

Pharmacokinetics and tumor uptake of a derivatized form of paclitaxel associated to a cholesterol-rich nanoemulsion (LDE) in patients with gynecologic cancers

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Abstract *Objective:* A cholesterol-rich nanoemulsion termed LDE concentrates in cancer tissues after injection into the bloodstream. The association of a derivatized paclitaxel to LDE showed lower toxicity and increased antitumoral activity as tested in a B16 melanoma murine model. Here, the pharmacokinetics of LDE–paclitaxel oleate and the ability of LDE to concentrate the drug in the tumor were investigated in patients with gynecologic cancers. *Methods:* Either LDE–paclitaxel oleate doubly labeled with [¹⁴C]-cholesteryl oleate and [³H]-paclitaxel oleate or [³H]-paclitaxel-cremophor was intravenously injected into eight patients. Blood samples were collected over 24 h to determine the plasma decay curves. Fractional clearance rate (FCR) and pharmacokinetic parameters were calculated by compartmental analysis. Also, specimens of tumors and the corresponding normal tissues were excised during the surgery for radioactivity measurement. *Results:* The LDE and paclitaxel oleate FCR were similar (0.092 ± 0.039 and 0.069 ± 0.027 h⁻¹, respectively, $n = 5$, $P = 0.390$). FCR of paclitaxel oleate asso-

ciated to LDE was smaller than that of paclitaxel-cremophor (0.231 ± 0.128 h⁻¹, $P = 0.028$). Paclitaxel oleate $T_{1/2}$ and AUC were greater than those of paclitaxel-cremophor ($T_{1/2} = 14.51 \pm 3.23$ and 6.62 ± 2.05 h and $AUC = 2.49 \pm 0.35$ and 1.26 ± 0.40 , respectively, $P = 0.009$, $P = 0.004$). The amount of paclitaxel and LDE-radioactive labels in the tumor was 3.5 times greater than in the normal tissues. *Conclusion:* Paclitaxel oleate associated to LDE is stable in the bloodstream and has greater plasma half-life and AUC than those for paclitaxel-cremophor. LDE concentrates 3.5 times more paclitaxel in malignant tissues than in normal tissues. Therefore, association to LDE is an interesting strategy for using paclitaxel to treat gynecologic cancers.

Keywords Paclitaxel · Emulsions · Low-density lipoprotein (LDL) receptors · Drug targeting · Cancer chemotherapy

Introduction

Low-density lipoprotein (LDL) is the main carrier of cholesterol in the plasma. LDL is removed from circulation into the cells by specific receptors on the plasma membrane that recognize the LDL unique protein, apolipoprotein (apo) B100. In cancer cells, the LDL receptor is upregulated [8]. We have previously shown that a cholesterol-rich nanoemulsion termed LDE is taken up by the LDL receptors [10–12]. LDE is manufactured without protein, but in contact with plasma it acquires apo E that is also recognized by the LDL receptors allowing endocytosis of the nanoemulsion. LDE can thus be used to target antineoplastic drugs against cancer cells that overexpress LDL

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receptors. It was demonstrated in patients that after intravenous injection, LDE can concentrate in leukemia cells or in solid tumors such as ovarian and breast carcinomas that overexpress those receptors [1, 7, 12]. Recently, the association of antineoplastic drugs to LDE, such as carmustine and derivatized compounds of etoposide and paclitaxel, was tested [13, 16, 20]. Derivatization with oleic acid was used as a strategy to improve the association yield and the stability of the drug when complexed with LDE. The association with the drugs does not alter the biological properties of the microemulsion, such as the ability to bind to LDL receptors. Stable LDE–drug complexes with preserved cytotoxic activity of the associated drugs were obtained and it was demonstrated in *in vitro* and *in vivo* studies that the side effects of the antineoplastic drugs were remarkably reduced [13, 16, 20].

Paclitaxel, one of the most effective anticancer drugs, has activity against ovarian, breast, head, neck, non-small lung and prostatic cancers. The drug is commercially available as a 1/1 mixture of Cremophor EL and ethanol. However, this vehicle can induce histamine release, thereby leading to severe hypersensitivity reactions [19]. Indeed, the lack of tolerability of Cremophor EL is still a major issue concerning paclitaxel use and may certainly compromise the overall results of chemotherapy. Recently we conducted studies on the association of a derivatized form of paclitaxel to LDE in melanoma tumor-bearing mice. Compared with commercial paclitaxel, the preparation showed greater therapeutic index, achieved not only by diminishing the toxicity but also by increasing the anticancer action.

