DISCOVERY OF THERAPEUTIC AGENTS

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Structural Biology

X1-001 MOLECULAR BIOLOGICAL APPROACHES TO DRUG DESIGN FOR G PROTEIN COUPLED RECEPTORS, Catherine D. Strader, Mari R. Candelore, Michael R. Tota, Tung M. Fong, and Dennis Underwood, Merck Research Laboratories, Rahway, NJ 07065.

Receptors whose mechanism of action is mediated through the activation of G proteins share structural, as well as functional, similarities. Molecular models of these receptors, based on electron diffraction data on rhodopsin, postulate seven hydrophobic transmembrane helices connected by hydrophilic loops. The majority of the primary sequence homology among these receptors involves residues within the transmembrane domain. Site-directed mutagenesis of the ß-adrenergic receptor has shown that the ligand binding domain is contained within the transmembrane core of the receptor, with most of the binding energy provided by an ion pair formed between the amine group of the catecholamine ligand and the carboxylate side chain of Asp113 in the third transmembrane domain of the receptor. G protein activation is triggered by interactions of the catechol ring of the ligand with residues in helices 5 and 6 of the receptor. In contrast to the relatively compact ligand binding domain of the catecholamine receptors, the undecapeptide substance P binds to both the transmembrane and extracellular regions of the NK1 neurokinin receptor. Small molecule antagonists of this receptor have recently been described, and specific interactions between these antagonists and residues in the transmembrane domain of the NK1 receptor have been identified. The location of the non-peptide antagonist binding site in the NK1 receptor is similar to that of the biogenic amine binding site in the ß-adrenergic receptor. These data suggest the existance of a generally conserved binding site for small molecules in the transmembrane domains of G protein coupled receptors, with the ligand specificity being determined by the chemical properties of amino acid side chains at key positions within this binding pocket. The combination of molecular biological and receptor modeling approaches allows the identification of critical functional groups within receptor binding pockets that can serve as targets for medicinal chemistry.

Biopharmaceuticals

X1-002 DNA Vaccines: Discovery and Development Margaret A. Liu, John Shiver, Donna Montgomery, Jeffrey Ulmer, and John Donnelly, Merck Research Laboratories, West Point PA 19486

DNA vaccines, which are non-replicating plasmids encoding proteins of various pathogens, are capable of generating protective immunity in animal models of several diseases. Their discovery arose by a synthesis of findings from different scientific disciplines including immunology, gene therapy and virology. These vaccines present the appealing ability to generate both antibody responses and cellular immunity, the type of immunity that hitherto has required the use of a live infectious agent to induce. In animal models of various viral diseases, immune responses and protection against viral challenge have been seen after immunization with DNA encoding viral proteins. Neutralizing antibodies, helper T cells (Th1) and cytotoxic T cells were generated. The protection has included both homologous protection as well as cross-strain protection, in which protection is seen against a strain different than the one from which the gene was cloned.

While the vaccines represent a novel approach to immunization, the technology involved is relatively simple. Nevertheless, to make DNA vaccines into effective clinical entities involves addressing a number of process and safety issues that are quite different from those of traditional vaccines or pharmaceuticals. Preclinical immunogenicity and protection results will be presented as well as a comparison of this technology with traditional vaccine approaches. The unique process and safety issues will be discussed along with issues relevant to collaborative efforts necessary for optimizing rapid development of a new technology.

Converting Data into Predictive Tools

X1-003 **QSAR AND MODELLING IN DRUG DESIGN: A TODAY'S PERSPECTIVE, Hugo Kubinyi**, Drug Design, BASF AG, D-67056 Ludwigshafen, Germany.

Specifically acting drugs bind to certain biological targets, *i.e.* receptors, enzymes, ion channels, signal and transport proteins, even to DNA. For binding, a complementarity of the surface properties of the ligand and its binding site is most important. In contrast, lipophilicity plays a predominant role for drug absorption and distribution into different organs and tissues, typically in the form of a nonlinear dependence. In the case of acids and bases, dissociation and ionization determine the partitioning of these substances.

QSAR methods correlate biological activities with physicochemical properties, while 3D QSAR approaches correlate them with molecular fields. Especially CoMFA (Comparative Molecular Field Analysis) has become a powerful tool to derive quantitative relationships for heterogeneous series of compounds, considering their 3D structures. In addition, similarity indices have been used to derive quantitative structure-activity relationships.

An intrinsic feature of all QSAR and 3D QSAR studies is the alignment of molecules in their correct conformation, according to their similarity and a hypothetical pharmacophore. However, similarity is a highly undefined property in cases where no information on the 3D structure of the binding site is available. Several examples of multiple binding modes and unexpected structure-activity relationships demonstrate that the alignment is the most critical point in a QSAR study. In many cases of "rational" alignment, intuition and reality are far apart.

Another promising development in computer-aided drug design are *de novo*-approaches, like LUDI, which use the 3D structure of a protein to search molecules which fit the binding site. Subsequently these lead structures are modified in an appropriate manner, either by the program or by the chemist.

Despite all the progress in QSAR, 3D QSAR and drug design, scientific work in this field is often based on arbitrary hypotheses. Only 3D structures of ligand-protein complexes, resulting from X-ray crystallography and, hopefully soon, from advanced NMR techniques, give solid information on the true background of quantitative structure-activity relationships.

