Cancer Chemother Pharmacol (1992) 29: 396–400

Cancer Chemotherapy and Pharmacology

Treatment of cancer patients with a low-density-lipoprotein delivery vehicle containing a cytotoxic drug

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Received 1 May 1991/Accepted 14 October 1991

Summary. A cytotoxic drug (vincristine, VC) was incorporated into low-density lipoprotein (LDL) and given to cancer patients for the first time by repeated intravenous injection. Individuals presenting with ovarian or endometrial cancer received four or five weekly doses of 1.4 mg/m^2 LDL/VC. The uptake of LDL/VC by the adrenal cortex and the liver was minimised by concurrent administration of prednisolone and chenodeoxycholic acid. No febrile, allergic or other reaction attributable to the LDL occurred, and no side effect on haemopoietic, adrenal or liver functions was observed. The neurotoxic side effects commonly seen during VC therapy appeared to be reduced. These results suggest that directed cytotoxic therapy might be achieved in humans through the use of LDL as a carrier. Thus, dose-range and comparative studies using LDL/VC vs $VCSO_4$ are warranted in malignancies in which treatment with the latter drug has been established.

Introduction

One of the major problems associated with the treatment of malignant disease with cytotoxic drugs is the lack of selectivity of such drugs, which may give rise to a variety of often severe side effects. The severity of these side effects may require dose modifications which render the achievement of a therapeutic response difficult. One theoretical possibility of reducing the toxicity and simultaneously improving the therapeutic response is to target the drug at cancer cells using a suitable carrier. Several carriers, including antibodies and liposomes, have been proposed but their use involves disadvantages such as limited carrying capacity and uptake by the reticuloendothelial system. An endogenous carrier such as low-density lipoprotein (LDL) might overcome these difficulties and has thus been suggested for this role [2, 9, 14, 17, 18].

LDL contains about 70% in cholesterol of the plasma and serves as the major transport system for this metabolite. LDL is bound to cells via specific surface receptors, then internalised and degraded in lysosomes, whereby its cholesterol is released. This is one mechanism by which cells obtain adequate amounts of cholesterol for the synthesis of cell membranes and, in some cases, steroid hormones and bile acids.

Several studies have suggested that the receptor-mediated uptake of LDL is higher in tumour cells than in normal tissue. In patients presenting with acute myeloid leukaemia, measurement in vivo of the uptake of radiolabeled LDL by leukaemic cells showed a strong correlation with the higher levels of LDL-receptor activity determined in vitro in these cells as compared with normal leukocytes and bone marrow cells. The consequent increase in the uptake of LDL was mirrored by a decrease in plasma levels of cholesterol. During cytotoxic therapy the level of plasma cholesterol increased in parallel with the effect on the malignant cells [1, 14, 16, 18]. Many solid tumours also exhibit increased levels of LDL-receptor activity as compared with normal tissue [2]; for example, in lung-tumour tissue a 2- to 3-fold increase in the uptake in vivo of radiolabeled LDL has been observed [19].

There are several methods for the incorporation of lipophilic cytotoxic substances into LDL particles [7, 10, 12], and LDL functions as a carrier of these agents to cultured tumour cells [9, 15, 17, 20]. One such substance is vincristine (VC). In mice bearing solid tumour implants (MAC29), the intravenous injection of LDL/VC complexes resulted in a reduction in tumour growth significantly greater than that achieved using $VCSO_4$ at the same dose (Double, personal communication).

VC produces characteristic neurological side effects that represent the most common dose-limiting toxicity [4, 6], among which paraesthesia and loss of deep tendon reflexes are the most frequently observed. Due to these easily characterised side effects and because VC has been

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Table 1. Patients' characteristics

Patient number	Age (years)	Histopathological diagnosis	Clinical stage	Previous operation	Previous radiotherapy
1	56	Adenocarcinoma	III Ovarian carcinoma	Partial hysterectomy and salpingo-oophorectomy 28 months before study	Cobalt 60 therapy 27 months before study: upper field, 35 Gy/tumour lower field, 45 Gy/tumour
2	42	Cystadenocarcinoma	I Ovarian carcinoma	Total hysterectomy 2 months before study	–
3	62	Cystadenocarcinoma	I Ovarian carcinoma[a]	Total hysterectomy 2 months before study	–
4	60	Adenocarcinoma	II Endometrial carcinoma	Radical hysterectomy 7 months before study	Intravaginal radium (22 mCidestruid) and cobalt 60 therapy (40 Gy/tumour) 7 months before study
5	36	Adenocarcinoma	III Ovarian carcinoma	Total hysterectomy 13 months before study	–
6	48	Adenocarcinoma ville	III Ovarian carcinoma	Partial hysterectomy, hemi-colectomy, left adnexectomy and right oophorectomy 2 months before study	–
7	51	Cystadenocarcinoma	III Ovarian carcinoma	Bilateral adnexectomy and partial resection of omentum 1 month before study	–
8	67	Adenocarcinoma	III Ovarian carcinoma	Explorative laparatomy 5 months before study	–
9	43	Adenocarcinoma	III Ovarian carcinoma	Right adnexectomy and partial resection of omentum 3 months before study	–

