative efficiency on this cell line. In contrast, a low cytostatic effect was observed in normal cells. Colorectal cancer (CRC) is the second most common cause of death from cancer in France and the fourth most commonly diagnosed cancer in the world, so we choose to demonstrate the proof of the Quilamine concept on this kind of cancer.

In the present work, our aim was to investigate the impact of HQ1-44 on cell proliferation and the mechanisms involved as well as assessing its efficiency and tolerance *in vivo* as an antitumoral treatment in athymic nude mice xenografted with HCT116 cells, by comparison with *cis*-platin.

2. Materials and methods

2.1. In vitro *study*

2.1.1. *Chelator solutions*

All reagents were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France) at the highest available grade. Stock solutions of the Quilamine HQ1-44 (10 mM) were prepared in water. Cells were supplemented with iron by exposure to 20 μM exogenous iron-citrate complexes (1:10) for 72 h.

2.1.2. *Cell studies*

The involvement of the PTS in the selective uptake of Quilamine HQ1-44 was investigated in the HCT116 cell line, derived from human colon carcinoma (ATCC number 91091005). We previously demonstrated the higher sensitivity of this cell line to Quilamines, compared to other carcinoma cells [30].

These cells were grown in McCoy's medium (Life Technologies, Invitrogen™, Saint Aubin, France), supplemented with 10% fetal calf serum (Eurobio, Les Ulis, France), 100 units/mL penicillin and 50 μg/mL streptomycin, at 37 °C in 5% humidified CO_2.

2.1.3. *Cell treatment*

For experiments, cells were harvested with trypsin and seeded 24 h before the treatments in microplates (Becton Dickinson, Oxnard, CA, USA) at a density of 10,000 cells/cm^2. In these conditions, cells reached confluency in 5–6 days. Chelator exposure was performed one day (D1) or one week (D8) after cell seeding in proliferating or confluent HCT116 cells, respectively. The effect of Quilamine on cell viability was also tested in the presence of DFMO (1 mM), a selective inhibitor of ODC, which induced a depletion of intracellular putrescine and spermidine and thus activated the PTS. In this case, preincubation, in the presence of the ODC inhibitor, was performed prior to the Quilamine treatment. In other experiments, 50 μM of spermidine was added to the culture medium to perform a competitive inhibition of Quilamine uptake through the PTS. Aminoguanidine (1 mM), an inhibitor of the serum amine oxidase, was added to the cell culture medium to prevent the oxidation of exogenous polyamine.

For the caspase 3/7 activity assay, Quilamine uptake quantification, polyamine concentration determination and iron status evaluation, cell lysates were obtained after 72 h of treatment. After supernatant collection for biochemical analysis, cells were washed twice with 1 mL of ice-cold phosphate-buffered saline solution (PBS). Cells were collected by scraping in 1 mL PBS, and then centrifuged for 5 min at 2000 × *g*. The cell pellet was sonicated for 30 min at 4 °C in 500 μl of pure water. Protein content in cell extracts was measured according to a method adapted from Bradford (1976) (Bio-Rad Protein Assay, Bio-Rad, Ivry sur Seine, France) and absorbance was read at 595 nm.

2.1.4. *Measurement of cytostatic and cytotoxic effects*

Treatments was performed one day (D1) or one week (D8) after cell seeding in proliferating or confluent HCT116 cells, respectively. After 72 h of incubation at 37 °C, cell supernatants were collected for cytotoxicity evaluation (cytotoxicity detection kit – LDH, Roche, Penzberg, Germany). Results were reported as a percentage of extracellular LDH activity with respect to the control value.

Cell viability was determined by cell nuclei counting after Hoechst 33342 staining and the dose-effect curves were analyzed according to an established procedure [30]. The number of cell nuclei was reported as a percentage of the value obtained in control conditions. Due to their biphasic feature, the dose–response curves were fitted as the sum of two sigmoids (double 4-parameter fit) according to Rodbard [31]. The various parameters, including the percentage and the IC_{50} values of each sigmoid, were deduced from these fits. Three independent replicates were performed for each experiment, which was repeated three times.

2.1.5. *Measurement of DNA synthesis*

DNA synthesis was assessed by tritiated thymidine incorporation. Twenty-four hours before cell harvesting, [^{3}H] methyl-thymidine (Amersham, Uppsala, Sweden) was added to the culture medium at a final concentration of 0.5 μCi/mL. Cells were washed twice with PBS and lysed in 0.1 M NaOH. DNA precipitation was achieved with 30% trichloroacetic acid (TCA), followed by two washes with 10% and 5% TCA successively, and dissolution in formic acid. The radioactivity of 200 μl of this formic solution was counted in 5 mL of Instagel using a Packard Tricarb 2100TR scintillation counter β (Perkin Elmer, Waltham, MA, USA) and expressed as cpm/g of protein.

2.1.6. *Analysis of the cell cycle*

The cell cycle was assessed by flow cytometry. Cells were detached with trypsin and nuclei were stained with propidium iodide (Cycletest Plus Kit, Becton Dickinson, Le Pont de Clais, France). Data were acquired and analyzed with a FACSCalibur flow cytometer (Becton Dickinson) using Cell Quest software (Becton Dickinson).