Antineoplastic potency of arylchloroethylurea derivatives in murine colon carcinoma

Elisabeth Miot-Noirault1, Jean Legault2, Florent Cachin1, Emmanuelle Mounetou1, Françoise Degoul1, René C. Gaudreault2, Nicole Moins1 and Jean Claude Madelmont1
1UMR 484 INSERM-Université d’Auvergne-Centre Jean Perrin, Rue Montalembert BP 184, 63005 Clermont-Ferrand, France; 2Centre de recherche, Centre Hospitalier Universitaire de Québec, Hôpital Saint-François d’Assise, 10 rue de l’Espinay, Quebec City, Quebec, G1L 3L5, Canada

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Summary
In a search for new antineoplastic agents the lead compound N-(4-tert-butylphenyl)-N′-(2-chloroethyl)urea (CEU-22) of a series of 1-aryl-3-(2-chloroethyl)ureas and its iodinated bioisostere CEU-98, were previously selected on the basis of their cytotoxicity and the potent tropism for the intestinal tract (evidenced for CEU-22). In this study, we investigated the antitumour profile of these two drugs for the indication of colon cancer. In vitro, we found that micromolar concentrations of both CEU-22 and CEU-98 inhibited proliferation of DLD-1, Caco-2, HT-29, SW-948 and CT-26 lines. In vivo, a high inhibition of tumour growth and a life span increase were observed when BALB/c mice grafted subcutaneously with CT-26 cells received 5 daily intratumoural injections of each drug. When administered by the intraperitoneal route according to an intermittent schedule starting Day 1 or Day 7 post-implant, only CEU-98 demonstrated antitumour activity (T/C = 29% for the Day-1,5,9-treatment versus 40% for the Day-7,11,15-treatment) and a life span increase around 40% for the two protocols. These results make CEU-98 a candidate for further investigations with a view to developing an efficacious treatment of colorectal cancer.

Introduction
The lack of selectivity of most anticancer agents, the occurrence of intrinsic or acquired resistances of tumour in response to chemotherapy cures and poor therapeutic index, are major problems in gastroenterologic oncology. Indeed, colorectal cancers are a major public health problem in industrial countries. Every year, roughly 1 million new malignant cases are diagnosed worldwide, and over 500 000 patients die from this disease [1]. Some 30% of patients have advanced disease at diagnosis, and therefore have an unfavourable prognosis. The first-line chemotherapy for colorectal cancers mainly relies on a few potent drugs such as 5-fluorouracil and its biochemical modulators, and more recently, on irinotecan. However, the 5-year survival rate of advanced colorectal cancer patients treated with optimised combined therapy still remains below 5% [2].

In work to improve the management of cancer patients, medical research has focused on finding new selective targeted therapies with a lower capacity to induce resistant phenotypes of tumour cells, and on improving therapeutic index. To that end, we have developed in the past decade a new class of antineoplastic agents, 1-aryl-3-(2-chloroethyl)ureas (CEUs) derived from the biofunctional aromatic moiety of chlorambucil and the un-nitrosated pharmacophore of Carmustine [3, 4]. Among the series of CEU derivatives that have been synthesised, we focused on two drugs, the prototypical N-(4-tert-butylphenyl)-N′-(2-chloroethyl)urea (CEU-22)
and its iodinated bioisostere N-(4-iodophenyl)-N′-(2-chloroethyl)urea (CEU-98). Both CEU-22 and CEU-98 were found to be more cytotoxic than their parent drugs in a wide panel of cell lines including human breast carcinoma (MDA-MB-231), human colon adenocarcinoma (LoVo), murine lymphocytic leukaemia (P388D1, L1210), and chronic myelogenous leukaemia (K562) [5, 6]. Although deriving from DNA alkylators, both drugs were, interestingly, found to be unreactive towards DNA and glutathione and to maintain their highly cytotoxic potential against lines expressing acquired resistance to conventional chemotherapeutic agents [7]. A study of the mechanism of action underlying the cytotoxicity of CEU-22 has demonstrated that this drug is a microtubule-disrupting agent that covalently binds to specific cysteiny1 residues of β-tubulin, preventing microtubule assembly, and leading to cytoskeleton dissolution and cell death [8]. Also, after intraperitoneal administration in healthy mice, [14C]-CEU-22 was observed to concentrate in the intestinal tract [9].

The original mechanism of action of CEUs, as well as the potential tropism of CEU-22 for the intestines, lend CEUs new potent candidates as colorectal cancer treatment drugs. The purpose of this study was therefore to assess, in vitro and in vivo, the antitumour potency of both CEU-22 and CEU-98 in a colon cancer animal model. We first compared the cytotoxic potency of each drug on human and murine colon carcinoma cell lines. Their antineoplastic activity was assessed in vivo on the murine CT-26 colon carcinoma subcutaneously grafted in BALB/c mice, treated by intratumoral and systemic routes. Dose-schedule behaviour and limitations were also determined. The results indicate that CEU-98 may deserve further investigation as an agent for the treatment of colorectal cancer.

Materials and methods

Drugs

N-(4-tert-butylphenyl)-N′-(2-chloroethyl)urea (CEU-22) and N-(4-iodophenyl)-N′-(2-chloroethyl)urea (CEU-98) (Figure 1) were prepared as previously described [3, 6]. The CEUs were stored at 4°C in the dark until use. CEUs molecules are highly water insoluble and thus preventing their formulation in hydrophilic vehicles. Fresh solutions were prepared 30 minutes before use according to the procedures described as follows:

For in vitro studies, CEU-22 or CEU-98 was dissolved in DMSO.

For in vivo studies, each of the two drugs was dissolved in a mixture of Labrafils® M1944 Cs (Gattefosse, France), dimethylacetamide (Sigma-Aldrich, France), and Tween 80® (Sigma-Aldrich) (89: 9: 1%, vol/vol). This specific formulation was critical in determining the drug administration route for the in vivo experiments. Indeed, the viscosity of the vehicle used for solubilizing the CEUs also prevented their administration by the intravenous route at volumes higher than 50 µl. Considering the solubility limit of CEUs for the vehicle, a volume of 50 µl was unable to reach the therapeutic doses required in our in vivo experiments.

Intraperitoneal administration was therefore chosen as an alternative route for systemic delivery of the drugs.

In vitro studies

Cell culture conditions. The human colorectal adenocarcinoma cell lines DLD-1, Caco-2, SW-948 and HT-29 were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, United Kingdom). The CT-26 mouse colon cell line was obtained from Dr. I.J. Fidler (MD Anderson Cancer Center, USA). Cell lines were maintained as a monolayer culture in minimum essential medium with Earle’s salts (MEM®, Gibco-BRL, France) supplemented with 10% foetal calf serum (Sigma-Aldrich), 1% solution of vitamins