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Phase I trial of interleukin-2 and high-dose arginine butyrate in metastatic colorectal cancer

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Abstract Introduction: Interleukin-2 (IL-2) and sodium butyrate allow rats to be cured of peritoneal carcinomatosis from colon cancer. We performed a phase I trial of IL-2 and high-dose arginine butyrate (ArgB) in patients with advanced metastatic colorectal cancer. **Patients and methods:** From April to July 1997, six patients were included in the trail; they had a median age of 52 years, four had a performance status of 0, two had a performance status of 1 with normal biological functions. All patients had received at least two prior lines of chemotherapy. A fixed dose of 18 MIU/m^2 IL-2,was administered by subcutaneous injection and ArgB was delivered via continuous intravenous infusion on days 1–6 with escalating doses starting at 2 g kg^{-1} day^{-1}. **Results:** The planned dose escalation was not possible because of toxicities. A daily ArgB dose of 2 g/kg was delivered for nine cycles. Level 2 (4 g/kg) could not be delivered in three of the six patients because of liver toxicity. The dose-limiting toxicities were fatigue and liver function disturbances. The maximum tolerated dose for ArgB was 3 g kg^{-1} day^{-1}, in combination with IL-2 at 12 MIU m^2 day^{-1}. No clinical response was seen. Pharmacokinetic analysis showed large intra- and interindividual variations. **Conclusion:** This schedule with a high dose of ArgB proved to be highly toxic with liver insufficiency. We will be running another trial with lower doses of ArgB calculated from the schedule used in the experimental model, starting at a dose of 20 mg kg^{-1} day^{-1} for ArgB and 200 000 UI kg^{-1} day^{-1} IL-2, every 8 h.

Key words Apoptosis · Immune stimulation · Cell differentiation · IL-2 · Arginine butyrate · Colorectal cancer

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Introduction

Colorectal cancer is a major public health problem in economically developed nations. In the European community, this malignancy is the commonest cancer with about 170 000 new cases diagnosed each year [4]. The first-line chemotherapy in advanced colorectal carcinoma currently is represented by 5-FU modulated with folinic acid. Recently new chemotherapeutic agents have been developed, including the topoisomerase I inhibitor irinotecan (campto) and a new platinum derivative, oxaliplatin (L-OHP). These allow a second-line chemotherapy to be prescribed for patients who have failed to respond to previous 5-FU-based chemotherapy. Unfortunately, and despite a significant improvement in quality of life with new chemotherapy regimens, the results in terms of survival remain very poor and a cure is exceptional for patients with evolutive metastatic colorectal carcinoma. Other therapeutic methods, such as immunotherapy, consist in increasing immune responsiveness against the tumour. Interleukin-2, the main growth factor of $CD8^+$ lymphocytes and natural killer (NK) cells, has been evaluated in patients with metastatic colorectal carcinoma but the results have not been conclusive [12]. Tumour cells can avoid the host's immune response by various means, including lack of antigen presentation and/or absence of accessory molecules involved in cell adhesion. Thus, a new combination, IL-2 and sodium butyrate has been studied in the laboratory. Sodium butyrate, a short-chain fatty acid, is an effective inducer of differentiation and

apoptosis in a variety of tumour cells, particularly in colon carcinoma cells [5]. In the laboratory, syngenic BDIX rats were injected intraperitoneally (i.p.) with colon carcinoma cells from the PROb clone, a weakly immunogenic model, as are human colon tumours [10]. Four groups of rats, with established tumours (day 10 after i.p. engraftment), were formed: a control group, which was injected i.p. with phosphate-buffered saline; the second and third groups receiving with i.p. treatment by sodium butyrate or IL-2 alone; the fourth group receiving the combination IL-2/sodium butyrate.

The rats in the untreated group died on about day 40 with widespread peritoneal carcinomatosis, whereas 60% of rats in the group receiving the combined treatment were still alive at day 200, free from disease and considered as cured with specific protection against the re-engraftment of PROb cells.