Chemical Diversity

X1-004 THE IMPACT OF COMBINATORIAL CHEMICAL LIBRARIES ON DRUG DISCOVERY, John C. Chabala, John J. Baldwin, Jonathan J. Burbaum, Daniel Chelsky, Lawrence W. Dillard, Ian Henderson, Gregory Kirk, Ge Li, Michael H. J. Ohlmeyer, Troy R. Randle, John C. Reader, Nolan H. Sigal, Pharmacopeia, Inc., Princeton, NJ

Combinatorial synthesis of libraries containing large numbers of pharmaceutically relevant heterocycles and other small molecules is now feasible using binary-encoding split synthesis technology which provides a virtually limitless source of diverse compounds needed for the identification of new lead structures. The availability of such large combinatorial sources of compounds will have several profound effects on drug discovery. First, the rate at which new lead structures are identified will increase. This will require that higher throughput assays than those employing 96-well microtiter plates be developed to efficiently screen the millions of new compounds within combinatorial libraries, and several approaches employing techniques such as FACS sorting and gel permeation have already been developed. With higher diversity of molecules and higher screening rates, a series of targets for which selective small molecule effectors have been difficult to identify will be addressable, including such targets as phosphate-handling proteins and tight protein-protein interactions, as well as proteins identified from mapping the human genome. Second, combinatorial approaches have already been employed in the accelerated, semi-automated definition of structure-activity relationships (SAR) and selectivity, and the integration of such approaches into drug optimization will likely become routine. Combinatorial drug optimization will free medicinal chemists to attack more difficult problems such as complex natural product manipulation and optimization of *in vivo* properties. In addition, combinatorial chemistry will permit a more intelligent choice of which lead to pursue among multiple lead structures. The generation of enormous amounts of SAR data from assaying combinatorial libraries will demand that new computational paradigms be evolved that will complement drug design to further accelerate rates of drug optimization. Third, assaying large numbers of diverse structures will provide broader and deeper patent protection, which, in combination with more rapid lead discovery and optimization, will provide longer market exclusivity.

X1-005 NEW STRATEGIES FOR GENERATING CHEMICAL DIVERSITY, Walter H. Moos, et al., Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608.

A new field of research, "molecular diversity", has exploded in the '90s. In the preparation of drug candidates, the automated and combinatorial use of natural building blocks, such as the standard L-amino acids, as well as unnatural building blocks like N-substituted glycines, now allows the generation and screening of unprecedented numbers of compounds. Drug discovery -- better, faster, cheaper? Witness that diversity-based discovery programs are currently producing literally billions of potential lead compounds every year. Indeed, more compounds have been made and screened in the last several years than in the entire, previous history of the pharmaceutical industry. Of course, diversity is more than a game of numbers. Critical elements of variety, complexity, spatial features, and multiple physicochemical parameters contribute to diversity. And, combinatorial synthesis begs for new assay schemes to be established, including affinity selection techniques, tagging methodologies, and brute-force deconvolution strategies. The resulting demands placed on bioinformatics can be staggering. Immense datasets are now amassed in a period of months, and innovative tools for data handling and analysis are essential. Lead discovery is often followed by lead optimization, although newer diversity libraries based on well-known medicinal pharmacophores have potential to produce development candidates directly. Where optimization is required, diversity can again play a role in speeding and enhancing the research process, yielding better drug candidates, faster. Discovery and development can be contrasted in relation to diversity libraries. One might predict that any suitably diverse set of building blocks may yield potent, selective leads. In contrast, orally available, once-a-day therapies can restrict the viable chemistries to those producing low molecular weight compounds, with stability in biological milieus, appropriate safety characteristics, etc. There are noteworthy ramifications of the diversity game beyond targeted pharmaceutical research, namely, in the renewed interest in polymer-supported organic chemistry, in novel ways of representing multi-dimensional physicochemical parameter space in our limited 5-dimensional world (3-dimensions, x, y, and z, plus color and time), in biophysics and spectroscopy applied to assays, for example, mass spectrometric identification of leads and fluorescence-activated cell sorting techniques, and in other disciplines too. Thus, an integrated approach to exploiting molecular diversity, taking into account both discovery and development considerations, is ultimately required for optimal success. Some concluding remarks... The field of molecular diversity is now sweeping the industry, pharmaceutical and biotechnology companies alike. Those companies who hesitate will be left behind for sure. However, like all new tools, molecular diversity is just one additional armament to add to the arsenal of drug hunters, alongside structure-based design, genetic engineering, and other past and future advances in pharmaceutical discovery strategies. Molecular diversity is not a miracle unto itself, and a balanced perspective on these advances remains critical to avoiding the catastrophes that arrogance often brings.

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2. "Discovery of nanomolar ligands for 7-transmembrane G-protein coupled receptors from a diverse N-(substituted)glycine peptoid library," R. N. Zuckermann, et al., J. Med. Chem. **1994**, 37, 2678-2685.

In vitro Models for Predicting Metabolism and Pharmacokinetics

X1-006 DEVELOPING *IN VITRO* MODELS TO PREDICT HUMAN DRUG TRANSPORT AND METABOLISM, Ronald T. Borchardt, Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.

A major challenge confronting medicinal chemists in the future will be to design drug candidates having molecular characteristics that permit the molecule to optimally interact with the pharmacological target (e.g., enzyme, receptor) (rational drug design). In addition, the drug candidate must have the molecular characteristics that allow the molecule to circumvent the biological barriers (e.g., intestinal mucosa, blood brain barrier) which could limit its access to the pharmacological target *in vivo* (rational drug delivery). To assist medicinal chemists in conducting rational drug delivery, our laboratory has developed and validated *in vitro* cell culture systems that mimic the intestinal mucosa and the blood brain barrier (BBB). The model of the intestinal mucosa consists of a human colon carcinoma cell line (Caco-2) grown onto microporous membranes. Caco-2 cells have been shown to undergo spontaneous enterocytic differentiation in culture ultimately exhibiting morphological and biochemical characteristics similar to small intestinal absorptive cells (1). The model of the BBB consists of primary cultures of bovine brain microvessel endothelial cells (BBMEC's) grown onto microporous membranes. In culture, BBMEC's exhibit morphological and biochemical characteristics similar to the endothelial cells that constitute the BBB *in vivo* (2). Both of these cell culture systems have been used by pharmaceutical scientists to evaluate rational drug delivery strategies (3,4). In this presentation, examples will be given to illustrate how these cell culture systems have been used to evaluate chemical and formulation strategies designed to minimize the metabolism and/or maximize the permeability of drug candidates.

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X1-007 STUDIES WITH CYTOCHROME P450 SUBTYPES: CLUES TO HUMAN METABOLISM. Dennis A. Smith, Department of Drug Metabolism, Pfizer Central Research, Sandwich, Kent CT13 9NJ, U.K.

