Administration of reduced glutathione in FOLFOX4 adjuvant treatment for colorectal cancer: effect on oxaliplatin pharmacokinetics, Pt-DNA adduct formation, and neurotoxicity

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Oxaliplatin is a promising drug for cancer therapy and the oxaliplatin/5-fluorouracil/leucovorin (FOLFOX) regimen has become the standard adjuvant treatment for colorectal cancer. However, the oxaliplatin-induced neurotoxicity still represents a clinical problem leading to a discontinuation of the therapy. Many strategies have been proposed in order to manage the neurotoxicity, but their effect on antitumoral efficacy is still unclear. In this study, we investigated the effect of reduced glutathione administration on neurotoxicity, oxaliplatin pharmacokinetics, and platinum-DNA (Pt-DNA) adduct formation in patients affected by colorectal cancer treated with FOLFOX4 adjuvant regimen. Twenty-seven patients were randomized to receive GSH 1500 mg/m^2 or saline solution before oxaliplatin infusion. Evaluation of neurotoxicity, pharmacokinetics of plasmatic total and ultrafiltered Pt, and determination of Pt-DNA adduct formation on white blood cells was performed during the 5th, 9th, and 12th cycles. At the end of all cycles of therapy, the patients in the GSH arm showed a statistically significant reduction of neurotoxicity ($P=0.0037$) compared with the placebo arm. There were no significant differences in the main pharmacokinetic parameters between the two arms except a lower area under the plasma concentration–time curve and a smaller apparent steady-state volume of distribution (V_{ss}) when GSH was coadministered. This difference can be explained by the natural function of GSH in the detoxification of oxaliplatin and by its ability to remove the Pt bound to plasma proteins. The determination of Pt-DNA adduct formation shows no statistically significant differences between the two arms. In conclusion, this study indicates that coadministration of GSH is an effective strategy to reduce the oxaliplatin-induced neurotoxicity without impairing neither the pharmacokinetics of oxaliplatin, nor the Pt-DNA adduct formation. *Anti-Cancer Drugs* 20:396–402 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

In recent years, oxaliplatin-based chemotherapy protocols, particularly oxaliplatin in combination with infusional 5-fluorouracil/leucovorin (FOLFOX), have emerged as the standard of care in adjuvant treatment and in first-line and second-line therapy of advanced-stage colorectal cancer [1–3].

After intravenous administration, about one-third of oxaliplatin binds irreversibly to erythrocytes and one-third forms complexes with albumin and other plasma proteins [4]. The remaining, one-third, free fraction of oxaliplatin undergoes a series of nonenzymatic biotransformations and forms complexes with DNA, which block DNA replication and transcription, and endogenous S-donor ligands such as glutathione (GSH), methionine, and cysteine [5]. The reaction with S-donor ligands plays an important role in the process of detoxification from platinum (Pt) and could even act as a drug reservoir for platination of DNA, as suggested by a number of studies carried out *in vitro* and *in vivo* with cisplatin and oxaliplatin [6,7].

Oxaliplatin is characterized by a spectrum of activity and toxicity different from that of cisplatin and carboplatin. It exhibits no nephrotoxicity and moderate hematotoxicity at clinical doses, but it causes both a reversible acute, cold-related dysesthesia, occurring in more than 90% of patients, and a dose-limiting, cumulative, chronic peripheral sensory neuropathy, that occurs in 10–18% of patients after 4–6 months, when the cumulative dose of oxaliplatin approaches 800 mg/m^2. In about three-fourths of patients, neurotoxicity is reversible with a median time to recovery of 13 weeks after treatment discontinuation [6,8–10].

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Preclinical and clinical trials studied the effect of coadministration of different agents (e.g. calcium–magnesium infusions, antiepileptic drugs, and GSH) against neurotoxicity induced by oxaliplatin: many agents have shown some activity in the prophylaxis and treatment of neuropathy, however, their effect on cumulative neurotoxicity is still not completely defined [6].