As LDE was shown to concentrate in gynecologic cancers such as ovarian carcinoma [1] in which paclitaxel is a first-line drug, this study was designed to verify the complex stability in human plasma compartment, to investigate the ability of LDE to concentrate paclitaxel in gynecologic malignant tissues and to compare the pharmacokinetics of paclitaxel associated with LDE with that of the commercial drug.

Methods

Patients

The study was conducted on eight consecutive patients with gynecologic carcinoma (four ovarian, three endometrium and one cervix carcinoma). They were enrolled for the study upon admission at the gynecology ward of the Medical School Hospital of the

University of São Paulo. All patients had been scheduled for surgical procedures (bilateral oophorectomy, omentectomy and/or radical hysterectomy and lymphadenectomy). Their age ranged from 26 to 86 years (mean 52.6 ± 17.5 years). Four patients were postmenopausal. The plasma cholesterol concentration ranged from 124 to 287 mg/dl (210 ± 45 mg/dl), LDL cholesterol from 63 to 187 mg/dl (125 ± 35 mg/dl), HDL cholesterol from 36 to 73 mg/dl (50 ± 12 mg/dl) and triglycerides from 104 to 243 mg/dl (169 ± 47 mg/dl). Diagnoses were confirmed by histologic analysis. The experimental protocol was approved by the Ethics Committee of the hospital and an informed consent was obtained from each participant.

Blood determinations

Total plasma cholesterol and triglycerides (TG) of the subjects were determined after a 12 h fast with the aid of enzymatic kits (CHOP_PAP, Merck and Abbott, respectively). HDL cholesterol was determined using the same method, after precipitation of LDL and VLDL with $MgCl_2$ and phosphotungstic acid. LDL was calculated using the Friedewald equation [4].

Preparation of LDE and LDE–paclitaxel oleate

In brief, LDE was prepared from a lipid mixture composed of 40 mg cholesteryl oleate, 20 mg egg phosphatidylcholine, 1 mg triolein and 0.5 mg cholesterol. Emulsification of lipids by prolonged ultrasonic irradiation in aqueous media and the procedure of two-step ultracentrifugation of the crude emulsion with density adjustment by addition of KBr to obtain LDE microemulsion was carried out by the method described previously [5] modified by Maranhão et al. [11]. LDE was dialyzed against saline solution and passed through a 0.22 μm filter for the experiments. When necessary, trace amounts of [^{14}C]-cholesteryl ester (Amersham, Buckinghamshire, UK) were added to the initial solution. Paclitaxel oleate was incorporated into LDE by solubilization of paclitaxel oleate in ethanol and adding it into the emulsion. The solution was sonicated for 30 min at 70°C using a Branson Sonifier 450 (Danbury, CT, USA), equipped with a 1 cm flat titanium probe. LDE–paclitaxel oleate was centrifuged at 3,000 rpm for 15 min to separate the unbound paclitaxel oleate. LDE–paclitaxel oleate was then passed through a 0.22 μm pore polycarbonate filter and kept at 4°C until use. When necessary [3H]-paclitaxel oleate (Moravek, Brea, CA, USA) was added to the initial solution. The yield of each batch was assayed before

use. LDE–paclitaxel oleate was always prepared on the same day of the experiments.

Paclitaxel-cremophor-ethanol based formulation (paclitaxel-cremophor)

Paclitaxel (1.25 mg; Calbiochem, La Jolla, CA, USA) was dissolved in 100 μ l pure grade ethanol and 100 μ l Cremophor EL; the solution was stirred for 10 min and to that added 10 μ l [3 H]-paclitaxel (Moravek, Brea, CA, USA). Prior to patient administration the paclitaxel-cremophor-ethanol based formulation was diluted in 0.9% sodium chloride (1.8 ml) and passed through a 0.22 μ m pore polycarbonate filter.