Traditionally, Drug Metabolism input to the discovery process has been largely on an empirical case by case basis[1]. Considerable advances in our understanding of drug disposition have been made. Basic rules can be applied to the behaviour of a compound, in terms of absorption and elimination, by man and animals, based on physico-chemistry and structure. This is particularly true in the area of the cytochrome P450, the principal enzymes involved in the primary clearance of drugs. The major human forms, CYP2D6, CYP2C9 and CYP3A4 all have distinct substrate preferences[2]. These are being catalogued and rationalised in terms of the topography of the active site, the steric hindrance of the access of the iron-oxygen complex to the possible sites of metabolism and the ease of electron or hydrogen abstraction from the various carbons or heteroatoms of the substrate. Whilst actual tertiary structure data is not available on these enzymes valuable insights have been made using data from soluble, bacterial cytochrome P450's, structural overlay of substrates etc. Binding of substrates and inhibitor to CYP2D6 and CYP2C9 require the formation of an ion pair and a hydrogen bond respectively. In contrast, the major interaction with substrate and CYP3A4 appears to involve lipophilic binding. These differences in binding mechanism translate to the actual affinity of binding. CYP2D6 substrates in particular tend to have higher affinity (lower Km's) for the enzyme than those of CYP3A4. Moreover, whilst CYP3A4 can metabolise a molecule at a number of sites distant from one another, CYP2D6 and CYP2C9 are far more constrained in their regioselectivity. Such insights not only impact on the understanding of the behaviour of existing drugs. Since the enzymes and systems remain the same, these understandings will be applied to the design of molecules with improved pharmacokinetic properties, whilst the structure activity relationships of the biological targets are being researched and revealed.

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Strategic Alliances

X1-008 THE ROLE OF STRATEGIC ALLIANCES IN THE NEW DRUG DISCOVERY PROCESS, Wendell Wierenga, Ph.D., Parke-Davis Pharmaceutical Research, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

R&D collaborations, or corporate partnerships as they are often referred to, between the large pharmaceutical organizations and "biopharma" are becoming increasingly commonplace and, presumably, increasingly important. The 1980's witnessed corporate partnerships which were in large part product driven--a licensing deal. The 1990's however are exhibiting a plethora of R&D driven alliances based on access to technology and small molecule drug discovery (screening, chemical/natural product libraries, drug design). This follows a paradigm shift from "biotech" to "neopharma". These alliances also represent a shift in business models from "fully integrated" to "virtually integrated". This evolution in strategy recognizes shared risk/shared reward, two-way technology, and flexible, yet complex structures. Furthermore, these new structures have necessitated creative designs for collaborative roles, decision-making, and financing. Representative examples will be presented.

Gene Manipulation

X1-009 IN VIVO GENE TRANSFER FOR THE SYSTEMIC DELIVERY OF BIOPHARMACEUTICALS, Sandeep Tripathy, Eugene Goldwasser, Eliav Barr, and Jeffrey M. Leiden. The University of Chicago, Chicago, IL 60637

The development of an *in vivo* gene transfer approach to provide stable delivery of physiological levels of recombinant proteins to the systemic circulation would represent a significant advance in our ability to treat a large number of human diseases including pituitary dwarfism, hemophilias A and B, and the erythropoietin-responsive anemias. We have previously reported that genetically modified skeletal muscle stem cells (myoblasts) can be used to produce physiological levels of human growth hormone in the systemic circulation of mice for at least 3 months following intramuscular (IM) implantation. Although effective, such an *ex vivo* gene therapy approach is both labor intensive and expensive because it requires the isolation, growth, and transfection of primary myoblasts from each patient to be treated. We now report the development an adenovirus-mediated *in vivo* gene transfer protocol into skeletal muscle that results in the stable delivery of physiological levels of recombinant proteins to the systemic circulation. A replication-defective adenovirus expressing the human erythropoietin (hEpo) cDNA under the transcriptional control of the cellular EF1 promoter and the 4F2HC enhancer was constructed using standard techniques. Cultured primary human myocytes infected with 5pfu/cell of this virus secreted 1000 mU/ml of hEpo which was both appropriately glycosylated and fully bioactive *in vitro*. Neonatal CD1 or adult SCID mice injected IM once with 10^7-10^9 pfu of this virus displayed significant, dose-dependent increases in serum hEpo levels and hematocrits which were stable over the 150 day time course of these experiments. The injected adenovirus remained localized to the site of injection and there was no evidence of either systemic infection or local muscle pathology. In contrast, adult immuocompetent CD1 mice injected with this same vector displayed only transient elevations in hematocrits. The transient recombinant gene expression observed in these immunocompetent animals reflected both a cytotoxic T cell response directed against the adenovirus-infected myocytes and a humoral immune response against the human Epo. We conclude that IM injection of replication-defective adenoviruses represents a technically feasible and efficient *in vivo* gene transfer method for the stable delivery of physiological levels of recombinant proteins to the circulation. However, the use of this system for the treatment of human serum protein deficiencies will require the development of less immunogenic viral vectors and/or the use of immunosuppressive regimens to prolong recombinant gene expression in vivo.

Legal Issues: Patents

X1-010 ACCESS TO RESEARCH TOOLS: WHO SHOULD BE LICENSED UNDER THE BAYH-DOLE ACT? Joshua A. Kalkstein, Pfizer Inc, Eastern Point Road, Groton, Connecticut 06340.

The framers of the Constitution considered patents important enough to empower Congress to enact laws establishing them. However, neither the Federalist Papers nor the Antifederalist Papers contain any discussion of the provision. In enacting Bayh-Dole in 1980 and amending it in 1984, Congress similarly provided little in the way of legislative history or statutory definition to teach the National Institutes of Health, for example, how the statute was to be administered. While the Congressional intent to enable universities to take title to their federally funded patented inventions and to grant exclusive licenses to those inventions for commercial purposes emerges from the legislative history, there is no discussion of the pre-existing public policy demanding broad and rapid dissemination of taxpayer-funded inventions to those scientists in the private, academic and governmental sectors able to employ them in their research. Nevertheless, the plain meaning of language in the statute that is neither addressed in the scant legislative history nor, to a great degree, in the definitions, compels the conclusion that Congress intended the federally funded licensor to grant exclusive commercial licenses to make, use and sell and non-exclusive research licenses to make and use, but not to sell, simultaneously. Moreover, public policy going back to the Constitutional prescription "To promote the Progress of Science ..." supports the idea that taxpayer funded science should be employed to as great a degree as possible to enhance the advance of science in the public interest. Furthermore, given the fact that the holder of a non-exclusive research license cannot commercialize anything that would infringe the patent, the holder of the exclusive commercial license is not penalized.