Chronic oxaliplatin-induced neurotoxicity is attributed to the accumulation of Pt-based compounds in the dorsal root ganglia, followed by the dorsal root and peripheral nerves, because of a lower clearance of the drug rather than an increased cellular uptake [6,8,9]. These findings suggested the coadministration of reduced GSH to patients treated with oxaliplatin in order to prevent the initial accumulation of Pt adducts in the dorsal root ganglia and thus reducing the neurotoxicity.

The efficacy of coadministration of GSH for the prevention of cisplatin-induced neurotoxicity, without reduction of the clinical activity, was established in a series of preclinical and clinical studies [7,11–14]. The effect of GSH on Pt-protein binding and the formation of Pt-DNA adducts was evaluated in ex-vivo studies in whole blood: the results showed that GSH is able to reduce the Pt-protein binding formation and promote the release of Pt from proteins, moreover GSH slightly reduces Pt-DNA adducts formation [15]. Recently, a randomized, double-blind, placebo-controlled trial to assess the efficacy of GSH in the prevention of oxaliplatin-induced neurotoxicity, was conducted by Cascinu *et al.* [16]. In this study, 52 patients treated with the FOLFOX regimen, were randomized to receive GSH or saline solution coadministered with oxaliplatin; the results showed a statistically significant reduction of neurotoxicity in the GSH arm, together with a statistically significant reduction of sural sensory nerve conduction in the placebo arm but not in the GSH arm.

The results obtained by Cascinu *et al.* [16] suggest that GSH could be a promising drug for the prevention of oxaliplatin-induced neuropathy without apparently reducing the clinical activity of oxaliplatin. However, the major limit of this study is the lack of unambiguous evidence that the activity of oxaliplatin (e.g. the ability of Pt to react with tumor DNA) is not affected by GSH administration.

The aim of this work is to study the effect of coadministration of GSH in patients treated with the FOLFOX4 adjuvant regimen, both on the main pharmacokinetic parameters of total (protein bound and unbound) and ultrafiltrable (unbound) Pt, and on the formation of Pt-DNA adducts. As tumor tissue is not easily available, the amount of Pt-DNA adducts in the white blood cells (WBC) has been considered as model for the amount of adducts in tumor tissue: this choice is supported by several studies that showed a correlation between Pt-DNA adducts in leukocyte and disease response in patients receiving Pt-based chemotherapy [17,18]. A differentiation of WBC is not required, as the adduct formation is independent from the relative amount of lymphocytes, granulocytes, and monocytes [19].

Methods

Patient population

A randomized trial was conducted in 27 patients treated with the FOLFOX4 adjuvant regimen after curative resection of colorectal cancer in order to investigate the effects of GSH administration on pharmacokinetics of Pt and formation of Pt-DNA adducts. Patients who had undergone complete resection of histologically proven stage T1-4N1M0 colon cancer, were eligible. Treatment had to be started within 7 weeks after surgery. Other eligibility criteria included an age of 18–75 years; a Karnofsky performance status score of at least 60; a carcinoembryonic antigen level of less than 10 ng/ml; the absence of prior chemotherapy, immunotherapy, or radiotherapy; and adequate blood counts and liver and kidney function. Patients were excluded if they had clinically established neuropathy, diabetes mellitus, alcoholic disease, or other neurological diseases. All patients signed a written informed consent. The study was conducted in accordance with the Tokyo–Helsinki Declaration and Good Clinical Practices, and was approved by the local ethics committee.

Pretreatment and follow-up evaluations

General assessment before treatment included medical history and physical examination, including a detailed neurological and hematological examination, blood chemistry (measurement of carcinoembryonic antigen level, creatinine, urea, glucose, transaminases, γ-glutamyl transferase, alkaline phosphatase, ionogram, bilirubin, protidogram, and electrophoresis), urine, electrocardiogram, chest radiograph, and abdominal ultrasonography or computed tomography. Blood tests were carried out weekly throughout the course of therapy. Patients were monitored for adverse effects throughout the treatment period and until 28 days after the last cycle of chemotherapy, unless treatment-related adverse effects required additional follow-up. Neurological examination was carried out during the 5th, 9th, and 12th cycles or if the patient showed symptoms, and repeated 3 and 6 months after the last cycle of treatment. Neurological adverse effects were assessed with the use of the neurosensory section of the Common Toxicity Criteria of the National Cancer Institute, Version 3.

