DRUG DELIVERY: BARRIERS TO DRUG TRANSPORT AND THE DESIGN OF NOVEL THERAPEUTIC AGENTS

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Plenary Sessions *Page*

January 8

Transport of Novel Therapeutics in Tissues 164

Epithelial, Lymphatic, and Interstitial Transport of Novel Therapeutics 165

January 9

Mucosal Vaccines and Delivery of Proteins to Mucus Epithelia 166

Delivery of Antibody-Based Therapeutics 166

January 10

Delivery of DNA and RNA in Cells and Tissues 167

Gene Therapy and Nucleic Acid Therapeutics 168

January 11

Drug Delivery to the Brain and Novel Strategies for Drug Development 168

January 12

Delivery of Cells and Particulates in Tissues 168

Late Abstracts 170

Poster Sessions

January 8

Targetting and Transport in Drug Delivery (A4-100-110) 171

January 9

Mucosal Vaccines and Delivery of Proteins to Mucus Epithelia;
Delivery of Antibody-Based Therapeutics (A4-200-210) 174

January 10

Delivery of DNA and RNA in Cells and Tissues; Gene Therapy and
Nucleic Acid Therapeutics (A4-300-308) 177

Transport of Novel Therapeutics in Tissues

A4-001 PHYSIOLOGICAL BARRIERS TO TRANSPORT IN TISSUES, Rakesh K. Jain, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

For a blood-borne therapeutic agent to reach its target cells in a tissue, it must accomplish three critical tasks: distribution through blood vessels, transport across vessel walls and through the interstitium. We have examined these transport processes in normal and tumor tissues *in vivo* using various macroscopic and microscopic approaches. Macroscopic approaches include a tissue-isolated tumor that is connected to the host with a single artery and a single vein. Microscopic approaches include various transparent tissue preparation, e.g., the mouse tail, the rabbit ear chamber, the dorsal skin fold chamber and cranial windows in rodents. Using in vivo microscopy, these preparations permit us to monitor noninvasively and over a long term various microscopic events: angiogenesis and blood flow, metabolic microenvironment, transvascular and interstitial transport, and leukocyte-endothelial interactions. These experimental approaches, coupled with mathematical models, have helped us gain insights into the physiological barriers to the delivery of (a) molecules of different size, charge and configuration, (b) particles such as liposomes and (c) effector cells. [See abstracts by L. Baxter, D. Berk, Y. Boucher, R. Melder and F. Yuan, and *Scientific American*, **271**:58-65, 1994].

In this presentation, I will focus on our studies on the interstitial convection, diffusion and binding of macromolecules using fluorescence recovery after photobleaching (FRAP). This approach is based on the analysis of sequential fluorescence microscopy images following a laser-induced photobleaching event (*Proceedings of the National Academy of Sciences*, **86**:5385-5389, 1989). The digitized images are analyzed by a spatial Fourier transform method that is insensitive to the effects of out-of-focus and scattered light (*Biophysical Journal*, **65**:2428-2436, 1993). The fluorescence recovery is interpreted according to a diffusion-reaction model. We used this experimental approach to investigate the transport of monoclonal antibodies (MAb) in the interstitium of human tumor xenografts (LS174T colon adenocarcinoma implanted in dorsal skinfold chambers in athymic nude mice) (*Cancer Research*, **52**:6553-6560, 1992). Fluorescein-labeled tumor-specific MAb (ZCE025) or a nonspecific control MAb (S1) were administered i.v. at doses ranging from 10^{-3} to 6 mg/g body weight, and the movement of the MAb within the interstitium was measured by FRAP at 24 and 100 h post injection. Control MAb was observed to diffuse at a hindered rate of ~1/3 compared to diffusion in free solution, and a fraction (~1/3) of these molecules was immobile within the timescale of the experiment. The relative reduction in diffusion coefficient was similar to that observed for a smaller macromolecule (bovine serum albumin). The immobile fraction, attributable to nonspecific binding, was independent of MAb concentration. In contrast, the specific MAb exhibited a dose-dependent mobility: at low doses (<0.01 mg/g), the immobile fraction approached 100%, but at high doses (>0.1 mg/g), the immobile fraction was the same as observed for control MAb.

A4-002 TRANSPORT OF MACROMOLECULES ACROSS THE MICROVASCULATURE: HETEROPOROUS MEMBRANE MODELS. Bengt Rippe, Department of Nephrology, University Hospital of Lund, 221 85 Lund, Sweden.

The exchange of small solutes and water across continuous microvascular walls occurs mainly through the spaces between endothelial cells, forming interendothelial junctions, denoted 'small pores' (functional radius ≈ 4-6 nm). How macromolecules traverse the microvascular endothelium is more controversial. The morphology of capillary walls offers three major possible routes: 1) wide interendothelial junctions, 'capillary leaks' (width ≈ 20-30 nm), 2) transport via plasmalemmal vesicles, i.e. transcytosis, and 3) channels formed by fused endothelial vesicles. From a physiological point of view there is much evidence supporting the notion of passive protein transport by convection through the endothelium: 1) Tissue cooling has been shown to reduce the transcapillary flux of radiolabelled proteins in proportion to the temperature dependent increase in fluid viscosity, but not to the marked extent that tissue metabolism was reduced. 2) Studies of the sieving characteristics of the blood-interstitial barrier in various vascular beds using lymphatic protein flux analyses are in line with a bimodal (two-pore) capillary selectivity. In such studies there is generally a coupling of large solute transfer to transcapillary volume flow (J_V) even for very large proteins. 3) There is evidence for convective protein transport from blood to tissue even when net J_V is zero, due to 'volume circulation' in the capillary wall.

'Volume circulation' can theoretically occur in heteroporous membranes. If there exist a few 'large pores' (radius 20-30 nm) in the capillary membrane besides a high number of 'small pores' (radius ≈ 4-6 nm), there will be a balance between the capillary Starling forces, i.e. the transcapillary hydrostatic (ΔP) and colloid osmotic pressure differences ($\Delta\pi$), across the small pores. Across the large pores, however, the effective $\Delta\pi$ is much too low to balance out the normal ΔP. Here there will always be a bulk filtration of plasma proteins from blood to tissue. In an isovolumetric state, protein-poor fluid enters the plasma from the tissue across the small pores and protein-rich fluid leaves the capillaries across the large pores. Hence, there is a net convective flux of proteins by solvent drag in the absence of a net convection. The magnitude of this flux is dependent on the $\Delta\pi$. Single-pore formalism, however, will tend to greatly overestimate the diffusive component of protein transfer. With homoporous concepts the coupling coefficient between protein transfer and lymph flow can still be adequately assessed at high J_V's.

Under specific conditions, the two-pore theory has to be extended to include the presence of the endothelial water-channels, the 'aquaporins'. When large crystalloid osmotic gradients are established across the microvasculature, such as across peritoneal capillaries in peritoneal dialysis, a large fraction of the total osmotic fluid flow will occur through these water-only pathways. Then, there will be marked sieving of small solutes across the capillary walls. Failure to account for the effects of aquaporins when crystalloid osmotic gradients are present may lead to (slight) underestimation of the capillary small-pore radius. In most instances, however, the two-pore model is sufficient to explain how transvascular solute clearances vary as a function of solute radius and to describe the coupling between large solute transfer and J_V.

A4-003 TRANSVASCULAR TRANSPORT IN NORMAL AND TUMOR TISSUE, Fan Yuan, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

The pharmacokinetics of blood-borne anti-tumor agents depends on transvascular transport. Hence the distinct properties of the tumor microvessel wall should be exploited for optimal drug delivery. A critical step in the optimization is to understand mechanisms or processes of transvascular transport. To this end, we have developed intravital fluorescence microscopy techniques to non-invasively measure and manipulate microvascular permeabilities in different tumors transplanted at two locations: dorsal skin-fold chambers and cranial windows in both mice and rats (1,2). We found that, in general, tumor vessels are more permeable to macromolecules than normal vessels, except for certain brain tumors (1,2). This is consistent with previous studies where the vessels in VX2 carcinoma transplanted in the rabbit ear chamber are seven times more permeable than those of granulation tissue (3). However, a human glioblastoma (HGL21) transplanted in cranial windows in severe combined immunodeficient (SCID) mice exhibits a lower microvascular permeability which is comparable with that of the blood-brain barrier (2). This is true for both macromolecules (e.g. albumin) and small molecules (MW<600). Microvascular permeability of both normal and tumor tissue can be influenced by the microenvironment (e.g. cytokines and inflammatory agents) and the size and charge of drugs. In a human colon adenocarcinoma (LS174T) transplanted in SCID mice, the permeability to albumin (MW=66,000) is $1.2\pm0.5\times10^{-7}$ cm/sec, which is about four times lower than that to ovalbumin (MW=45,000). Sterically stabilized liposomes (90 nm in diameter) used as drug carriers can also cross tumor microvessel wall, with the permeability of $2.0\pm1.6\times10^{-8}$ cm/sec (4). However, there is no extravasation of these liposomes in normal s.c. tissue, only minimal intramural accumulation in postcapillary and collecting venules has been observed. The largest size of liposome particles that can pass through the endothelium of LS174T in SCID mice is between 400 and 850 nm. No correlation between tumor size or age and microvascular permeability has been observed in our study.

1. Yuan, F., Leunig, M., Berk, D.A., and Jain, R.K., *Microvascular Research*, **45**: 269-289, 1993.
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4. Yuan, F., Leunig, M., Huang, S.K., Berk, D.A., Papahadjopoulos, D. and Jain, R.K., *Cancer Research*, **54**: 3352-3356, 1994.

Epithelial, Lymphatic, and Interstitial Transport of Novel Therapeutics

A4-004 LYMPHATIC TRANSPORT OF MACROMOLECULES, David A. Berk, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

The lymphatic system, recognized for its role in maintaining tissue fluid balance and as a locus for important immune system interactions, merits closer scrutiny from the standpoint of drug delivery. Strategies for optimal delivery of therapeutic or diagnostic agents, especially high molecular-weight agents, can benefit from a more detailed knowledge regarding the role of the lymphatics in clearing drug from tissue. Such knowledge will also allow evaluation of the lymphatic role during direct interstitial infusion of an agent. Physiologically-based pharmacokinetic models recognize the important relation between drug accumulation and lymphatic drainage, but detailed knowledge of lymphatic transport at the microscopic level is scant. For instance, it is likely that an absence of functional lymphatic vessels within solid tumors, coupled with a significant transvascular fluid flux, has profound physiological consequences for drug delivery. A recently developed animal model allows noninvasive observation of the initial lymphatic capillaries of mouse skin (1) and provides a complement to other experimental models for the measurement of vascular, transvascular, and interstitial transport. Fluorescently-labeled dextran (molecular weight 2 million), deposited intradermally ($\approx$ 2 µl volume) at the distal portion of the tail, is rapidly taken up by the skin lymphatics to reveal an extensive, highly regular, mesh-like network of lymphatic capillaries (mean diameter = 34 µm; mean distance between vessels = 380 µm). Local lymph velocities were measured by a fluorescence photobleaching technique (2), and an effective velocity of axial transport along the network was determined by photometric analysis of the timecourse of network staining. The labeled material is transported through this network proximally along the length of the tail at characteristic velocities of 1 to 20 µm/s (median velocity = 6 µm/s). No staining of lymphatic vessels occurred at sites of tumor cell (FSA II) implantation and growth, although the etiology of this disappearance (whether by a purely mechanical phenomenon or one involving cellular and biochemical factors) is still under investigation.

(1) Am. J. Physiol. 267 (Heart Circ. Physiol. 36) (in press), 1994.
(2) Biophys. J. 65: 2428-2436, 1993.

A4-005 INTERSTITIAL HYPERTENSION IN TUMORS AND ITS ROLE IN ANTIBODY DELIVERY, Yves Boucher, Massachusetts General Hospital and Harvard Medical School, Department of Radiation Oncology, Boston, MA 02114.

Compared to most normal tissues interstitial fluid pressure (IFP) is significantly higher in experimental and human solid tumors. We have shown in humans that interstitial hypertension was a pathophysiological characteristic of breast (mean ± SD; 15.0 ± 9.0 mm Hg), head and neck (19.0 ± 17.5 mm Hg), cervix (23.0 ± 20.5 mm Hg) colorectal (21.0 ± 12.0 mm Hg) and lung (10.0 ± 7.5 mm Hg) carcinomas as well as metastatic melanomas (14.5 ± 12.5 mm Hg). In normal skin and breast mean IFPs were respectively of 0.4 and 0.0 mm Hg. In comparison to tumors at other sites the IFP in brain tumors in humans was found to be significantly lower varying between - 0.5 and 8.0 mm Hg with a mean of 1.0 ± 2.5 mm Hg. The IFP in the brain surrounding the tumor was similar or slightly higher than in the tumor. A mathematical model of fluid transport in tumors was developed to identify the mechanisms governing interstitial hypertension and also to characterize how fluid movement determines the penetration of large macromolecules (e.g. monoclonal antibodies) in tumors (1, 2). The major parameters included in the model were the high vascular permeability and hydraulic conductivity of tumor vessels as well as the lack of a functional lymphatic circulation in solid tumors. The model predicted that the IFP was uniform throughout the tumor except for a steep drop in the periphery, and that the microvascular presssure (MVP) was the main driving force for tumor interstitial hypertension. In order to test the predictions of the model IFP profiles and MVP were measured with micropipets in mammary tumors transplanted in rats. The pressure profiles predicted by the model were confirmed in both subcutaneous and tissue - isolated tumors (3). In a tissue - isolated tumor the MVP was found to be similar to the IFP in the center of the tumor; in the region of the tumor periphery associated with the steep drop in IFP the MVP was significantly higher than the IFP (4). Since the extravasation of macromolecules is highly dependent on the filtration of fluids, the negligible pressure gradient in the center of tumors could be responsible for the poor accumulation of antibodies reported in several studies. In the tumor periphery the large difference in hydrostatic pressure between the microvascular and interstitial space leads to high filtration rates and a greater accumulation of antibodies. Strategies aimed at reducing tumor IFP and increasing pressure gradients between the microvascular and interstitial space will also be discussed.