LDE–paclitaxel oleate and commercial paclitaxel plasma kinetics

The association LDE–paclitaxel oleate labeled with [14 C]-cholesteryl oleate and [3 H]-paclitaxel oleate (15 mg total lipid mass and 1.25 mg paclitaxel oleate, at a volume of 2 ml) or [3 H]-paclitaxel-cremophor (1.25 mg, at a volume of 2 ml) was intravenously injected in a bolus 24 h before the beginning of the surgical procedure scheduled for the patients. Blood samples were collected from a vein of the contralateral arm relative to the injection at pre-established intervals during 24 h (0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h). Blood was centrifuged and the radioactivity contained in 1.0 ml of plasma was measured by liquid scintillation counting (Packard 1660 TR, Meriden, CT, USA). Removal of [14 C]-LDE-[3 H]-paclitaxel oleate or [3 H]-paclitaxel from the plasma was evaluated by the fractional clearance rate (FCR). The FCR was calculated according to the method described by Matthews [14], where a_1 , a_2 , b_1 and b_2 were estimated from biexponential curves obtained from the remaining radioactivity found in plasma after injection, fitted by least squares procedure, as $y = a_1e^{-b_1t} + a_2e^{-b_2t}$, where y represents the radioactivity plasma decay.

Pharmacokinetic parameters were calculated according to a multicompartmental model using a computer and software from PK Solutions (Ashland, OH, USA). The log plasma concentration versus time curves was fitted by biexponential equations and the half-lives ($t_{1/2\beta}$) calculated by dividing 0.693 by the rate constant for each phase. Total plasma clearance (CL) was calculated by dividing the dose by the AUC. The volume of distribution at steady state (V_{dss}) was estimated graphically from trapezoidal total area measurements.

LDE–paclitaxel oleate uptake by tumor and normal tissues

During the surgical procedure, at a time roughly between 24 and 27 h after the injection of the preparation, the excised tissues of interest were provided by the surgeon. Fragments of the tumor and of the normal tissues were put into 0.9% cold saline solution and immediately transported to the laboratory for analysis. The fragments of normal and cancer tissues were then chopped, and lipids and drug from 1.0 g of both tissues were extracted with chloroform/methanol (2:1, v/v) and the radioactivity measured in a scintillation solution [3].

The dose injected into each patient, evaluated according to the guidelines of the International Commission on Radiological Protection [18], was well below the annual limit of ingestion dose of 50 mSv.

Statistical analysis

The differences in the emulsion FCRs were evaluated using Graf Pad Instat, Version 3.0. The differences between the data on neoplastic and normal tissues were evaluated by Mann–Whitney test. In all analysis, a P value < 0.05 was considered significant.

Results

Figure 1 shows the decay curves of [14 C]-cholesteryl oleate and [3 H]-paclitaxel oleate obtained after the injection of the labeled LDE–paclitaxel oleate in five patients. Both curves show a biexponential aspect. It can be seen that the curve of the LDE cholesteryl oleate does not substantially differ from that of paclitaxel oleate, as both run rigorously in parallel and, accordingly, the FCR of both LDE and paclitaxel oleate did not differ (0.092 ± 0.039 and 0.069 ± 0.027 h^{-1} , respectively, $P = 0.390$).

Figure 2 shows the decay curves of [3 H]-paclitaxel oleate associated to LDE injected in five patients and [3 H]-paclitaxel-cremophor injected in three patients. Both curves show a biexponential aspect. The curve of paclitaxel oleate is slower than that of paclitaxel-cremophor. Accordingly, the FCR of paclitaxel oleate is smaller than that of paclitaxel-cremophor (0.069 ± 0.027 and 0.231 ± 0.128 h^{-1} , respectively, $P = 0.028$).