Treatment plan

Treatment consisted of oxaliplatin 85 mg/m^2 on day 1, given as a 2-h infusion in 250 ml of 5% dextrose, concurrent with the 6-*S* stereoisomer of leucovorin 200 mg/m^2 as a 2-h infusion followed by bolus 5-fluorouracil 400 mg/m^2 and a 24-h infusion of 5-fluorouracil 600 mg/m^2/day for two

consecutive days. Therapy was repeated every 2 weeks, for a maximum of 12 cycles. GSH was given at a dose of 1500 mg/m^2 in 100 ml of physiological saline over a 15-min period immediately before each oxaliplatin administration to GSH-randomized patients, whereas physiological saline solution was administered to placebo-randomized patients. Routine antiemetic prophylaxis with dexamethasone 8 mg and a 5-hydroxytryptamine-3 receptor antagonist was used for both treatment arms. Adverse effects were graded according to the Common Toxicity Criteria of the National Cancer Institute, Version 3. Dose reductions were based on the worst adverse effects observed during the previous cycle. The dose of oxaliplatin was to be reduced to 75 mg/m^2 in the event of persistent (at least 14 days) paresthesias, temporary painful paresthesias, or functional impairment. Oxaliplatin was discontinued in cases of persistent painful paresthesias or functional impairment. Together with reductions in the dose of oxaliplatin, the bolus dose of fluorouracil was reduced to 300 mg/m^2 and the infusion to 500 mg/m^2 in the event of grade 3 or 4 neutropenia or thrombocytopenia (or both), diarrhea, stomatitis, or other drug-related adverse effects of grade 3. Only the dose of fluorouracil was scheduled to be reduced in the event of skin-related adverse effects of grade 3 or 4. Treatment was delayed by up to 3 weeks until the patient recovered from various adverse effects, the neutrophil count exceeded 1500/mm^2, and the platelet count exceeded 100 000/mm^2. Chemotherapy was discontinued in the event of cardiac or neurocerebellar adverse effects or grade 3 or 4 allergic reactions.

Blood samples

During the 5th, 9th, and 12th cycles, blood samples (10 ml) were collected in heparinized tubes from a large vein in the arm not receiving the drug infusion, immediately before GSH or placebo administration, at the end of oxaliplatin infusion as well as 1, 2, and 48 h after the end of oxaliplatin infusion.

Each sample of 3 ml was immediately centrifuged at 2500*g* for 10 min at 4°C to separate the plasma. The fraction of ultrafiltered plasma was obtained by ultrafiltration in Centricon 10 membranes (Amicon Division, Beverly, Massachusetts, USA). Plasma and ultrafiltered plasma samples were immediately frozen and stored at −70°C until analysis. The remaining blood was used to prepare WBC for analysis of Pt-DNA adducts (see below).

Analytical procedures

Analysis of Platinum in total and ultrafiltered plasma

A 3030-Z Zeeman spectrometer, equipped with an AS-60 autosampler (Perkin Elmer, Fremont, California, USA), was used to analyze Pt in total and ultrafiltered plasma. Pt hollow-cathode lamp operated at 30 mV voltage. During the atomization stage, absorbance was monitored with Zeeman correction and peak heights of absorbance profiles were recorded at 265.9 nm.

Determination of platinum-DNA adducts in white blood cells

DNA platination in WBC was determined by a four-step procedure consisting of isolation of WBC from whole blood, separation of DNA, quantification of DNA, and quantification of Pt bound to DNA using a modification of the method by Kloft *et al.* [20].

In brief, WBC were isolated using density-gradient centrifugation (Polymorphprep; Axis-Shield, Oslo, Norway) and two bands (mononuclear and polymorphonuclear cells) were harvested and pooled. Then, the cells were washed twice with ice-cold PBS to remove other blood components and the gradient medium.