1) Cancer Res., 48: 7022 - 7032, 1988.
2) Microvascular Res., 37: 77 - 104, 1989.
3) Cancer Res., 50: 4478 - 4484, 1990.
4) Cancer Res., 52: 5110 - 5114, 1992.

A4-006 ENHANCED AND SUSTAINED DELIVERY OF NOVEL THERAPEUTIC AGENTS THROUGH ORAL MUCOSAE BY TRANSMUCOSAL SALIVA-ACTIVATED MUCO-ADHESIVE DELIVERY SYSTEM: **Y.W. Chien[1], K. Yukimatsu[2], Y. Nazaki[2], M. Kakumoto, M. Ohta[2] and Y. Lee[1]; [1]Rutgers University, Controlled Drug-Delivery Research Center, Piscataway, N.J. 08854, USA, [2]Toyobo Pharmaceuticals Development Center, Otsu, Shiga, Japan.**

Parenteral administration is currently the delivery route of choice for many novel therapeutic agents, including peptide-/protein-based therapeutic agents, which are subject to extensive hepato-gastrointestinal "first-pass" elimination. However, the majority of these therapeutic peptides/proteins cannot accomplish their full range of medical benefits when administered parenterally as limited by the extremely short duration of their therapeutic activities. Recently, active research programs have been initiated to explore the feasibility of using various easily-accessible absorptive mucosae as the alternative routes for the non-invasive systemic delivery of novel therapeutic agents that have low systemic bioavailability due to high "first-pass" clearance.

To achieve the enhanced and sustained delivery of these therapeutic agents through oral mucosae, a transmucosal saliva-activated muco-adhesive delivery system (TmMDS) has been developed. The TmMDS is a bi-layer device comprising of a fast-release layer and a sustained-release layer. The fast-release layer is capable of providing a rapid release of a therapeutic agent, with or without a combination of stabilizers and permeation enhancers, while the sustained-release layer is capable of adhering to the alveolar/gingival mucosa and of releasing the therapeutic agent (the maintenance dose) continuously for prolonged duration. Using this TmMDS as the delivery device, organic-based therapeutic agents, like isosorbide dinitrate and nefidipine, have successfully overcome the oral mucosa barrier and promptly achieved the therapeutic levels required for the prevention/treatment of angina pectoris/hypertension for up to 12 hours. The systemic bioavailability attained is 4-5 times higher than that obtained by the conventional sustained-release oral dosage form. Long-term clinical efficacy was demonstrated. The TmMDS was also applied to the transmucosal delivery of peptide-based therapeutic agents. Using the naturally-occurring Luteinizing hormone-releasing factor (LHRF) as the model peptide, the feasibility of transmucosal delivery was also studied. The effect of various formulation variables on the release profile of LHRF and its transmucosal permeation kinetics was investigated systematically, using a specially-designed clinically-relevant transmucosal permeation cell apparatus, and the results will also be discussed.

Mucosal Vaccines and Delivery of Proteins to Mucus Epithelia

A4-007 INDUCTION AND REGULATION OF IMMUNE RESPONSES TO BIOENGINEERED MUCOSAL VACCINES, Jerry R. McGhee, Mariarosario Marinaro, Raymond J. Jackson, Ichiro Takahashi, Takachika Hiroi, Steven Chatfield, John VanCott, David Pascual, Frits Van Ginkle, Kohtaro Fujihashi, Masafumi Yamamoto, John Clements, Ken Bost and Hiroshi Kiyono, The Mucosal Immunization Research Group and Immunobiology Vaccine Center, University of Alabama at Birmingham, Birmingham, AL 35294; Medeva, London, England; Tulane University, New Orleans, L.A.

The mucosal immune system is a vast network of tissues, cells and molecules which function in a highly regulated manner for host protection and is ideally suited for vaccine development. This system is unique in that it is anatomically separated into inductive lymphoid tissues such as gut-, bronchus- and nasopharyngeal-associated lymphoreticular tissues (GALT, BALT, and NALT) where initial antigen uptake via specialized epithelial cells, termed M cells takes place. For example, oral immunization with vaccine proteins result in the formation of antigen-specific B and T cells in GALT, e.g., the Peyer's patches (PP) and antigen-specific B and T cells rapidly migrate to mucosal effector sites such as the lamina propria regions of the gastrointestinal (GI), upper respiratory and genitourinary tracts and to exocrine glands where actual differentiation of lymphoid cells occur and result in antibody synthesis, largely of the IgA isotype. Mucosal secretory IgA (S-IgA) as well as serum IgG subclasses, IgA and IgE responses to protein-based vaccines are dependent upon T helper (Th) cells and our Mucosal Immunization Research Group (MIRG) has focused on the nature of Th cell-mediated B cell responses following oral immunization with various antigen delivery systems. Our studies include assessment of oral adjuvants such as cholera toxin (CT) and CT-B as well as the related *E. coli* heat labile enterotoxin (LT) and LT-B molecules. We have used a model antigen, tetanus toxoid (TT) with CT or LT as well as delivery in microspheres or by recombinant *Salmonella* expressing fragment C of TT. Our studies show that oral immunization with TT and CT as adjuvant induces strong Th2 cell responses, which result in mucosal S-IgA as well as serum IgG1, IgA and IgE responses. The induction of IgE responses to oral vaccines is of course potentially detrimental, and alternate strategies are also followed. For example, oral TT given with LT as adjuvant result in Th1 followed by Th2 cell responses and a more "balanced" antibody profile ensues. In addition to mucosal S-IgA, serum IgG of all subclasses occur with IgA and low IgE responses. Oral administration of microspheres induces serum IgG responses largely of IgG1 subclass with low IgA and no IgE antibodies. On the other hand, oral immunization with r*Salmonella*-Tox C induces strong CMI responses as well as serum IgG antibodies; however in this case the subclass is largely IgG2a. These studies indicate that oral immunization with most antigens, and especially with CT as adjuvant, results in Th2-type responses. On the other hand, r*Salmonella* induce Th1-type responses when given by the oral route. Thus, the nature of delivery system and adjuvant affects the outcome of mucosal and systemic immune responses to vaccine proteins. Other key areas under investigation include : 1) the analysis and quantitation of virus-specific cytotoxic T lymphocytes (CTLs) and derived cytokines following mucosal immunization, 2) the regulation of cytokine production by epithelial cells in normal immunity and in intestinal inflammation (e.g., IBD), 3) the potential regulatory roles for epithelial cell interactions with intraepithelial lymphocytes (IELs) as effector cells in mucosal immunity, and 4) the role of neuropeptides in the regulation of antigen-specific mucosal immune responses. This work was supported by NIH-NIAID-DMID Contract AI 15128, and NIH grants AI 18958, DE 04217, DK 44240, AI 30366 AI 35932, AI 35544, CA 54430 and DE 09837.

A4-008 CONTROLLED DELIVERY OF PROTEINS TO A MUCOSAL SURFACE, W.M. Saltzman, J.K. Sherwood, M.R. Parkhurst, T.L. Wyatt, K.J. Whaley, and R.A. Cone, The Johns Hopkins University, Departments of Chemical Engineering and Biophysics, 3400 N. Charles Street, Baltimore, MD 21218.

The high rates of occurrence of sexually transmitted diseases (STD) and unwanted pregnancy are catastrophic international problems that require novel solutions. Since the lower reproductive tract of the female is the site of heterosexual STD transmission and sperm migration, it is also a convenient and appropriate site for delivery of prophylactic or therapeutic agents. Antibodies in the mucus secretions provide the first line of defense against the entry of pathogens. One method for protecting an individual is to increase the number of pathogen-specific antibodies in the mucus secretions. While systemic delivery of these antibodies may provide protection in some cases, it is reasonable to expect that antibodies must accumulate in the mucus layer of the epithelial tissue for protection.

We developed a topical approach for passive immunoprotection: long-term delivery of monoclonal antibodies from a controlled release polymer in direct contact with the mucus secretions. We tested this new method of antibody delivery by inserting polymer rings containing antibodies into the vaginas of mice [1]. Small polymer rings released significant quantitites of active antibody into buffered water for 30 d; antibodies released from the polymer were able to diffuse freely through unstirred layers of human cervical mucus [2]. When these same rings were inserted into the vaginas of mice, active antibody was continuously released into the cervical/vaginal mucus for 30 d. Preliminary studies, using antibodies against herpes virus [3], suggest that this approach can protect mice from genital herpes infections. Since significant absorption of many substances, including macromolecules, can occur through the vaginal epithelium, we also determined the rates of elimination for topically delivered antibodies, and the extent of antibody entry into body. Although our initial studies have focused on the use of topically delivered antibodies for protection against STD, this same approach may be useful for passive immunocontraception and stimulation of active mucosal immunity in the vagina [4].

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Delivery of Antibody-Based Therapeutics

A4-009 PHARMACOKINETICS AND REGIONAL DELIVERY, Robert L. Dedrick[1], Paul F. Morrison[1], Michael F. Flessner[2], Douglas W. Laske[1], Edward H. Oldfield[1], [1]National Institutes of Health, Bethesda, MD 20892, [2]University of Rochester, Rochester, NY.

The pharmacokinetics of regional drug delivery have been described for body regions with uniform concentrations[1]. It is easily shown for a linear system with constant parameters that the pharmacokinetic advantage, R_d, associated with administration of a pharmacologic agent directly into a body cavity or tissue is given by $R_d = 1 + CL_{TB}/(K(1 - E))$, where CL_{TB} is the total body clearance, K is the intercompartment transport parameter and E is the fraction of the drug that is irreversibly removed by the infused compartment. Thus, a low value of K, which is often cited as an obstacle to penetration of a drug from the systemic circulation, offers a potentially exploitable opportunity if the agent can be delivered directly to the target. This concept is illustrated by recent theoretical and experimental work on the intraperitoneal administration of a monoclonal antibody to rats bearing a human tumor xenograft[2] and to the administration of proteins directly into brain tissue[3,4]. Penetration of ^{111}In-MAb 96.5 into tumors derived from a human melanoma cell line (FEMX-II) was studied by quantitative autoradiography. The tumors were implanted in the anterior abdominal wall of athymic (RNU nude) rats, and the MAb is specific for antigen p97 associated with FEMX-II cells. Following 200 min of exposure, the MAb tumor concentration was one-tenth of the surface concentration at a depth of about 0.5 mm. Interestingly, although the transport cannot be described by a simple diffusion model and appears to be primarily convective, manipulation of water transport by the use of hypertonic dialysate had little or no effect on penetration. The surface concentration excess of the tumor compared with adjacent normal tissue was consistent with binding parameters obtained *in vitro* on tissue slices. The importance of convection compared with diffusion in the interstitial transport of large molecules provides an opportunity for well controlled delivery of macromolecular agents to tissue by intratissue infusion. The theoretical validity of this concept was established for isotropic brain tissue (gray matter) by calculations employing a transport model including convection, diffusion, chemical reaction and capillary permeation. Results suggested that a slowly degraded 180-kDa macromolecule infused at a rate of 3 μl/min would penetrate about 1.5 cm in 12 hr and produce quite uniform tissue exposure. The advantage of convection compared with diffusion was illustrated by simulations in which the infusion rate was reduced to approach a diffusionally dominated process. Comparison of the two procedures by means of both threshold-concentration and area-under-the-concentration-curve (AUC) metrics showed a 5- to 10-fold volume advantage for the high-flow technique associated with much more uniform tissue exposure. The plausibility of high-flow microinfusion for the delivery of proteins to the brain was supported by the bilatral infusion of ^{111}In-labeled transferrin for 1-4 hr into the corona radiata of cats. The tissue volume exposed to at least 1 % of the infusate concentration as determined by quantitative autoradiography at the end of the infusion increased linearly with infusion volume over the range from 75 to 600 μl with a slope of 6 μl tissue/μl infusate.

(1) Dedrick RL. J Pharm Sci 75:1047-1052, 1986; (2) Flessner MF, Dedrick RL. Cancer Res (in press); (3) Morrison PF et al. Am J Physiol 266:R292-R305, 1994; (4) Bobo H et al. Proc Natl Acad Sci (USA) 91:2076-2080, 1994.