Tables 1 and 2 show the pharmacokinetic parameters of paclitaxel oleate associated with LDE and of paclitaxel-cremophor, respectively. The paclitaxel oleate half-life and AUC are greater than those of

Fig. 1 Plasma decay curve of LDE–paclitaxel oleate, doubly labeled with [^3H]-paclitaxel oleate (filled circle) and [^{14}C]-cholesteryl ester LDE (filled square). Plasma samples were taken over 24 h for radioactive counting in scintillation vials. Results are presented as means \pm SD (bars)

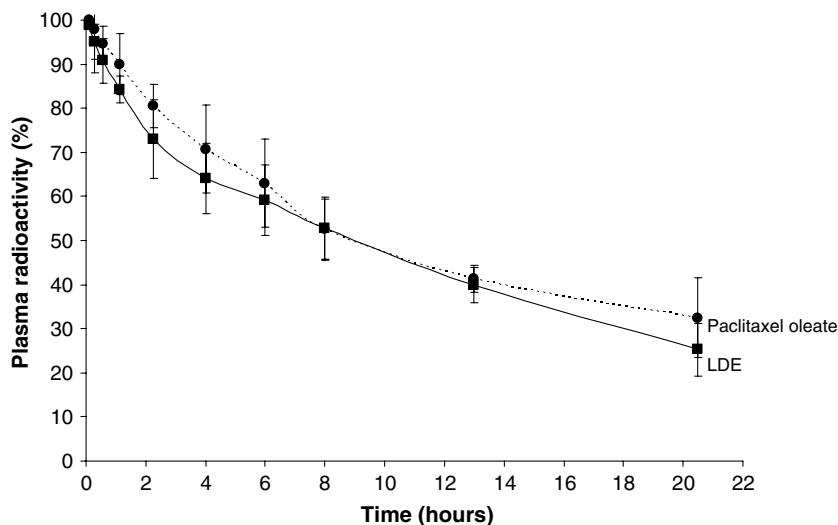


Fig. 2 Plasma decay curve of LDE-associated paclitaxel oleate labeled with [^3H]-paclitaxel oleate (filled circle) and commercial labeled [^3H]-paclitaxel (open square). Plasma samples were taken over 24 h for radioactive counting in scintillation vials. Results are presented as means \pm SD (bars)

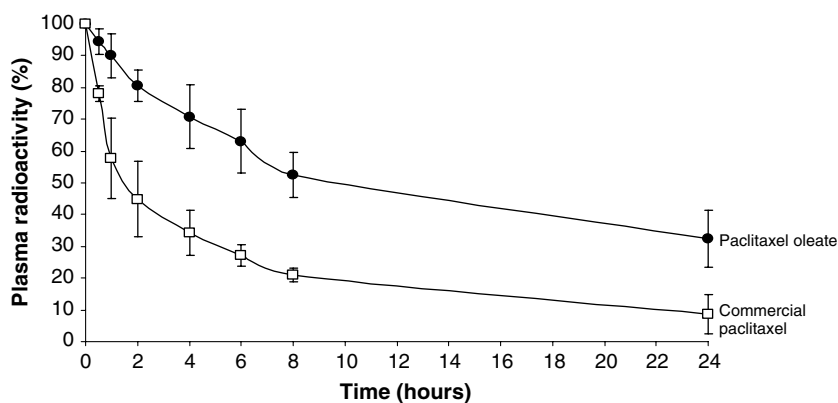


Table 1 Pharmacokinetic parameters of paclitaxel oleate following bolus infusion of [^3H]-paclitaxel oleate associated with LDE

Patient	$T_{1/2}$ (h)	AUC (ng/h/l)	V_{ss} (l)	CL (l/h)	FCR (h^{-1})
1	17.16	2.32	31.98	1.29	0.067
2	8.13	3.32	17.65	1.50	0.088
3	12.81	2.24	41.33	2.24	0.045
4	17.95	2.53	79.46	3.07	0.098
5	16.48	2.02	35.27	1.48	0.046
Mean \pm SD	14.51 \pm 3.23	2.49 \pm 0.35	41.14 \pm 15.41	1.92 \pm 0.59	0.069 \pm 0.027

Table 2 Pharmacokinetic parameters of commercial paclitaxel following bolus infusion of [^3H]-paclitaxel

Patient	$T_{1/2}$ (h)	AUC (mg/h/l)	V_{ss} (l)	CL (l/h)	FCR (h^{-1})
1	8.96	1.50	42.95	3.32	0.097
2	5.11	0.80	45.56	6.18	0.247
3	5.80	1.47	17.05	2.03	0.350
Mean \pm SD	6.62 \pm 2.05	1.26 \pm 0.40	35.19 \pm 15.76	3.84 \pm 2.12	0.23 \pm 0.13

paclitaxel-cremophor ($P = 0.0097$ and 0.0038 , respectively). However, V_{ss} and CL are not different ($P = 0.6186$ and 0.0926).

