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PRECLINICAL STUDIES

N-4-iodophenyl-*N*′-2-chloroethylurea, a novel potential anticancer agent with colon-specific accumulation: radioiodination and comparative in vivo biodistribution profiles

Emmanuelle Mounetou · Elisabeth Miot-Noirault · René C. Gaudreault · J. Claude Madelmont

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Summary In a search for more selective anticancer drugs, we have designed nitrogen mustard and nitrosourea conjugates leading to a series of *N*-4-aryl-*N*′-2-chloroethylureas (CEUs). The iodinated derivative *N*-4-iodophenyl-*N*′-2-chloroethylurea (4-ICEU) has demonstrated significant antineoplastic and antiangiogenic potency in preclinical evaluations. In this study, 4-ICEU was radiolabelled with [^{125}I]iodide in order to carry out a comparative study of its in vivo behavior profile. 4-[^{125}I]-ICEU was synthesized by direct electrophilic radioiodination with 80% radiochemical yield and 97% radiopurity. Three different routes of administration (intraperitoneal (ip), intravenous (iv) and intratumoral (it)) were tested in mice bearing subcutaneously implanted CT-26 murine colon carcinoma. The results clearly established that 4-ICEU was more stable to biotransformation than previously studied CEUs congeners. It was readily bioavailable and reached the CT-26 colorectal tumor regardless of the route of administration. Additionally, the colon mucosa was an important target tissue where 4-ICEU accumulated and remained largely untransformed. In conclusion, these results justify further investigations for developing 4-ICEU as a new chemotherapeutic agent for colorectal cancer.

Keywords Chloroethylurea · Anticancer agent · Colon specificity · Radioiodination

E. Mounetou (✉) · E. Miot-Noirault · J. C. Madelmont
INSERM UMR 484, EA 4231, BP 184,
63005 Clermont-Ferrand, France
e-mail: emmanuelle.mounetou@inserm.fr

E. Mounetou · E. Miot-Noirault · J. C. Madelmont
Université Clermont-Ferrand 1,
63001 Clermont-Ferrand, France

R. C. Gaudreault
Centre de Recherche, CHUQ, Hôpital Saint-François d'Assise,
10, rue de l'Espinay,
Québec, QC G1L3L5, Canada

Introduction

N-Aryl-*N*′-(2-chloroethyl)ureas (CEUs) are a new class of soft alkylating agents exhibiting potent antiangiogenic and antitumoral activities. We designed these compounds as conjugates of the aromatic ring of nitrogen mustards (e.g. chlorambucil) as the biofunctional moiety and the 2-chloroethylurea group of aliphatic nitrosoureas (e.g. carmustine) as the pharmacophore (Fig. 1) [1–3]. Our goal was to develop new drugs offering both a higher therapeutic index and a lower capacity to induce tumor chemoresistance in response to two majors challenges in modern cancer chemotherapy [4–6]. We previously reported that CEUs are not only more cytotoxic than the parent drugs (i.e. chlorambucil and carmustine) against a wide panel of tumor cell lines but also remain active in cell lines that have developed resistance to several antineoplastic agents [7–10]. In addition, CEUs do not exhibit mutagenicity [9]. Their main target is microtubules which are crucial components of the cytoskeleton in cancer cells and are involved in mitosis [11, 12]. CEUs act as antimitotic agents, preventing microtubule assembly through β-tubulin alkylation *via* covalent binding to the 198 glutamic acid residue leading to the arrest of cell cycle progression t in G2/M phase, and to apoptosis[13–15].

Preclinical evaluations carried out with the first lead compound of the series, i.e. *N*-(4-*tert*-butyl)phenyl-*N*′-

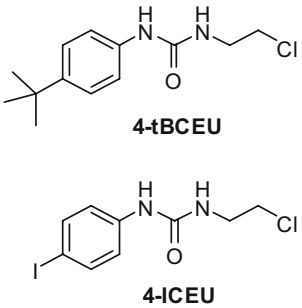


Fig. 1 Chemical structures of *N*-(4-*tert*-butyl)phenyl-*N*′-(2-chloroethyl)urea (4-tBCEU) and *N*-4-iodophenyl-*N*′-(2-chloroethyl)urea (4-ICEU)