In the monotherapy groups, the median survival was significantly increased to 80 days ($P < 0.01$) but only by IL-2 treatment, and no rats were cured. The conclusion of these experiments was that sodium butyrate enhanced immunogenicity of the cancer cells by increasing the expression of MHC I and ICAM-1 in vitro. These phenotypic modifications also allowed colon cancer cells to be recognized by immune effectors, lymphokine-activated killer (LAK) cells and $CD8^+$ lymphocytes, stimulated and recruited by IL-2. Further results have shown that sodium butyrate treatment induces apoptosis in the colon cancer cells, which could play an important role in the activation of a specific immune response [1]. Moreover, sodium butyrate leads to the arrest of cell growth in G1 phase and this synchronization promotes cellular accumulation in the IL-2-sensitive phase of the cell cycle [8].

On the basis of these preclinical data, we designed a non-randomized phase I dose-escalating study of IL-2/arginine butyrate (ArgB) in metastatic colorectal cancer. The main objective of this study was to evaluate the toxicity of such a combination in an intrapatient escalating schedule, to determine the dose-limiting toxicity and maximum tolerated dose and possibly to recomand a dosing schedule in a phase II trial. Efficacy was a secondary end-point of the present trial; the pharmacokinetics of ArgB was measured and its immune function monitored (data not shown).

Patients and methods

Patient selection

Patients with a history of advanced colorectal carcinoma, not accessible to surgery, and progressing after one or more cytotoxic metastatic regimens, were candidates for this study.

Eligibility criteria also included age between 18 and 75 years, WHO performance status 0–2, a life expectancy of at least 3 months and a delay between discontinuation of the last chemotherapy and enrolment in this trial of a least 4 weeks. Adequate haematological function (absolute neutrophil count at least 2000/μl, haemoglobin level above 10 g/dl and platelet count superior to 100 000/μl), normal liver tests [total bilirubin level no more than 1.25 times the upper normal limits, aspartate aminotransferase (AST) and (ALT) levels at least twice the upper normal limits], and normal kidney parameters (serum creatinine below 150 μmol/l) were required. The left ventricular ejection fraction was calculated by radionuclide angiography and the normal index was greater than 50%. Before treatment, a central venous catheter was implanted subcutaneously. Patients with brain metastases or a past history of a prior malignancy (other than non-melanoma skin cancer or cervical carcinoma in situ) were not eligible. Concomitant treatment with corticosteroids was not allowed. Patients had to have at least one bidimensionally measurable lesion (one diameter of 20 mm or more), measurable by computed tomography (CT) scan according to WHO criteria. Measurable lesions had to be outside a previous radiotherapy field. The protocol was approved by the local ethics committee and all patients gave written informed consent before treatment.

Therapeutic agents

ArgB is a whitish-yellow compact mass with the typical strong smell of butyrate acid, very soluble in water or dilute alcohol but hardly soluble in ethanol. ArgB was provided by Aguettant laboratories (BP 7144, Lyon France) in glass bottles as a clear solution containing 250 g ArgB diluted in 1000 ml sterile water. The solution has a pH of 7.7 and contains 1770 mosmol/l. Recombinant human IL-2 (Proleukin) was supplied by Chiron laboratories, Suresnes, France. Before injection, 18 MIU IL-2 was reconstituted with 1.2 ml sterile water.

Treatment schedule (Table 1)

A fixed dose of 18 MIU/m^2 IL-2, divided into two daily doses separated by 14 h, was administered by subcutaneous injection on days 1–5. ArgB was delivered via continuous intravenous infusion over 6 h, twice daily, and was started 4 h after each injection of IL-2. On day 6, a continuous 24-h intravenous infusion of ArgB completed each cycle. The induction treatment was composed of four cycles: IA (days 1–6), IB (days 15–20), IIA (days 43–48) and IIB (days 57–62). The rationale of phase I trials is to increase doses of the drug under investigation with the aim of determining the dose-limiting toxicity and the maximum tolerated dose. Although low-dose ArgB combined with IL-2 seems to be adequate in the animal model for the antitumoral effect, we decided to follow the methodology of phase I studies with an intrapatient dose escalation of ArgB. At the time when the protocol was formulated, the determination of dose-limiting toxicity and maximum tolerated dose constituted the primary endpoint of the trial with the aim of defining the optimal dose for a future phase II trial. The planned dose-escalation scheme was to start with a dose of 2 g kg^{-1} day^{-1} ArgB and double this at each cycle (2, 4, 8, 16 g kg^{-1} day^{-1}). Doses of ArgB were escalated if no dose-limiting toxicities were seen during the previous cycle. Toxicity was assessed before each cycle