Delivery of DNA and RNA in Cells and Tissues

A4-010 RETROVIRAL GENE DELIVERY, Robert G. Hawley, Division of Cancer Research, Sunnybrook Health Science Centre and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M4N 3M5, Canada.

A safety-modified retroviral vector system has been developed for potential use in gene therapy protocols (1). The system is based on the MSCV (murine stem cell virus) vector (2) that has proved to be highly efficient at transducing functional copies of exogenous genes into murine bone marrow cells capable of long-term hematopoietic repopulation of lethally-irradiated recipients (2-4). Safety-modified MSCV vectors include a mutated packaging signal for generation of high-titer virus stocks that is incapable of encoding any viral proteins and they are devoid of all *env* sequences so as to reduce the possibility of generating replication-competent viruses by recombination with helper proviruses in amphotropic packaging cell lines. In the standard configuration, the gene of interest in cDNA form is driven by a variant long terminal repeat (LTR) and a selectable marker [either the neomycin phosphotransferase (*neo*) gene, the hygromycin B phosphotransferase (*hph*) gene, the puromycin *N*-acetyltransferase (*pac*) gene or the β-galactosidase (*lacZ*) gene] is expressed from an internal phosphoglycerate kinase (pgk) promoter. Both the variant LTR, in association with a novel 5' leader region lacking the Moloney murine leukemia virus stem cell silencer element, and the pgk promoter direct constitutive expression in a wide range of cell types. Moreover, down-regulation of the LTR, which has been reported to occur with other "double-copy" retroviral vectors and suggested to be due to the negative influence of a second transcriptional unit in the vector or the presence of the *neo* gene, has not been observed in MSCV derivatives. Variations of the MSCV design for expression of multiple genes in specific effector cells will be described, and results obtained in murine models of systemic and localized delivery of therapeutic gene products will be presented.

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A4-011 NOVEL POLYMERS FOR THE DELIVERY OF PEPTIDES, OLIGONUCLEOTIDES AND PROTEINS, Jeffrey A. Hubbell[1,2], Jennifer L. West[2], Sanghamitra M. Chowdhury[1], and Randall C. Dunn[3], [1] Department of Chemical Engineering and [2] Biomedical Engineering Program, University of Texas, Austin, TX 78712-1062, and [3] Obstetric and Gynecological Associates, 7550 Fannin Rd., Houston, TX 77054.

The delivery of biological macromolecules, which are usually hydrophilic, is challenging from the hydrophobic resorbable polymers typically in use. Further, it is difficult to deliver these agents locally, upon the surface of a tissue to be treated. We sought to develop biodegradable polymeric materials that were suitable for local delivery upon tissues, as well as for use as barriers upon tissue surfaces for the control of wound healing. We have explored the photopolymerization of water-soluble macromers from aqueous solutions to form crosslinked gels upon tissue surfaces. To form gels that are adherent upon tissue surfaces, we have used photopolymerization in situ, directly upon the tissue. The macromer consists of a central chain of polyethylene glycol (PEG), with flanking regions of oligomers of degradable ester links, such as oligo(lactic acid), further end-capped with photopolymerizable groups, such as acrylic groups (1). We have utilized both long wavelength ultraviolet (2) and visible light (3) photoinitiation systems. The process of photopolymerization upon the tissue results in adhesion of the hydrogel to the tissue, presumably via the flow of the liquid precursor in to texture on the tissue. Drugs may be delivered from the gel to the tissue surface by incorporation in the macromolecular precursor solution and gelation, resulting in entrapment of the drug within the gel. If the drug is large relative to the distance between crosslinks (i.e., with a large drug, or a small PEG molecular weight), release is determined by the degradation of the gel. If the drug is small relative to the distance between crosslinks, release may precede degradation of the polymer. As an example, we have evaluated the use of fibrinolytic proteins to reduce postoperative adhesions in an ischemia and serosal injury model in the rat (2). Tissue plasminogen activator (tPA, 3 mg/ml, 1.5 ml/dose per rat), urokinase plasminogen activator (uPA, 1.8 mg/ml), or streptokinase (SK, 1.2 mg/ml) were incorporated within a gel derived from a 15% solution of an 8 kDa PEG with α- and ω-flanking lactic acid oligomers (DP approx. 5), further modified α- and ω-terminally with acrylate groups. The fraction of the length of the injured uterine horn tissue that resulted in adhesions was quantified 7 days after injury and treatment. As a comparison, the same amount of drug was delivered by intraperitoneal injection for 3 days. This gel has been previously shown to degrade over a 3-4 day period.

Drug, in Gel	Number of Animals	% Adhesions, Mean ± SEM	Drug, Free	Number of Animals	% Adhesions, Mean ± SEM
None	7	22 ± 3	None	7	77 ± 6
tPA	7	4 ± 1	tPA	7	49 ± 8
uPA	7	6 ± 3	uPA	7	78 ± 7
SK	7	45 ± 9	SK	6	83 ± 5

(1) A.S. Sawhney et al., *Macromolecules* **26**:581-587 (1993); (2) J.L. Hill-West et al., *Obstet. Gynecol.* **83**:59-64 (1994); (3) J.L. Hill-West et al., *Proc. Nat. Acad. Sci. USA* **91**:5967-5971 (1994).

A4-012 BIODISTRIBUTION AND METABOLISM OF ^{3}H-INTERNAL LABELED OLIGONUCLEOTIDES: COMPARISON OF A PHOSPHOTHIOATE TO BOTH 3' 5' BLOCKED AND UNBLOCKED PHOSPHODIESTERS. H. Sands*, L. Gorey-Feret, S. P. Ho, Y. Bao, A. J. Cocuzza, D. Chidester, G. L. Trainor, and F. W. Hobbs, Dupont Merck Pharmaceutical Co. Wilmington, DE 19880 and *Lexin Pharmaceutical Corp., Horsham, PA 19044.

Biodistribution and metabolism of oligonucleotides were determined using a ^{3}H-labeled 20 nucleotide phosphorothioate and its unblocked and 3' 5' end blocked phosphodiester analogs. The oligonucleotides were radiolabeled by ^{3}H-methylation of an internal deoxycytidine with *HhaI* methylase and S-[^{3}H] adenosylmethionine. Biodistribution studies were conducted after intravenous injection of 6 mg/kg (5 uCi) of each oligonucleotide. Metabolism was determined by paired-ion high performance liquid chromatography (HPLC). After unblocked phosphodiester injection radiolabel rapidly cleared from the blood. Relative initial concentrations were as follows: Kidney>blood>heart>liver>lung>spleen. Radiolabel in spleen peaked at 1 hr and remained elevated for 24 hrs. At 2 hrs the concentration in all organs, except spleen, was equal to that in blood. HPLC analysis of the kidney, liver and spleen extracts and urine indicated extremely rapid metabolism to monomer. Results of studies after the injection of the phorphorothioate differed from those using the unblocked phosphodiester. Despite its rapid clearance from the blood, phosphorothioate accumulated rapidly in all tissues, especially kidney. Kidney uptake increased over time, remaining high for 24 hrs. Ratios of organ to blood concentrations at 2 hrs for all organs were 5:1 or greater. Kidney and liver ratios were 84:1 and 20:1 respectively. Analysis of the kidney and liver extracts and urine indicate that slow metabolism occurred. The 3' 5' end blocked phosphodiester oligonucleotides were protected by circularization or by incorporation of either phosphorothioate or methylphoshonate linkages. Although some of these modifications protected the blocked oligonucleotides from degradation by exoducleases present in mouse serum, they were much less stable in vivo than the all phosphorothioate, and only marginally more stable than the unblocked oligonucleotide. In other respects, the 3' 5' end blocked phosphodiester oligonucleotides also resembled the unblocked phosphodiester oligonucleotides: radiolabel cleared rapidly from the blood, there was little evidence for tissue accumulation, HPLC analysis of tissue extracts showed extremely rapid degradation to monomer and only mononucleotides metabolites were present in urine. In summary, unblocked and 3' 5' end blocked phosphodiester oligonucleotides were rapidly attacked in vivo by endonucleases and therefore are relatively unattractive candidates for drug development.

Gene Therapy and Nucleic Acid Therapeutics

A4-013 SYSTEMIC GENE TRANSFER AND EXPRESSION, Tim Heath[1], Denny Liggitt[2], Yong Liu[3], Guanghuan Tu[3], Donald McDonald[3], and Robert Debs[3], [1]University of Wisconsin, Madison WI., [2]University of Washington School of Medicine, Seattle, WA 98195, and Cancer Research Institute, University of California, San Francisco, CA,94143.

We are investigating cationic liposome-based intravenous (iv) gene and transfer expression to attempt to both create somatic cell transgenic animals and to develop efficient and safe approaches for gene therapy. Currently, we are maximizing the level, duration and cellular-specificity of gene expression produced by cationic liposome-mediated iv gene delivery by optimizing multiple different parameters. These include the cationic liposome formulation, expression vector construction and host milieu. To date, we have shown that a single iv injection of human cytomegalovirus (CMV)-chloramphenicol acetyltransferase (CAT) expression plasmid:cationic liposome complexes into mice can transfect a wide variety of tissues and cell types. Specifically, we found significant CAT gene expression in all 20 of the tissues we assayed. There was no physiologic or histopathologic evidence of host toxicity in mice injected once or repeatedly with these DNA:liposome complexes. Immunohistochemical analysis revealed two discrete patterns of CAT gene expression at the cellular level. In one group of tissues, CAT gene expression was detected in large numbers of vascular lining cells, primarily in small vessels of the venous system. In another group of tissues, primarily belonging to the reticuloendothelial system, CAT antigen was detected not only in vascular lining cells, but also in some extravascular parenchymal cells. CAT antigen was still detectable in significant numbers of cells in some tissues 63 days after a single iv injection of the DNA:liposome complexes. Using immunohistochemistry, we have also shown that liposome-mediated gene expression can be preferentially targeted in animals at the cellular level, by linking the CAT gene to 3.8 kb of the human cystic fibrosis transmembrane conductance regulator promoter region. Some degree of tissue-specific targeting can be achieved either by regional administration of DNA:liposome complexes or by changing the specific cationic liposome formulation injected. Methods for targeting the expression of delivered genes to endothelial cells are currently being explored.

Drug Delivery to the Brain and Novel Strategies for Drug Development

A4-014 ENZYMES IN THE SYNTHESIS OF PHARMACEUTICALS, Jonathan S. Dordick, Pramod P. Wangikar, Yuri L. Khmelnitsky, Vikram M. Paradkar, Joseph O. Rich, Brett D. Martin, and Xiamao Chen. Department of Chemical and Biochemical Engineering, University of Iowa, Iowa City, IA 52242.

Enzymes have found increasing importance and use in the pharmaceutical industry for the preparation of new drugs, drug intermediates, and novel matrices for drug delivery systems. The most important aspects of enzyme catalysis in the generation of biologically active compounds are the high degree of stereo- and regioselectivity afforded by the biocatalyst, and the ability of enzymes to be used in nonaqueous media. Examples of direct interes to the pharmaceutical industry include the regioselective acylation of sugars for the synthesis of sugar-containing hydrogels (for use as macroporous drug delivery matrices), the selective modification of sugars in the synthesis of nucleoside derivatives, and the rational modification of enzyme stereo- and regioselectivity for general use in the synthesis of bioactive compounds.

Delivery of Cells and Particulates in Tissues

A4-015 NANOPARTICLES IN DRUG DELIVERY, Elaine Merisko-Liversidge[1], M. J. Shaw[1], G. McIntire[1], P. Sarpotdar[1], E. Cooper[1], G. Wolf[2], T. Corbett[3] and Gary G. Liversidge[1]. 1. Pharmaceutical Sciences, Sterling Winthrop, Malvern, PA. 2. CIPR Mass. General Hospital, Charlestown, MA, 3. Wayne State University, Detroit, MI.

Frequently in drug discovery a highly biologically active agent is identified but due to its poor solubility in water (<1mg/ml) is difficult to evaluate and develop beyond an initial discovery phase. Oral administration of such agents is frequently associated with poor bioavailability; very limited range of dose versus bioavailability proportionality; and large fed-fasted variability in bioavailability. For parenteral, the conventional pharmaceutical method for dealing with such agents is developing a formulation containing a high percentage of co-solvents. On administration this oftentimes is responsible for anaphylaxis, pain at the site of injection and, paradoxically, precipitation which results in poor bioavailability and emboli formation.

To address the drawbacks associated with poorly water soluble compounds, we have developed a widely applicable method of formulating these agents as dispersible particles. Using wet-bead milling technology a series of molecules with diverse chemistries and applications have been formulated as a fine particle suspension having a sufficient amount of adsorbed surface modifier to maintain an effective average particle size of less than 400nm. The methods of preparation of these nanocrystalline dosage forms will be presented together with an overview of current and future application of the technology.

