Project Information

2R44ES015641-02

Project Number: 2R44ES015641-02
Title: VALIDATION OF HUMAN VAGINAL TISSUE ASSAY FOR ENDOCRINE DISRUPTORS

Contact PI / Project Leader: AYEHUNIE, SEYOUM
Awardee Organization: MATTEK CORPORATION

Abstract Text:

DESCRIPTION (provided by applicant): Environmental or occupational exposure to a broad variety of chemical agents can alter normal endocrine function. The effects of these "endocrine disruptors" (ED) can have serious health implications including deleterious effects to reproductive capacity, fetal development, the immune system, and carcinogenesis. Current animal tests are expensive, use a large number of animals, and are not necessarily applicable to humans. Thus, a validated, human in vitro method to identify ED is an area of great importance. This research project will validate an organotypic EpiVaginal tissue model for Tier 1 screening of chemicals with endocrine disrupting potential. Phase I research will validate MatTek's organotypic vaginal-ectocervical (EpiVaginalTM) tissue model for use in identifying ED. A battery of 75 model compounds with known ED activity will be selected from the revised ICCVAM list of recommended substances. The production of estrone by the tissue model and changes to tissue morphology and gene expression will be monitored as biomarkers of ED. A prediction model for ED with be finalized and the test method will undergo formal validation in a multi-center, GLP study. In addition, reproducibility of the assay method and adaptation of the method to a high throughput screen format will be investigated. If successful, the proposed method will have high impact in environmental chemical safety programs and ultimately will reduce the effect of these chemicals on human health. PUBLIC HEALTH RELEVANCE: Validation an in vitro organotypic tissue based assay to screen for endocrine disrupting potential is important to minimize hazards to humans and wildlife exposed to chemicals that interfere with normal hormonal regulation. The assay method will be adapted to a high throughput format to allow rapid and low- cost screening of chemicals. The organotypic tissue based in vitro screening method will have enormous environmental and public health significance.

Public Health Relevance Statement::

Validation an in vitro organotypic tissue based assay to screen for endocrine disrupting potential is important to minimize hazards to humans and wildlife exposed to chemicals that interfere with normal hormonal regulation. The assay method will be adapted to a high throughput format to allow rapid and low- cost screening of chemicals. The organotypic tissue based in vitro screening method will have enormous environmental and public health significance.

NIH Spending Category:

Biotechnology; Estrogen

Project Terms:

Adverse effects; Advisory Committees; Affect; Agonist; Agriculture; Androgens; Animal Testing; Animals; Area; base; Biological Assay; biomarker; Blinded; carcinogenesis; Cell Culture Techniques; Cells; Chemical Agents; Chemical Exposure; Chemicals; cost; Development; Dose; Drug Industry; Economics; Endocrine; Endocrine disruption; Endocrine Disruptors; Endocrine system; environmental chemical; Environmental Exposure; Environmental Health; Environmental Pollution; Estrogen Receptors; Estrogens; Estrone; Evaluation; Exposure to; Fetal development of the mammalian embryo or fetus; Food Processing; Gene Expression; hazard; Hazard Identification; Health; high throughput screening; Histology; Hormone Receptor; hormone regulation; Hormones; Human; human tissue; Immune system; In Vitro; in vitro Assay; Interagency Coordinating Committee on the Validation of Alternative Methods; Laboratories; Malignant Neoplasms; Methods; Modeling; Monitor; Morphology; Occupational Exposure; operation; Phase; Production; progesterone receptor B; programs; public health medicine (field); Reproducibility; reproductive; Reproductive system; Research; Research Project Grants; response; Rodent; Safety; Screening procedure; Structure; System; Testing; Thyroid Gland; Tissue Model; Tissues; United States Environmental Protection Agency; Vagina; Validation; validation studies; Women's Health;

Project Information

1R43ES019378-01

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DESCRIPTION | DETAILS | RESULTS | HISTORY | SUBPROJECTS | SIMILAR PROJECTS BETA | NEARBY PROJECTS BETA | LINKS BETA | NEWS AND MORE BETA

Project Number: 1R43ES019378-01
Title: HIGH THROUGHPUT MITOCHONDRIAL NEPHROTOXICANT ASSAY

Contact PI / Project Leader: BEESON, CRAIG CANO
Awardee Organization: SCHNELLGEN, INC.