(2-chloroethyl)urea (4-tBCEU; Fig. 1), showed that although its antineoplastic activity on animal models was limited mainly due to metabolic inactivation via hydroxylation of the *tert*-butyl group, 4-tBCEU exhibited a particular tropism for the gastrointestinal tract organs, notably the colon [16, 17]. This observation prompted us to carry out further investigations to assess the potential of CEUs for colorectal cancer treatment. We recently focused our efforts on the iodinated bioisostere *N*-4-iodophenyl-*N*′-(2-chloroethyl)urea (4-ICEU; Fig. 1) which exhibits not only improved antiproliferative and antiangiogenic activity in various cancer cell lines but also increased antitumoral activity and life-span in murine colon carcinoma [14, 17, 18]. We hypothesized that replacing the *tert*-butyl group with the bioisosteric iodine atom should increase in vivo stability. In addition, the presence of an iodine-substituted phenyl ring allowed its radioiodination. Indeed, the radioiodinated 4-ICEU would be a useful tool for confirming that the colon is a relevant target organ. ^{125}I radioisotope was chosen for its appropriate range of energy decay (35 keV) and half-life time ($T_{1/2}$=60 d) compatible with exploring in vivo tissue distribution *via* quantitative whole-body autoradiography [19, 20].

As part of the preclinical development of 4-ICEU as a potential anticancer agent, we studied the radiosynthesis of 4-[^{125}I]-ICEU and comparative pharmacokinetic profiles in CT-26 murine colon carcinoma-bearing mice with particular emphasis on in vivo stability and organ targeting. Three different administration routes were tested, i.e. intraperitoneal (ip), intravenous (iv) and intratumoral (it). These crucial data on the bioavailability of the drug candidate substantiated the anticipated colon tropism, and confirmed that the colon is a relevant target for future development of 4-ICEU in anticancer therapy.

Experimental

Chemistry

General

Proton nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard. Infrared (IR) spectra were recorded on a Bruker Vector 22 FTIR spectrometer. Melting points (mp), uncorrected, were determined on an electrothermal melting point apparatus. Elemental analyses were performed by the CNRS Service Central d'Analyses (Vernaison, France). Electrospray ionization mass spectrometry (ESI-MS) was performed on a Brüker ESQUIRE-LC mass spectrometer. Chemicals were supplied by Sigma-Aldrich Chimie SARL (St Quentin Fallavier, France). Sodium [^{125}I] iodide as no carrier added in solution reductant-free aqueous sodium hydroxide was purchased from Amersham Biosciences UK (Little Chalfont, England). Analytical thin layer chromatography (TLC) was conducted on precoated silica gel plates (SDS, 60 F254, 0.25 mm thick) with both detection by ultraviolet light at 254 nm and visualization by iodine. Radioanalysis of TLC plates was carried out by scanning with an Ambis 400 detector (B. Braun Scientec). High pressure liquid chromatography (HPLC) analysis were performed on a HP 1100 System with a UV/visible detector (Hewlett Packard, Les Ulis, France) equipped with a reverse phase Lichrocart column (Lichrosphere® RP18-e-, 5 μm, 125×4 mm, Hewlett Packard, Les Ulis, France) and connected to a 500TR flow scintillation analyser (Packard Instrument SA, Rungis, France). The flow rate was 1 mL/min with a gradient mobile phase starting from an acetonitrile/water mixture:30/70 (*v*/*v*) to 100/0 at 25 min. Specific activity was evaluated using an automated Packard 5530 gamma counter (EGG Instruments, Evry, France).

The standard *N*-4-iodophenyl-*N*′-2-chloroethylurea (4-ICEU) and the precursor *N*-phenyl-*N*′-2-chloroethylurea were synthesized as previously described [7].

Synthesis of N-4-[^{125}I]iodophenyl-N'-2-chloroethylurea (4-[^{125}I]ICEU)

To a 1 mL conical reaction vial fitted with a Teflon lined stopper containing a suspension of *N*-phenyl-*N*′-2-chloroethylurea (3 mg, 5 μmol) in a 0.25 M phosphoric acid aqueous solution (100 μL) was added sodium [^{125}I] iodide (37 MBq, 1 mCi) as a 0.15 M sodium iodide aqueous solution (50 μL) and a 0.15 M chloramine-T aqueous solution (50 μL). The mixture was magnetically stirred at 80°C for 20 min and the reaction was quenched by the addition of a 80 μM aqueous solution of sodium metabisulfite (50 μL). After cooling at 4°C, the vial was centrifuged at 3,000 rpm for 5 min. The precipitate was isolated after removal of the supernatant and several washings with water to afford 4-[^{125}I]ICEU in 80% radiochemical yield. The radioiodinated product comigrated with the unlabeled standard (*Rf*=0.25, eluent: ether/cyclohexane: 50/50, *v*/*v*). Radiochemical and chemical purity