A4-016 DELIVERY OF EFFECTOR CELLS TO NORMAL AND TUMOR TISSUES, Robert J. Melder, Massachusetts General Hospital and Harvard Medical School, Department of Radiation Oncology, Boston, MA 02114

The localization of either adoptively transferred or naturally circulating effector cells within tumors depends on several factors. These may be grouped into three principal categories: 1) the biophysical or phenotypic properties of the effector population, 2) the properties of the tumor vasculature, and 3) the characteristics of blood flow through the systemic circulation of the host. Within the first category, factors such as the visco-elastic characteristics, size, adhesive capability and specificity of the effector population must be considered. The characteristics of the tumor vasculature, such as architecture, flow rates, uniformity of perfusion and cellular adhesion molecule expression also contribute to delivery and retention of effector populations within the tumor vessels. Lastly, the systemic distribution of effector populations is subject to the characteristics of blood flow and vascular architecture in each of the normal organs. The combination of these factors will influence how many cells will actually enter the tumor vessels and adhere, or how many redistribute throughout the normal tissue. Consequently, the efficacy of a cellular mediated therapy and its potential for deleterious effects may be largely dependent upon successful localization in the relevant tissue. Multiple technologies have aided us in understanding elements of this process, including: micropipet manipulation of single cells (*Cell Biophysics*, **20**: 161-176, 1992), Positron Emission Tomography (PET; *Cancer Research*, **53**: 5867-5871, 1993), Dynamic flow-chamber studies (*Biophysical Journal*, **67**: 889-895, 1994), and observations of cranial window preparations in normal and tumor bearing mice (*Cancer Research*, **54**: 4564-4568, 1994). Studies with PET tracking of ^{11}C labeled activated natural killer (A-NK) cells showed high localization (up to 50 %) in the pulmonary vasculature within an hour after injection (*J. Immunol. Meth.* **175**: 79-87, 1994). Administration of labeled cells directly into the local circulation of a murine fibrosarcoma grown in the tail via the communicating veins resulted in tumor specific retention of the injected cells, although the rate of uptake and retention of the cells was highly heterogeneous. A second tumor model was developed which would permit microscopic observation of cells injected into the arterial blood supply of a spontaneous mammary carcinoma (MCaIV) grown in the cranial window of a mouse. Localization of A-NK cells was observed in the tumor vessels with binding efficiencies ranging from 0 to greater than 90% (25.8±3.3, Mean±SE). Low level retention of cells in normal tissue was associated with deformation of the cells, suggesting mechanical entrapment in pial capillaries. Flow chamber studies of NK cells on activated endothelium indicated the importance of VCAM/VLA4 in A-NK adhesion under dynamic conditions, however, the role of this adhesion mechanism in tumor specific binding is unclear. Measurement of the rigidity of IL2-activated NK cells has previously shown significant increases in the resistance of cells to deformation by an external force (micropipet aspiration). Recent studies (*J.Immunol.Meth.*, In Press) have shown that treatment with thioglycollate can be used to reduce cellular rigidity at least two-fold without a loss of viability or cytotoxicity in the treated effector cells, which may be useful to decrease the entrapment of injected cells in normal vasculature.

A4-017 NEW DEVELOPMENTS IN LIPOSOMAL DELIVERY: STERIC STABILIZATION AND ACCUMULATION IN TUMORS, D. Papahadjopoulos, et al., Cancer Research Institute and Department of Pharmacology, University of California, San Francisco, CA,94143

Liposomes stabilized by the inclusion of lipids with bulky hydrophilic groups have a pronounced effect on tissue distribution and produce a large increase in the pharmacological efficacy of encapsulated Doxorubicin against mouse tumors. This anti-tumor effect is associated with prolonged circulation time in blood, a markedly decreased uptake by liver and spleen, and a corresponding increased accumulation in implanted tumors (1-2).To examine the localization of liposomes in various tissues at the cellular level, we have produced liposomes labeled with encapsulated colloidal gold or rhodamine-dextran (3). The colloidal gold was detected by optical microscopy after silver chloride enhancement and staining of fixed tissue. Silver-enhanced colloidal gold was found predominantly within Kupffer cells in normal liver and within macrophages in bone morrow. Within the implanted tumors, silver-enhanced gold particles could be seen in small blood vessels and focally beyond the endothelial layer, in the extracellular space around tumor cells. Extravasation and perivascular accumulation of liposomes was also observed by fluorescence microscopy in a Human Tumor Xenograph(4). Since sterically stabilized liposomes do not bind and are not endocytosed by tumor cells (5), we have conjugated on the liposome surface an antibody fragment (rhu MAbHER 2 Fab') recognizing the HER-2 receptor, which is overexpressed in many breast cancer cells. Such targeted liposomes bind avidly to the target tumor cells, are rapidly endocytosed and can produce strong growth inhibition and cytotoxicity due to encapsulated Doxorubicin (6). Pharmacokinetic and therapeutic experiments in nude mice inoculated with the target human breast tumor cells (BT-474 and SK-BR-3) are in progress.More recently, we have been able to formulate thermosensitive sterically stabilized liposomes with high differential release of encapsulated Doxorubicin between 37° and 42° in the presence of 50% human plasma (7). Finally, we have shown that local hyperthermia applied to mouse tumors growing SC enhances the deposition of sterically stabilized liposomes into the tumor tissue, and significantly increases their anti-tumor efficacy against C-26 colon carcinoma (8).

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A4-018 *Abstract Withdrawn*

Late Abstracts

POLYMERS AS AN INTRACRANIAL IMPLANTABLE CONTROLLED DRUG DELIVERY SYSTEM.

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The blood-brain barrier limits the usefulness of many drugs for applications in the central nervous system. One promising method for bypassing the blood-brain barrier is clinically implantable biocompatible polymers. Several polymer-drug devices have been developed for intracranial implantation to release drugs to the brain over extended periods of time. The polymers deliver higher concentrations of drug to the brain than can be achieved with systemic drug administration while minimizing systemic exposure to the drug. This technology has been used to treat patients with malignant brain tumors.

A phase III clinical drug trial assessing the effectiveness of BCNU loaded polymers in patients with recurrent malignant gliomas has recently been completed. Novel chemotherapeutic drugs, immunotoxins, and angiogenesis inhibitors have been delivered against gliomas in the laboratory. Dexamethasone delivered from an implantable polymer has also been investigated as a method for treating cerebral edema. The polymer produced significantly lower blood drug levels than occurred with systemically administered drug, suggesting that there should be fewer side effects relative to systemically administered drug. Thus, biocom-patible polymers are a novel way to administer drugs to the central nervous system and may have applications for a variety of other solid tumors. Surgicallyimplanted biodegradable polymers are able to deliver high drug concentrations over prolonged periods of tumor directly to the site of tumor resection.

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THERAPY OF CANCER METASTASIS BY SYSTEMIC ACTIVATION OF MACROPHAGES,

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Despite continuous improvements in early diagnosis, surgical techniques, general patient care, and local and systemic adjuvant therapies, most deaths from cancer are still due to metastases that are resistant to conventional therapies. In the majority of cancer patients, metastasis has occurred by the time of diagnosis of the primary tumor, and metastases can be located in different lymph nodes and visceral organs. Different organ environments can modify the response of a metastatic tumor cell to systemic therapy and thus alter the efficiency of anticancer agents. The major barrier to the treatment of metastases is the biological heterogeneity of cancer cells in primary tumors and metastases which is exhibited in characteristics such as cell surface receptors, enzymes, karyotypes, cell morphologies, growth properties, sensitivities to various therapeutic agents, and ability to invade and produce metastasis. The heterogeneous nature of neoplasms and the resistance of variant cells imply that the successful therapy of disseminated cancer must include a modality that can overcome these obstacles. The systemic activation of macrophages with liposomes containing biological agents can meet these challenges.

Tumoricidal macrophages selectively bind to and destroy malignant cells *in vitro* and *in vivo* while leaving nonneoplastic cells unharmed. Moreover, macrophage-mediated lysis of tumor cells is not associated with the development of significant tumor cell resistance. Intravenously administered liposomes are removed from the circulation by phagocytic cells. The endocytosis of liposomes containing activation signals activates macrophages *in situ*. Multiple administrations of such liposomes produce the destruction of cancer metastases in many murine tumor systems in spontaneous canine osteosarcoma and in humans with relapsed osteogenic sarcoma lung metastasis. The stimulation of host antitumor responses by liposome-encapsulated macrophage activators does not require the help of T lymphocytes or natural killer cells. The outcome of macrophage-tumor interaction *in vivo* is a function of factors such as the macrophage-to-tumor cell ratio, the activation state of the macrophages, and the rate of tumor cell proliferation. The therapy of metastases by macrophage activation therefore will be most successful when the metastases are small, slow to proliferate, and infiltrated by a large number of tumoricidal macrophages. The critical limitation to treatment of disseminated metastases by systemic macrophage activation, however, appears to be tumor burden. A heterogeneous disease such as cancer cannot be treated by a single modality: the rational integration of macrophage-directed therapy in the multimodality management of the cancer patient should contribute to the therapy of cancer metastasis.

RGD PEPTIDE-BASED DELIVERY,

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We have used phage display libraries to search for peptides that bind to the ligand-binding site of integrins. The integrins are heterodimeric membrane receptors that mediate cell adhesion. Many of the 20 or so integrins known at this time reorganize the tripeptide sequence RGD in their ligand proteins, which include extracellular matrix proteins and fibrinogen. Peptides containing the RGD sequence are of considerable interest, because they can be used to probe integrin functions and in the supporting or inhibiting various cell adhesion processes.

The peptide libraries used in our recent work contain a random peptide insert flanked by a cysteine residue on each side, an arrangement that results in the expression of the random sequence as in a disulfide-bonded loop. Screening of a library with a heptapeptide insert (CX_7C library) for phage that bind to the $\alpha_5\beta_1$ integrin yielded primarily RGD-containing inserts confirming the central role of the RGD sequence in the ligand-binding of this fibronectin receptor (Koivunen *et al., J. Cell Biol.* 124:373-380, 1994). An interesting observation made in these studies was that essentially all of the phage that bound to the integrin bound to its ligand-binding site, indicating that this site has properties that are quite different from the rest of the molecule. In addition to peptides containing the RGD sequence, we found peptides that appeared to mimic auxiliary binding sites for the integrin---these peptides tended to have low affinities. As the stringency of the screening procedure was increased to enhance the yield of high affinity binders, the sequences flanking the RGD in the inserts became more restricted, indicating a preferential sequence context for the RGD in $\alpha_5\beta_1$ binding. Similar screening with other RGD-directed integrins has revealed distinct flanking sequence specificities for each of them, indicating that RGD peptides that are selective for the individual integrins can be identified by this procedure.

One of the $\alpha_5\beta_1$-binding sequences identified did not contain an RGD sequence, but bound with a high affinity. The corresponding cyclic peptide CRRETAWAC inhibits $\alpha_5\beta_1$-mediated cell attachment to fibronectin and is essentially specific for $\alpha_5\beta_1$ among the integrins. For some tumor cells, binding of $\alpha_5\beta_1$ to a fibronectin matrix is necessary for these cells not to undergo apoptosis. By preventing $\alpha_5\beta_1$ binding to fibronectin, the CRRETAWAC peptide can enhance tumor cell apoptosis. The potential of this peptide in tumor therapy animal models is being assessed.

Targetting and Transport in Drug Delivery

A4-100 INCREASED CELLULAR UPTAKE OF THE HIV-1 TAT PROTEIN AND CARGO PROTEINS CONJUGATED WITH TAT AFTER MODIFICATION WITH BIOTIN. Ling Ling Chen,‡ Alan D. Frankel,§,† Jennifer L. Harder,† Stephen Fawell,‡ James Barsoum,‡ and Blake Pepinsky‡. ‡Biogen Inc., 14 Cambridge Center, and †Whitehead Institute for Biomedical Research, Cambridge, MA 02142; §Department of Biochemistry and Biophysics, and Gladstone Institute of Virology and Immunology, UCSF, San Francisco, CA 94141

The HIV-1 Tat protein can efficiently enter cells when added exogenously in tissue culture and can carry other molecules into cells when added as conjugates. By using transactivation of a CAT reporter gene under control of the HIV LTR as a measure of intracellular delivery, we found that the addition of hydrophobic groups to Tat potentiated its uptake. Biotin was the most promising of the reagents tested and we characterized this effect in more detail. When coupled through a cysteine thiol, the addition of a single biotin to Tat increased CAT activity by about 6-fold. Increased activity was only seen with reducible biotin analogs, as modification with non-cleavable analogs is known to block Tat transactivation activity. Biotin had no effect on Tat uptake when mixed with Tat without cross-linking. To test if biotin also increased the uptake of heterologous proteins that were conjugated to tat, we used the cytotoxic activity of tat-RNase conjugates as a model system for uptake. Biotin also increased the delivery of a Tat_{37-58} RNase conjugate. The evaluation of other systems, including domain III of *Pseudomonas* exotoxin and the E2 repressor from human papillomavirus are in progress. The increased uptake of Tat and Tat conjugates by addition of hydrophobic groups may significantly enhance the usefulness of Tat as a delivery vehicle, and the approach may be applicable to other systems.

