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for Breast Cancer Chemotherapy

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A platform technology has been established, based on reconstituted high density lipoprotein (rHDL) nanoparticles, to facilitate the delivery of intravenously administered drugs (including cancer chemotherapeutic agents). We have prepared rHDL/Taxol® complexes with a consistent composition and molecular weight that exhibited exceptional stability upon repeated gel chromatography and ultracentrifugation. Studies in mice led to the observation that about *ten times as much HDL encapsulated Taxol®* remained in the circulation, 1 hr post injection, compared to free Taxol®.

We have examined a number of cancer cell lines for their ability to incorporate cholesterol esters and Taxol® from rHDL complexes. The delivery of cholesteryl esters and paclitaxel was nearly as efficient to cancer cells as it was to a normal control (ovarian granulosa cells). We also showed that the uptake efficiency of cholesteryl esters and paclitaxel by cancer cells were closely correlated ($r^2=0.89$; $p<0.04$). Finally, we have found that the uptake of paclitaxel from an rHDL/paclitaxel formulation by cancer cells is inhibited by HDL₃ in a concentration dependent manner. These findings strongly suggest that the uptake of chemotherapeutic agents from the rHDL/drug complexes is facilitated by a receptor-mediated mechanism and thus should provide an improved formulation for targeted chemotherapy.

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INTRODUCTION

Adjuvant cancer chemotherapy has long been the accepted strategy for the treatment of several types of malignant tumors, especially those involving metastases (1). To enhance the efficacy of chemotherapy, multiple agents, longer and more intensive treatment, and combination therapy have been used. Although these modified approaches have resulted in improvements, the severity of side effects (2) and the gradual resistance developed by tumors during therapy (3) continue to hinder the efficacy of cancer chemotherapy. Consequently, more efficient delivery systems are needed to reduce the toxicity associated with cancer chemotherapy, while preserving the effectiveness of the respective chemotherapeutic agents.

In order to reduce the toxicity of chemotherapeutic agents, delivery vehicles including liposomes have been investigated (4,5). Some of the data emerging from these studies have shown that the efficacy of the drug delivery by liposomes was inversely related to the diameter of the particle (6). However, even the smallest liposomes have a diameter five times larger than the average high density lipoprotein (HDL) particle, a hydrophobic lipid micelle core with surface components of phospholipid and apolipoproteins (7). Because the overall properties of HDL are consistent with a potentially superior hydrophobic drug transporter, we have undertaken the investigation of rHDL particles as delivery vehicles for cancer chemotherapeutic agents.

KEY RESEARCH ACCOMPLISHMENTS

This is a "Concept Award" report. Due to the limited funding, the accomplishments are correspondingly modest. Nevertheless, we are pleased to report numerous exciting findings, two poster presentations at national meetings and some extended funding for this project.

The following scientific accomplishments can be documented during the second year of this award.

1. Three new formulations have been developed involving paclitaxel that are all superior to the earlier preparation reported earlier (8). The major advantage of these new formulations is the ability to exclude cremophor EL for the solubilization of paclitaxel, Cremophor EL is toxic upon intravenous administration and thus its presence is highly undesirable in formulations for cancer chemotherapy
2. Studies with Rhodamine-123, a chemotherapeutic agent currently in Phase I trial show that the rHDL formulation substantially enhances the drug's cytotoxicity against cancer cells.
3. Preliminary studies with drug resistant cell lines suggest that the rHDL formulation is highly effective in delivering anti-cancer drugs to resistant cells and in preventing the subsequent efflux of the anti-cancer agent from these cells

Studies in Progress:

We are currently beginning studies with valrubicin (AD32) a derivative of adriamycin (doxorubicin) that is considerably more effective against cancer cells and has reduced cardiotoxicity compared to doxorubicin.

REPORTABLE OUTCOMES

We have had an article published in *Stem Cell and Targeted Therapy*; K.A. Dickey and A. Keating Eds. *Garden Jennings (Pubs, 2003)*. -see Appendix

We will present our recent findings at the ASBMB meeting in Boston in June 2004.

We are filing an additional patent with the US Patent Office to expand our original application .

We have been submitting proposals to the DOD-CDMRP Breast cancer Research Program IDEA grant competition nearly every year. Unfortunately, the reviewers have been consistent in finding reasons to turn our applications down. Surprisingly, the comments made by the reviewers included criticism regarding the lack of progress as the resulting from the funding of our *Concept Award*.

CONCLUSIONS

We have completed and exceeded all the projected goals that were outlined for this project in the "Concept proposal". The next phase of our studies involves translational studies with animals that will require substantially higher level of funding. Accordingly, we have been submitting proposals to the DOD Breast Cancer Program, the National Institutes of Health and to the Susan G. Komen Breast Cancer Foundation. We are hopeful that these efforts will result in additional funding and the continuation of our work.

