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Liposomal Oxaliplatin in the Treatment of Advanced Cancer: A Phase I Study

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Abstract. ***Background:*** *Lipoxal is a liposomal oxaliplatin, which reduces the cytotoxic agent's adverse reactions without reducing effectiveness. Our objectives were to determine the adverse reactions, dose limiting toxicity (DLT) and the maximum tolerated dose (MTD) of Lipoxal.* ***Patients and methods:*** *Twenty-seven patients with advanced disease of the gastrointestinal system were included in the study. All patients had been pretreated with standard chemotherapy according to established guidelines. At entry, all patients had recurrent or progressive disease (stage IV gastrointestinal cancers: colorectal, gastric and pancreatic). Six lipoxal dose levels (100 mg/m^2, 150 mg/m^2, 200 mg/m^2, 250 mg/m^2, 300 mg/m^2 and 350 mg/m^2) were set and at least 3 patients were included at each level. Eight patients were treated at 300 mg/m^2 (MTD). The treatment was given once weekly for 8 weeks.* ***Results:*** *No serious side effects were observed at the first four dose levels (100-250 mg/m^2). At levels 5 and 6 mild myelotoxicity and nausea were observed. The most common adverse reaction was grade 2-3 peripheral neuropathy, observed in all 4 patients treated at 350 mg/m^2. The 350 mg/m^2 dose level was therefore, considered as DLT and the 300 mg/m^2 level as the MTD. Of the 27 patients, three achieved partial response and 18 had stable disease for 4 months, (range 2-9 months).* ***Conclusion:*** *The most common toxicity was peripheral neuropathy at the 300 and 350 mg/m^2 dose levels. Lipoxal was well tolerated and greatly reduced all of the other side effects of oxaliplatin, especially myelotoxicity and GI tract toxicities. These preliminary results showed adequate effectiveness in pretreated patients.*

Oxaliplatin, an analog of cisplatin, has shown a wide antitumor effect *in vitro* and *in vivo* and a better safety profile than cisplatin (1-7). The main adverse reactions are neurotoxicity and hematological and gastrointestinal toxicity (8). No nephrotoxicity has been observed, in contrast to cisplatin, and no hydration is needed during its administration. Kidney tubular necrosis has been rarely observed (8). The alkaline hydrolysis of oxaliplatin produces the oxalato monodentate intermediate complex (pKa 7.23) and the dihydrated oxaliplatin complex in 2 consecutive steps. The monodentate intermediate is assumed to rapidly react with endogenous compounds (9). The crystal structures of oxaliplatin bound to a DNA dodecamer duplex with the sequence 5'-d (CCTCTGGTCTCC) has been reported (10). The platinum atom forms a 1,2-intrastrand cross-link between two adjacent guanosine residues bending the double helix by approximately 30 degrees toward the major groove. Crystallography has provided structural evidence for the importance of chirality in mediating the interaction between oxaliplatin and duplex DNA (10). With oxaliplatin, like cisplatin, adduct lesions are repaired by the nucleotide excision repair system. Oxaliplatin, like cisplatin, is detoxified by glutathione (GSH)-related enzymes. ERCC1 and XPA expressions were predictive of oxaliplatin sensitivity in 6 colon cell lines *in vitro* (11). Oxaliplatin combined with 5Fluorouracil and folinic acid improved the response rate and progression-free and overall survival of patients with advanced colorectal cancer (12). The dose-limiting adverse reaction of oxaliplatin is neurotoxicity (sodium channel inactivation) and the kinetics are altered after exposure of animals to oxaliplatin. The results from preliminary clinical studies indicate that the sodium channel blockers carbamazepine and gabapentin may be effective in preventing neurotoxicity (13).

Liposomal encapsulation of oxaliplatin was achieved using Regulon's platform technology (data not shown). The new drug (lipoxal) has completed stability and chemical testing and one formulation was chosen for preclinical studies in January 2003.