Future Role of Rational Design

X1-011 FUTURE DIRECTIONS IN DRUG DESIGN, Brian W. Metcalf, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Three approaches to drug discovery will be discussed, examples being taken from ongoing programs at SmithKline Beecham. Future directions for these approaches will then be considered. The first of these, peptidomimetic strategies, will be illustrated by the discovery of orally active antagonists of the endothelin receptors, ET_A and ET_B. The approach involved the conceptual linkage of molecules discovered by empirical screening with the experimentally derived 3 D structure of the endothelin-1. Future directions concern the use of site directed mutagenesis of the receptors in order to develop models of receptor-ligand interactions and guide design of receptor-subtype selective antagonists. The second example of peptidomimetic strategies will describe RGD mimetics, used to antagonize the platelet GpIIb/IIIa receptor and potentially useful as antithrombotics. Future directions include extrapolation to other integrins that bind the RGD motif.

The second approach to be described relates to the use of a pharmacologically active molecule of unknown mechanism, to define a new molecular target. In this case, photoaffinity approaches deployed using proprietary small molecules that suppress cytokine production have led to the discovery of a novel kinase involved in IL-1 and TNFsynthesis.

The third approach involves large scale sequencing of cDNA libraries from individual cell types in order to identify novel targets. In this case sequencing of a human osteoclast library has identified a novel cysteine protease. In situ hybridization experiments point to the extremely limited tissue expression of this protease, suggesting that its inhibition should lead to a selective effect in the cell type of interest.

X1-012 MOLECULAR MECHANISMS FOR BACTERIAL RESISTANCE TO THE ANTIBIOTIC VANCOMYCIN, **Christopher T. Walsh, Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115**

Significant clinical resistance has arisen in gram positive bacteria to the antibiotic Vancomycin. High level, inducible resistance is conferred by five plasmid borne genes, Van R, Van S, Van H, Van A, Van X. Each of the five encoded proteins has been subcloned, expressed in and purified from *E coli* to homogeneity to assess function and analyze the necessary role each plays in antibiotic resistance. Van S and Van R form a two component sensing and response regulator system in which Van S is a transmembrane sensor with a cytoplasmic kinase domain and Van R a transcription factor, activated when phosphorylated by Van S, to turn on transcription of the Van H, A, and X genes. Van H and A act to produce D-ala-D-lactate rather than the normal D-ala-D-ala as an intermediate in peptidoglycan biosynthesis. It turns out that Vancomycim binds with 1000 fold lower affinity to cell wall targets terminating in D-ala-D-lactate rather than D-ala-D-ala, explaining the antibiotic resistance phenotype. Van X is a D-ala-D-ala dipeptidase that hydrolyzes D-ala-D-ala, while leaving D-ala-D-lactate to be taken forward for cell wall biogenesis. All five Van genes need to be expressed for high levels of resistance.

Late Abstract

PROSPECTS FOR NEW BIOPHARMACEUTICALS, Alison Taunton-Rigby, Mitotix, Incorporated, Cambridge, MA

The techniques of biotechnology have been used for the last fifteen years in drug discovery and development. Biotechnology companies have developed some notable new drugs based on human proteins (interferon, erythropoietin, etc.) and have an extensive pipeline of potential drugs in clinical development (~250 in clinical trials, with ~50 in Phase III).

Over 1200 new companies have been established to exploit this new technology. In addition, all large pharmaceutical companies now use biotechnology as an integral part of drug discovery. However, some issues have emerged as a result of biotechnology:

- How should drug development be financed?
- Can biopharmaceuticals be developed more quickly than traditional chemical entities?
- What are the success rates of biopharmaceuticals and chemical entities in clinical research?
- Should there be a separate biotechnology industry?
- How will large pharmaceutical companies and the biotechnology industry partner with each other?

Of fundamental importance are questions of who will be involved in the discovery, development, marketing and sales of highly successful drugs in the future; which of the new technologies such as gene therapy or antisense will succeed; and which fields of research such as the cell division cycle, apoptosis, signal transduction, etc. will be highly productive. The companies who understand biotechnology and these issues thoroughly will make the best choices.

Discovery of Therapeutic Agents

X1-100 HUMAN ENDOTHELIAL CELL LINES AS ALTERNATIVE IN VIVO PHARMACODYNAMIC MODELS,

Edwin W. Ades, Francisco J. Candal, Velma G. George, James M. Pruckler, Diane C. Bosse, and Thomas J. Lawley, Centers for Disease Control and Prevention and Emory University, Atlanta, GA 30333

Human umbilical vein cells (HUVEC) have played a pivotal role in almost all studies of human endothelium. Endothelial cells are very important in leukocyte trafficking, inflammation, wound healing, tumor metastasis, hemostasis, and cytokine production. However, it has become increasingly evident that not all endothelial cells are alike. For example, large vessel and small vessel (ie.. microvascular) endothelial cells differ in several respects including anatomical site and function, in vitro growth requirements and the differential expression and regulation of cell adhesion molecules. The lifespan of all primary isolates of endothelial cells in culture is limited and there is variability inherent in the utilization of cells from different donors which can introduce variations in experimental results within and between experiments that can prove to be vexing. Therefore, in order to gain more consistency and reproducibility of results and to better dissect the biological differences in both levels of differentiation and function between umbilical vein endothelial cells and microvascular endothelium cells; we developed an immortalized human microvascular cell line as well as an umbilical vein cell line. These cells show no signs of senescence (passage 75 or greater), exhibit typical cobblestone morphology, take up acetylated LDL, form tube-like structures on Matrigel™, express von Willibrand's Factor and maintain an endothelial cell phenotype similar to freshly cultured, non-transformed cells. These transfected cells express biologic responses to selected cytokines and growth factors with functional similarity to freshly cultured, non-transformed cells. These cells obviate the need for repeated isolation and repeated exposure of laboratory personnel to tissue from unknown sources as well as any variability that might be introduced through multiple tissue source differences. Since these similarities are established, these immortalized cells can now be utilized with an appropriate level of confidence in subsequent biologic studies.