Abstract Text:

DESCRIPTION (provided by applicant): The kidney is a target of toxicity from drugs, and industrial and environmental chemicals because of its high blood flow, numerous transporters, and reliance on aerobic metabolism. Not surprisingly, mitochondria are a common intracellular target of chemicals in multiple organs, leading to decreased aerobic metabolism and ATP, and cell death. Current in vitro models of nephrotoxicity and mitochondrial damage are inadequate for many of the same reasons: cultured cells are very glycolytic with minimal aerobic metabolism, and there are no moderate or high-throughput real-time metabolomic assays. Consequently, new cellular models and metabolomic methodologies are needed to evaluate nephrotoxicity and mitochondrial damage. We have developed primary cultures of renal proximal tubular cells (RPTC) that exhibit in vivo levels of aerobic metabolism, are not glycolytic, and retain higher levels of differentiated functions. In conjunction, we have a new technology (Seahorse Extracellular Flux Analyzer) to measure cell metabolism (oxygen consumption and acid extrusion) in real time in 24-well plates. The long-term goal of this proposal is to merge the RPTC model and the Seahorse technology to develop a quantitative high-throughput assay (qHTS) to measure the effects of toxicants on renal mitochondrial function. Phase I of the proposed research has two aims: (1) respiratory measurements for RPTC will be optimized for sensitivity and precision in a 96-well format; (2) the optimized metabolic assay integrated with automated imaging will be tested against a selection of clinically relevant nephrotoxicants and non-nephrotoxicants. Phase II of the research will use these results to develop a 96-well based qHTS format and validate it with 1400 TOXNET compound library. This assay system will identify nephrotoxicants with mechanism- based criteria for assessment of new drugs, consumer products, and environmental agents. PUBLIC HEALTH RELEVANCE: The final results of the proposed research will be a quantitative high-throughput assay that can assess new drugs, consumer products, and environmental agents for their potential to cause kidney damage in humans.

Public Health Relevance Statement::

The final results of the proposed research will be a quantitative high-throughput assay that can assess new drugs, consumer products, and environmental agents for their potential to cause kidney damage in humans.

NIH Spending Category:

Biotechnology; Kidney Disease

Project Terms:

Acids; Address; Annexins; Apoptosis; base; Binding (Molecular Function); Biological Assay; Blood flow; Buffers; Cell Death; cell injury; Cell membrane; Cell model; Cell Respiration; Cells; cellular imaging; Chemicals; clinically relevant; Concentration measurement; consumer product; Cultured Cells; Dose; Drug toxicity; Electron Transport; environmental agent; environmental chemical; Evaluation; Exhibits; extracellular; fluorophore; Genomics; Genus Hippocampus; Goals; Health Sciences; high throughput screening; Human; Image; In Vitro; in vitro Model; in vivo; Information Systems; instrument; Kidney; kidney cell; Libraries; Measurement; Measures; member; Metabolic; Metabolism; metabolomics; Methodology; Methods; Mitochondria; nephrotoxicity; new technology; novel; Oligomycins; Organ; Oxygen Consumption; Pharmaceutical Preparations; Phase; Proxy; public health relevance; Reliance; Research; Respiration; respiratory; Screening procedure; Staining method; Stains; Stress Tests; System; Technology; Testing; Time; Toxic effect; toxicant; Toxicology Data Network; Tubular formation; United States National Institutes of Health; Validation;

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Project Information

5R44ES015646-03

Project Number: 5R44ES015646-03
Title: GENETICALLY DIVERSE EMBRYONIC STEM CELL LINES FOR REPRODUCTIVE TOXICOLOGY
Contact PI / Project Leader: CHOI, TED
Awardee Organization: PREDICTIVE BIOLOGY