[a] With liver metastases

Table 2. Treatment procedure and evaluation of the effects of treatment with LDL/VC and $VCSO_4$ on ovarian or endometrial cancer

	Weeks						
	0	1	2	3	4	5	6
Treatment:							
VC/LDL		x	x	x	x		
$VCSO_4$						x	
Prednisolone		x x x x x x x x x x x x x x					
Chenodeoxycholic acid	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx						
Examinations:							
Physical and neurological examination	x	x	x	x	x	x	x
Ultrasonogram	x					x	
Chest X-ray	x					x	
Laboratory tests:							
VC in plasma	x				xxx	xxx	
VC in urine					x	x	
Blood chemistry	x	x	x	x	x	x	x
Urine chemistry	x					x	

successfully incorporated into human LDL without changing the physical or biological properties of the particles and the resultant complexes have been found to be effective in the treatment of animal tumours in vivo, LDL/VC was chosen as the first example of this type of formulation for study in humans. LDL has previously been given to patients as a single intravenous injection to ascertain its distribution through the tissues [13] or to follow its uptake by haematological and solid tumours [18, 19]; however, to the best of our knowledge, it has not been given to humans by repeated injection or in complexes containing cytotoxic drugs. The primary objective of the present study was therefore to examine the feasibility of the administration of LDL/drug complexes to patients by repeated injection. Our secondary goal was to determine whether such treatment would result in a reduction in the side effects produced by VC, as this might indicate a diminution in the uptake of the drug by neural tissue.

Patients and methods

The investigation was an open study, approved by a committee of independent research workers in the Bialystok Medical Academy, in patients suffering from advanced ovarian or endometrial cancer who exhibited disease progression following previous treatment with surgery or radiotherapy. Conventional chemotherapy for ovarian cancer was not available for these subjects at the commencement of the study. Patients who were too ill to participate or whose expected survival amounted to <1 month were excluded. Informed consent was obtained from the nine patients who were entered in the study; their characteristics are listed in Table 1.

Preparation of LDL-drug complexes. LDL was isolated by the ultracentrifugation of plasma from a hyperlipidaemic individual in the United Kingdom that had been screened to exclude human immunodeficiency virus (HIV) and hepatitis. Incorporation of the cytostatic drug was performed according to the method of Masquelier et al. [7, 8]. Briefly, purified LDL was lyophilised in the presence of sucrose as a stabilising agent, and a solution of VC in methylene chloride was added at 0.25 mg/mg lipoprotein. After incubation at room temperature, the solvent was completely removed by evaporation under nitrogen gas and the

LDL/VC complexes were dissolved in phosphate-buffered saline (PBS, pH 7.4). Non-incorporated drug was removed and the LDL/VC solution was sterilised by filtration through 0.22 μm membrane filters (Millex G. V., Millipore S. A., Molsheim, France). The VC content of the solution in the final containers was analysed by high-performance liquid chromatography (HPLC).

Treatment and evaluation of effects. The scheme of treatment and side-effect evaluation is summarised in Table 2. Because the liver is the major organ responsible for the metabolism of LDL, it displays high levels of LDL-receptor activity, which can be down-regulated by the administration of bile acids. Consequently, chenodeoxycholic acid (Chenofalk, Thames Laboratories Limited, Wrexham, UK) was given for protection of the liver at a daily dose of 7.5 mg/kg beginning at 1 week prior to the first LDL/VC treatment. As the adrenal cortex also exhibits high levels of LDL-receptor activity due to its need for cholesterol for hormone synthesis, prednisolone (Precortisyl, Roussel Laboratories Ltd., Uxbridge, UK) was given as a protective agent at a daily dose of 30 mg starting at 1 day prior to each LDL/VC injection; thereafter, the dose was reduced by 5 mg every other day until the day preceding the next treatment. LDL/VC was given to patients once a week over a 4-week period by intravenous injection of 1.4 mg/m^2 body surface area up to a maximum of 2 mg/injection. To enable a preliminary pharmacokinetic comparison, an equivalent dose of $VCSO_4$ was given during the 5th week.

Physical and neurological examinations were performed at each weekly visit, and toxicities were classified according to WHO criteria [21]. Blood and urine samples were taken for laboratory examination at the times indicated in Table 2. The blood tests included determinations of haemoglobin values, haematocrit, red and white blood cell and platelet counts, and levels of serum transaminases and alkaline phosphatase. Urine chemistry included measurements of glucose and albumin content. Chest X-rays and ultrasonograms were obtained from all patients before and after the course of treatment.