Table 1 Initial treatment schedule. Interleukin-2 (*IL-2*) was administered by subcutaneous injection in two daily doses separated by 14 h on days 1–5. Arginine butyrate (*ArgB*) was delivered via continuous intravenous infusion over 6 h, twice daily, on days 1–5 and via continuous 24-h intravenous infusion on day 6

Cycle	Days	Drug regime
IA (week 1)	1–5	IL-2 = 18 MIU m^2 day^{-1}
	1–6	ArgB = 2 g kg^{-1} day^{-1}
IIA (week 3)	15–19	IL-2 = 18 MIU m^2 day^{-1}
	15–20	ArgB = 4 g kg^{-1} day^{-1}
IIA (week 7)	43–47	IL-2 = 18 MIU m^2 day^{-1}
	43–48	ArgB = 8 g kg^{-1} day^{-1}
IIB (week 9)	57–61	IL-2 = 18 MIU m^2 day^{-1}
	57–62	ArgB = 16 g kg^{-1} day^{-1}

and recorded according to WHO toxicity criteria. Three consolidation cycles were scheduled at the maximum tolerated dose of ArgB for patients with complete or partial response at the time of response evaluation. Patients with progressive or stable disease after induction treatment were withdrawn from the study.

Pretreatment evaluation and follow-up studies

On entry into the study, a complete medical history was taken for all patients, who also received a full physical examination.

Laboratory procedures performed at baseline were a complete blood cell count with differential and platelet counts. A panel of biochemical markers was evaluated, including electrolytes, with calcium, phosphorus, magnesium and blood urea nitrogen, creatinine, total protein, albumin, uric acid, glucose, liver function tests, thyroid hormones (triiodothyronine, thyroxine, thyroid-stimulating hormone) and the tumour marker carcinoembryonic antigen. Viral serodiagnosis for hepatitis B, C and HIV was mandatory. A baseline ECG and radionuclide ventriculography were also obtained. The extent of metastatic disease was documented by thoracic and abdominal CT scans and bone scintigraphy. Following baseline evaluation, tumour sites were re-evaluated after cycle IIB (week 12) by CT imaging for determination of the size of measurable lesions. The follow-up during the infusion of ArgB included the recording of blood pressure, pulse and temperature, which was measured every 2 h and diuresis over 24 h was also determined. Complete blood cell counts with differential and platelet counts, liver (AST, ALT, alkaline phosphatase, γ-glutamyltranspeptidase and total bilirubin) and renal function tests (blood urea nitrogen and creatinine) were performed and electrolytes, osmolarity and prothrombin index were assayed every 48 h.

Toxicity assessment and dose adaptation

IL-2 and ArgB were witheld if the following toxic effects of grade 3 or 4 were noted, particularly in relation to IL-2 therapy: (1) hypotension resistant to intravenous vasopressor treatment, (2) cardiac dysfunction or ischaemia, (3) agitation or mental confusion, (4) respiratory distress, (5) hyperbilirubinaemia grade 3 or 4, (6) creatininaemia above 400 μmol/l, and (7) a prothrombin index below 40%. IL-2 was resumed at full dose when toxicities had resolved, and ArgB resumed at the dose of the previous cycle. If a new episode of toxic effects grade 3 or 4 occurred, doses of IL-2 and ArgB were reduced.