X1-101 NEUROPROTECTIVE USE-DEPENDENT BLOCKERS OF ION CHANNELS CONTROLLING GLUTAMATE RELEASE

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Blockers of the voltage-activated ion-channels controlling the presynaptic release of glutamate are potentially suitable as therapies for indications, such as brain damage resulting from high risk cardiovascular surgery, where both focal and global ischemic brain damage may occur. We have originated a family of N,N'-disubstituted guanidines that preferentially block the voltage-gated Ca^{2+} and/or Na^{+} channels governing glutamate release under conditions of persistent depolarization, relative to their ability to block glutamate release elicited by brief, transient depolarizations characteristic of normal physiological release events in non-ischemic brain. Using a rapid superfusion measurements of neuroptransmitter release from brain synaptosomes [Turner and Goldin (1989) Biochem. **28**, 586] and electrophysiological analysis of cloned neuronal Na channels expressed in CHO cells, we have differentiated these actions from those of venom peptide antagonists of presynaptic Ca^{2+} channels governing neurotransmitter release and toxins which block Na channels such as tetrodotoxin (TTX). The results indicate that CNS 1237 and other members of our compound series are use-dependent blockers of the voltage-activated ion channels governing glutamate release.

In vivo studies of CNS 1237 in the rat MCAO focal stroke model have indicated efficacy comparable to that observed by the same laboratory (Graham et al) for the glutamate release blocker BW619C89 (Burroughs-Welcome). Maximal efficacy is achieved at doses (3 mg/kg bolus followed by a 4 hour infusion of 0.75 mg/kg) severalfold lower than that required for maximal protection by BW1003C89. Neuroprotective doses of CNS 1237 produced no significant reduction of blood pressure, and a modest dose-dependent reduction in heart rate, establishing the value of our novel strategy for discovery of neuroprotective agents.

X1-102 NOVEL TARGETS FOR ANTIMICROBIAL DRUGS IN ß-LACTAM RESISTANT STREPTOCOCCUS PNEUMONIAE,

Regine Hakenbeck, Thorsten Grebe, Jan Krauß, Marc van der Linden, Peter Reichmann, and Dorothea Zähner, Max-Planck Institut für molekulare Genetik, D-14195 Berlin

Penicillin and multiple antibiotic resistant *S. pneumoniae* represent a growing therapeutic problem worldwide. Penicillin resistance in *S. pneumoniae* has been believed to be only due to alterations in penicillin target enzymes, the penicillin binding proteins (PBPs). Between one to three point mutations in e.g. PBP 2x reduce the affinity to cefotaxime considerably and in one case abolish binding almost completely. Since overproduction of a soluble derivative of PBP 2x has been achieved, such mutant proteins can be used as test systems for ß-lactams with unusual and novel activities [1]. A completely different approach is based on the recent discovery that also non-PBP genes are affected in ß-lactam resistant laboratory mutants, and that all higher resistant laboratory mutants investigated so far are also defective in natural competence [2,3]. The first non-PBP gene identified in cefotaxime resistant mutants demonstrates this connection especially clear: one single point mutation in a histidine kinase CiaH confers both, decrease of cefotaxime susceptibility and at the same time complete loss of transformability [2]. *ciaH* is suggested to be part of an operon that includes the corresponding response regulator *ciaR*. In several other mutants as well as in some resistant clinical isolates, certain steps of resistance increase are neither due to mutations in PBP genes nor in the *cia* operon. Identification of the respective mutations, in addition to the genes regulated by the *cia* system, will help toward a new interpretation for penicillin action and to identify new potential antibiotic targets.

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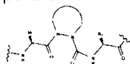
X1-103 A RECOMBINANT HUMAN STROMELYSIN CATALYTIC DOMAIN IDENTIFIES TRYPTOPHAN DERIVATIVES AS HUMAN STROMELYSIN INHIBITORS.

Donald Hupe, Lynn Hupe, Qi-Zhuang Ye, Linda Johnson, Ian Nordan. Departments of Biochemistry and Biotechnology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

The human stromelysin catalytic domain (SCD) has been expressed in *E. coli* and purified to homogeneity. We have used this recombinant SCD for inhibitor screening and identified tryptophan derivatives as competitive inhibitors of SCD. Both Cbz-L-Trp-OH (IC_{50} 2.5 µM, K_i 2.1 µM) and Boc-L-Trp-OH (IC_{50} 10 µM, K_i 8 µM) showed good inhibitory activity. Modification at the indole nitrogen with formyl group (IC_{50} 34 µM, K_i 28 µM) or mesitylene-2-sulfonyl group (IC_{50} 63 µM, K_i 52 µM) showed reduced activity. The amide Cbz-L-Trp-NH_2 was not active, but esters Cbz-L-Trp-OSu (IC_{50} 13 µM, K_i 11 µM) and Boc-L-Trp-OSu (IC_{50} 102 µM, K_i 84 µM) showed activity. Aromatic amino acid derivatives Cbz-L-Tyr-OH (IC_{50} 24 µM, K_i 20 µM) and Cbz-L-Phe-OH (IC_{50} 40 µM, K_i 33 µM) were also active, but other amino acid derivatives had no activity. Although Cbz-D-Trp-OH (IC_{50} 86 µM, K_i 71 µM) was active, the L-configuration is consistently preferred for inhibitory activity. Some of the SCD inhibitors were tested on full length human stromelysin purified from cultured human cells, and they showed the same potency rank order. These results demonstrate the usefulness of recombinant DNA technology in generating the authentic human protein with improved properties for drug discovery.