Regarding the overall potential impact of our research, we believe that it has unlimited potential. Accordingly, our research has wide applications in breast cancer chemotherapy because, in addition to taxol, many of the frequently employed drugs are poorly water soluble and their application would thus be substantially improved by the rHDL delivery system. In addition, because of the vast potential for targeting, via modifications of its protein or lipid components, the future application of the rHDL drug delivery system could revolutionize chemotherapy via tumor specific targeting and potentially overcoming drug resistance. The proposed approach has the additional potential to substantially improve the delivery of anticancer drugs to hormone resistant breast tumors and thus enhance the prognosis for the survival of breast cancer patients.

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APPENDICES:

Paper published in *Stem Cell and Targeted Therapy*; K.A. Dickey and A. Keating Eds. Garden Jennings (Pubs, 2003).

Abstract to be presented at the ASBMB 2004 meeting.

NOVEL DELIVERY SYSTEM FOR TARGETED CANCER THERAPY

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Abstract. Reconstituted high density lipoprotein (rHDL) particles have been developed that are able to incorporate water insoluble drugs as core components and thus are excellent targeted drug delivery vehicles. Specifically, we have prepared stable paclitaxel (TX) containing rHDL particles that exhibit the expected molecular weight, lipid and protein composition. The rHDL/ TX complexes were toxic to cancer cells as the drug was avidly taken up from the rHDL/TX complexes, apparently by a receptor mediated mechanism.

Background. Although the primary course of initial management for most malignant tumors is surgery, patients often require adjuvant or neo-adjuvant chemotherapy. To enhance the impact of chemotherapy, multiple agents have often been utilized, resulting in improved survival and cure rates. Recent strategies have also involved combinations of cisplatin, taxol or doxorubicin in order to overcome the drug resistance of malignant tumors. However, despite these positive developments, the solubility and toxic side effects of many chemotherapeutic agents remain a serious concern in the management of cancerous tumors.

The drug delivery system described here (Figure1) offers substantial advantages over conventional approaches for cancer chemotherapy.

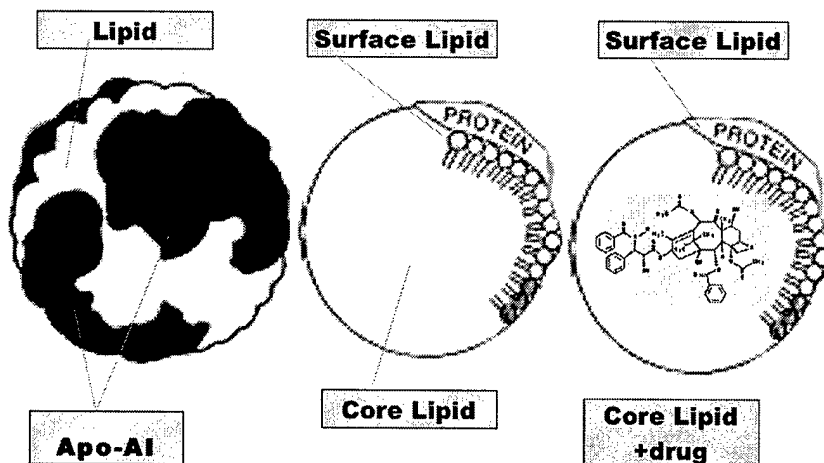


Figure 1.
Schematic view
of the rHDL drug
delivery system.
Protein = apoA-I.

The rHDL particles are considerably smaller than liposomes and thus offer substantially increased stability and improved pharmacokinetics over the conventionally used liposome system (1). Using the rHDL system, lower doses of the anti-cancer drug may be used during therapy and thereby limit the risk of side effects during intravenous administration. In addition, the rHDL delivery system has the potential for overcoming drug resistance (2) as the lipoprotein/drug complex is likely to facilitate the entry of anti-cancer drugs into the cancer cells by avoiding the membrane associated pump system (that is responsible for multi-drug resistance). Because of the very broad potential for targeting, via modifications of its protein or lipid components, the development and application of the rHDL drug delivery system has the potential to revolutionize the intravenous administration of drugs, including cancer chemotherapy.

Another advantage of the rHDL formulation is based on uptake of HDL core components, facilitated by a specific cell surface receptor (Figure 2).