Animal Data

Animal studies of a liposomal oxaliplatin (lipoxal) have shown the following. Intraperitoneal (*i.p.*) injection of lipoxal, or oxaliplatin as a control, in rats was used to study tissue biodistribution from 10 min to 7 days post-injection. The maxima levels of total platinum (Pt) in the plasma at a

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Key Words: liposomal oxaliplatin (lipoxal), gastrointestinal cancer.

Table I. *Lipoxal dose escalation*

Dose Level	Number of Patients	Lipoxal (mg/m^2 per week)
First	3	100
Second	3	150
Third	5	200
Fourth	4	250
Fifth	4+4	300
Sixth	4	350

dose of 15 mg/kg were 14.0 μg/ml after lipoxal injection compared to 7.5 μg/ml after oxaliplatin treatment; these levels were attained at 7-20 min after injection. Similar plasma pharmacokinetic behavior was observed for kidney tissue; the plasma and kidney had the highest levels of platinum during the first 20 min after injection. Spleen tissue exhibited over 2 times higher levels of platinum after oxaliplatin treatment, compared to lipoxal at the same dose level during an extended period of 40-190h post injection. Following 11 repetitive administrations of lipoxal in rats, the spleen attained astonishingly high levels of total Pt in all tissues examined (100 μg/g tissue). The liver exhibited similar pharmacokinetics of Pt accumulation as a function of time after oxaliplatin *versus* lipoxal treatment (data not shown). The liposomal form of oxaliplatin (lipoxal) reduces the adverse reactions of the encapsulated cytotoxic agent without reducing effectiveness.

The present study was a clinical trial with liposomal oxaliplatin (lipoxal) with the following primary objectives: a) to define the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of escalating doses of weekly lipoxal administration and, b) to detect the toxicity profile and pharmacokinetics of lipoxal monotherapy in pretreated advanced GI tract cancer patients. The secondary objectives were to determine efficacy and survival.

Patients and Methods

The study was a phase I cohort, dose-escalation trial of lipoxal. The study protocol was reviewed and approved by our Institutional Review Board. An informed consent document satisfying all institutional requirements was read by the patients and signed as a condition of their registration.

Eligibility criteria. All patients were required to meet the following criteria: confirmed histological or cytological diagnosis of cancer, at least one bidimensionally measurable or evaluable disease, WHO performance status 0-2, a life expectancy greater than 3 months, previous treatment by standard chemotherapy at the time of entry and to have been refractory to any prior cytotoxic treatment. Patients were eligible if they had had 2 or 3 previous courses, provided that they had been off treatment for at least 3 weeks

Table II. *Patients' characteristics at baseline*

		No.	%
Total number of patients		27	100
Age (yr)			
Median	62		
Range	32-78		
Gender			
Male		18	66.7
Female		9	33.3
Performance Status			
0		2	7.4
1		14	51.9
2		11	40.7
Stage IV		27	100
Primary Tumor			
Colorectal		12	44.4
Pancreas		8	29.6
Stomach		4	14.8
Biliary		2	7.4
Liver		1	3.7
Histology			
Adenocarcinoma		27	100

Assessment. The eligible patients over 18 years of age were required to have adequate hematological, renal and hepatic functions as defined by WBC count $3.5x10^9$/l, absolute neutrophil count $1.5x10^9$/l, platelet count $100x10^9$/l, hemoglobin level 9 g/dl, total bilirubin level 1.5 mg/dl, ALT and AST twice the upper normal limit in the absence of liver metastases or 5 times the upper normal limit in case of documented liver metastasis, and creatinine level 1.5 mg/dl. A medical history, physical examination, assessment of vital signs, electrocardiogram, chest and abdominal computed tomography (CT) or ultrasound were performed before treatment. During treatment (1 day before each course) blood count, blood urea and sugar, serum creatinine and uric acid tests, and ECG were done. CT scan assessments were done after at least 8 weekly drug infusions, or earlier in the case of disease progression.