The isolation of DNA from WBC was performed by solid-phase extraction with QIAamp DNA-blood kits (Qiagen, Hilden, Germany). The isolation procedure consisted of the lysis of WBC and adsorption of DNA onto a silica membrane followed by two washing steps in order to remove other cell components. In the last step, DNA was eluted from the column. All DNA samples were stored at −20°C until further analysis. The DNA concentrations and the purity of the isolated DNA were determined by ultraviolet spectrometry measuring the absorption at 260, 280, and 320 nm. This method was validated and met the requirements on bioanalytical methods.

The quantification of Pt bound to DNA was performed by a validated adsorptive stripping voltammetry method. This highly sensitive method, described by Weber *et al.* [21], allowed the determination of Pt with a lower limit of quantification of 0.4 pg/ml [22,23]. In brief, the residue of the dried eluate was decomposed to mineralization using a high-pressure asher (Kürner, Rosenheim, Germany). A detailed description of the mineralization process is given by Messerschmidt *et al.* [23]. Then, Pt was quantified by adsorptive stripping voltammetry as described by Pieck *et al.* [24]. On the basis of the DNA and Pt concentrations, the Pt/nucleotide ratio was calculated using the relative atomic mass of Pt (A_r (Pt) = 195.1) and the relative molecular mass of nucleotides (M_r (nucleotide) = 330).

The between-day precision for the whole method consisting of DNA isolation, DNA and Pt quantification was 11.8% (relative standard deviation). Based on this result, the method was regarded to be suitable to characterize Pt-DNA adduct formation and its interindividual variability in clinical samples.

Pharmacokinetic and statistical evaluation

Noncompartmental pharmacokinetic analysis of plasmatic total and ultrafiltered Pt was performed during the 5th,

9th, and 12th cycles using Kinetica 2000 4.1.1 software (InnaPhase Corp., Philadelphia, Pennsylvania, USA). The only assumption was that the terminal elimination phase could be approximated by an exponential equation, so that a straight line can approximate the logarithmic transformation of data belonging to the terminal elimination process. The main pharmacokinetic parameters determined in this study were C_{max}, AUC_{tot}, k_{el}, $t_{1/2}$, CL, and V_{ss}. C_{max} is the maximum observed plasma concentration; AUC_{tot} is the area under the plasma concentration–time curve from $t = 0$ to $t = \inf$ extrapolated by the software; the elimination rate constant k_{el} was estimated from the terminal portion of the log-transformed plasma concentration–time curve. The elimination half-life '$t_{1/2}$' was calculated as $\ln 2/k_{el}$; total plasma clearance 'CL' was calculated as CL = dose/AUC; the apparent steady-state volume of distribution 'V_{ss}' was calculated using a noncompartmental first-moment method, as $V_{ss} = AUMC \times D/AUC^2$, where AUMC = area under the first moment curve, D = dose.

Statistical evaluation was performed using Instat 3.05 software (Graphpad, San Diego, California, USA). The Kruskal–Wallis nonparametric test was used to analyze the total Pt accumulation among the 5th, 9th, and 12th cycles and the Mann–Whitney nonparametric test was used to compare pharmacokinetic parameters and Pt-DNA adduct amount between GSH-treated patients and control group.

Results

Patient characteristics

Twenty-seven patients (18 males, 9 females; median age 61 years, range 44–75), affected by colon adenocarcinoma classified as T1-4N1M0 and subjected to curative resection, were enrolled and treated with FOLFOX4 in adjuvant setting. The patients were randomized in two arms to receive either GSH (14 patients) or physiological saline solution (13 patients) before oxaliplatin, following the FOLFOX4 regimen until 12 cycles maximum.

After a median overall follow-up period of 12 months, we did not observe a relapse in any of the two arms. On account of the short follow-up period, it was not possible to evaluate progression-free or overall survival.