was >97% as determined by HPLC analysis (one peak at 12.1 min). Analytical data for the unlabeled compound obtained with non-radioactive sodium iodide: mp 194–196°C; ^{1}H-NMR (DMSO-d_6) δ 3.40–3.44, 3.60–3.66 (2 m, 4H, CH_2CH_2Cl), 6.42 (broad s, 1H, N*H*CH_2, exchanges with D_2O), 7.21–7.54 (dd, 4H, Ar–H), 8.75 (broad s, 1H, Ar*N*H, exchanges with D_2O); ^{13}C-NMR (DMSO-d_6) 41.96 (NCH_2), 45.09 (CH_2Cl), 84.62, 120.80, 137.97, 140.94 (Ar–C), 155.62 (CO); IR (KBr) ν 3320 (NH), 1635 (C=O) cm^{-1}. MS (ESI): *m*/*z* 323.90 ([M^+], $C_9H_{10}ClIN_2O$, calcd 323.95). Anal. ($C_9H_{10}ClIN_2O$) C, H, N.

Pharmacokinetics studies

Six-week-old male BALB/c mice (Charles River Company, Lyon, France) were injected subcutaneously in the right flank with 2.5×10^5 mouse colon carcinoma CT-26 cells obtained from Dr. I.J. Fidler (MD Anderson Cancer center, USA). After 10 days, the studies were performed after iv, ip or it injection of 4-[^{125}I]-ICEU (185 kBq i.e. 10 mg kg^{-1}, 740 kBq i.e. 47 mg kg^{-1} and, 666 kBq i.e. 36 mg kg^{-1} respectively).

Blood kinetics and tissue distribution

Animals were sacrificed by CO_2 inhalation at various time intervals after 4-[^{125}I]-ICEU administration, and rapidly frozen by immersion in liquid nitrogen. They were then embedded in a 2% gel of carboxymethyl cellulose, frozen in liquid nitrogen and were sagittally sectioned at −22°C with a Reichert-Jung Cryopolycut cryomicrotome (Heidelberg, Germany). For each mouse, eight body sections selected at different levels were taken using no. 810 Scotch band tape (3 M, Saint Paul, MN, USA), dried for 48 h at −22°C and analyzed with an Ambis 4000 detector (B. Braun Sciencetec), which allows visualization and quantification of the radioactivity distribution in whole-body sections. The results were expressed as the percentage of the injected dose of radioactivity per gram of tissue (% ID/g). For the quantitation of radioactivity uptake within the colon mucosa, mice were sacrificed by CO_2 inhalation at various time intervals after administration. Colon was dissected out. Its content was eliminated and the mucosa washed. Radioactivity of mucosa aliquots was measured using an automated Packard 5530 gamma counter (EGG Instruments, Evry, France). The results were corrected for radioactive decay and expressed as the percentage of the injected dose of radioactivity per gram of tissue (% ID/g).

In vivo stability

Plasma, urine, rehydrated feces and organs homogenized in a Potter were sampled and extracted three times with methanol by magnetic stirring for 1.5 h at room temperature. The extracts were combined and centrifuged at 2,000×*g* for 10 min at 4°C. The supernatant volume was measured, aliquots taken and radioactivity determined. The recovery of radioactivity from extraction procedures was in the range of 80–85%. After evaporation of the extracts under reduced pressure of the extracts, the dry residues were dissolved in the HPLC mobile phase (acetonitrile/water:30/70 (*v*/*v*)) and filtered through Dynagard syringe filters (Spectrum Microgon, Laguna Hills, CA, USA). HPLC analysis was performed using the aforementioned HPLC conditions.

Excretion of the radioactivity

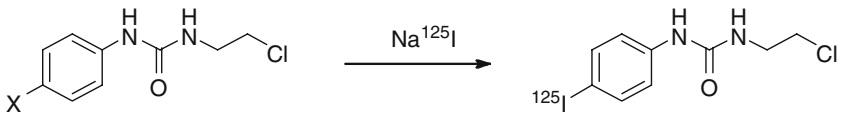
Animals (8) were housed in metabolic cages (Iffa-Credo, L'arbresle, France) enabling separate collection of feces and urine. Urine and feces were collected 24, 48, 72 h after administration. Radioactivity of urine and dried feces samples was directly measured using an automated Packard 5530 gamma counter (EGG Instruments, Evry, France).