Treatment plan. Lipoxal was supplied by Regulon Inc. Biotechnology Company, 715 North Shoreline Blvd, Mountain View, California 94043, USA. Drug characteristics: provided in 3 mg/ml, 50 ml per glass vial, 150 mg of oxaliplatin per glass vial; stored at 4°C, opaque appearance characteristic of a liposomal drug. Lipoxal is diluted in 1 L 5% dextrose and given in a 3-hour intravenous infusion once weekly for 8 consecutive weeks. In case of side-effects and, in particular, myelotoxicity or neurotoxicity, a delay of treatment by one week would take place. No pre- or post hydration was needed. No other drugs, such as antiemetic or antiallergic, were planned to be given prophylactically. In case of nausea or vomiting, support by antiemetics (ondansetron) or antiallergic (dexamethasone) were to be given

Table III. *Plasma pharmacokinetic parameter estimates for lipoxal in patients*

Dose (mg/m^2)	C_{max} (μg Pt/ml)	AUC (μg Pt*h/ml)	CI ($L/h*m^2$)	K_{el} (l/h)	$t_{1/2}$ (h)	V_{ss} (L/m^2)
250	9.175	424.4	0.289	0.028	24.3	9.7
350	12.087	782.3	0.219	0.020	35.5	10.9

Abbreviations and parameters as in the text

In preceding animal studies, approximately 400 $mg/m^2$to 600 mg/m^2were defined as the MTD. In humans, a dose of 100 mg/m^2 was introduced for level one and the dosage increase was decided at 50 mg/m^2 per level. The dose escalation of lipoxal per group is shown in Table I.

Drug-related toxicities were evaluated during each cycle of therapy and graded according WHO criteria. The DLT was defined as any grade 3 or 4 toxicity, with a neutrophil count <500 ml associated with fever persisting longer than 72 h, in 50% of the patients. Other grade 2-3 toxicities, in particular neurotoxicity, were also considered as DLT if observed in at least 50% of the patients. One dose level lower than that of DLT was defined as the MTD. Cohorts of a minimum of 3 patients were scheduled for entry at each dose level. The dose was escalated to the next higher level after all 3 patients had received at least one cycle. The treatment was discontinued with the occurrence of a DLT and the patient continued at one dose level below

Pharmacokinetics. For the pharmacokinetic study, blood samples were taken at the following hours: 0 (before drug infusion) and after the start of the infusion at 1, 3, 6, 24, 98, 120 (5 days) and 168 (7 days) hours. Three ml of blood were drawn into EDTA or heparincontaining tubes and were then centrifuged and refrigerated at 2°C and eventually sent for laboratory analysis for total platinum levels. Blood samples from 10 patients at the 250 mg to 350 mg/m^2 dose levels were used for the pharmacokinetic studies. The platinum levels (total and serum ultrafiltrates) were measured by atomic absorption (Perkin Elmer AA 700 Graphite Furnace Atomic Absorption Spectrometer at Regulon A.E.); the area under the plasma concentration-time curve (AUC) and the C_{max} (maximum concentration of total platinum in serum) were calculated. The total body clearance (Cl) was calculated by $CL=D_{iv}/AUC$, where D_{iv} is the intravenous dose of lipoplatin and AUC the relative Area Under Curve for a specific dose. The K_{el} (elimination rate constant) was calculated by linear regression analysis of the logarithmic plasma concentration-time curve by the formula $K_{el}=[Ln(C_{p1})Ln(C_{p2})]/(t_2-t_1)$, where $t_1$and $t_2$are the starting and ending time points of measurements and $C_{p1}$and C_{p2} the starting and ending concentrations of total platinum in serum for $t_1$and t_2, respectively.

The $t_{1/2}$(elimination half-time) was calculated by the formula $t_{1/2}=0.693\ (1/K_{el})$; $1/K_{el}$ is the MRT (mean residence time), the statistical moment analogy to half-life $t_{1/2}$(14). In effect, the MRT represents the time for 63.2% of the administered dose to be eliminated.