Pharmacokinetic analysis

Blood samples for the pharmacological investigations were drawn from a separate i.v. catheter into heparinized tubes and samples were centrifuged at 1000*g* for 10 min at 4 °C. The resulting plasma supernatants were transferred to individual polypropylene tubes and stored frozen at −20 °C. Butyric acid in plasma was measured on days 1 and 5 during the first 6 h of infusion, after 1 h, 3 h, 6 h, and 2, 5, 10 and 15 min after infusion. Butyric acid concentrations were measured by HPLC after derivatization with 2,4′-dibromoacetophenone employing crown ether catalysis [7]. The limit of sensitivity was 8 μg/ml. Intra- and interassay variations were lower than 10%. Pharmacokinetic parameters were estimated using MICROPHARM software [9] by a one-compartment model. The analysis focused on the area under the plasma concentration/time curve (AUC), the total plasma clearance and the half-life.

Results

Patient characteristics (Table 2)

Six patients with advanced colorectal carcinoma were entered into the study from April to July 1997; their relevant characteristics are listed in Table 2. Their median age was 52 years (range 45–73 years), their performance status was 0 (range 0–1) and all patients had received at least two prior chemotherapy treatments (range 2–4). The six patients had evolutive visceral disease: two with liver metastasis (patients 1 and 2) and four with liver and lung metastasis (patients 3, 4, 5 and 6). All patients received at least two courses of IL-2/ArgB and were assessable for toxicity. A total of 18 courses were delivered to six patients at six dose levels.

The median number of courses administered per patient was 3 (range 2 to 4). Because of severe side-effects, the planned dose escalation schedule was not possible and only two patients completed 4 courses, two received 3 courses while two completed 2 cycles only.

Toxicity

Dose-limiting toxicity: liver toxicity (Tables 3, 4)

In the first two patients enrolled, one developed a disseminated intravascular coagulation syndrome with thrombopenia grade 3, fibrinopenia of 0.7 g/l and prothrombin index of 7% at a dose level of 6 g kg^{-1} day^{-1} ArgB. This consumption coagulopathy was associated with hepatic cholestasis and cytolysis grade 4. The second patient had received 3 cycles but no dose escalation was performed because of hepatic toxicity (bilirubinaemia

Table 2 Patient characteristics

Characteristic	Patients					
	1	2	3	4	5	6
Age (years)	65	73	52	45	45	48
Sex	M	M	F	M	F	M
Performance status	0	1	0	0	0	1
Year of initial diagnosis	93	94	96	96	96	96
Site of primary disease	Sigmoid	Sigmoid	Sigmoid	Rectum	Sigmoid	Sigmoid
Site of metastases	Liver	Liver	Liver lung	Liver lung	Liver lung	Liver lung
Number of previous chemotherapy lines	2	4	2	2	3	2
Delay between last chemotherapy and first cycle of IL-2/ArgB	25 weeks	29 weeks	4 weeks	4 weeks	7 weeks	8 weeks

Table 3 Dose-limiting toxicity. *G* grade

Dose		No. of patients/courses	Dose-limiting toxicity
IL-2 (MIU m^2)	ArgB (g/kg)		
18	2	2/4	Bilirubinaemia G3 (2 cycles) and G4 (1 cycle)
	4	1/1	0
	6	1/1	Disseminated intravascular coagulation syndrome with hepatic cytolysis G4 and cholestasis G4
12	2	4/5	Prothrombin index: 47% (1 cycle)
	3	1/2	Prothrombin index: 47% (1 cycle)
	4	2/3	Prothrombin index: 16% (1 cycle)
	5	1/1	Bilirubinaemia G3
9	1	1/1	0