X1-104 A MODEL PEPTIDE INCORPORATING THE TETRAHYDROPHTHALAZINE NUCLEUS, A CONSTRAINED AZA ANALOGUE OF PHENYLALANINE **Toni Kline, Ellen Sieber-McMaster, Luciano Mueller, and Chester A. Meyers. The Bristol-Myers Squibb Pharmaceutical Research Institue, Lawrenceville, NJ 08543**

Replacement of the α carbon with a nitrogen in α amino acids gives rise to azamino acids, a useful addition to the repertoire of backbone isosteres for peptidomimetics. Most examples of azamino acids that have been incorporated



into peptides are linear analogues, in which conformational effects are restricted to the immediate vicinity of the urea bond. In a sense, the utility of the linear azamino acids is the absence of overall structural perturbation to the peptide; Sidechain functions are not dramatically displaced. The urea linkage permits a configuration midway between D and L, leading to a somewhat extended planarity of the pseudoamide bond with the ureido-residue. In contrast to the linear azamino acids, the heterocyclic analogues might be expected to exhibit stronger conformational preferences. Examples of this class of azamino acids are very limited. Recently, azaproline has been synthesized and shown to be an effective inducer of a highly rigid geometry such that the preceding residue could be accommodated readily by a β turn whereas the following residue is forbidden from engaging in a β turn. As part of our exploratory program in peptidomimetic structural motifs, we prepared a tetrahydrophthalazine as a constrained Phe analogue that could then be incorporated into a stable, fully deprotected peptide. The tetrahydrophthalazine nucleus has been reported in mechanistic chemistry studies, but it has never been utilized in a peptide context. We now report the synthesis of N-(([N-alanyl]-1,2,3,4- tetrahydro-2-phthalazinyl)carbonyl)-L-alanine (**1**) as a model target. NMR studies of **1** will be presented showing the influence of the heterocycle on pseudopeptide secondary structure.

X1-105 CONSTRUCTION OF A MANNOSE RECOGNIZING E-SELECTIN MUTANT, Timothy P. Kogan, Brian Dupre, Bob Bjercke, Sid Sherwood, Ron Tilton, Mitch Revelle and Pamela J. Beck, Department of Molecular Biology and Department of Medicinal Chemistry, Texas Biotechnology Corporation, 7000 Fannin, Suite 1920, Houston, Texas 77030

E-selectin (ELAM-1) is a member of the selectin family of cellular adhesion molecules. This family of proteins has an amino-terminal Ca^{++}-dependent lectin or carbohydrate-recognition domain that is essential for ligand binding. The E-selectin ligand is the sialyl $Lewis^x$ carbohydrate antigen (sLe^x) [Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc]. We have developed a model of E-selectin binding to the sLe^x tetrasaccharide using the recently published x-ray crystallographic structure of E-selectin (ligand unbound) together with the ligand bound crystallographic structure of a related C-type animal lectin, the mannose-binding protein. Analysis of this model indicated that the alteration of one E-selectin amino acid to the analogous lysine residue found in the mannose-binding protein might shift binding specificity from sLe^x to mannose. Using site-directed mutagenesis, we have changed E-selectin alanine 77 to lysine and assessed binding specificity. Instead of sLe^x binding, this mutant protein displays preferential binding to mannose containing oligosaccharides, a result that confirms the modeled selectin-ligand binding interactions and offers the first positive molecular genetic confirmation of the selectin-carbohydrate ligand interaction. The recent publication of the x-ray structure of the E-selectin lectin domain has facilitated improvements in this model of protein-carbohydrate interactions, and has resulted in the design and synthesis of non-carbohydrate inhibitors of selectin mediated cellular adhesion. Some results of this work will be presented along with both *in vitro* and *in vivo* data of a new class of selectin inhibitors that have potential as novel anti-inflammatory agents.

X1-106 PLANT CELL CULTURE MANIPULATION AS A SOURCE OF NOVEL CHEMICAL DIVERSITY FOR DRUG DISCOVERY, Christopher J. Pazoles, Angela M. Stafford and Malcolm Morville, Phytera, Inc., Worcester, MA 01605

Plants represent an important and largely untapped source of broad and novel chemical diversity. While roughly 25% of the pharmaceuticals sold today are plant-related, only a small fraction of plant species have been systematically evaluated for medicinal utility, making plants an almost certain source of new drugs and drug leads. The challenge in realizing this potential is to rapidly and effectively access and evaluate the extensive range of simple and complex chemicals which plants are capable of producing. Phytera's unique strategy is to combine rational, worldwide sourcing of plant material with advanced techniques in plant cell culture and, in particular, manipulations of these cultures which greatly expand the diversity of chemicals produced compared to that found in the intact plant. Such treatments, which can only be efficiently used in the plant cell culture setting, include hormones (e.g. methyl jasmonate), genetic manipulations (e.g. DNA derepression) and environmental stress factors (e.g. infectious agents). These can be used alone or in combinations to generate more diverse phytochemical arrays than can be accessed via the native plant. This unique approach to generating chemical diversity is being coupled to modern technologies for targeted, high throughput biological screening and rapid chemical isolation/identification (e.g. computer-aided dereplication, LC/mass spec). Together, these tactics are capable of identifying novel leads/candidates, for a wide range of therapeutic applications, which are not accessible via more traditional synthetic or natural product starting points.

X1-107 APPLICATION OF HPLC/MS ANALYSIS OF PLATELET ACTIVATING FACTOR (PAF) AND RELATED PHOSPHOLIPIDS TO A MODEL FOR THE STUDY OF DRUG EFFECT ON LEUKOCYTE-ENDOTHELIAL CELL INTERACTION. S. Rizea[1], L. Silvestro[1], G. Montrucchio[2], G. Camussi[3], [1]Res Pharma Pharm. Res. s.r.l., Via Belfiore 57, 10125 Torino Italy, [2]Lab. Immunopatologia, Medical School, Univ. di Torino, [3]Cattedra di Nefrologia II Facoltà. di Medicina, Univ. di Pavia, Varese, Italy.

The development of an "in vitro" model combined with an adequate analytical tool is important to study drugs acting on PAF. The widely used bioassay on platelets is sensitive for PAF but unable to measure phospholipids like lyso-PAF, lyso-PC and acyl-PAF that, even if inactive on platelet aggregation, possess other relevant biological properties. GC-MS, the only alternative analytical technique widely used for PAF, has restrictions with other phospholipids while HPLC-MS/MS showed to be a more flexible tool for the analysis of such lipids. In this study we present the results obtained by this technique in an "in vitro" model to evaluate the drug effects on polimorphonuclear neutrophils (PMN)-endothelial cells (EC) interaction ; the analyses were performed on EC or PMN samples extracted with chloroform-methanol-water. The first experiments showed the presence of isobaric phospholipids ; the use of specific phospholipases and derivatizations allowed to overcame the analytical ambiguities. Changes in the pattern of cell-associated or released phospholipids were observed after cell stimulation and/or treatment with selected drugs. The results obtained indicate that this model is a valuable tool to deepen the interactions between PAF, related phospholipids and drugs.