Results

Patients. The patients' characteristics are shown in Table II. In total, 27 patients were enrolled (age range 32-78 years, median 62 years, males 18, females 9, performance status (PS) 0-2). All patients had undergone previous chemotherapy treatment.

Toxicity. Lipoxal GI tract toxicity was negligible. Without antiemetics (ondansetron), nausea or mild vomiting was observed, but with ondansetron administration, no nausea/vomiting was observed nor was diarrhea. Mild, grade 1 myelotoxicity (neutropenia) was only seen in 2 patients (7.4%) at the highest dose level (350 mg/m^2). There was no hepatotoxicity, renal toxicity or cardiotoxicity, nor was alopecia seen. Mild asthenia was observed in 3 patients.

The main side effect was neurotoxicity, which was observed after at least 3 infusions of the agents; grade 1 neurotoxicity was seen at the 3rd and 4th dosage levels, grade 2 at the 5th level and grades 2-3 in 100% of the patients at the 6th level.

On the basis of these results, grade 2-3 neurotoxicity was considered as the DLT which was observed in 100% of patients treated with 350 mg/m^2 of lipoxal; therefore 300 mg/m^2 was defined as the MTD. The lipoxal dose escalation and the number of patients treated at each of the 6 levels are presented in Table I.

Pharmacokinetics. The results regarding the pharmacokinetics are presented in Table III and in Figures 1 and 2. The halflife of oxaliplatin in the plasma was found to be 24.3 h at 250 mg/m^2 and 35.5 h at 350 mg/m^2 (Table III).

Compliance with treatment. A total number of 104 infusions (cycles) were administered, with a median of 4 cycles per patient (range 2-15). The median interval between cycles was 7 days. The dose intensity was 100% of that planned. No patient had a delay in treatment due to grade 3 or 4 hematological toxicity; however, patients at a dosage of 350 mg/m^2 after a maximum of 4 or 5 infusions (cycles) had a

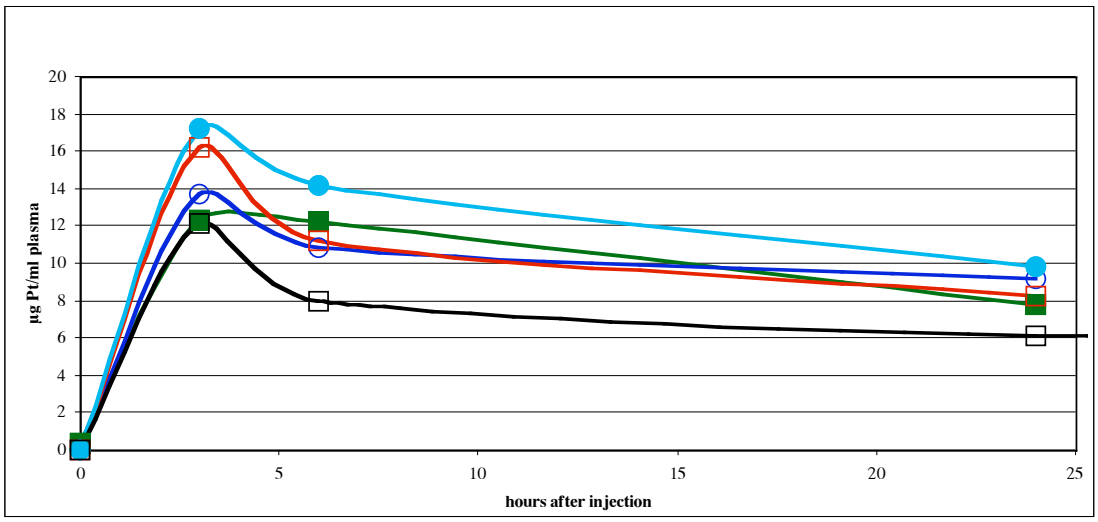

Figure 1. *Pt levels of 5 patients during lipoxal chemotherapy (0-25 hours). Lipoxal dose: 350 mg/m^2.*