Results

Chemistry

As high specific activity was not required, the most straightforward method for radioiodination of small molecules, i.e. iodine exchange, was first assessed [19, 20]. To that end, the reaction of 4-ICEU with sodium [^{125}I]-iodide was carried out at 135°C in citrate buffer in the presence of a catalytic amount of copper sulfate (Table 1, entry 1) [21]. However, radio-TLC analysis of the medium did not confirmed the formation of 4-[^{125}I]-ICEU. Another strategy using the well-described oxidative iododestannylation reaction was therefore devised [19]. Using the tributyltin group allows the regiospecific radioiodination of aryl rings in high radiochemical yield [22]. The widely used tri-*n*-butylstannylaryl precursor is prepared by tetrakis (triphenylphosphine)palladium-mediated stannylation of the aryl iodide in the presence of hexabutylditin in refluxing toluene [23]. However, this procedure yielded only 30% of the desired stannane, even after 24 h of reaction. Finally, the direct electrophilic iodination of *N*-phenyl-*N*′-2-chloroethylurea by sodium [^{125}I]-iodide in the presence of chloramine-T as oxidizing agent was attempted in aqueous medium under various pH conditions (Table 1, entries 2–4). Reaction time and temperature were also optimized. The iodination was initially carried out at the mg scale using non-radioactive sodium iodide to assess the regiospecificity of the reaction. The ^{1}H-NMR spectrum exhibited a doublet of doublets at 7.21–7.54 ppm that

Table 1 Radiosynthesis of 4-[^{125}I]-ICEU



Entry	*X*	Method	Conditions	Yield[a] (%)
1	^{127}I	Isotopic exchange	$CuSO_4$/citrate buffer, 135°C/1 h	0
2	H	Electrophilic iodination	Chloramine-T/H_2O/phosphate buffer, 80°C/30 min	50
3	H	Electrophilic iodination	Chloramine-T/H_2O/H_3PO_4, 60°C/20 min	65
4	H	Electrophilic iodination	Chloramine-T/H_2O/H_3PO_4, 80°C/20 min	80

[a] Radiochemical yield determined by radio-TLC analysis

confirmed the para-substitution of the aromatic ring and was in agreement with our previously reported NMR data on 4-ICEU [7]. In all the experiments, the labelled compound was identified by comparing its chromatographic behavior (TLC, HPLC) with that of the authentic non-radioactive standard prepared as previously described [7]. Finally, the reaction conducted in phosphoric acid at 80°C for 20 min gave optimal yield (Table 1, entry 4). After purification, 4-[^{125}I]-ICEU was obtained in 80% radiochemical yield and, 97% radiopurity, with a specific activity of 1.5 TBq mmol^{-1}.

Pharmacokinetic studies

Mice subcutaneously implanted with CT-26 murine colon carcinoma received an iv, ip or it injection of 4-[^{125}I]-ICEU (185 kBq, i.e. 10 mg kg^{-1}; 740 kBq, i.e. 47 mg kg^{-1} and, 666 kBq, i.e. 36 mg kg^{-1} respectively).

Blood kinetics Radioactivity levels in the blood of mice treated with either administered ip, iv or it 4-[^{125}I]-ICEU are shown in Fig. 2. In mice injected by the iv route, blood radioactivity kinetics displayed an initial stable phase with levels of around 4–4.5%ID/g up to 2 h post injection (pi) followed by a sudden and rapid clearance between 2 and 3 h pi and a final stabilization phase at around 1–2%ID/g until 6 h pi. Radioactivity was totally eliminated at 24 h after administration. In mice treated ip route, radioactivity concentration rapidly reached values of around 4%ID/g and remained relatively constant, with a maximum of 5.30±0.93%ID/g at 3 h pi. From four hours pi, the level of radioactivity decreased slowly, reaching 1.5±0.5%ID/g after 24 h.

Whereas blood radioactivity levels were initially similar in ip- and iv-treated mice, they decreased more rapidly over time in iv-treated mice as illustrated at the 3 h time-point (1.10±0.50%ID/g vs. 5.30±0.93%ID/g respectively). Blood radioactivity concentrations in of it-route-injected mice were lower than in ip- or iv-treated mice but were still significant (e.g., 1.84±0.27%ID/g at 2 h pi) even at early time-points, allowing a rapid whole-body distribution of the radioactivity (Table 2).