Table 3 shows the data of tissue uptake of the labels [^{14}C]-cholesteryl oleate and [^3H]-paclitaxel oleate of the LDE–paclitaxel oleate association. The uptake of

Table 3 Uptake of LDE and paclitaxel oleate radioactivity by normal and neoplastic tissues

Patient	Tumor site	Uptake of [¹⁴ C]-LDE by the tissue (cpm/g)			Uptake of [³ H]-paclitaxel oleate by the tissue (cpm/g)		
		Tumor	Normal	Tumor/normal	Tumor	Normal	Tumor/normal
1	Ovarian	1,997	348	5.71	4,563	941	4.85
2	Cervix	695	234	2.97	2,929	603	4.86
3	Ovarian	507	353	1.43	394	236	1.67
4	Endometrium	264	53	4.47	198	33	5.82
5	Endometrium	1,246	459	2.71	347	315	1.10
Mean ± SD		942 ± 692	290 ± 154	3.5 ± 1.7*	1,686 ± 1,968	426 ± 353	3.6 ± 2.1*

* $P = 0.9025$

both cholesteryl oleate and paclitaxel oleate by the tumor was greater than that of the normal tissues. The uptake by the tumor/uptake by the normal tissue ratio of cholesteryl oleate was similar to that of paclitaxel oleate (3.5 ± 1.7 and 3.6 ± 2.1 , $P = 0.936$).

Discussion

The current study shows that the association of paclitaxel to LDE is stable in the bloodstream of cancer patients and pronouncedly alters the pharmacokinetic profile of the drug when compared to the commercial formulation. The concentration in gynecologic carcinomas of both LDE and paclitaxel oleate is several fold greater than in the corresponding normal tissue.

An important prerequisite to achieve a selective therapy is the stability in the blood of the drug-carrier complex. In this regard, it has previously been demonstrated that after LDE-paclitaxel oleate was injected into the bloodstream of B16 melanoma-bearing mice, most of the paclitaxel oleate remained stably associated to LDE [16]. In the current study, the lack of dissociation of paclitaxel from LDE was confirmed in patients by the similar removal rates and identical decay curves of both the drug and the nanoemulsion labeled lipid. The stability of the LDE-drug complex in patients has, in fact, been demonstrated recently for another derivatized compound, namely etoposide oleate [2]. Together with LDE-carmustine that was previously tested in cancer patients [13] and LDE-etoposide oleate [20], the development of LDE-paclitaxel oleate suggests that the LDE system is flexible enough to include several anticancer agents so that combined chemotherapy could be performed with different LDE-drug preparations.

Despite the LDE-paclitaxel oleate complex being proven stable, the question remains whether the addition of the drug to the nanoemulsion damages the biological properties of the latter, specially the ability

to internalize into the cells through the LDL receptor endocytic pathway. The experiments in which LDE uptake by the tumor, where the receptors are upregulated, is measured comparatively to the uptake of normal tissues strongly supports the assumption that LDE-paclitaxel oleate retains the ability of the microemulsion to bind to receptors and concentrate in the neoplastic tissues with receptor upregulation. This is suggested by the 3.6 times greater uptake measured in the tumor compared to normal tissues. The internalization of LDE-drug complex by cells that overexpress LDL receptor has been previously confirmed in vitro, through double-labeled LDE-drug uptake experiment [13, 16, 20], and in vivo by the finding that both LDE and drug were internalized into the tumor cells together in a B16 melanoma animal model.