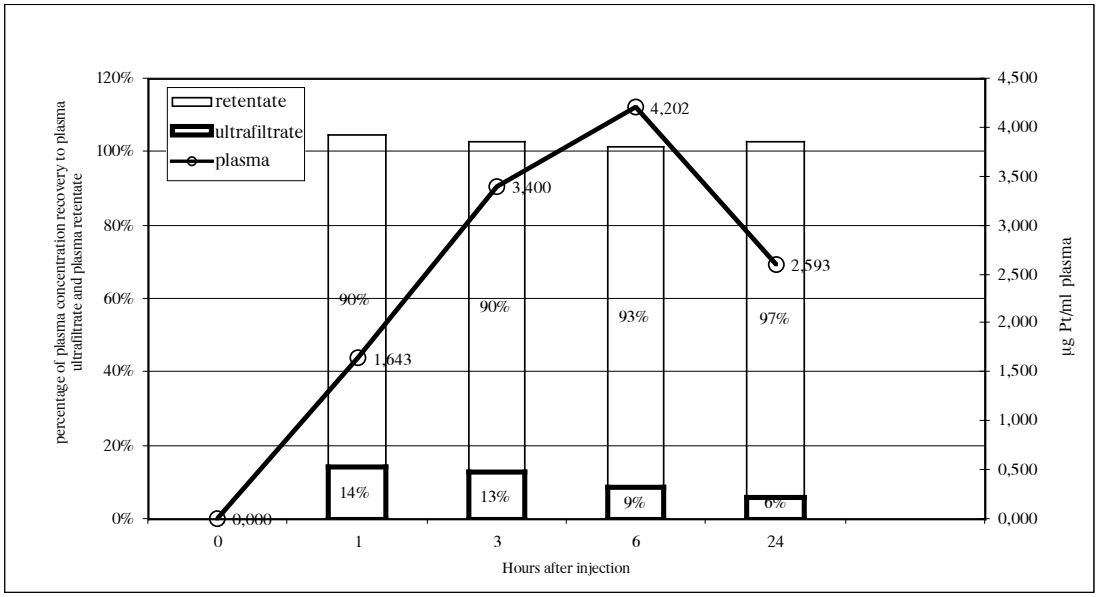

Figure 2. *Distribution of Pt levels in plasma during lipoxal chemotherapy treatment. Lipoxal dose: 420 mg (250 mg/m^2).*

two-week interval before the lower dose of 300 mg/m^2 was administered. Seventeen (63%) patients stopped treatment due to disease progression after 4-6 cycles. Twelve patients (44.4%) were still alive at the end of the study.

Response to treatment. Responses were analyzed on an intention-to-treat basis. There were no complete responses. Three out of 27 patients (11.1%) achieved a partial response;two of these patients had gastric cancer, one of whom had pleural effusion and the other had bone metastases; the third was a patient with liver metastases from colon carcinoma. The determination of a partial response was based on a CTscan for the first patient, a bone scan for the second patient and a CT-scan and a bilirubin serum level value for the third patient. The third patient was treated while the serum bilirubin level was 51 mg/dl and after 2 courses of treatment the level dropped to 8 mg/dl and lasted for 5 weeks.

The duration of response was 4, 7 and 2 months for each of the above patients, respectively. Eighteen (66.7%) patients achieved stable disease with a median duration of 4 6 months (range 2-9 months). Six patients showed disease progression. In all 3 responders there was also a reduction of

50% or more of the marker CA-19-9 and PS improved from 2 to 1.