Tissue distribution Tables 2, 3 and 4 show the quantitative biodistribution of total radioactivity at time points from 15 min to 24 h pi in representative tissues of mice treated with 4-[^{125}I]-ICEU. These data were determined by quantitative whole-body autoradiography. However, γ-counting of tissue aliquots was used as a complementary method, especially for the evaluation of radioactivity levels in the colon, since it allowed isolation of the colon mucosa, thus avoiding contamination by its contents.

Following ip administration of 4-[^{125}I]-ICEU, radioactivity was widely distributed into most tissues. All tissues showed high uptake rapidly achieved 30 min pi. The

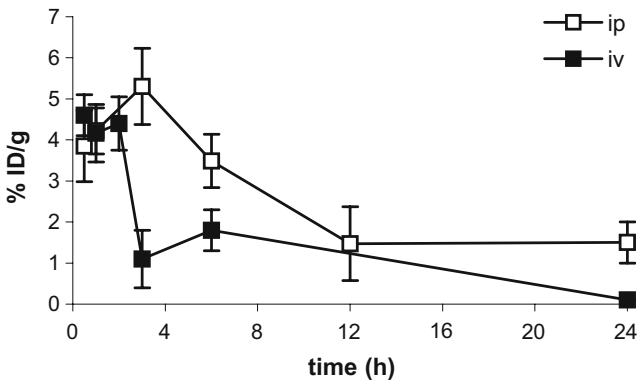


Fig. 2 Blood kinetics of total radioactivity following administration of 4-[^{125}I]-ICEU by ip or iv route in BALB/c mice implanted with murine CT-26 colon carcinoma. Values are expressed as the percentage of the injected dose per gram of blood and correspond to the mean±standard deviation for two animals for each time-point

Table 2 Biodistribution data for 4-[^{125}I]-ICEU after intraperitoneal (ip) administration to BALB/c mice implanted with murine CT-26 colon carcinoma

Tissue	Percentage of injected dose per gram of tissue (%ID/g)						
	15 min	30 min	1 h	3 h	6 h	12 h	24 h
Brain	1.13±0.44	2.12±0.23	2.21±0.52	2.72±0.30	0.28±0.25	–[b]	–[b]
Lung	2.53±0.20	5.38±2.32	4.73±0.99	5.01±0.15	3.15±0.93	–[b]	–[b]
Thyroid	–[b]	14.92±2.00	16.05±2.80	17.66±4.79	17.55±9.73	31.07±14.90	–[c]
Liver	3.85±0.31	7.18±1.97	5.94±0.89	6.68±0.56	2.51±0.68	1.47±0.25	2.44±0.59
Kidney	4.58±1.85	3.62±1.89	4.61±1.21	4.72±0.82	3.31±0.2	–[b]	–[b]
Spleen	–[b]	8.03±2.06	3.46±1.09	6.06±0.02	1.77±0.02	1.12±0.28	–[b]
Stomach	2.52±0.02	6.09±0.43	9.13±1.09	14.71±1.97	18.92±7.57	15.34±2.54	5.67[c]
Colon[a]	31.16[c]	12.96±1.43	10.51[c]	12.42±3.63	9.85[c]	6.32[c]	2.98±0.71
Tumor	1.29±0.19	2.61±0.53	2.64±0.54	3.27±0.32	2.40±0.46	2.40±0.30	2.57±0.61
Muscle	1.29±0.02	1.91±0.59	1.98±0.51	2.32±0.39	0.66±0.02	0.71±0.5	–[b]

Values are the mean±standard deviation for the quantification of eight whole-body sections from 11 animals
[a] Quantified using a γ counter after collection of the organ, elimination of the contents, and washings (see experimental section)
[b] Could not be quantified
[c] Quantified in one animal

radioactivity remained stable or increased slightly up to 3 h pi. It was cleared rapidly until 6 h pi and more gradually thereafter. The highest concentrations were found in the colon mucosa (12.96±1.43%ID/g), spleen, lung and liver (Table 2). The lowest radioactivity levels were observed in the brain and muscles. At 24 h pi, most of the radioactivity has been eliminated from the tissues, except for the stomach, liver and colon mucosa. In contrast, tumor uptake did not vary significantly over the time frame studied, and remained at around 2–3%ID/g globally, with. tumor (3.27± 0.32%ID/g), kidney and heart exhibiting intermediates values. Moreover, as generally observed with molecules directly radiolabelled with iodine, radioactivity accumulated in the thyroid and stomach.