A4-101 DISTINCT INFLUENCE OF PEGylation ON THE TUMOUR LOCALISATION OF TRANSFERRIN AND A TUMOUR-SPECIFIC Fab FRAGMENT (F9), Cristina Delgado*, Angel Herraez*, Ajay K. Agrawal*, R. Barbara Pedley$, Derek Fisher*, Richard H.J. Begent$ and Gillian E. Francis*, Molecular Cell Pathology* and CRC Targeting and Imaging Group$, Royal Free Hospital School of Medicine, University of London, England.

PEGylation of proteins has proven an effective means to increase their plasma half-life, reduce their immunogenicity/antigenicity and to increase their resistance to proteolytic degradation. Since some of the biological activity is retained (depending on the coupling method used) PEGylated proteins have advantages over their unmodified counterparts as therapeutic agents. However, the basis for the altered pharmacokinetics and biodistribution of PEGylated proteins (including the reported enhanced tumour localisation of a PEGylated $F(ab')_2$) remains obscure. We have studied the influence of PEGylation in the biodistribution of two proteins, a Fab fragment of A5B7 (an anti-CEA antibody) and transferrin, in nude mice bearing the human colon carcinoma LS174T (which bears both CEA and receptors for transferrin). PEGylation increased the AUC for F9 in all tissues (blood > tumour > colon > lung > liver > muscle > spleen > kidney). In contrast, PEGylation of transferrin led to qualitative and quantitative different changes to the AUC: increased (blood > liver > lung = spleen > kidney), unchanged (tumour) and reduced (muscle > skin > colon). In order to establish which changes to the rates of entry (k_{in}) and exit (k_{out}) to and from the tissue were occurring, the pharmacokinetic data has been fitted to a simple two-compartmental model in which a numerical solution for k_{in} and k_{out} for each tissue is obtained by linear regression of the data for concentration in tissue, AUC for blood and AUC for tissue at every time point (AH, manuscript in preparation). PEGylation produced a general reduction in both rates for the two proteins in all tissues with the exceptions of F9 to and from the liver (modest increases of both rates, with greater increment for k_{out} than for k_{in}) and of k_{out} for F9 from the spleen (modest increase). The contrast in the changes of k_{in} and k_{out} for F9 and transferrin, give insight into PEG-specific and protein determined factors in the biodistribution of PEG proteins. The contrasting effects on these rates for tumour and normal tissues for F9, provide the basis of the improved tumour localisation. The implications of these results for the potential application of PEGylated proteins in tumour targeted therapy will be discussed.

A4-102 INSULIN ANALOGUES AIMED AT THE LIVER, Richard H.Jones, Fariba Shojaee-Moradie, Heike Eckey, Peter H. Sönksen and Dietrich Brandenburg, Department of Medicine, UMDS of Guy's and St. Thomas 's Hospitals, London, SE1 7EH. UK.

Current methods of insulin adminstration in the treatment of diabetes fail to reproduce the relatively greater hepatic than peripheral exposure to insulin which results from physiological delivery into the hepatic portal vein. Insulin analogues with an intrinsically greater effect on the liver than in adipose tissue or muscle could offer a more physiological approach to the treatment of diabetes even after conventional subcutaneous injection.

Having shown in glucose clamp protocols using D-[3-^{3}H]glucose that relative to insulin, ($M_r \approx 6000$), covalent insulin dimers ($M_r \approx 12000$) exhibited 5.6-19.2 fold higher potencies in reducing hepatic glucose production (Ra), than increasing glucose utilisation (Rd) we suggested that the endothelial barrier of peripheral tissues could be exploited to target insulin analogues selectively to the liver.

We have designed and prepared analogues of insulin which include a thyroxyl group and can bind to thyroid binding proteins after absorption from subcutaneous sites into the circulation. Glucose Ra and Rd were measured during intravenous infusion of insulin (Ins), $N^{\alpha B1}$thyroxyl-insulin (T4-Ins) and $N^{\alpha B1}$thyroxyl-aminohexanoyl-insulin (T4-AHA-Ins) into 4 anaesthetised beagles. Variable infusion of D-glucose and D-[3-^{3}H]glucose was used to maintain euglycaemia and specific activity.

Values for the effects on Ra and Rd were calculated as area between the basal values and the subsequent values plotted against time (AUC) For Ins,T4-Ins and T4-AHA-Ins, AUCs for Ra were -385±51, -347±57 and -403±77 mg/kg respectively (mean±SEM), (no significant differences). For Rd, values were 1056±129, 565±91 and 744±136 mg/kg, both analogues different from Ins $p<0.05$.

These results support the proposition that these analogues retain access to hepatic insulin receptors after binding to thyroid hormone binding proteins. We suggest that reduced transcapillary endothelial transport to peripheral insulin receptor sites can explain their relatively lower capacity to stimulate glucose Rd.

A4-103 CONTROLLED DELIVERY OF GROWTH FACTORS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS, Christine E. Krewson and W. Mark Saltzman, Department of Chemical Engineering, Johns Hopkins University, Baltimore, MD 21218

Neurotrophic factors have been shown to prevent degenerative changes in axotomized neurons of the central nervous system, and to enhance neurite extension, regeneration, and survival of peripheral neurons. Unfortunately, protein drugs are generally considered to be poor candidates for treating CNS diseases because they penetrate the blood brain barrier very slowly following systemic administration. Long-term, localized delivery of growth factors may help regenerate lost structure and function in diseased or traumatized tissues.

To approximate the *in vivo* delivery of growth factors in a more controlled environment, a three-dimensional *in vitro* system was designed in which PC12 cells were suspended in a collagen matrix. In the presence of a poly(ethylene-co-vinyl acetate) (EVAc) polymer matrix impregnated with nerve growth factor, a gradient of NGF was established within the gel. The extent of neurite outgrowth from the cell aggregates at any position in the gel depended on both the time following polymer insertion and distance from the polymer. In gels with varying concentrations of PC12 cells, levels of NGF in both the gel and the overlying medium was found to depend on cell concentration, suggesting that metabolism by cells has an important influence on local NGF concentration.

Similar polymer disks were implanted into the brains of adult male rats, and NGF concentration in the surrounding brain tissue was determined 2 days and 1 week post-implantation. NGF concentrations greater than 1 ng/ml were found within 2-3 mm rostral and caudal to the polymer disk edges in the ipsilateral hemisphere at both times. Picogram levels of NGF could be detected throughout the entire brain. Maximum NGF levels were found in the brain sections that had contained the polymer, reaching levels of 160 ng/ml after 2 days, and 60 ng/ml after 1 week. NGF released into buffered saline from polymer disks could be changed by a factor of about 5 by altering the formulation of the polymer. However, implantation of these different polymers into the brains of rats did not cause any variation in the NGF concentration detected in the tissue. These results suggest that protein drugs are readily eliminated from the CNS, and that to treat a neurodegenerative disorder effectively with such an agent, the protein must be delivered almost directly to the site of action.

A4-104 DIGITONIN ENHANCES THE UPTAKE OF CARBOPLATIN IN LIVER TUMOR AFTER ADMINISTRATION IN THE HEPATIC ARTERY.

Per G. Lindnér*, Peter L.J. Naredi*, Stephen B Howell◊, Dennis Heath◊, and Larsolof R. Hafström*, *Department of Surgery, Sahlgrenska Hospital, University of Göteborg, S-413 45 Göteborg and ◊UCSD Cancer Center, San Diego U.S.A.

One reason for lacking therapeutic effect of cytotoxic drugs is that the intracellular concentration of the drug is insufficient. Platinumcontaining drugs enter the cell slowly and have a poor tissue penetration. Increasing the permeability of the cell membrane might increase the intracellular drug concentration. Digitonin, a detergent, that increases the cell permeability through binding to cholesterol molecules in the cell membrane can, *in vitro*, increase platinum accumulation and reduce tumor growth rate.

Aim: To investigate if digitonin increases drug uptake, *in vivo*, in an experimental tumor system.

Material and methods: In LH rats a hepatoma was implanted in the liver. 7 days after implantation a catheter was inserted in the gastroduodenal artery so that infusions could be given in the hepatic artery without altering hepatic blood flow. 0,2 ml 400 μM digitonin solved in DMSO or saline in the control group) was infused in the hepatic artery and 10 minutes later carboplatin(CBDCA) 25 mg/kg was infused. One hour later the animals were sacrificed and biopsies from tumor and liver parenchyma were obtained.

Results: The concentration of platinum in liver tumor was 135±30 ng/mg (n=7, mean±SEM) in the digitonin-DMSO group and 12±4 ng/mg (n=7) in the control group ($p<0,01$). In liver parenchyma the concentration was 9±2 ng/mg in the digitonin-DMSO group and 13±3 ng/mg in the control group. Measured with the 133Xenonclearance, digitonin did not alter the tumor blood flow.

Conclusion: These findings demonstrates that pretreatment with digitonin-DMSO increases the uptake of CBDCA.

A4-105 DRUG TARGETING TO THE PULMONARY ENDOTHELIUM: AN APPROACH EXPLORING THE MONOCLONAL ANTIBODY TO ACE.

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Angiotensin-converting enzyme, ACE is an ectopeptidase localized on the luminal surface of the vascular endothelium. Lungs contain a large amount of ACE accessible to the circulation. We have suggested to the use of a monoclonal antibody to ACE as an affinity carrier for drug targeting to the pulmonary endothelium. We have generated Mab 9B9, mouse IgG1 monoclonal antibody to human ACE, which does not fix complement, does not inhibit ACE enzymatic activity and does not damage endothelium *in vitro* or *in vivo*. Mab 9B9 cross-reacts with rat, cat, monkey and hamster enzyme; radiolabeled Mab 9B9 accumulates selectively in the lungs of these animals after systemic administration regardless on the route of injection. Pulmonary uptake of Mab 9B9 labeled with 111-In was visualized with a gamma-camera. Therefore, radiolabeled Mab 9B9 may be used for gamma-immunoscintigraphy of the pulmonary vasculature. Our experiments in cultivated human endothelium and in perfused isolated rat lungs demonstrated that endothelial cells in both models effectively internalize Mab 9B9 without marked degradation (about 60% of cell-bound antibody comes inside the cell, 30% remains surface-associated and less than 8% of radiolabel appears in the medium as products of intracellular degradation and dissociation of surface-bound antibody). Thus, Mab 9B9 may be explored as a tool for intracellular delivery of drugs or genetic material. Biotinylated Mab 9B9 (b-Mab 9B9) demonstrated high antigen-binding capacity *in vitro* and pulmonary uptake *in vivo*. B-Mab 9B9 provides effective pulmonary uptake of radiolabeled streptavidin (15% of ID/g), with a lung/blood ratio 20. We have biotinylated two antioxidant enzymes, catalase and superoxide dismutase (SOD) without significant loss of their enzymatic activity. Tri-molecular complexes b-SOD/streptavidin/b-Mab 9B9 and b-catalase/streptavidin/b-Mab 9B9 both accumulated in the rat lungs selectively after systemic injection (10% of ID/g; lung/blood ratio to 20). Conjugation with streptavidin and b-Mab 9B9 not only offers selective pulmonary delivery of enzymes, but also prolongs dramatically their half-life in the circulation. These results strongly support high potential of Mab 9B9 as an affinity carrier molecule for drug targeting to the pulmonary endothelium.

A4-106 PHARMACOKINETICS OF ANTIBODIES IN THE FEMALE MOUSE REPRODUCTIVE TRACT,

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The World Health Organization estimates that 100 million acts of sexual intercourse occur daily worldwide.[1] This results in 350,000 transmissions of sexually transmitted diseases (STD) as well as 910,000 conceptions daily, at least 16% of which are unwanted. To address these problems, we have been investigating the use of controlled release devices for localized antibody delivery to the lower female reproductive tract for passive immunization against STDs and sperm.

Since it is well documented that significant absorption of many substances, including macromolecules, can occur through the vaginal epithelium,[2] it was important to determine the rates of antibody elimination from the lower female reproductive tract and the extent of antibody absorption through the vaginal epithelium into the body. We have studied the fate of antibodies experimentally in mice and with pharmacokinetic models that relate *in vitro* and *in vivo* antibody release from a vaginal device. These controlled release devices were made from poly(ethylene-co-vinyl acetate) (EVAc) and contained mouse IgG antibodies as well as Ficoll as a pore-forming agent. These devices continuously release freely-diffusing active antibodies *in vitro* over 30 days.[3]

Similar devices containing monoclonal antibodies (mAb) against herpes virus were also implanted in female mice, which were then locally challenged with active herpes virus 3 or 7 days post-implantation. Control mice received devices containing polyclonal mouse IgG. These studies have shown that the mAb controlled release devices offer significant protection.

1. World Health Organization. June 25, 1992. Reproductive Health, a Key to a Brighter Future. *New York Times.*
2. Okada, Hiroaki. 1991. *Peptide and Protein Drug Delivery.* Markel Dekker Publishers, New York. p. 633-666.
3. Sherwood, JK, RB Dause, and WM Saltzman. 1992. Controlled Antibody Delivery Systems. *Bio/Technology.* 10: 1446-1449.