Neurotoxicity

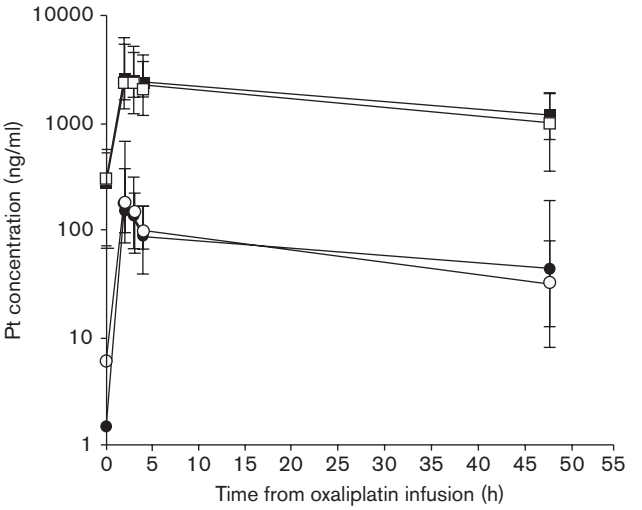
A total of 259 cycles were administered, the mean number of cycles per patient was 10.0 (range 5–12) in the GSH arm and 9.2 (range 5–12) in the control arm, the mean total oxaliplatin dose per patient was 841 mg/m^2 (range 415–1020) in the GSH arm and 772 mg/m^2 (range 425–1020) in the control arm. For all 27 patients, the neurotoxicity was evaluated after the 5th, 9th, and 12th cycles using the Common Toxicity Criteria of the National Cancer Institute, Version 3. At the end of treatment, in the GSH arm we observed only moderate neurotoxicity with grade 1 in seven patients (50%) and grade 2 in seven patients (50%), whereas in the placebo arm the neuro toxicity observed was from moderate to severe, with grade 2 in nine patients (69%) and grade 3 in four patients (31%), no grade 4 neurotoxicity was observed in none of the two arms. The difference of neurotoxicity between the two arms, analyzed by the nonparametric Mann–Whitney test, is statistically significant ($P = 0.0037$). The other chemotherapy toxicities are comparable between GSH and the placebo arm and similar to previously reported ones [10].

Pharmacokinetics of total and ultrafiltered platinum

Pharmacokinetics of total and ultrafiltered Pt was evaluated during the 5th, 9th, and 12th cycles on a total of 25 evaluable cycles for the GSH arm and 20 evaluable cycles for the placebo arm.

Pt total levels at early sampling times showed a cumulative increase from the 5th to the 12th cycle, as already described by previous studies [25]. However, the difference, analyzed with the nonparametric Kruskal–Wallis test for independent data, was not statistically significant between the 5th, 9th, and 12th cycles neither for the placebo nor for the GSH arm (P=0.0619 and 0.6052, respectively) and does not affect the pharmacokinetic parameters. The pharmacokinetic profile of either total or ultrafiltered Pt in each arm was comparable among the three different cycles and the data were used to determine the median pharmacokinetic curves irrespective of the cycles. The mean plasma levels of total and ultrafiltered Pt are presented in Fig. 1, modest interpatient variability was observed in the plasma concentration levels at the different sampling points. Pharmacokinetics of total and ultrafiltered Pt is characterized by a high peak value at

Fig. 1



Median platinum (Pt) concentration–time curve in total (□) and ultrafiltered plasma (○). Black symbols represent the control arm and white symbols represent the glutathione arm.

the end of oxaliplatin infusion, followed by a biphasic decay: an initial rapid phase, mainly related to the distribution process, and a terminal phase, that is shorter for the ultrafiltered with respect to total Pt because of the time dependency of the binding in plasma. The profile of the concentration–time curve of either total or ultrafiltered Pt is similar in GSH and control arm and is comparable with results already obtained by our group [26,27]. The median value of main pharmacokinetic parameters, summarized in Tables 1 and 2, is comparable with previously reported ones [5,25–27]. The differences in the main pharmacokinetic parameters between the two arms were analyzed by the nonparametric Mann–Whitney test: the only statistically significant difference seem to be the AUC_{tot} of total Pt, that is lower in the GSH arm compared with placebo arm (127 260 ng × h/ml and 166 950 ng × h/ml, respectively, $P = 0.0356$) because of a moderate increase of Pt clearance, and the V_{ss} of ultrafiltered Pt that is lower in the GSH arm compared with placebo arm (822 396 and 1 320 600 ml, respectively, $P = 0.0066$).