After iv administration, tissues radioactivity kinetics generally followed four phases: rapid distribution in a large number of tissues, followed by stabilization at peak levels between 1 and 2 h, fast elimination between 2 and 3 h, and a final more gradual elimination up to 24 h (Table 3). The highest activities were detected in well-perfused tissues, particularly the lung (15.2±3.00% ID/g at 1 h pi) and the colon mucosa (18.90±2.60%ID/g at 1 h pi). At late time-points, high retention was still observed in these organs compared with the other tissues. High levels of radioactivity (8–10%ID/g) were also found in liver at 1 h pi, kidney and stomach at 2 h pi. Tumor and spleen levels of radioactivity rapidly became substantial (2.5–3.5%ID/g) and remained constant until 2 h post-administration.

Table 3 Biodistribution data for 4-[^{125}I]-ICEU after intravenous (iv) administration to BALB/c mice implanted with murine CT-26 colon carcinoma

Tissue	Percentage of injected dose per gram of tissue (%ID/g)					
	30 min	1 h	2 h	3 h	6 h	24 h
Brain	1.10±0.50	1.90±0.8	2.10±0.60	0.20±0.06	0.10±0.06	–[b]
Lung	14.50±2.20	15.2±3.0	12.60±2.50	2.80±0.70	5.00±1.30	0.80±0.20
Liver	7.20±1.70	10.9±3.20	7.80±2.10	1.40±0.90	1.80±0.30	0.40±0.20
Kidney	6.10±1.40	7.40±0.60	8.40±1.00	1.60±0.60	1.50±0.40	0.10±0.02
Spleen	2.40±0.50	3.90±0.80	4.10±0.60	0.70±0.30	1.30±0.50	0.30±0.20
Stomach	4.30±1.20	3.00±0.50	9.20±1.90	2.70±0.40	–[b]	–[b]
Colon[a]	5.70±1.50	18.9±2.60	13.6±3.40	3.00±1.80	4.00±1.60	0.10±0.01
Tumor	2.70±0.40	3.60±0.40	3.00±0.60	0.40±0.10	0.80±0.20	0.10±0.05
Muscle	1.50±0.60	2.30±1.30	2.10±0.20	0.30±0.10	0.40±0.10	–[b]

Values are the mean±standard deviation for the quantification of eight whole-body sections from eight animals
[a] Quantified using a γ counter after collection of the organ, elimination of the contents, and washings (see experimental section)
[b] Could not be quantified

Table 4 Biodistribution data for 4-[^{125}I]-ICEU after intratumoral (it) administration to BALB/c mice implanted with murine CT-26 colon carcinoma

Tissue/fluid	Percentage of injected dose per gram of tissue (%ID/g)		
	1 h	2 h	3 h
Blood	1.81±0.66	1.84±0.27	1.31±0.73
Lung	7.01±1.34	4.09±0.89	2.94±0.73
Thyroid	–[a]	10.96[b]	–[a]
Liver	3.83±1.05	1.83±0.39	1.48±0.15
Kidney	2.00±0.56	1.88±0.43	1.32±0.15
Stomach	0.46±0.01	3.25[b]	–[a]
Colon	7.90±1.90	29.36±10.20	23.15±4.40
Tumor	29.4±15.70	40.87±12.50	19.00±7.10
Muscle	1.28±0.63	0.36±0.11	0.50±0.45

Values are the mean±standard deviation for the quantification of eight whole-body sections from two animals
[a] Could not be quantified
[b] Quantified in one animal

Conversely, brain and muscles displayed the lowest uptakes at any given time.

Following it administration of 4-[^{125}I]-ICEU, more than 50%ID/g was distributed in all the organs examined at 2 h pi (Table 4). The peak radioactivity concentrations were observed in the lung, liver and, kidney at 1 h pi and in the stomach, thyroid, colon at 2 h pi. There was a slow elimination after this time-point. However, a particularly high accumulation of radioactivity in the colon was noted over the period examined (23.15±4.40%ID/g at 3 h pi).

In vivo stability The blood and target organs extracts collected from mice treated with 4-[^{125}I]-ICEU were analyzed by HPLC to determine the percentage of 4-[^{125}I]-ICEU in total radioactivity. The data obtained are presented in Fig. 3. The chromatographic profiles of the samples analyzed were very similar. Under the used HPLC conditions, 4-[^{125}I]-ICEU eluted at 12.0 min. The metabolites detected were more polar, with retention times of around 3.0 min and 8.0 min, and are still unidentified.