A4-107 APPLICATION OF STREPTAVIDIN-BIOTIN BINDING IN TWO-STEP ANTIBODY-BASED TUMOR TARGETING PROTOCOLS

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We have applied pharmacokinetic models to compare protocols that utilize monoclonal antibodies for tumor localization of a radionuclide. The protocols are (1) injection of a monoclonal antibody directly labeled with a radionuclide (one-step protocol); (2) injection of radiolabeled streptavidin after pretargeting with biotinylated antibody; and (3) injection of radiolabeled biotin after pretargeting with streptavidinylated antibody. Three distinct physiological spaces are described: (i) a 300 μm diameter, prevascular tumor nodule in the lung, (ii) the normal lung surrounding the tumor, and (iii) the plasma. The plasma kinetics, transcapillary transport into normal lung interstitium, diffusion into and out of the nodule, lymphatic outflow from the normal tissue, binding of MAb to tumor-associated antigen (Ag), internalization of MAb-Ag complex, and binding of the second component to the pretargeted MAb are the processes explicitly modeled. Parameters in the model are based on published experimental data and scaled, if necessary, to account for molecular weight and interspecies differences. The spatial distribution and average concentration of the radiolabeled species in the tumor nodule, the tumor:blood and tumor:lung ratios, the relative exposure and the mean residence time form the basis for comparison of the three protocols. Two dose regimes are investigated, as are the effects of internalization and degradation of the MAb-Ag complexes. The calculations indicate that, in the absence of internalization and degradation, the streptavidinylated antibody/radiolabeled biotin protocol offers considerable pharmacokinetic advantage over the other protocols.

A4-108 TIME DEPENDENCY OF TWO NASAL ABSORPTION ENHANCERS ON INTRANASALLY ABSORPTION OF GROWTH HORMONE IN VIVO, Charlotte Vermehren, Harald S. Hansen and Mads K. Thomsen, Department of Biology Science, Royal Danish School of Pharmacy, Copenhagen, Denmark.
The phospholipid, didecanoylphosphatidylcholine (DDPC), and alpha-cyclodextrin (alpha-CD) may be used as absorption enhancers to promote the absorption of peptide drugs across the nasal mucosa. The aim of this study was to investigate whether the enhancer efficacy was time dependent. Furthermore, the effect of alpha-CD and DDPC was investigated on the electrophysiological parameters of rabbit nasal epithelium mounted in a Ussing chamber. Powders of the enhancers were i.n. delivered to rabbits at t=0. At different time points (0.5, 1 and 3h) thereafter, the rabbits were dosed with a growth hormone (GH) powder and the plasma concentration of GH was followed with time. In this way the amount of GH absorbed was determined. Rabbits dosed with GH and enhancers at the same time were used as controls. The enhancer preparation of DDPC plus alpha-CD, showed GH plasma concentration AUC and Cmax values, which significant decreased with time after enhancer administration. This may indicate recovery of mucosal leakiness. Similar findings were obtained with the alpha-CD preparation, whereas the preparation containing only DDPC showed no recovery of leakiness within 3h. Fifteen min incubation of DDPC or alpha-CD with isolated nasal mucosas in Ussing chambers induced concentration dependent reductions in the electrophysiological parameters, i.e. the resistance, short circuit current and potential difference. Low concentrations (0.1%, 0.5%) of DDPC and (1%, 3%) alpha-CD, respective, showed complete or partial reversability of the three parameters, whereas high level (2%) DDPC and (8%) alpha-CD, showed only a trend toward recovery. These results suggest that alpha-CD alone or in combination with DDPC has a reversible effect on the mucosal leakiness in vivo. The in vitro reduction of the electrophysiological parameters suggested impaired sodium transport across the tissue and loosening of tight junctions. Paracellular absorption may thus contribute to nasal absorption of GH to the bloodstream.

A4-109 TARGETING LIPOPHILIC PRODRUGS TO BRAIN, LUNG, AND SPLEEN, M.B. Yatvin, W. Li, Y. Chen, N.R. Zearfoss and M.H.B. Stowell*, Division of Radiation Biology, Department of Radiation Oncology, Oregon Health Sciences University, Portland, OR 97201; *Department of Chemical Biology, California Institute of Technology, Pasadena, CA 91125
Covalently linked lipophilic prodrugs attain higher intracellular drug concentrations than free drug and can be targeted to specific tissues. Oral or intravenous administration of methotrexate covalently linked to sphingosine via hexanoic acid (MC) resulted in a 13x higher brain concentration than free MTX. *In vitro* studies show that for drug action, free MTX must be released from the lipid component. Since MC is not hydrolyzable, it lacks activity. Methotrexate linked via an ester bond to sphingosine formed the ceramide prodrug ME_6C. Oral intubation of ME_6C produced a 100 fold increase in brain ME_6C in fed mice and a 300 fold increase in fasted mice over MTX. The prodrug, which had a half-time of approximately 18 hours, could serve as a drug reservoir in the brain, providing a continuous supply of free-MTX through ester bond hydrolysis. In contrast, the brain concentration of free MTX was too low for its half-time to be determined. No concomitant increases in the ME_6C levels in the liver and kidney were observed. Linking MTX to sphingosine via a salicylic acid/ester bond formed the prodrug MSC, which, when administered intravenously, concentrated minimally in the brain. In the lung and spleen, MSC levels were 25-125x greater than the MTX levels between 1 and 48 hours after administration, with the concentration of MSC in liver and kidney being only 4x that of free MTX. The following conclusions can be drawn: (1) lipophilic methotrexate-prodrugs cross the blood brain barrier and accumulate to a greater concentration in the brain than free drug; (2) for functional activity, methotrexate must be released from the prodrug by ester bond hydrolysis; (3) the type of ester linkage affects the tissue distribution of the prodrug. Thus, depending on the prodrug employed, MTX can be targeted to brain, spleen, or lung, without corresponding increases in liver and kidney, in which MTX toxicity can be a limiting factor.

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A4-110 AEROSOL DELIVERY OF APROTININ: A NOVEL THERAPY FOR ACUTE RESPIRATORY DISEASES. O.P. Zhirnov, L.S. Kirzhner, A.V. Ovcharenko, and N.A. Malyshev. The D. I. Ivanovsky Institute of Virology, Moscow Clinics of Infectious Diseases N1, Federal Center of Chemi-Biological & Ecological Research, Moscow 123098, Russia. The basic proteinase inhibotor from bovine organs, aprotinin (an active ingredient of Trasylol) has been shown to suppress virus replication and to protect from Influenza and paramyxovirus bronchopneumonia of mice [Antiviral Res. (1994) 23:107 -118]. Here we show the therapeutic efficacy of a small-particle aerosol inhalations of aprotinin against human respiratory diseases caused as by influenza virus, parainfluenza viruses, adenoviruses, so by mixed infections. ~100 patients were examined. The overall improvement of patient state has been documented by aprotinin aerosol treatment day 1. The proposal aprotinin delivery was associated with a ~2-fold reduction in the duration of systemic and respiratory symptoms compared to placebo treatment. Being well tolerated generally aprotinin inhalations caused neither local irritant complications nor any allergic reactions. Both hepatic and hematopoetic toxicity have not been documented. These features of aprotinin aerosol inhalations make them a very promising drug for therapy of Influenza and acute respiratory diseases.

Mucosal Vaccines and Delivery of Proteins to Mucus Epithelia; Delivery of Antibody-Based Therapeutics

A4-200 CONVERSION OF ADENOVIRAL-TRANSDUCED CELLS INTO RECOMBINANT ADENOVIRAL PRODUCER CELLS, R.I. Garver, Jr.[1], K.T. Goldsmith[1], D.T. Curiel[1], J.A. Engler[2], Departments of [1]Medicine and [2]Biochemistry, UAB School of Medicine and Birmingham VAMC, Birmingham, Alabama.
In vivo gene transduction strategies have utilized the administration of retroviral producer cells as a means of increasing the efficiency of gene transfer, but the lytic viral vectors such as adenovirus are not amenable to such an approach using conventional adenovirus packaging cell lines. It was hypothesized that cells targeted for adenoviral gene transduction could be converted into recombinant adenovirus-producing cells by codelivery of the virus and a replication-enabling plasmid. The adenovirus used, AdCMV-luc, contained a luciferase expression cassette within an E1A/E1B deletion. A plasmid containing Ad5 E1A/E1B sequences, pE1A, was made to supply the deleted E1 functions in trans. Polylysine was covalently attached to the AdCMV-luc exterior to function as an ionic linker between the virus and the pE1A. Complexes of AdCMV-luc/pE1A were added to the human prostate carcinoma line, PC-3, followed by extensive washing; controls were identically treated with complexes of AdCMV-luc/pUC 13. Lysates and supernatants from the pE1A cotransduced PC-3 cells were capable of transferring luciferase activity to virgin cells. Titers of virus in the pE1A cotransduced PC-3 cells were in the 10^6 range compared to titers of 10^2 measured in control supernatants. Viral DNA from the pE1A cotransduced cells was shown by restriction endonuclease and southern blot analysis to be identical to the AdCMV-luc viral stock. In summary, cotransduction of an E1-deleted adenovirus with an ionically linked plasmid containing the deleted E1 sequences resulted in the production of new, functional, recombinant adenovirus as evidenced by the ability of the virus to transfer the luciferase expression cassette, the marked increase in viral titer from cells receiving the E1 sequences, and by genomic analysis of the new virus produced.

A4-201 **CELLULAR TARGETING OF ANTISENSE COMPOUNDS USING OLIGONUCLEOTIDE CONJUGATES.**
Muthiah Manoharan, Kathleen L. Tivel & P. Dan Cook
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We have employed Conjugation methodologies with an aim to improve the absorption and distribution of oligonucleotides by cells. These include the conjugation of various pendant moieties to the oligonucleotide to affect its overall physical properties. Pendent moieties may mediate absorption by binding to certain internalizing cell receptors; the same moieties may also affect the hydrophobicity, negative charge and amphipathicity of the oligonucleotides. Our laboratory has prepared conjugates of oligonucleotides with cholic acid, cholesterol, polyamines and polyethylene glycols, biotin and various lipophilic alkyl groups (adamantane, long alkyl chains and di-*O*-hexadecyl-*rac*-glycerol lipid) to study enhanced absorption of antisense agents.

The *in vitro* antisense effects imparted by these ligands and attempts to delineate the uptake pathway of the conjugates will be presented. The effect of conjugation on biophysical properties (T_m, lipophilicity etc.,) and nuclease resistance of oligonucleotides will also be addressed. *In vivo* experiments of some of the conjugates in mouse will be compared to the *in vitro* results.

A4-202 RETROVECTORS AS DRUG DELIVERY VEHICLES, Margaret D. Moore, Jim Respess, Nick De Polo, Linda Karavodin, David Hsu, Joan Robbins, Douglas J. Jolly and Steven Chang, Viagene, Inc, 11055 Roselle St., San Diego, CA 92121
One of the challenges of drug delivery is introduction of specific information or drug to a target cell type or tissue. Gene therapy is the introduction of genetic information to a cell or tissue. Traditional modes of therapy, including those using engineered proteins, require the transport of the active agent from the outside to the inside of a tissue or cell; in contrast, gene therapy works from the inside out by synthesis of gene products *in situ*. This has implications for the efficacy of these agents, dosage, pharmacokinetics and likelihood of toxic side effects. The real challenge in gene therapy is to directly administer the vector, have the genes reach the appropriate cells and there exact a therapeutic effect. The following issues have a large effect on the feasibility of this approach: 1) efficient expression of the therapeutic agent 2) targetability 3) specific integration and 4) evading the immune system. We will describe ongoing research on the use of retroviral vectors (retrovectors) for *in vivo* gene delivery as anti-cancer and anti-infectious disease agents. We will be specifically discussing our results in engineering retrovectors to evade the immune system. Also, we will update our ongoing research to achieve targetability with retrovectors.

A4-203 EVALUATION OF ZYN-LINKERS® AS OLIGONUCLEOTIDE DELIVERY SYSTEMS, K.A. Muirhead, C-E. Lin, S.P. Duprey, J.F. Burns, E.B. Jones, S.M. DiGiorgio, B.D. Gray, G.A. Kopia, Zynaxis, Inc., Malvern PA 19355.
K. Jayaraman, T. Hill, H. Vu, N. Chaudhary, Triplex Pharmaceutical Corp., Houston, TX 77380

Rapid, efficient *in vivo* delivery still remains a major hurdle for development of oligonucleotides (ODN) as therapeutic agents. Zyn-Linkers, lipid-like amphiphiles under development for local drug delivery, have previously been conjugated to a variety of therapeutic agents including antineoplastics, peptides, and carbohydrates. Conjugation to Zyn-Linkers facilitates rapid association of coupled drugs with cell membranes, facilitating intracellular delivery without the need for specific cell surface receptors. Selection of appropriate conjugation chemistry also allows retention and controlled release of therapeutic agent at the site of delivery for extended periods of time.