In a previous study [1], it was shown that after the injection of radioactive LDE into surgical patients 24 h before the oophorectomy procedure, the concentration of nanoemulsion by the ovarian carcinoma was on average eight times greater when compared to the contralateral normal ovary as calculated by the radioactive counting means and tenfold greater as calculated by the individual tumor/normal tissue uptake. In those experiments LDE was not associated to an anticancer drug and our concern in the current study was to verify whether the paclitaxel oleate association could somehow disturb the ability of the nanoemulsion to target the neoplastic tissue. Here, the LDE lipid label was found to concentrate in the tumor 3.5 times relative to the normal organ tissue, which is a remarkable result but inferior to what we had obtained in ovarian carcinoma. However, the current study did not include ovarian carcinoma only: two patients had endometrium and one had cervix carcinoma, whereas two patients had ovarian carcinoma. In the two ovarian carcinoma cases, the tumor/normal tissue uptake ratio was very different: one had a ratio of 1.4 and the other 5.7. In our mentioned study [1], the ratio varied from 2.9 to 27.0. Therefore, the smaller relative uptake observed

here compared to our previous results can be ascribed to differences in the expression of the LDL receptors not only among neoplasias of different organs but also among the tumors of the same organ that in the case of ovary comprises a number of different tumor types.

In this study, a clear-cut demonstration that LDE indeed performs as a drug targeting vehicle is given by the finding of a 3.6 times greater uptake of paclitaxel oleate associated to the microemulsion in the neoplastic compared to the normal tissues. This figure is practically equal to the 3.5 times concentration of the LDE-radioactive cholesteryl esters and is a convincing counterproof to the curves of the plasma kinetics of the complex. Taken together, both the plasma and tissue data show that LDE carries the drug in the plasma and delivers to the tumor tissue without substantial dissociation.

Another important finding in this study is that the modification and association of paclitaxel to LDE changed the pharmacokinetics of the drug, as compared with the cremophor-based formulation. Two measures were taken to warrant a reliable comparison between the LDE and the cremophor-based formulation. First, because the proportion of cremophor used to solubilize paclitaxel elicits pharmacokinetic changes [19], in the current experiment this proportion was rigorously that of the commercially available formulations. Second, to standardize the procedure, both preparations were injected in a bolus and in equally minimal amounts.

Paclitaxel oleate associated to LDE has longer half-life and greater AUC when compared to the commercial presentation. This suggests that the same dose of paclitaxel associated to the nanoemulsion is maintained at greater concentrations for a longer period. Because paclitaxel therapeutic action is highly schedule-dependent and its cell killing ability is more dependent on the duration of exposure [6], the longer time circulation in the bloodstream attributed to the LDE–paclitaxel oleate formulation may offer an additional advantage. Furthermore, Seidman et al. proposed that prolonged exposure to low paclitaxel concentrations may produce antitumor activity against disease that progressed during short taxane exposure. Therefore, the new drug pharmacokinetics reached by association with LDE may enlarge its pharmacologic action spectrum [21].

The utilization of paclitaxel in gynecologic malignancy has been spread to new standard regimens, other than the primary indications. The use of paclitaxel in association with carboplatin as a radiosensitization agent for locally advanced cervix cancer, instead of carboplatin alone, showed less intolerance [17].

Paclitaxel alone or in combination with other anti-neoplastic agents has shown to improve the treatment of persistent or recurrent endometrial carcinoma that has failed prior chemotherapy [9]. Our previous studies showed that LDE–paclitaxel oleate is pronouncedly less toxic in mice and now it is shown in cancer patients that LDE has the ability to concentrate the drug at the tumor sites. Taken together, these results pave the way for future clinical trials to confirm the potential advantages of LDE–paclitaxel in the treatment of gynecologic neoplasias.