Comparing the administration routes, the stability of 4-[^{125}I]-ICEU was in the order it > ip > iv. For example, the percentages of unchanged ICEU in the liver at 1 h pi were 91%, 45% and 12% following it, ip and iv administration respectively. The highest rate of biotransformation was observed in the blood, and the percentage of 4-[^{125}I]-ICEU was invariably below 5% from 3 h after ip or iv administration. The fraction of 4-[^{125}I]-ICEU was higher in the tumor and colon, and declined more slowly than in blood at all time-points. In these organs, the levels of the intact form were in the 40–60% range at 1 h pi and decreased to 20–30% at 2–3 h pi. However, 4-[^{125}I]-ICEU remained significantly present in the tumor and colon at later time-points accounting for 15–20% of total radioactivity (i.e. 6 h pi for the iv route). Finally, metabolization in the colon was generally slower than in the tumor.

Excretion of radioactivity The radioactivity in urine and feces of mice given ip 4-[^{125}I]-ICEU was gradually and mainly eliminated in urine (>50% in 48 h). Fecal excretion was low (25% in 48 h). Finally, over 80% of the administrated dose was excreted within 72 h, with about 10% remaining in the carcasses.

HPLC analysis of fecal extracts and urine samples collected over 48 h indicated that intact 4-[^{125}I]-ICEU accounted for only 10% of total radioactivity.

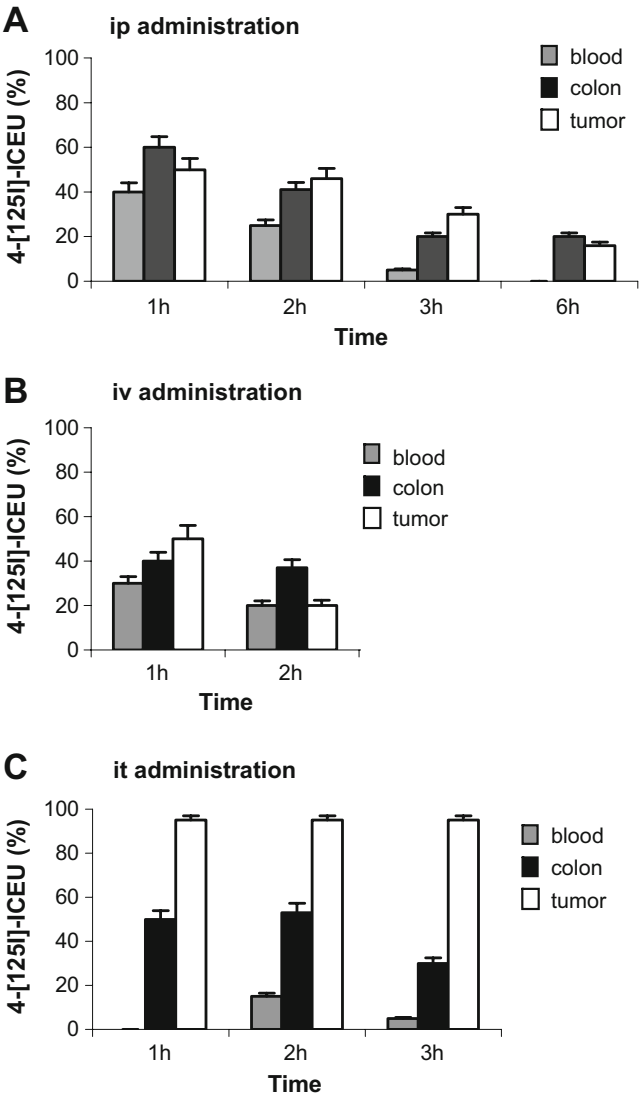


Fig. 3 Stability of 4-[^{125}I]-ICEU after administration to BALB/c mice implanted with murine CT-26 colon carcinoma. **a** ip route; **b** iv route; **c** it route

Discussion

The pharmacokinetics data presented here indicates that 4-ICEU is rapidly and widely distributed to most organs and tissues, whatever the route of administration. Additionally, blood and tissues kinetics displayed highly similar patterns. The peak concentrations were reached at 2 h pi for it- or iv-treated mice. For ip-treated mice, peak concentration were reached at 3 h pi, after which. elimination then occurred gradually over 72 h pi. Excretion was mainly urinary. As the biostability evaluation indicated that, 4-[^{125}I]-ICEU was stable in vivo up to 3 h pi, whatever the route of administration used, the radioactivity detected until that time can be considered as related to 4-[^{125}I]-ICEU. The HPLC analysis of blood and target organs demonstrated that the biotransformation of 4-ICEU was slow and not as extensive as for its previously studied congener 4-tBCEU [16]. These results confirmed that 4-ICEU possesses promising properties for future therapeutic development.