X1-108 A PARADIGM FOR DRUG DISCOVERY EMPLOYING ENCODED COMBINATORIAL LIBRARIES.
Nolan H. Sigal, Jonathan J. Burbaum, Michael H. J. Ohlmeyer, John C. Reader, Ian Henderson, Lawrence W. Dillard, Ge Li, Troy L. Randle, Daniel Chelsky, and John J. Baldwin. Departments of Biology and Chemistry, Pharmacopeia, Inc., Princeton, NJ 08540

It is now possible to synthesize very large libraries of small molecules on solid supports, and to identify individual library members using chemical tags. To test directly the potential of these tag-encoded libraries in drug discovery, we chose to target carbonic anhydrase as a model. Two libraries consisting of a total of 7,870 members were synthesized, and structure-activity relationships based on the structures predicted by the tags derived. To validate this approach, two active representatives of each library [2-(*N*-(4-sulfamoylbenzoyl)-4'-aminocyclohexane-spiro)-4-oxo-7-hydroxybenzopyran and (*N*-(4-sulfamoylbenzoyl)-L-leucyl)piperidine-3-carboxylic acid] were re-synthesized and shown to have K_D values of 15 nM and 4 nM, respectively. Further, a sublibrary of 217 sulfamoylbenzamides was analyzed for isozyme selectivity, and we see a clear, testable structure-activity relationship that can be used to direct future synthetic efforts toward more isozyme-selective carbonic anhydrase inhibitors. The success of the combinatorial approach emphasizes the power of techniques for the rapid synthesis and evaluation of large numbers of possible structures, and anticipates a new paradigm of drug discovery and optimization.

X1-109 IN VITRO CARDIAC CELL MODEL COMBINES FUNCTIONAL AND BIOCHEMICAL ASSAYS : USE OF VIDEO-COMPUTERIZED APPARATUS AND MASS SPECTROMETRY.
L. Silvestro[1], T. Lampidis[2], M. Macario[1], [1]Res Pharma Pharm. Res. s.r.l., Via Belfiore 57, 10125 Torino Italy [2]Dep. Cell Biol. and Anatomy, School of Medicine, Univ. of Miami, USA.

Effective techniques have been developed to grow spontaneously beating myocites "in vitro" using heart tissues from newborn animals. This model has been employed in pharmacologic, morphologic and electro-physiological studies however, due to sample limitations, few biochemical studies have been performed.

We have been using a video-computerized system, for the measurement of cardiac cell beating, to investigate the cardiotoxicity by the anti-tumor agent Adriamycin on a similar model. Recently, to investigate biochemical events which may correlate with functional cardiac changes, an HPLC/MS has been employed as analytical tool on cell extracts. The interest was to investigate the intracellular levels of phosphocreatine, ATP, ADP, AMP and cyclic nucleotides during ischaemia as well in anthracycline toxicity. Thus cell beating was studied concurrently with the above biochemical parameters. The conditions to obtain ischaemia (gas, pH control, temperature) were critical to reproduce the damages following an ischemic injury. The results on the reversal of anthracycline damage by hexogenous phosphocreatine were particularly striking. This model permitted us to study accurately the biochemical events involved in these effects with samples of only a few million cells. We consider that this model is useful for studies on the pharmacodynamic of drugs acting on the myocites.

X1-110 **NOVEL NATURAL PRODUCTS WHICH CIRCUMVENT MULTIPLE DRUG RESISTANCE.**
Charles D. Smith, Gregory M. L. Patterson and Richard. E. Moore, Department of Pharmacology, Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111; and Department of Chemistry, University of Hawaii, Honolulu, HI 96822.

The success of cancer chemotherapy programs is often limited by the development of tumor cell resistance to both the original therapeutic agent and other seemingly unrelated drug, i.e. multiple drug resistance (MDR). P-glycoprotein is a transmembrane protein which acts as an energy-dependent drug efflux pump, actively removing a variety of structurally diverse compounds, including most currently utilized anticancer drugs. We have initiated a program to identify and characterize new compounds from cyanobacteria (blue-green algae) which overcome P-glycoprotein-mediated MDR. Screening of a collection of ~2000 extracts allowed samples to be classified into 4 groups which: 1) were neither cytotoxic nor reversed MDR; II) were cytotoxic to drug-sensitive cells but not drug-resistant cells; III) were cytotoxic to both drug-sensitive and -resistant cells; or IV) reversed MDR. Bioactivity-directed fractionation led to the isolation of several compounds which antagonized P-glycoprotein-mediated MDR (e.g. tolyporphin, hapalosin, N-methylwelwitindolinone C isothiocyanate), and novel cytotoxins which are not subject to transport by P-glycoprotein (e.g. cryptophycin, tolytoxin, welwitindolinone C isothiocyanate). Interestingly, each of the cytotoxins indicated above are selective cytoskeletal poisons which maintain activity against MDR cells. Further development of these compounds should define their potential usefulness in the treatment of drug-resistant tumors.