Plasma sampling and vincristine assay. Plasma samples for estimations of VC concentration were taken prior to the commencement of LDL/VC therapy and both immediately before and at 0.5, 2 and 6 h after the fourth LDL/VC injection and the $VCSO_4$ injection given during the 5th week. The assay of VC levels in plasma was performed essentially as previously described [5]. Briefly, the enzymeimmunoassay (EIA) system consisted of Maxisorb microtiter plates, rabbit IgG_2 [diluted 1 : 500 (v/v) in carbonate buffer (pH 9.6)], VC-alkaline phosphatase conjugate (about 1 μg/ml), standards or samples in PBS, PBS-Tween and 1 mg (3.8 μmol) *p*-nitrophenyl phosphate dissolved before use in 1 ml 1.25 M diethanolamine buffer (pH 9.8).

The assay procedure was modified slightly in that 15-μl samples of centrifuged plasma were diluted with 10 μl PBS prior to their addition to the plates. Incubation with *p*-nitrophenyl phosphate was carried out at room temperature until the absorbance of the zero sample had reached about 1 at which point the entire plate was read. Blanks were prepared by including 15 μl reference plasma (no VC) in the assay, and the absorbance value obtained was used to correct those determined for the samples. Each reaction was performed in triplicate and Student's *t*-test was used for statistical analysis of the data.

Table 3. Pharmacokinetic data on patients 2–5 following the administration of LDL/VC and $VCSO_4$

Parameter	LDL/VC	$VCSO_4$
c_{max} (ng/ml)	24.4 ± 4.2	19.6 ± 0.8
t_{cmax} (h)	0.9 ± 0.8	0.5 ± 0.0
$t_{1/2}$ (h)	5.6	5.1
AUC (ng h ml^{-1})	104.0 ± 23.4	73.5 ± 13.2

Duplicate plasma samples were taken at 0, 0.5, 2 and 6 h; assays were carried out in triplicate as described in Patients and methods

Results

Within 48 h of the first administration of LDL/VC, one subject (patient 1), with a history of gallbladder disease in whom multiple stones were revealed by ultrasound examination, developed acute cholecystitis necessitating a cholecystectomy. She elected to withdraw from the study. The remaining eight patients received four weekly injections of LDL/VC and one of $VCSO_4$ (weekly maximum, 2 mg) without showing evidence of febrile or allergic reactions during the observation period. None of the haematological or liver-function test parameters showed any deviation from normal physiological values, and no abnormality in urine chemistry was observed. Radiography of the chest before and after the course of therapy revealed no abnormality in the heart or lungs.

For technical reasons, plasma samples were available from only four individuals (patients 2–5). The results of the pharmacokinetic analyses for these four subjects are shown in Table 3. The maximal concentration (c_{max}) values measured following LDL/VC administration ranged from 18 to 28 ng/ml, and those determined after the injection of $VCSO_4$ varied between 18 and 20 ng/ml. The AUC values were 80–127 and 60–86 ng h ml^{-1} for LDL/VC and $VCSO_4$, respectively. However, the differences were

Table 4. Time of development and severity of the toxic effects encountered

Patient number	Peripheral neurotoxicity		Abdominal pain		Constipation		Alopecia	
	WHO[a]	Week[b]	WHO	Week	WHO	Week	WHO	Week
2	3	5	1	2	0	–	2	3
3	1	5	1	2	0	–	3	5
4	1	3	1	4	0	–	3	5
5	1	3	1	1	0	–	2	3
6	1	5	1	2	0	–	0	–
7	1	4	1	2	0	–	0	–
8	0	–	1	4	1	5	3	3
9	1	4	1	3	1	4	0	–

Toxicity was assessed weekly according to the 5-point WHO scale [21] during patients' visits to the clinic for therapy

[a] WHO scale 0–4

[b] Week during which the maximal effect was observed (0–6 weeks)

not statistically significant. No VC was detectable in urine samples taken at 6 h after the administration of either LDL/VC or $VCSO_4$.

The most frequently observed side effects were alopecia, peripheral neuropathy/paraesthesia and abdominal pain. The numbers of patients experiencing the side effects and the degrees of severity are shown in Table 4. Only one person (patient 2) experienced WHO grade 3 paraesthesia and tendon-reflex loss. All subjects suffered mild and transient abdominal pain and two patients developed mild constipation, but there was no progression to paralytic ileus and no case of hoarseness, ptosis, double vision or lethargy was encountered.