To determine whether Zyn-Linkers could deliver ODN, our initial goals were i) to synthesize a Zyn-Linker:ODN conjugate and ii) to determine if Zyn-Linkers could enhance uptake of ODN as compared with a standard agent (Lipofectin). A Zyn-Linked conjugate of 18-mer anti-*c-myc* ODN was prepared by derivatization of a bifunctional 3'-amino-modifier CPG support with 5-(N-hydroxysuccinimidyl p-aminomethylene terephthalate) -1-didocosanyl-3,3,3',3'-tetramethyl-indocarbocyanine chloride, followed by solid phase synthesis, cleavage, and deprotection using standard methods. Anion exchange chromatography yielded a conjugate (Zyn-ODN) with appropriate absorbance characteristics (ODN at 260 nm, Zyn-Linker at 550 nm). A similarly prepared 5' fluorescein-labeled conjugate (Zyn-ODN-F) exhibited appropriate fluorescence characteristics (fluorescein at 520 nm, Zyn-Linker at 572 nm and contained <1% contaminating ODN or unconjugated Zyn-Linker. An initial quantitative fluorescence microscopy study using rabbit aortic smooth muscle cells showed substantially increased ODN uptake when delivered via Zyn-Linker vs. Lipofectin™, suggesting that further evaluation of Zyn-ODN as defined molecular delivery systems is warranted.

A4-204 HYDROPHOBIZATION OF VIRUS-SPECIFIC ANTIBODIES AND ANTISINSE OLIGOS PROMOTES THEIR INTRACELLULAR TRANSPORT. A. Ovcharenko, N. Melik-Nubarov, A. V. Kabanov, Y. Suzdaltseva, & O. P. Zhirnov. Federal Center of ChemiBiological & Ecological Research, The D. I. Ivanovsky Institute of Virology; Research Center of Molecular Diagnostics, Moscow 113149, Russia.

The principle of artificial hydrophobisation was employed to enhance the delivery of antivirals to the viral intracellular targets. Two classes of molecules: (i) the monoclonal ab against internal influenza virus proteins, M1 and NP; (ii) the oligo (TTG ACG AAA TT), which is complementary to the loop-forming site of the PB2 virus polymerase gene, were tested. The Mabs were made hydrophobic with covalent-linked stearic residues, the oligo was combined with the undecyl residue added to the 5-terminal phosphate. The Mabs containing 1-3 residues of stearic acid per molecule were shown to retain their functional activity. Both modified drugs suppressed the Flu A/PR/8/34 virus replication and inhibited the synthesis of the virus polypeptides in MDCK cells. Similar results were obtained with A/Aichi/68, and A/Chile/83 viruses. Under the same conditions, the non-modified Mabs and oligo did not affect the virus development. The effective antiviral concentrations of the hydrophobised Mabs and oligo in maintenance culture medium were 0.25 nM and 50 uM, respectively. These data show that artificial hydrophobisation of antivirals may be applied as a promoter of their antiviral targeting.

A4-205 *Abstract Withdrawn*

A4-206 ON THE INTERACTION OF CATIONIC LIPID COMPLEXES WITH NUCLEIC ACIDS. Garry B. Takle, Sandra M. Flynn and Lisa White. Innovir Laboratories, Inc., 510 E 73rd St., New York, NY 10025.

Cationic liposomes are emerging as highly efficient and relatively non-toxic vehicles for the delivery of nucleic acids. In order for these particulates to become usable for the delivery of nucleic acid therapeutics to humans, characteristics of the nucleic acid-lipid interaction need to be determined. We have been developing numerous assays for the investigation of this interaction using various cationic liposome formulations. Data from experiments reported here give information on such parameters as efficiency of nucleic acid binding, protection of nucleic acids from nucleases, cell binding and uptake, cellular localization and transfection efficiency.

A4-207 HIGH ABSOLUTE FREQUENCY OF HOMOLOGOUS RECOMBINATION IN VITRO AND IN VIVO, Nancy S. Templeton, Leon Baltrucki, and Cornelia M. Gorman, Gene Expression and Delivery, MEGABIOS Corp., San Carlos, CA 94070

The frequency of homologous recombination per electroporated cell is defined as the absolute frequency. This frequency in mouse embryonic stem (ES) cells is extremely low, approximately 10^{-4} - 10^{-6}, by current procedures. We have developed a method for gene targeting in mouse ES cells that produces an absolute frequency of 10^{-1}. The protocol uses micro-electroporation chambers and an optimized electroporation procedure previously described that produces insignificant cell death. After electroporation, cells are plated at an optimum density for cell growth. Plating density and efficiency of gene delivery appear to be the critical factors required for high absolute frequency of homologous recombination. Because this frequency is extremely high, it is possible to perform gene targeting without the use of selectable markers. In addition, the absolute frequency of gene targeting *in vivo* is high. Mutant transgenic mice homozygous for the p53 null allele were targeted with mouse genomic DNA that corrects the mutant allele. Gene targeting was performed without the use of selection. DNA:lipid complexes were delivered intravenously by tail vein injection. The results show that 1/50 cells of the lung were corrected at the p53 locus. Other tissues contained 1/500 cells that were corrected. These tissues include brain, spleen, thymus, muscle, heart, kidney, colon, and liver. Data from transient expression of CMV-CAT *in vivo* show far greater levels of CAT in the lung as compared to other tissues. These studies suggest that high efficiency gene targeting can be achieved *in vivo* with optimized gene delivery. Methods for increasing DNA delivery to more cells of all tissues *in vivo* are being tested.

A4-208 SYNTHESIS, PURIFICATION, AND CHARACTERIZATION OF NOVEL ANTISENSE OLIGONUCLEOTIDE CONJUGATES

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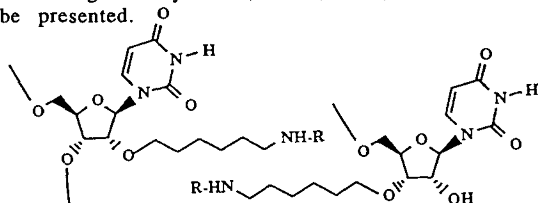
To improve the pharmacokinetic properties of antisense oligonucleotides, several functionalities have been conjugated to the 2'- or 3'-position of uridine via a 2'- or 3'-*O*-alkylamino linker, including cholesterol, biotin, di-*O*-hexadecyl-*rac*-glycerol, and other alkyl groups. These nucleosides were subsequently converted to a phosphoramidite or attached to controlled pore glass and incorporated into oligonucleotides by automated DNA synthesis.

Physical and biological characteristics of the conjugates were examined and compared to those of unmodified analogs. Synthesis and purification schemes for the monomers and oligomers and analysis of the oligomers by HPLC, PAGE, NMR, MS and CE will be presented.



R=Alkyl groups, biotin, cholesterol or di-*O*-hexadecyl-*rac*-glycerol

A4-209 BIOCHEMICAL APPROACHES TO ENHANCE TRANSDERMAL DRUG DELIVERY. I. EFFECT OF LIPID SYNTHESIS INHIBITORS,

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The permeability barrier resides in the intercellular domains of the stratum corneum, where it is mediated by an approximately equimolar mixture of three lipids, cholesterol, free fatty acids, and ceramides. Transdermal transport of lipophilic and amphipathic compounds is thought to occur through the same domains, but in a direction opposite to transepidermal water loss (TEWL). Prior studies have shown that all three lipids (cholesterol, ceramides, and fatty acids) are required for barrier function; e.g., inhibition of either cholesterol, fatty acid, or ceramide synthesis after barrier perturbation delays barrier recovery rates, as assessed by TEWL. In this study, we have investigated the potential of lipid synthesis inhibitors to enhance transdermal drug delivery (lidocaine and/or caffeine) in relation to the delay in barrier recovery. After acetone disruption of the barrier, treatment with either 5-tetradecyloxy-2-furancarboxylic acid (TOFA), a fatty acid synthesis inhibitor, or fluindostatin (FLU) or cholesterol sulfate (CS), both cholesterol synthesis inhibitors, significantly increase lidocaine HCl absorption during a 2 h period by 2- to 3-fold vs a standard propylene glycol/ethanol/water (1:3:1) vehicle. Moreover, coapplications of TOFA and CS cause an additional increase in lidocaine uptake (~ 5-6 fold). Furthermore, when the barrier was disrupted initially with DMSO, instead of acetone, coapplications of TOFA and FLU also increase plasma drug levels up to 8-fold vs standard vehicle 1 h post-application. Although these metabolic inhibitors also were shown to favor the partitioning of drugs from the vehicle into the stratum corneum by thermodynamic mechanisms, our results demonstrate that modulations in the epidermal lipid biosynthetic repair response, following application of conventional penetration enhancers, permeabilize the barrier by independent biochemical mechanisms. These strategies point to a novel, biochemical /metabolic means to enhance transdermal drug delivery.

A4-210 USE OF CYCLODEXTRIN AS CARRIER FOR OLIGONUCLEOTIDES,

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The use of antisense oligonucleotides as tools for modulating gene expression represents a novel strategy for designing drugs for various diseases. In addition to various other parameters, cellular uptake and internalization of oligonucleotides is an important parameter for its effectiveness as antisense agents. We have used cyclodextrin and its analogs as carriers for oligonucleotides to increase cellular uptake. The studies were carried out using ^{35}S-labeled or fluorescent-labeled phosphorothioate oligonucleotide in human T cell leukemia H9, CEM and MOLT-3 cell line. Cellular uptake of oligonucleotide phosphorothioate in presence of cyclodextrin was found to be dose and time dependent. Using various cyclodextrin analogs, e.g., 2-hydroxypropyl β-cyclodextrin (HPCD), hydroxyethyl β-cyclodextrin (HECD) and mixture of various hydroxypropyl β-cyclodextrin (Encapsin), increase in oligonucleotide uptake, up to two to three fold in 48 hours, was observed. Cyclodextrin itself was not toxic at the concentration being used. In order to further increase the uptake, we studied oligonucleotides covalently linked with moieties which have strong affinity for cyclodextrin, e.g., adamantane. In comparative studies, oligonucleotide phosphothioate-adamantane conjugate, more increase in uptake was observed in presence of cyclodextrin. These studies suggest that cyclodextrin and its analogs may be a class of promising novel carrier for oligonucleotides and analogs.

Delivery of DNA and RNA in Cells and Tissues; Gene Therapy and Nucleic Acid Therapeutics

A4-300 A NON-IONIC SURFACTANT VESICULAR FORMULATION OF AMPHOTERICIN B: A PRELIMINARY STUDY OF ANTILEISHMANIAL ACTIVITY, Alan J. Baillie[a], Denise M. Williams[a] and K. Christine Carter[a], Departments of Pharmaceutical Sciences[a] and Immunology[b], University of Strathclyde, Glasgow, UK. G4 ONR.
Amphotericin B (AMB) will form complexes with surface active agents which are of interest in drug delivery. We have investigated non-ionic surfactants as a means of solubilising AMB for use in the treatment of visceral leishmaniasis. A vesicle formulation of AMB was produced using a mixture of a chemically defined non-ionic surfactant, cholesterol or ergosterol and dicetyl phosphate in a 5:4:1 molar ratio. AMB was incorporated into the vesicles either in powder form when it was melted with vesicular constituents (2 minutes at 100ºC) prior to hydration with a citrate phosphate buffer pH 6.0 or in solution form when melted vesicular constituents were hydrated with amphotericin B citrate phosphate buffer solution pH 6.0 containing 0.025% dimethylsulfoxide cosolvent. After hydration unentrapped drug was removed by centrifugation and the vesicular pellet resuspended in 'free' drug solution (0.05mg/ml in citrate phosphate buffer). Mean vesicle diameter (nm) was measured using laser photon correlation spectroscopy and vesicle drug content determined using a *Saccharomyces cerevisiae* bioassay. Antileishmanial activity of the vesicle suspensions was investigated using *in vitro* and *in vivo* models. Vesicle suspensions were more active that free amphotericin B solution treatment against *Leishmania* parasites in both models.

A4-301 THE INFLUENCE OF DRUG CONCENTRATION AND SURFACTANT CHOICE ON THE *IN VIVO* ANTIPARASITIC EFFICACY OF SODIUM STIBOGLUCONATE NON-IONIC SURFACTANT VESICULAR FORMULATIONS IN AN ACUTE MODEL OF MURINE VISCERAL LEISHMANIASIS, K. Christine Carter[a], Denise M. Williams[a] and Alan J. Baillie[b], Departments of Immunology[a] and Pharmaceutical Sciences[b], University of Strathclyde, Glasgow, UK, G4 ONR. The effect of treatment with sodium stibogluconate loaded non-ionic surfactant vesicle (NIV) formulations prepared using six surfactants (Surfactants V-X) on splenic, hepatic and bone marrow parasite numbers was assessed in a murine model of acute visceral leishmaniasis. Vesicle suspensions were prepared by hydration of a melt of surfactant: cholesterol: dicetyl phosphate (5:4:1 molar ratio) with 33.3, 66.6, 100 or 133mg Sb^V/ml sodium stibogluconate solution. Unentrapped drug was removed (by centrifugation) and the vesicular pellet resuspended in free stibogluconate solution (0.97mg Sb^V/ml). Mean vesicular diameters (nm) were determined using laser photon correlation spectroscopy and antimony content of the vesicles analysed by flame atomic absorption spectrophotometry. NIV suspensions had a surfactant specific antileishmanial effect which depended on the hydrating drug concentration. Paradoxically suspensions hydrated with the lowest drug concentration were the most effective for all surfactants with Surfactants VII and VIII producing the most active formulations (based on observed suppression of liver, spleen and bone marrow parasite burdens).