Discussion

Discussion Liposomal oxaliplatin (lipoxal) was tested in the present trial as monotherapy in patients with advanced cancer of the gastrointestinal system. All patients had undergone standard pretreatment and all the included colorectal cancer patients had also been treated with oxaliplatin. The treatment with lipoxal has only had prior testing in preclinical studies and no other clinical trial has been previously performed. The present trial was based on preclinical study data and data on non-liposomal oxaliplatin. The latter mainly helped in focusing our present trial on estimating the similarities or differences of liposomal oxaliplatin versus standard oxaliplatin; GI tract and hematological side-effects were not observed with the former. The only side-effect that remained without any difference i.e. any reduction, was neurotoxicity. Neurotoxicity was often seen analogously with the increase in dosage of the agent; this became the only or main criterion for defining DLT. The MTD defined dose was 300 mg/m^2 administered weekly. There was also cumulative neurotoxicity as is also the case with non-liposomal oxaliplatin. With respect to effectiveness, the 11% response rate observed in pretreated patients refractory to previously established tumors could be meaningful in future trials in a combined chemotherapy modality. It is also important to point out that the cancer types selected for this trial are not those which are the most sensitive to chemotherapy.

Liposomal oxaliplatin (lipoxal) is a well tolerated agent. The dose of 300 mg/m^2 was established as the MTD but further investigation is needed, particularly with other agents in combination. GI tract and bone marrow toxicities are very much reduced, compared to the standard form of oxaliplatin. The only adverse reaction was neurotoxicity which defined DLT.

References

1. De Gramont A, Vignond J, Tournigand C, *et al*. Oxaliplatin with high-dose leucovorin and 5-fluorouracil 48-h continuous infusion in pretreated metastatic colorectal cancer. Eur J Cancer 1997; *33*: 214-219.
2. Giaccheti S, Perpoint B, Zidani R, *et al*. Phase III multicentre randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first line treatment of metastatic colorectal cancer. J Clin Oncol 1997; *18*: 136-147.
3. De Gramont A, Figer A, Seymour M, *et al*. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000; *18*: 2938-2947.
4. Souglakos J, Mavroudis D, Kakolyris S, *et al*. Triplet combination with irinotecan plus oxaliplatin plus continuous-infusion fluorouracil and leucovorin as first-line treatment in metastatic colorectal cancer: a multicenter phase II trial. J Clin Oncol 2002; *20*: 2651-2657.
5. Scheithauer W, Kornek GV, Raderer M, *et al*. Randomized multicenter phase II trial of two different schedules of capecitabine plus oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2003; *21*: 1307-1312.
6. Goldberg RM, Sargent DJ, Morton RF, *et al*. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2004; *22*: 23-30.
7. Sorbye H, Glimelius B, Berglund A, *et al*. Multicenter phase II study of Nordic fluorouracil and folinic acid bolus schedule combined with oxaliplatin as first-line treatment of metastatic colorectal cancer. J Clin Oncol 2004; *22*: 31-38.
8. Cassidy J, Misset JL. Oxaliplatin-related side effects: characteristics and management. Semin Oncol 2002; *29 (Suppl 15):* 11-20.
9. Jerremalm E, Eksborg S, Ehrsson H. Hydrolysis of oxaliplatin-evaluation of the acid dissociation constant for the oxalato monodentate complex. J Pharm Sci 2003; *92*: 436-438.
10. Spingler B, Whittington DA, Lippard SJ. 2.4 crystal structure of an oxaliplatin 1,2-d (GpG) intrastrand cross-link in a DNA dodecamer duplex. Inong Chem 2001; *40*: 5596-5602.
11. Arnould S, Hennebelle I, Canal P, Bugat R, Guichard S. Cellular determinants of oxaliplatin sensitivity in colon cancer cell lines. Eur J Cancer 2003; *39*: 112-119.
12. De Vita F, Orditura M, Matano E, *et al*. A phase II study of biweekly oxaliplatin plus infusional 5-fluorouracil and folinic acid (FOLFOX-4) as first-line treatment of advanced gastric cancer patients. Br J Cancer 2005; *92 (9)*: 1644-1649.
13. Lersch C, Schmelz R, Eckel F, *et al*. Prevention of oxaliplatin-induced peripheral sensory neuropathy by carbamazepine in patients with advanced colorectal cancer. Clin Colorectal Cancer 2002; *2*: 54-58.
14. Gibaldi M, Perrier D. Non-compartmental analysis based on the statistical moment theory. In: Pharmacokinetics. 2nd edition. New York: Marcel Dekker 1982; 409-417.

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