Tumor uptake was confirmed by tumor/blood ratios in the range of 0.6–0.9 before significant metabolization occurred at 2 and 6 h pi for ip and iv injection, respectively. Moreover, metabolization rate was generally slower in the tumor than in blood. These data are in accordance with our previous results [17–18]. The antitumor activity of it-administered 4-ICEU was reproduced in ip administered, with high tolerance and efficacy (around 60% tumor growth inhibition and 45% life-span increase). Representative slices showing the localization of 4-[^{125}I]-ICEU in the tumor are presented in Fig. 1. The highest levels of radioactivity were generally found in blood-rich tissues (lung, spleen) and in the metabolization and elimination organs (liver, kidney). As is usual for iodinated compounds, the thyroid and stomach displayed higher concentrations of radioactivity at later time-points. Conversely, the brain and muscles showed no accumulation of radioactivity at any time-points, whatever the route of administration.

There was important accumulation of radioactivity in the colon mucosa, irrespective of the route of administration. Radioactivity levels were significantly higher in colon mucosa than in non-target organs (brain, muscles) or blood. Colon/blood ratios ranged between 2 and 3, and metabolization was slower in the colon mucosa than in blood, independently of the route of administration used. The drug showed stability and retention in the colon, particularly after ip administration (20% of unchanged form remained at 6 h pi). Mice whole-body autoradiograms corroborated the biodistribution profile reported above and clearly showed a dense signal corresponding to the intestinal tract, more specifically the colon (Fig. 4). The substantial concentration of radioactivity detected in the colon at 2–3 h post it injection also confirmed the colon-specific localization and accumulation. These results are in agreement with the particular tropism for the gastrointestinal tract previously found for the first lead compound, 4-tBCEU [16].

In conclusion, 4-[^{125}I]-ICEU was obtained in high radiochemical purity and high yield by direct electrophilic iodination of *N*-phenyl-*N*′-2-chloroethylurea. Radiolabelled 4-ICEU proved a useful tool for assessing the in vivo behavior of the drug in mice bearing subcutaneous CT-26 colon carcinoma, consequently validating its potential for the treatment of colorectal cancers. Biopharmaceutical profiles for the three routes of administration tested showed that: (1) compared with a *tert*-butyl group, an iodine atom at the 4-position of the phenyl ring enhanced the biostability of the CEU, (2) 4-ICEU was biodistributed into the CT-26 colon carcinoma tumor, and (3) 4-ICEU accumulated specifically in the colon mucosa. Taken together, these properties combined with the previously demonstrated antineoplastic activity [14–17] clearly suggest that radiolabelled 4-ICEU is a candidate for colorectal cancer therapy, offering potential for developing new selective therapies for this resilient cancer [24]. Since the data presented here appear of utmost importance, further investigation on both the antitumor efficacy and biodistribution profiles of 4-ICEU will be performed in mice bearing orthotopically transplanted CT-26 colon carcinoma tumors [25].

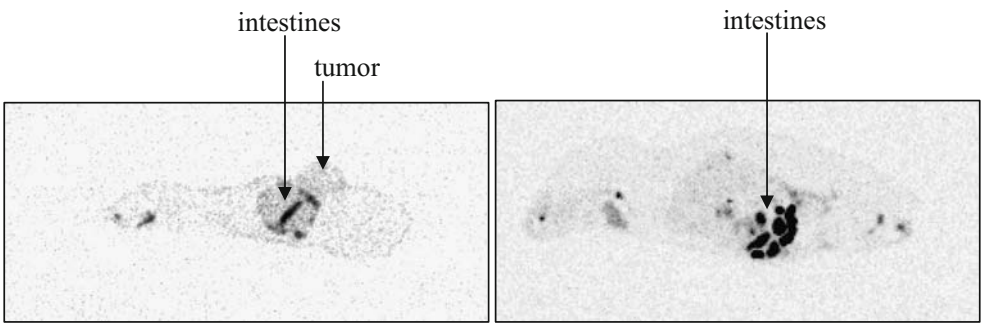


Fig. 4 Representative two-dimensional whole-body sagittal section autoradiograms of BALB/c mice implanted with murine CT-26 colon carcinoma at 2 h post-ip administration of 4-[^{125}I]-ICEU

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