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References

- Ades A, Carvalho JP, Graziani SR, Amancio RF, Souen JS, Pinotti JA, Maranhão RC (2001) Uptake of a cholesterol-rich emulsion by neoplastic ovarian tissues. *Gynecol Oncol* 82:84–87
- Azevedo CHM, Carvalho JP, Valduga CJ, Maranhão RC (2005) Plasma kinetics and uptake by the tumor of cholesterol-rich microemulsion (LDE) associated to etoposide oleate in patients with ovarian carcinoma. *Gynecol Oncol* 97:178–182
- Folch J, Lees M, Stanley HS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509
- Friedewald WT, Levy RI, Fredricson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultra centrifuge. *Clin Chem* 18:499–502
- Ginsburg GS, Small DM, Atkinson D (1982) Microemulsions of phospholipids and cholesterol esters. Protein-free models of low density lipoprotein. *J Biol Chem* 57:8216–8277
- Goble S, Bear HD (2003) Emerging role of taxanes in adjuvant and neoadjuvant therapy for breast cancer: the potential and the questions. *Surg Clin North Am* 83:943–971
- Graziani SR, Igreja FAF, Hegg R, Meneghetti C, Brandizzi LI, Barboza R, Amâncio RF, Pinotti JA, Maranhão RC (2002) Uptake of a cholesterol-rich emulsion by breast cancer. *Gynecol Oncol* 85:493–497
- Ho YK, Smith RG, Brown MS, Goldstein JL (1978) Low density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. *Blood* 52:1099–1114
- Lincoln S, Blessing JA, Lee RB, Rocereto T (2003) Activity of paclitaxel as second-line chemotherapy in endometrial carcinoma: a gynecologic oncology group study. *Gynecol Oncol* 88:277–281
- Maranhão RC, Garicochea B, Silva EL, Llacer PD, Pileggi FJ, Chamone DA (1992) Increased plasma removal of microemulsions resembling the lipid phase of low-density lipoproteins (LDL) in patients with acute myeloid leukemia: a possible new strategy for the treatment of the disease. *Braz J Med Biol Res* 25(10):1003–1007

11. Maranhão RC, Cesar TB, Pedrosa MTB, Hirata MH, Mesquita CH (1993) Metabolic behavior in rats of a nonprotein microemulsion resembling LDL. *Lipids* 28:691–696
12. Maranhão RC, Garicochea B, Silva EL, Dorlhiac-Llacer P, Cadena SMS, Coelho IJC, Meneghetti JC, Pileggi FJC, Chamone DAF (1994) Plasma kinetics and biodistribution of a lipid emulsion resembling low-density lipoprotein in patients with acute leukemia. *Cancer Res* 54:4660–4666
13. Maranhão RC, Graziani SR, Yamaguchi N, Melo RF, Latrilha MC, Rodrigues DG, Couto RD, Schreier S, Buzaid AC (2002) Association of carmustine with a lipid emulsion: *in vitro*, *in vivo* and preliminary studies in cancer patients. *Cancer Chemother Pharmacol* 49:487–498
14. Matthews CME (1957) The theory of tracer experiments with ¹³¹I-labeled plasma proteins. *Phys Med Biol* 2:36–44
15. Rao GG, Rogers P, Drake RD, Nguyen P, Coleman RL (2005) Phase I clinical trial of weekly paclitaxel, weekly carboplatin, and concurrent radiotherapy for primary cervical cancer. *Gynecol Oncol* 96:168–172
16. Rodrigues DG, Maria DA, Fernandes DC, Valduga CJ, Couto RD, Ibañez COM, Maranhão RC (2005) Improvement of paclitaxel therapeutic index by derivatization and association to a cholesterol-rich microemulsion: *in vitro* and *in vivo* studies. *Cancer Chemother Pharmacol* 55:565–576
17. Seidman AD, Hochhauser D, Gollub M, Edelman B, Yao TJ, Hudis CA, Francis P, Fennelly D, Gilewski TA, Moynahan ME, Currie V, Baselga J, Tong W, O'Donoghue M, Salvaggio R, Auguste L, Spriggs D, Norton L (1996) Ninety-six-hour paclitaxel infusion after progression during short taxane exposure: a Phase II pharmacokinetic and pharmacodynamic study in metastatic breast cancer. *J Clin Oncol* 4:1877–1884
18. Sowby FS (1984) (ed) Radiation protection. Part I. Limits for intakes of radionuclides by workers. Pergamon Press, Oxford
19. ten Tije AJ, Verweij J, Loos WJ, Sparreboom A (2003) Pharmacological effects of formulation vehicles: implication for cancer chemotherapy. *Clin Pharmacokinet* 42(7):665–685
20. Valduga CJ, Fernandes DC, Lo Prete AC, Azevedo CHM, Rodrigues DG, Maranhão RC (2003) Use of a cholesterol-rich microemulsion that binds to low-density lipoprotein receptors as vehicle for etoposide. *J Pharm Pharmacol* 55:1615–1622
21. Woo HL, Swenerton KD, Hoskins PJ (1996) Taxol is active in platinum-resistant endometrial adenocarcinoma. *Am J Clin Oncol* 19:290–291