After the initial course of treatment comprising four weekly doses of LDL/VC and one dose of $VCSO_4$, patient 2 received one further dose of $VCSO_4$ followed by a dose of LDL/VC; patients 3 and 6 each received one additional dose of $VCSO_4$. Although none of the three elected to receive any further treatment, they were observed regularly. At 7 months after treatment, all were feeling well and showed no sign of disease progression. Patient 5, who exhibited clinically palpable abdominal masses on her admission into the study at 12 months after a hysterectomy, received the initial course of treatment followed by one dose of $VCSO_4$ and four additional doses of LDL/VC over a 10-week period; 4 months later she underwent a laparotomy because of intestinal obstruction. There was no macroscopic evidence of neoplastic tissue, and histopathological examination revealed that none of the biopsy samples contained malignant cells.

Discussion

This is the first study reporting on the repeated administration to humans of a cytotoxic drug incorporated into LDL. The results indicate that VC incorporated into human LDL by the method of Masquelier et al. [7, 8] can be given to patients without undue side effects being caused by the carrier. One patient received a total of eight injections of LDL/VC, i.e. a cumulative dose of 16 mg VC in 64 mg LDL, over a period of 17 weeks. No febrile, allergic or other immediate reaction occurred in connection with the LDL/VC injections. Moreover, no effect on liver or adrenal function was caused by the present therapeutic regimen, which included the concurrent administration of bile acid and prednisolone. Animal studies have indicated that steroids down-regulate the high uptake of LDL by the adrenal cortex, thus reducing the potential risk of toxic damage to this organ [3]. The same study found that the administration of bile acids down-regulates LDL-receptor activity in the liver, and it has also been found that tumour growth in animals is accompanied by a reduction in the LDL-receptor activity in the liver (Kuhn, personal communication).

Patient 1 had been diagnosed as having gallstones on ultrasound examination prior to the initiation of therapy. In view of the short interval between this diagnosis and the occurrence of the acute cholecystitis that caused her to drop out of the study, it is unlikely that the development of the latter was attributable to LDL/VC administration.

Pronounced neurological symptoms, particularly paraesthesia, are extremely common during VC therapy [6] and represent the dose-limiting toxicity. Because of our experience with this drug, especially in the assessment of its side effects, we considered it reasonable to compare the severity of the neurological symptoms experienced by the patients in the present study with our previous experience and with reports in the literature. In a recent study by Jackson et al. [4], 36% of patients experienced moderate to severe paraesthesia that required dose modification after three weekly administrations of $VCSO_4$ at 70% of the dose used in the present study. In our subjects, who received VC incorporated into LDL, comparatively few and mostly mild side effects occurred. In particular, only one patient developed WHO grade 3 peripheral neuropathy associated with severe paraesthesia and mild tendon-reflex loss. In fact, the major problem for most of our subjects was alopecia (WHO grade 2 or 3 in five of eight patients). It is conceivable, albeit unlikely, that the possible reduction in side effects was a consequence of the concurrent administration of prednisolone. This drug is widely used in combination with VC and other cytotoxic drugs, albeit at a dose higher than that given in the present study, but no beneficial influence on the management of VC neurotoxicity has been reported [6]. However, it is likely that this glucocorticoid contributed to the overall well-being experienced by our patients, and it may have had a suppressive effect on febrile and allergic reactions to the LDL.

The observed differences in plasma concentrations of VC did not reach statistical significance, presumably due to the limited number of patients from whom data were obtainable in this initial study. However, differences in uptake by tissues may occur when $VCSO_4$ is used or when VC incorporated into LDL is given. VC is normally eliminated rapidly from plasma by protein binding and by concentration into thrombocytes, which possess "non-classic" LDL receptors that may not mediate the uptake of LDL/VC [11]. It is therefore likely that VC in the form of LDL/VC would remain in plasma longer than $VCSO_4$, as the former would be shielded from protein binding and would be removed from the circulation by LDL-receptor-mediated uptake, which is slower than passive diffusion. The fate of the drug delivered in the LDL/VC complex cannot be determined on the basis of the present results, since estimations of the VC content in tumour tissue were not possible in this study. However, other workers [2] have shown that cells from gynaecological tumours exhibit high levels of LDL-receptor activity, suggesting that the concentration of drug carried by LDL will be higher in the actively growing parts of a tumour.

In conclusion, the present data demonstrate that LDL-encapsulated VC may safely be given to cancer patients and suggest that a more selective therapy of malignancies might be achievable using this treatment modality. These findings warrant further investigation in the form of a larger study comparing the detailed pharmacokinetics of LDL/VC and $VCSO_4$, their side-effect profiles and the uptake of drug by malignant and non-malignant tissues.

Acknowledgements. We are indebted to Assoc. Prof. C. Peterson and Dr. S. Vitols, Department of Clinical Pharmacology, Karolinska Institute, Stockholm, and to Prof. M. Szamatowicz, Department of Obstetrics and Gynaecology, University Hospital, Białystok, for their valuable advice and criticism regarding this study and to Mrs. R. Parry for her skillful technical assistance.

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