A4-302 LIPOSOMAL ENCAPSULATION DELIVERS FOSCARNET (PFA) TO SITES OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) AND CYTOMEGALOVIRUS (CMV) INFECTIONS AND IMPROVES ITS PHARMACOKINETICS. Nathalie Dusserre, Céline Lessard, Denis Beauchamp, André Désormeaux, Louise Poulin, Michel Tremblay and Michel G. Bergeron. Infectiologie, Centre de Recherche du CHUL, Université Laval, Québec, Canada.
PFA is approved by the FDA for use in treatment of CMV infections in AIDS patients. PFA has also demonstrated both *in vitro* and *in vivo* anti-HIV activity. However, high incidence of nephrotoxicity may limit its clinical use. In this study, PFA was encapsulated in liposomes prepared from a mixture of long saturated acyl chain phospholipids. These liposomes (165±30nm in diameter) were produced by reverse phase evaporation followed by polycarbonate filter extrusion. Biodistribution and plasma concentrations of free and liposome-encapsulated 14C labeled PFA were determined in female Sprague-Dawley rats (175-222g) following a 10mg/kg intravenous single bolus dose of PFA. At specific time points, rats were sacrified, blood and different tissues were collected. Encapsulation strongly altered PFA tissular distribution, in particular the accumulation of PFA in the spleen, liver and lungs. It also enhanced PFA concentrations in lymph nodes, reported to hide latent provirus in the primary stages of HIV-1 infection. Furthermore, it greatly improved PFA plasma half life, increasing area under the curve by 77 times, while lowering PFA kidney concentrations. The following table shows PFA distribution 30 minutes after injection. Values are expressed in µM. Each result is the mean (±SD) obtained from 5 to 6 animals, p values ≤ 0.01.

	Plasma	Spleen	Liver	Lungs	Lymph nodes	Kidney
Free PFA	**14.8** (±1.8)	**3.8** (±1.5)	**8.5** (±1.3)	**8.7** (±2.8)	**8.9** (±2.0)	**132.9** (±56.3)
Liposomal PFA	**691.1** (±63.2)	**257.4** (±29.9)	**96.9** (±21.8)	**87.8** (±11.0)	**20.4** (±4.8)	**69.2** (±23.0)

In conclusion, liposomal formulation of PFA was shown to prolong drug circulation time and to accumulate in the reticuloendothelial system. This new approach to PFA delivery targets macrophages, a reservoir of CMV and HIV-1, while diminishing renal drug concentration. Such liposomes, previously shown to enhance drug uptake by macrophage-related cell lines without altering anti-HIV-1 activity, could therefore be expected to increase therapeutic PFA efficacy.

A4-303 OLIGONUCLEOTIDES FORMULATED WITH CATIONIC LIPIDS HAVE ANTISENSE ACTIVITY IN CELL CULTURE, Brian P. Dwyer & David A. Schwartz, Chemistry; Susan Scanlon & Bob D. Brown, Biology; Genta Inc., San Diego, CA 92121
Antisense oligonucleotides are believed to exert their effects by binding to RNA in the cytoplasm or the nucleus. Naked oligonucleotides are taken up by cells, but they are sequestered into endosomes. Cationic lipids are capable of transfecting cells with plasmids as well as delivering oligonucleotides to their requisite site(s) of action. We are using two complementary assays for intracellular delivery of antisense oligonucleotides. Firstly, the intracellular distribution of fluorescein-labeled oligonucleotides is determined by fluorescence microscopy. Secondly, cultured cells are transfected with a plasmid containing the gene for chloramphenicol-acetyl transferase (CAT) and either antisense or mismatched oligonucleotides, and the CAT activity is quantified. Results will be presented that demonstrate antisense activity in cultured cells with phosphorothioates and Genta's methylphosphonate-containing chimeric oligonucleotides formulated with cationic lipids. Our data also suggest that the majority of antisense activity occurs in the nucleus.

A4-304 A three-dimensional cell attachment matrix created by cross-linking RGD peptide modified hyaluronic acid.

James Glass, Ken Dickerson, Lin-Shu Liu, Kim Stecker, Ronda Schreiber and James Polarek. Telios Pharmaceuticals, Inc., 4757 Nexus Centre Drive, San Diego, CA 92121.

ABSTRACT

We have created biodegradable polymer scaffolds modified with peptides that promote cell attachment and migration. The scaffolds are prepared using a bi-functional reagent, such as divinyl sulfone; DVS, to cross-link a naturally occurring high molecular weight polymer, hyaluronic acid, containing a covalently bound cell attachment peptide. Lyophilization of this material results in the formation of a porous matrix whose pore size can be modified by polymer density. Divinyl sulfone cross-linking of hyaluronic acid results in a stable matrix which can be maintained in tissue culture environments for at least one month. The cell attachment peptides used contain the tripeptide sequence Arg-Gly-Asp (RGD). Cultured MG63 cells rapidly attach and spread by integrin-mediated mechanisms to the RGD-peptide modified polymer. There is no cell attachment to a non-modified polymer and peptide mediated attachment can be competed by soluble RGD peptides. Cell attachment to the peptide matrix also results in integrin signalling as demonstrated by the activation of the focal adhesion kinase (FAK). This data supports the observation that the material is capable of promoting cell migration as demonstrated by migration of fibroblasts from the polymer onto fibronectin coated polystyrene or from Matrigel™ into the matrices. Cells proliferate with similar doubling times as those in monolayer and fill in the interstices of the matrix by synthesizing extracellular matrix components including fibronectin and collagen type I. This new biomaterial could prove useful in vivo to facilitate soft tissue repair or as a cell delivery matrix and in vitro as a well defined material for culturing cells in a three-dimensional environment.

A4-305 ANTI-TUMOR THERAPY BY LOCAL DELIVERY OF TAXOL FROM FIBRIN SEALANT. C. Hue, W. Drohan, and M. MacPhee, American Red Cross, Jerome H. Holland Lab, Rockville, MD 20855.

Systemic chemotherapy uses anti-proliferative agents which produce dose-limiting side-effects, resulting in lowered efficacy. Local/regional chemotherapy would minimize side-effects while raising drug dosage to the tumor, thus increasing efficacy. Attempts to accomplish this have been hampered by the lack of a suitable biocompatible, hemostatic, & adhesive delivery matrix. Fibrin Sealant (FS), formed by mixing fibrinogen & thrombin, has these properties. Taxol is an effective antineoplastic agent against human ovarian cancer cells (eg., Ovcar-3). We determined the kinetics of Taxol release from FS clots in 2 *in vitro* models; infinite sink (IS) & limited sink (LS). IS and LS model *in vivo* conditions with high & low fluid volume & flow respectively. In the LS model, FS clots were placed in 2ml of PBS. In the IS model, FS clots were agitated in 50ml of PBS. Taxol was loaded in FS in suspension. Clots were incubated @ 37°C in PBS which was changed daily & bioassayed for inhibition of Ovcar-3 growth. In the IS model, Taxol-FS eluates from up to day 57 inhibited the growth of Ovcar-3, while in the LS model, eluates from up to day 64 inhibited growth. Eluates from FS alone were not inhibitory. To determine the effects of Taxol-FS on tumors *in vivo*, $1x10^7$ Ovcar-3 cells were injected i.d. into athymic mice. 48hrs later, Taxol-FS or FS was injected either intra-tumorally or into the flank opposite the tumor. After 21 days, the median tumor volumes (in mm^3) were; FS: 747, contralateral Taxol-FS: 515, & Taxol-FS: 94* (*=p<.0045 compared to FS and contralateral Taxol-FS). Tumors from mice receiving Taxol-FS intra-tumorally were also less vascular than control tumors. In summary, we have shown that FS released Taxol for a sustained period *in vitro*, and in preliminary *in vivo* experiments Taxol-FS was effective at retarding tumor growth. Thus FS may serve as a matrix for the local drug delivery of antineoplastic agents.

A4-306 INHIBITION OF TUMOR GROWTH *IN VITRO* AND *IN VIVO* BY STABLE ANALOGS OF THROMBOSPONDIN TYPE 1 REPEAT PEPTIDES, David D. Roberts, Neng-hua Guo, Henry C. Krutzsch, and John K. Inman, Laboratory of Pathology, NCI, and Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda, MD 20892-1500

The type I repeats of human thrombospondin-1 (TSP) contain several biologically active peptide sequences. Peptides containing the heparin-binding motif Trp-Ser-Xaa-Trp inhibit growth and motility responses of endothelial cells to basic fibroblast growth factor. We now show that these peptides inhibit growth of MDA MB435 human breast carcinoma cells *in vitro* and in nude mice. Two approaches were used to increase stability of the TSP peptides. Peptides containing C-terminal Cys residues were covalently coupled to iodoacetamidopropionylaminoethylcarbamylmethylated polysucrose. The peptide conjugates exhibited increased affinity for binding to heparin and were 10 to 100-fold more active than free peptides for inhibiting proliferation of endothelial or breast carcinoma cells. Modified retro-inverso analogs of the TSP peptides were prepared to prevent enzymatic degradation. The retro-inverso analogs retained heparin binding activity and inhibited proliferation of endothelial cells *in vitro*. Polysucrose conjugates of the retro-inverso analogs showed similar enhancement of anti-proliferative activity *in vitro*. Following orthotopic implantation of MDA MB435 cells in the mammary fat pads of nude mice, intravenous injection of peptide conjugates or free retro-inverso TSP peptide inhibited tumor growth. In contrast to their relative activities *in vitro*, the free retro-inverso TSP peptide was a more potent inhibitor of tumor growth than the conjugate *in vivo*, possibly due to its increased diffusion out of circulation and direct interaction with the tumor cells.

A4-307 BIODEGRADABLE SYSTEMS FOR SUSTAINED DELIVERY OF DRUGS TO BRAIN TUMOURS, T.L.Whateley,R.Rampling,L.Robertson,I.M.Crossan,P.A.Fallon,J. A.Plumb and D.J.Kerr, Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW, Scotland.

Brain tumour therapy involving the sustained delivery of a cytotoxic agent from a biocompatible, biodegradable *polymeric* system (either a monolithic implant ,thin film or microspheres) implanted following surgical resection , is a novel approach.

This study has investigated the formulation and *in-vitro* release of biodegradable polymeric implants , thin films and microspheres containing dexamethasone (for treating cerebral oedema associated with brain tumours) or the cytotoxic drugs ,carboplatin and etoposide.The *in-vivo* performance of the implant systems has been evaluated.The biodegradable poly(D,L-lactide-co-glycolide) (PLGA) was chosen as it has been accepted by the regulatory authorities for parenteral use.

The rat C6 glioma cell line was used to determine the cytotoxicity of carboplatin and etoposide.The neurotoxicity of both drugs in mice was determined by direct intracerebral injection into the left hemisphere of 25 µL volumes with concentrations ranging from 1.0 - 10 mg mL^{-1} for carboplatin and 0.8 - 10 mg mL^{-1} for etoposide.

TIME/hr	CARBOPLATIN	ETOPOSIDE
4	4.5×10^{-2}	4.5×10^{-4}
8	2.8×10^{-2}	1.9×10^{-4}
24	7.0×10^{-3}	1.8×10^{-4}
48	1.6×10^{-3}	2.7×10^{-5}
72	2.6×10^{-4}	3.9×10^{-6}

The Table shows the LC50 concentrations (in mg mL^{-1}) for the C6 glioma cell line at various exposure times for carboplatin and etoposide.Following intracerebral injection in mice, concentrations of carboplatin and etoposide up to 2.0 and 1.2 mg mL^{-1} respectively, showed no neurotoxicity. PLGA implants in rat brain have shown no adverse effects and have degraded in ca. two months.Release rates have been determined.

A4-308 *LEISHMANIA DONOVANI* INFECTION OF THE BALB/c MOUSE: EFFECT OF TREATMENT WITH VESICULAR PAROMOMYCIN, Denise M. Williams[a], Alan J. Baillie[b] and K. Christine Carter[a], Departments of Immunology[a] and Pharmaceutical Sciences[b], University of Strathclyde, Glasgow, U.K, G4 ONR. The influence of vesicle recipe and drug concentration on the ability of two chemically defined and related non-ionic surfactants (Surfactants IX and X) to produce paromomycin vesicle suspensions using a 'melt method' was assessed. Optimal vesicular drug entrapment (measured using a *Staphylococcus aureus* bioassay) was dependent on the surfactant used, the molar composition of the vesicles and the hydrating drug concentration. Mean vesicle diameter (nm) was measured using laser photon correlation spectroscopy. The optimal formulations, in terms of drug entrapment and vesicle diameter, for both surfactants were tested for antileishmanial activity in *in vitro* (using murine *Leishmania donovani* infected resident peritoneal macrophages) and *in vivo* systems. *In vitro* treatment with paromomycin vesicle suspensions was more effective than treament with 'free' paromomycin solution and there was a correlation between parasite killing and the production of reactive nitrogen intermediates. Similarly treatment with paromomycin vesicle suspensions was more effective against hepatic parasite burdens compared to that observed in animals treated with 'free' paromomycin solution.