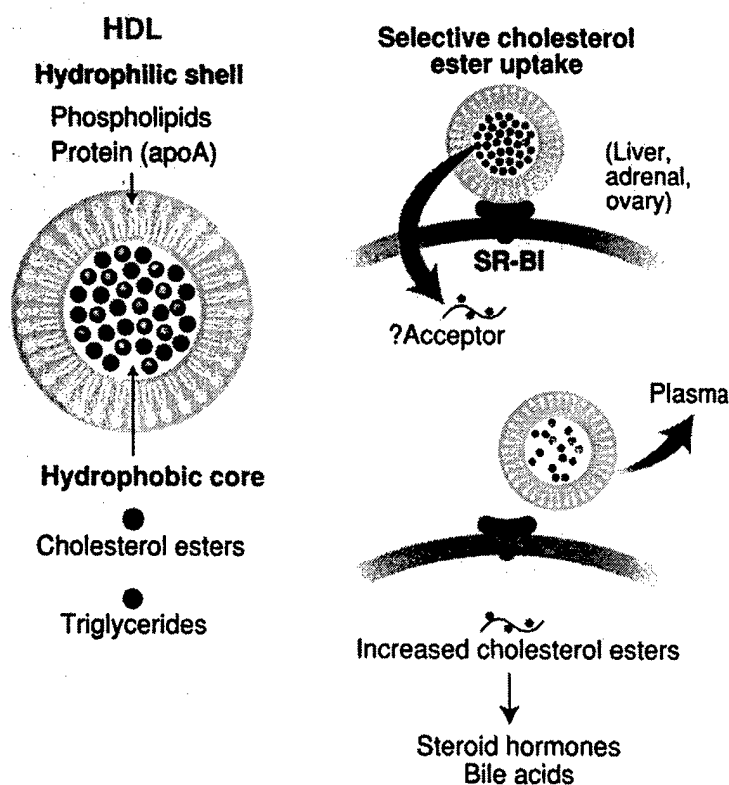


Figure 2

Mechanism for the cellular uptake of HDL core components (From: Steinberg, D. *Science*, 1996 271:460-1).

This mechanism has the potential to substantially improve the targeting efficiency of the formulations, delivered via the rHDL vehicles.

Results. During our initial studies (3), we have shown that paclitaxel (TX) and cholesteryl oleate were efficiently taken up by cancer cells when compared to a physiologically active ovarian cell line as a control (Table 1).

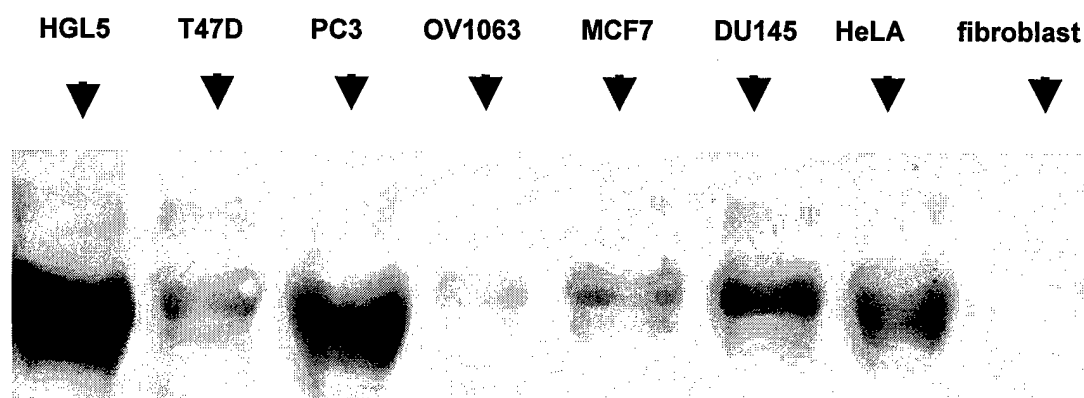
Table 1. Cellular incorporation of HDL-cholesteryl ester and paclitaxel from rHDL particles

CELL LINE		Cellular uptake of core component (%)	
		¹⁴ C-Taxol	³ H-cholesteryl ester
HGL5	Ovarian granulosa (control)	100	100
DU 145	Prostate carcinoma	67	75
MCF7	Mammary carcinoma	73	72
T47D	Mammary carcinoma	74	90
OV 1063	Ovarian carcinoma	74	75
PC3	Prostate carcinoma	83	91

These data show that the rHDL/drug complexes are able to deliver paclitaxel (an anti-cancer drug) with efficiency similar to that of cholesteryl ester (a natural HDL core component).

Immunoblotting studies, using antibodies against the SR-BI (HDL) receptor, show a relatively high expression of this receptor by cancer cells compared to a fibroblast cell line (Figure 3).

Figure 3. Immunoblot of the SR-B1 receptor in selected normal and cancer cell lines



In vivo administration of TX as part of the HDL complex show that the encapsulated preparation of TX had a substantially extended residence time in the circulation. After 30 minutes of injection into mice, about ten times more encapsulated TX remained in the circulation as compared to the free drug (Figure 4).