Table 4 Toxicities per patient implying dose modification of ArgB and IL-2. Patient 1, at a dose of 6 g kg^{-1} day^{-1} ArgB, developed a disseminated intravascular coagulation syndrome with hepatic cholestasis and cytolysis grade 4. Patient 2 received three cycles but no dose escalation was performed because of hepatic toxicity (bilirubinaemia G4). The dose of IL-2 was therefore reduced to 6 MIU m^2 day^{-1} on days 1–5, for patients 3, 4, 5 and 6 and no further dose of 6 g kg^{-1} day^{-1} ArgB was delivered. For patient 3, the dose of ArgB was reduced to 3 g kg^{-1} day^{-1} (cycles IIA and IIB) because of a decrease of the prothrombin index (*PI*). For patient 4, the dose of ArgB was reduced to 4 g kg^{-1} day^{-1} (cycle IIB) because of bilirubinaemia G3 (cycle IIA). For patient 5, the dose of ArgB was not increased because of a decrease of the prothrombin index. For patient 6, the decision to decrease the doses of ArgB and IL-2 (cycle IIB) was taken by the investigator owing to the toxic effects observed in the five previous patients. *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Patient	Cycle IA	Cycle IB	Cycle IIA	Cycle IIB
1	ArgB: 2 g kg^{-1} day^{-1}	ArgB: 4 g kg^{-1} day^{-1}	ArgB: 6 g kg^{-1} day^{-1} Bilirubinaemia G4 AST/ALT G4 PI: 7% Thrombopenia G3	
2	ArgB: 2 g kg^{-1} day^{-1} Bilirubinaemia G4	ArgB: 2 g kg^{-1} day^{-1} Bilirubinaemia G3	ArgB: 2 g kg^{-1} day^{-1} Bilirubinaemia G3	
3	ArgB: 2 g kg^{-1} day^{-1}	ArgB: 4 g kg^{-1} day^{-1} AST/ALT G3 PI: 16%	ArgB: 3 g kg^{-1} day^{-1}	ArgB: 3 g kg^{-1} day^{-1} PI: 47%
4	ArgB: 2 g kg^{-1} day^{-1}	ArgB: 4 g kg^{-1} day^{-1}	ArgB: 5 g kg^{-1} day^{-1} Bilirubinaemia G3	ArgB: 4 g kg^{-1} day^{-1}
5	ArgB: 2 g kg^{-1} day^{-1} PI: 62%	ArgB: 2 g kg^{-1} day^{-1} PI: 47%		
6	ArgB: 2 g kg^{-1} day^{-1}	ArgB: 1 g kg^{-1} day^{-1}		

grade 4) at the first dose level (2 g kg^{-1} day^{-1}). Thus, for the four subsequent patients, the dose of IL-2 was reduced to two treatments at 6 MIU m^2 day^{-1}on days 1–5, and no dose above 6 g kg^{-1} day^{-1} ArgB was delivered. Hepatic toxicity was the main side-effect of this regimen. Dose-limiting toxicity was reached in three of four patients with liver toxicity. Cholestasis and cytolysis grade 3–4 were seen in three (5 cycles) and two (2 cycles) patients respectively. Hepatic insufficiency was reflected by the prothrombin index dropping below 50% in 4/18 cycles (three patients). In these four cases, the perfusion of ArgB and subcutaneous injection of IL-2 were discontinued.

Haematological toxicity

Myelosuppression was moderate. Grade 3 anaemia occurred in 5 cycles and neutropenia grade 2 only in 1 cycle. Thrombopenia grade 3 was included in the disseminated intravascular coagulation syndrome experienced by patient 1 at 6 g kg^{-1} day^{-1} ArgB.

Hydroelectrolytic disorders

Hypokaliaemia (<3.5 mmol/l) developed during treatment in 14/18 cycles. This side-effect was easily managed by oral potassium supplementation. Hyperosmolarity tended to increase progressively with increasing of ArgB dose levels, and was explained by elevation of uraemia due to arginine metabolism. When the ArgB dose was 3 g/kg or more, hyperosmolarity required perfusion of a hypo-osmolar salt solution.

Clinical toxicities

All patients experienced mild to severe asthenia. Fever of grade 2–3 was reported in all cycles. An asymptomatic

decrease in blood pressure was observed with grades 3–4 in 11/18 cycles. Other clinical side-effects were rare and moderate, such as alopecia (grade 2 for three patients), skin toxicity (grade 2 for 3 cycles), diarrhoea (grade 1 for 4 cycles) and stomatitis (grade 1 for 3 cycles). Most striking was the liver pain during perfusion of ArgB in 8 cycles (5 grade 1 and 3 grade 2).