Determination of platinum-DNA adducts in white blood cells

Table 3 shows the median Pt-DNA adducts formation (expressed as Pt atoms/10^6 nucleotides) measured on DNA from leukocytes obtained at different time points (before, at the end, and 48 h after the oxaliplatin infusion) during the 5th, 9th, and 12th cycles. The amount of Pt-DNA adducts in each arm was comparable among the three different cycles and the data were used to determine the median value irrespective of the cycles: the evaluable cycles were 26 for the GSH arm and 20 for the placebo arm. The formation of Pt-DNA adducts shows no statistically significant difference between the two arms in none of the three time points. The measurable level of Pt-DNA adducts found at the 0 time (before the oxaliplatin infusion) is because of the Pt residue from the former cycles.

Discussion

In this study, we showed that GSH, administered to colorectal cancer patients treated with the FOLFOX4 schedule, is able to reduce the oxaliplatin-induced neurotoxicity, without substantial modification of main pharmacokinetic parameters of oxaliplatin, nor reduction of the amount of Pt-DNA adducts in WBC.

Table 3 Pt-DNA adducts measured in WBC

	Median (range) Pt-DNA adducts (Pt atoms/10^6nucleotides)		
Sample	GSH	Control	*P* value (Mann–Whitney)
0′ from oxaliplatin infusion	2.50 (0.48–20.17)	4.30 (0.33–15.70)	0.3865
End of infusion	4.56 (0.54–21.47)	4.24 (1.67–15.18)	0.7824
48 h from oxaliplatin infusion	4.90 (0.88–14.50)	4.80 (0.72–18.55)	0.5686

GSH, glutathione; Pt, platinum; WBC, white blood cells.
$P > 0.05$ nonsignificant difference, $P < 0.05$ significant difference.

Table 1 Oxaliplatin (total Pt) pharmacokinetic results

	Median (range)		
Pharmacokinetic parameters	GSH	Control	*P* value (Mann–Whitney)
C_{max} (ng/ml)	2474 (1853–6309)	2664 (1582–5532)	0.8550
AUC_{tot} (ng × h/ml)	127260 (88968.5–195557)	166950 (48114.7–255812)	0.0356
k_{el} (1/h)	0.018 (0.014–0.037)	0.017 (0.007–0.027)	0.2534
$t_{1/2}$(h)	37.45 (18.96–47.97)	40.11 (25.74–99.37)	0.2534
Clearance (ml/h)	1083 (757.2–1416)	964.7 (541.4–2889)	0.3854
V_{ss} (ml)	60462 (21166–86785)	67542 (26093–104696)	0.1852

AUC_{tot}, total area under the plasma concentration–time curve; C_{max}, maximum observed plasma concentration; GSH, glutathione; k_{el}, elimination rate constant; Pt, platinum; $t_{1/2}$, elimination half-life; V_{ss}, apparent steady-state volume of distribution.
$P > 0.05$ nonsignificant difference, $P < 0.05$ significant difference.

Table 2 Oxaliplatin (ultrafiltered Pt) pharmacokinetic results

	Median (range)		
Pharmacokinetic parameters	GSH	Control	*P* value (Mann–Whitney)
C_{max} (ng/ml)	196.0 (104.2–386.0)	197.3 (117.5–370.3)	0.8820
AUC_{tot} (ng × h/ml)	4486 (2994–8137)	4432 (2366–8589)	0.9818
k_{el} (1/h)	0.036 (0.010–0.070)	0.029 (0.010–0.050)	0.0550
$t_{1/2}$(h)	19.31 (10.00–46.36)	24.01 (14.71–54.23)	0.0550
Clearance (ml/h)	31060 (17083–55780)	33208 (18023–71858)	0.2349
V_{ss} (ml)	822396 (343257–2910430)	1320600 (716138–2344290)	0.0066

AUC_{tot}, total area under the plasma concentration–time curve; C_{max}, maximum observed plasma concentration; GSH, glutathione; k_{el}, elimination rate constant; Pt, platinum; $t_{1/2}$ elimination half-life; V_{ss}, apparent steady-state volume of distribution.
$P > 0.05$ nonsignificant difference, $P < 0.05$ significant difference.