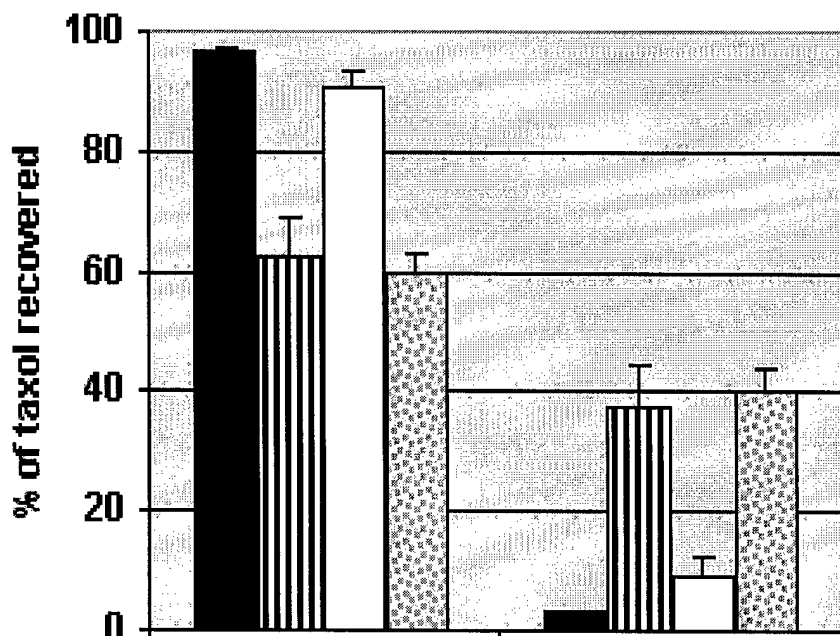


Figure 4. Taxol recovered when administered as the free drug-black bars (30 minutes post injection) and white bars (60 minutes post injection). As a core component of HDL-black striped bars (30 minutes post injection) and gray dotted bars (60 minutes postinjection). Preparations of taxol and HDL/taxol (labeled with ^{14}C taxol) were injected into mice ($n=5$) via the tail vein. Plasma and liver radioactivities were recorded post injection. The sum of the two pools was considered as 100%.

Conclusion. We have successfully concluded the "proof of principle" stage of our studies on the rHDL delivery delivery system. These studies show that a stable drug-containing lipid protein vehicle can be prepared that is able to facilitate the efficient delivery of anti-cancer drugs to cancer cells. Future studies addressing in vivo toxicity, tumor suppression in immunodeficient mice and subsequent clinical trials should provide the necessary data for the appropriate evaluation of this promising novel drug delivery system.

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at the University of North Texas Health Science Center by the American Cancer Society.

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Horse serum HDL as drug transporters. Johnson, S., Nair, M.P. Lacko, A.G. University of North Texas Health Science Center, Fort Worth TX 76107.

The purpose of this research was to evaluate horse serum HDL as a drug transporter, including the stability of the drug/HDL preparations. Our laboratory has developed a novel drug delivery system based on reconstituted and native HDL complexes that are highly effective in enhancing the solubility of hydrophobic drugs and may have particular utility in cancer. Horse serum because of its wide availability, its high HDL content and the absence of cholesteryl ester transfer protein (CETP) may serve as an attractive model system for studying HDL/drug complexes and their uptake by tumor cells

Horse serum HDL was prepared by a combination dodecylamine(DDA)-agarose chromatography followed by immunoaffinity chromatography utilizing an anti-serum albumin-agarose column. This method was considerably more efficient than the conventional ultracentrifugation procedure and resulted in a 60% vs a 8% yield of HDL cholesterol. The horse HDL was characterized regarding its chemical composition (lipid and apolipoprotein components) and molecular size. Horse HDL/drug complexes were prepared by incubating the isolated horse serum HDL with dilauroyl fluoresceine (DLF), a highly hydrophobic compound that becomes fluorescent upon removal of the fatty acyl chains. DLF is a good surrogate for anti-cancer drugs and was used to study stability of the HDL/drug complex and its uptake by cancer cells. Accordingly, the HDL/drug complexes were efficiently taken up by breast cancer (T47D and MCF7), prostate cancer (DU145) and ovarian cancer (OV1063) cells. These data show that: 1) the HDL delivery vehicle is likely to be effective as a drug delivery agent in cancer chemotherapy and 2) that dilauroyl fluoresceine is a suitable model compound for the study of the interactions between drugs and cancer cells.

Poster to be presented at the 2004 ASBMB meeting in Boston MA.