X1-111 ANTI-INFLAMMATORY PEPTIDE AGONISTS,
Holly A. Thomas and Edward T. Wei, School of Public Health, University of California, Berkeley, CA 94720

The discovery of new drugs often requires the explicit recognition of novel biological mechanisms that are suceptible to perturbation by exogenous chemicals. We summarized recent evidence (Annu. Rev. Pharmacol. Toxicol. 33:91-108, 1993) which suggests that certain peptides can act as agonists to inhibit the phase of the acute inflammatory response characterized by increased vascular permeability. In previous strategies for the discovery of anti-inflammatory drugs, agents were designed to act as antagonists of inflammatory mediators such as platelet-activating factor, leukotrienes, and bradykinin. Specific antagonists, by definition, work one-on-one against substances that promote inflammation, and the efficacy of a single antagonist may be limited if more than one mediator is released during tissue injury. An agonist, a term introduced by Reuse to describe a chemical that activates biological events, would be more efficacious than an antagonist if it could suppress convergent processes initiated by more than one inflammatory mediator. The concept of drugs as anti-inflammatory agonists was discussed by Svensjo & Persson in 1985. These authors showed that clinically applied asthma drugs such as the β_2-adrenergic agonist terbutaline and the xanthine drug theophylline acted on specific receptors in the microvasculature to shut off plasma protein leakage induced by the inflammatory mediators histamine and bradykinin. The studies reviewed here suggest that the immediate changes in vascular permeability after local injury can be inhibited by peptides belonging to the corticotropin-releasing factor and neurotensin superfamilies. These peptides act as anti-inflammatory **agonists** to reduce vascular leakage in the acute phase of inflammation and may be prototypes for the discovery of new therapeutics.

X1-112 EPIDERM- AN IN VITRO HUMAN SKIN MODEL FOR DRUG DESIGN,

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EpiDerm (MatTek Corporation, Ashland MA) is a synthetic human epidermis stratified on a microporous membrane in culture. This human epidermal model is grown at the air/liquid interface resulting in a cornified apical layer. We have used EpiDerm in both toxicity studies and drug design applications. In conjunction with the U.S. Army Medical Research Institute of Chemical Defense we have shown that this engineered tissue responds to toxic insult from the mustard, 2-chloroethylethyl sulfide (CEES). Alamar Blue, a non-invasive, metabolic fluorescent indicator revealed that EpiDerm could reflect mustard toxicity in a dose-dependent manner. Most importantly, however, Alamar Blue and EpiDerm revealed a latent toxicity that was apparent several days after exposure - an attribute similar to in vivo skin. In other unrelated studies we have used EpiDerm as a model tissue to assess and further develop HypoThermosol, a cold-storage solution. Data show that EpiDerm can be successfully stored for up to 9 days in Hypothermosol without loss in viability. EpiDerm stored in HypoThermosol also retains its ability to differentiate subsequent to warming. We suggest that EpiDerm will continue to be useful in the discovery of therapeutic and pharmaceutical agents.

X1-113 INTERACTIONS AND MODEL OF AN ENDOTHELIN RECEPTOR ANTAGONIST WITH TYR129 IN THE PUTATIVE RECEPTOR BINDING CAVITY,

M.L. Webb, P.S. Patel, P.M. Rose, E.C.K. Liu, D.A. Lach, S.M. Fisher, O. Hadjilambris, P.D. Stein, J. Barrish, T. Stouch, and S.R. Krystek Jr., Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543

Endothelin (ET) receptor antagonism has been considered a potential therapeutic intervention in the treatment of vascular diseases. To elucidate the mechanism of antagonist - ET receptor complex formation, the interactions of four chemically distinct antagonists were investigated using a combination of genetic and biochemical approaches. By site-specific mutagenesis we previously demonstrated that Tyr129 in the second transmembrane domain was important for high affinity, subtype-selective binding to the A subtype of ET (ET_A) receptors [Krystek et al., (1994) *J. Biol. Chem. 269*, 12383-12386]. Affinities of the constrained cyclic pentapeptide BQ-123, the pyrimidinyl benzenesulfonamide bosentan, the indanecarboxylic acid SB 209670 and the naphthalenesulfonamide BMS-182874 were decreased 20 to 1,000-fold to Tyr129Ala, Tyr129Ser, and Tyr129His ET_A receptor mutants. Substitution of Tyr129 with Phe or Trp did not alter the high affinity binding of BQ-123, bosentan or SB 209670. BMS-182874 binding affinity was decreased 10-fold to Tyr129Phe and Tyr129Trp ET receptors. These data indicate a role of specific aromatic interactions in the binding of these antagonists to ET_A receptors, and in the case of BMS-182874, also suggested a role for hydrogen bond formation. This hypothesis was supported by structure-activity data with analogs of BMS-182874 that varied the C-5 dimethylamino substituent on the naphthalene ring. On the basis of these data, a model of the docked conformation of BMS-182874 in the ET_A receptor is proposed as a starting point for further delineation of interactions that underlie antagonist -ET_A receptor complexes.

X1-114 LIPOPHILIC PRODRUG DISTRIBUTION AND RETENTION IN NORMAL AND TUMOR TISSUES,

M.B. Yatvin, W. Li, M. Leavitt*, and E.D. Werts*, Oregon Health Sciences University, Portland, OR 97201; *Allegheny General Hospital, Pittsburgh, PA 15212

Tissue targeting has been achieved using three prodrugs made by covalently linking methotrexate (MTX) to sphingosine. Methotrexate linked through hexanoic acid to ceramide (MC) was ineffective in inhibiting 3T3 cell growth, but attained a level 13x that of free MTX in mouse brain. The addition of an ester bond produced the prodrug ME_6C. Following oral intubation, the ME_6C concentration in the brain was 100x and 300x the level of free MTX in fed and fasted mice, respectively. The half-life of ME_6C in brain was 18 hours, indicating that the prodrug could act as a reservoir. No concomitant increase in ME_6C levels was observed in the dose limiting organs, liver and kidney. Methotrexate linked to sphingosine through a salicylic acid/ester bond (MSC) was directly cytotoxic to 3T3 cells, and the addition of mouse brain homogenate enhanced its cytotoxicity. The prodrug MSC accumulated in the lung rather than the brain.

When male Sprague-Dawley rats bearing a C6 glioblastoma were orally intubated with drug, brain accumulation of ME_6C was more than 150x that of MTX. Tumor ME_6C uptake equaled that in surrounding brain. Although MTX uptake was higher in tumor tissue than in normal brain tissue, it was strikingly lower than tumor ME_6C.

Our results indicate: (1) prodrug function is dependent on MTX release; (2) lipophilic prodrugs containing MTX readily cross the blood brain barrier; (3) prodrugs can serve as drug reservoirs; (4) targeting is altered by prodrug composition and conditions of administration; and (5) uptake of ME_6C into tumors is markedly greater than uptake of free MTX.

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