At the end of treatment, only a moderate neurotoxicity was reported in the GSH arm (50% grade 1 and 50% grade 2), whereas in the placebo arm the neurotoxicity resulted more severe (69% grade 2 and 31% grade 3): the difference was considered statistically very significative by nonparametric Mann–Whitney test ($P = 0.0037$). The results on neurotoxicity are comparable with those reported by Cascinu *et al.* [16].

Following the administration of oxaliplatin alone or with GSH coadministrated, most of the pharmacokinetic parameters of total and ultrafiltered Pt (representing the 'free' and pharmacologically active form of Pt) are comparable between the GSH and placebo arm, as indicated by the nonparametric Mann–Whitney test. The only statistically significant difference among the main pharmacokinetic parameters of total Pt is the AUC_{tot} that is higher in the placebo arm compared with GSH arm (166 950 and 127 260 ng × h/ml, respectively, $P = 0.0356$). The lower value of AUC_{tot} of total Pt in the GSH arm, may be explained by the natural function of GSH in the detoxification of oxaliplatin and other Pt compounds and by the ability of GSH to remove the Pt bound to plasmatic proteins, as shown by in-vitro study of Peleg-Shulman *et al.* [28] and by ex-vivo study of Brouwers *et al.* [15]. The releasing of Pt from proteins, promoted by GSH, might result in an increased rate of elimination of Pt from the body and, therefore, in a reduced toxicity. Moreover, the increase of Pt elimination rate, promoted by the GSH, does not affect the antitumoral activity of oxaliplatin as shown by the study of Pt-DNA adducts presented in this study, that unequivocally shows that the coadministration of GSH does not decrease the ability of the Pt to react with the DNA.

The increase in elimination rate of Pt, promoted by GSH administration, is confirmed by a decreased $t_{1/2}$ (19.31 h) and an increased k_{el} (0.036 1/h) in the GSH arm compared with the placebo arm (24.01 h and 0.029 1/h, respectively) with a difference near the statistical significance ($P = 0.0550$).

In this study, the Pt-DNA adducts formation on WBC, taken as surrogate of cancer cells, was measured with a new and very sensitive method that enabled the evaluation of ultratrace levels (low picogram range) of Pt in microgram amounts of isolated DNA from WBC [21,24]. The classical spectroscopic methods are not suitable for the evaluation of Pt-DNA adduct in clinical studies because of the very low level of Pt-DNA adducts formed in WBC of patients after treatment with 85 mg/m^2 of oxaliplatin (down to one Pt atom per 1 million nucleotides, or even lower) and of the limited sample volume of blood (about 10–15 ml, leading to an absolute amount of isolated DNA which is only in the microgram range).

The Pt-DNA adducts formation on WBC shows no statistically significant differences between the two experimental arms suggesting that there is no GSH influence on Pt-DNA adducts formation in tumor cells as well. Recently, an association between adduct levels in WBC and tumor response was reported [24], but further studies, designed to validate Pt-DNA adduct formation as a surrogate for antitumor activity during both adjuvant and palliative therapy, are required.

The ability of GSH to prevent the oxaliplatin-induced neurotoxicity without impairing Pt-DNA adduct formation in tumor cells (or in WBC taken as a model), could be explained by the pharmacokinetic properties of this compound. Exogenous GSH infusion, administered intravenously, is rapidly removed from the plasma compartment, but is not taken up by most of the cells (and probably even by the tumor cells) except for those tissues requiring high concentrations of antioxidant species [13]. Thus, high concentrations of GSH are found in the kidney [29] and in the cells strongly exposed to reactive oxygen species such as those of the central and peripheral nervous system [30–32]. This distribution could explain the protective effect of GSH against nephrotoxicity induced by cisplatin and neurotoxicity induced by oxaliplatin.

The lack of toxicity and interference with pharmacokinetics and effects of oxaliplatin suggest that GSH may be a promising drug for the prevention or delay of oxaliplatin-induced neuropathy in colorectal cancer patients.

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