Antitumour activity

Four patients were assessable for response because two received only 2 cycles, owing to liver toxicity. No tumour responses were noted with IL-2/ArgB treatment.

Pharmacokinetics

The pharmacokinetics of butyric acid was evaluated in 15 out of 18 cycles on day 1 and in 12 out of 18 cycles on day 5. A large variability of parameters was observed between patients. The mean total clearance and half-life were, on day 1, 1022 ± 575 l/h and 3.7 ± 0.9 min respectively, and on day 5 they were 756 ± 341 l/h and 5.1 ± 2.7 min respectively. AUC values remained stable over the dose range of ArgB (mean value: 141 ± 99 μg h ml^{-1}).

Discussion

The treatment investigated in this phase I study, based on preclinical data, proved to be highly toxic with poor clinical tolerance and liver insufficiency. The clinical toxicity included side-effects previously reported for intravenous IL-2 infusion: major fatigue, fever and arterial hypotension. The choice of 2 g kg^{-1} day^{-1} as the first dose level of ArgB relied on a phase I trial of ArgB in patients with β-globin disorders [11]. In this study, six patients were included and minimal side-effects were observed, there being a transient increase in serum aminotransferase concentrations in one patient. In the case of our protocol, ArgB was associated with IL-2, a potentially hepatotoxic cytokine, and all patients had evolutive liver metastasis. In addition, identical toxicities including vomiting, hepatomegaly and liver insufficiency have been described in infants with deficient in arginine-metabolising enzymes [2]. The liver toxicity is probably due to the high dose of arginine used in our trial, rather than being butyrate-related. The replacement of sodium butyrate, used for treatment in rat colon cancer, by ArgB was recommended in order to avoid a high sodium load when added to IL-2. Theoretically, the role of arginine seems also to have important implications for the immunotherapeutic treatment of malignant disease in humans. Indeed, supplementation of the diet of patients with colorectal cancer with L-arginine, before surgery, significantly enhances the NK and LAK cell activity [6]. During the 6 h of ArgB infusion, the plasma concentration of butyric acid showed inter- and intraindividual variations. AUC values remained stable over range of ArgB doses investigated, indicating no relationship between dose and pharmacokinetic parameters.

Nevertheless, the total clearance rate observed in the present study was high, the volume of distribution was large and the half-life was very short, confirming previously reported results [7]. The analysis of the immune effects induced by IL-2/ArgB treatment was identical to that following treatment with IL-2 alone: an increase of total lymphocytes, NK-type cells ($CD16^+/CD56^+$) and MHC-class-II-positive T lymphocytes (data not shown). It is interesting to note that IL-2 activation was not inhibited by ArgB.

In conclusion, because of high liver toxicity, the present phase I study was discontinued after the inclusion of six patients. As for other phase I studies in oncology, the objective was to determine the maximum tolerated dose in order to elaborate a phase 2 study with an optimal dose of ArgB. In this trial we have therefore applied the methodology used with chemotherapeutic agents: an escalating dose and analysis of the secondary effects. However, the action of the biological response modifiers may be different from the antimitotic effects of chemotherapy, and the methodology of phase I studies is probably not appropriate for this new type of therapy. The dose of sodium butyrate administered in rats was much lower (10 mg kg^{-1} day^{-1}). It is possible that this low dose would be effective in man and cause no toxicity. Therefore, another clinical trial with a similar patient population will be conducted using lower doses of ArgB calculated from the schedule used in the experimental model (starting at a dose of 20 mg kg^{-1} day^{-1} ArgB and three doses of 200 000 IU IL-2) with which impressive results were obtained in animals. The unique mode of action, with induction of apoptosis through ArgB, production of antigen-positive apoptotic bodies, phagocytosis by monocytes/macrophages, immune presentation to IL-2-stimulated lymphocytes and tumour cell lysis with specific immune memory, renders this therapeutic modality very attractive and should be further tested in humans after the failure of chemotherapy.

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