Abstract Text:

DESCRIPTION (provided by applicant): Determining the reproductive toxicity risk of compounds is vitally important to ensure the health of the general population. It is a difficult and complex task involving testing in animals. While it is well known that mouse strain background can have a profound effect on phenotype, understanding the role of strain background in toxicity testing remains a tremendous challenge. An in vitro genetic testing system contributes toward this end by offering the advantages of lower cost, higher throughput and no sacrifice of animals in testing. An in vitro genetic system can be a "first tier" testing platform, directing in vivo testing to mouse strains that maximize informativeness and minimize animal use. We propose to develop an in vitro, ES cell based system to assess the impact of genetic background in toxicity testing. ES cells are particularly attractive for this purpose since they can be propagated indefinitely in their pluripotent state while retaining the ability to contribute to all tissues of an animal. In Phase I we demonstrated the feasibility of this system by establishing ES cell lines from six genetically distinct mouse strains and showing that a measurable and significant variability due to genetic background can be measured in response to exposure to a reference compound, retinoic acid. In Phase II, a panel of approximately 100 genetically distinct ES lines will be established and tested with a larger panel of reference compounds, toward the development of a system to define, map and identify the genetic components of cellular response to environmental burden. This system will lead to more predictive reproductive toxicity testing by providing a platform to investigate the role of genetic background on toxicity risk. The testing platform will be made commercially available to the pharmaceutical and chemical industries, as well as to academic institutions. PUBLIC HEALTH RELEVANCE: We propose to develop a system that can help elucidate the role of genetics in reproductive toxicity testing. The broad and varied genetic backgrounds in the proposed testing system better reflects the genetic diversity of the US population, and may lead to more predictive testing that reduces environmental health risk to the population.

Public Health Relevance Statement::

We propose to develop a system that can help elucidate the role of genetics in reproductive toxicity testing. The broad and varied genetic backgrounds in the proposed testing system better reflects the genetic diversity of the US population, and may lead to more predictive testing that reduces environmental health risk to the population.

NIH Spending Category:

Genetics; Patient Safety; Stem Cell Research; Stem Cell Research - Embryonic - Non-Human

Project Terms:

Adipocytes; Adopted; animal tissue; Animals; base; Biological Assay; Biology; Cardiac Myocytes; cell type; Cells; Chemical Industry; Chromosome Mapping; Complex; cost; Data; Development; embryonic stem cell; Endothelial Cells; Ensure; environmental agent; Environmental Health; Environmental Risk Factor; ES Cell Line; Evaluation; Exhibits; Exposure to; Gender; Gene Expression; Gene Expression Profile; General Population; Genes; Genetic; genetic analysis; Genetic Identity; Genetic screening method; Government; Haplotypes; Health; Hepatocyte; In Vitro; in vivo; Institution; Islet Cell; Islets of Langerhans; Karyotype; Lead; Maps; Measurable; Measures; Mediating; Methodology; Methods; Mouse Strains; Mus; Neurons; Pathway interactions; Persons; Pharmacologic Substance; Phase; phase 1 study; Phenotype; Population; Process; public health relevance; Quantitative Trait Loci; reproductive; response; Risk; Role; safety testing; Screening procedure; System; Technology; Testing; Tissues; Toxic effect; toxicant; Toxicity Tests; Toxicology; Tretinoin; Validation; Variation (Genetics); Visual;

Project Information

1R43ES021688-01

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DESCRIPTION | DETAILS | RESULTS | HISTORY | SUBPROJECTS | SIMILAR PROJECTS BETA | NEARBY PROJECTS BETA | LINKS BETA | NEWS AND MORE BETA

Project Number: 1R43ES021688-01
Title: DEVELOPMENT OF A DIVERSITY OUTCROSS ES CELL PANEL FOR IN VITRO GENETICS OF TOXICO

Contact PI / Project Leader: CHOI, TED
Awardee Organization: PREDICTIVE BIOLOGY

Abstract Text:

DESCRIPTION (provided by applicant): Strain background can strongly influence the outcome of toxicity tests in animals. Time and cost constraints preclude in vivo approaches to longstanding concerns about the lack of genetic diversity in animal models. An in vitro platform is vastly more efficient for understanding the role of genetic background in toxicology testing, and sacrifices no animals. In this project we propose to build a large panel of genetically diverse ES cells from the Diversity Outcross line of mice. The DO mice are an advanced intercross of the 8 founder strains used for the Collaborative Cross. The DO strains capture almost all the sequence variants in laboratory strains, and harbor hundreds to thousands more recombinational breakpoints than F2 mice. They ideally suited for facilitate complex trait mapping. ES cells offer tremendous flexibility and is also ideal for an in vitro genetics platform. DO ES cell lines immortalize DO genomes, creating a renewable resource. In principle, ES cells can provide access to almost any cell type by directed differentiation. In this way, the DO ES panel is extremely versatile as a permanent, renewable resource. In Phase I, we will establish feasibility by deriving 90 independent, validated DO ES lines and estimate the genetic component of cytotoxicity observed for 15 model toxicant compounds in the Phase I panel. In Phase II we will complete the 600 ES line panel, identify compounds with a significant genetic component to cytotoxic response using a 1400 reference compound set, and map the loci mediating that genetic component in the identified compounds. PUBLIC HEALTH RELEVANCE: There is a longstanding interest in how genetics impacts toxicology, but cost effective, highly scalable tools to investigate this relationship were not available. This project will improve human health and reduce the risk of environmental toxicants by developing a high throughput in vitro platform for investigating the genetic basis of variable response to toxicant exposure.

Public Health Relevance Statement::

There is a longstanding interest in how genetics impacts toxicology, but cost effective, highly scalable tools to investigate this relationship were not available. This project will improve human health and reduce the risk of environmental toxicants by developing a high throughput in vitro platform for investigating the genetic basis of variable response to toxicant exposure.

Project Terms:

Animal Model; Animals; Area; base; Biological Assay; Biological Process; Biology; Cell Line; cell type; Cells; Chromosome Mapping; Complex; Contract Services; cost; cost effective; cytotoxic; cytotoxicity; Development; drug discovery; Embryo; embryonic stem cell; ES Cell Line; Evaluation; flexibility; Genetic; genetic analysis; Genome; Government; Head; Health; Housing; Human; improved; In Vitro; in vivo; Inbred Strain; Inhibitory Concentration 50; innovation; interest; Laboratories; Maps; Measures; Mediating; Modeling; Mus; Outcome; Persons; Phase; Provider; Resources; response; Risk; Role; Screening procedure; Services; Specific qualifier value; Testing; Therapeutic; Time; tool; Toxic effect; Toxic Environmental Substances; toxicant; Toxicant exposure; Toxicity Tests; Toxicology; trait; Validation; Variant; Variation (Genetics);

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Project Information

1R43ES021116-01

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Project Number: 1R43ES021116-01
Title: DNA REPAIR-ON-A-CHIP: SPATIALLY ENCODED MICROWELL ARRAYS

Contact PI / Project Leader: GEORGE, JAY
Awardee Organization: TREVIGEN, INC.

Abstract Text:

DESCRIPTION (provided by applicant): Human exposure to dangerous genotoxins is unavoidable, as DNA damaging agents are ubiquitous both in our environment and within our cells. DNA damaging agents and other genotoxins that arise from cellular metabolism, environmental sources or disease-related cellular defects contribute to cell death (e.g., neurodegeneration), gene mutations, gene rearrangements and in many cases, the onset of cancer, disease and aging phenotypes. In addition, many exogenous exposures such as chemotherapy and radiation treatment rely on the induction of tumor cell genotoxicity to mediate therapeutic response. Further, the ability to effectively and accurately repair spontaneous or induced DNA damage depends on the cellular DNA repair capacity. Therefore, the ability to quantify DNA damage and the rate of repair of the damage to the nuclear genome directly in human cells is critical in applications ranging from epidemiology to drug development. To address this technological need in the research community, to be better positioned to characterize the genotoxicity of newly developed pharmaceuticals, and to quantify DNA repair capacity without the need to identify specific DNA Repair gene defects, we propose the development of the next generation in DNA damage detection and quantification technology. This proposal, to develop the 'DNA Repair on a Chip' technology, combines the use of agarose-based Microwell arrays, spatially- encoded cellular recognition, human tumor cell lines with genetically-defined DNA repair status and extra-cellular matrix proteins to optimize, validate and commercialize a series of Spatially Encoded Microwell Arrays that will function as a tool to quantify DNA damage and measure cellular DNA Repair capacity at baseline and following genotoxin exposure on a single array or chip (DNA Repair on a Chip). The studies described in Aim 1 involve the development of a series of 24-well Spatially Encoded Microwell Arrays, with Microwells ranging from 10-50 5M in diameter and 20-50 5M in depth, suitable for gravity capture of a single cell of various sizes. Efficacy of the Microwell Arrays will be validated using radiation and small molecule inhibitors. Further, the sensitivity of the Microwell Arrays for analysis of cellular DNA Repair capacity will be evaluated using an isogenic panel of human tumor cell lines with defined defects in DNA Repair gene expression and following genotoxic stress. Iterative analysis and Microwell characterization will inform to finalize a set of 24-well Microwell Arrays for production and distribution. The studies described in Aim 2 involve additives to the Microwell Arrays that will enhance cell growth and attachment, providing optimal analysis of baseline DNA damage and most importantly, critical data on cellular capacity for in vivo repair post-damage. This technological advance opens the door to new strategies for drug discovery, genotoxicity testing, and environmental health research through objective, quantitative analyses. Phase II of the project will be expanded to offer 96-well capability, end-user software for spatial recognition and quantitation plus micro-well additive options for specialized cell growth and attachment. PUBLIC HEALTH RELEVANCE: We describe a new methodology that provides for robust, high-throughput DNA damage and repair analysis by exploiting gravity capture of single cells into a Microwell array. DNA damage levels are revealed morphologically by single-cell gel electrophoresis. The Microwell array enables fully automated DNA damage and DNA repair measurement of multiple experimental conditions simultaneously. This technological advance opens the door to new strategies for drug discovery, genotoxicity testing, and environmental health research through objective, quantitative analyses.

Public Health Relevance Statement::

We describe a new methodology that provides for robust, high-throughput DNA damage and repair analysis by exploiting gravity capture of single cells into a Microwell array. DNA damage levels are revealed morphologically by single-cell gel electrophoresis. The Microwell array enables fully automated DNA damage and DNA repair measurement of multiple experimental conditions simultaneously. This technological advance opens the door to new strategies for drug discovery, genotoxicity testing, and environmental health research through objective, quantitative analyses.

NIH Spending Category:

Cancer; Genetic Testing; Genetics

Project Terms:

Address; Adhesions; Aging; base; Biological Assay; Caliber; Cell Death; cell growth; Cell Line; cell type; Cell-Matrix Junction; Cells; chemotherapy; Comet Assay; Communities; Computer software; Cytoskeleton; Data; Defect; design; Detection; Development; Disease; DNA Damage; DNA Repair; DNA Repair Gene; drug development; drug discovery; Environment; Environmental Health; Epidemiology; expectation; exposed human population; Exposure to; Extracellular Matrix Proteins; Force of Gravity; Gel; Gene Expression; Gene Mutation; Gene Rearrangement; Genome; Genotoxic Stress; genotoxicity; Glass; Growth; Human; in vivo; inhibitor/antagonist; Joints; Life; Ligands; Malignant Neoplasms; Measurement; Measures; Mediating; Metabolism; Methodology; Mutagenicity Tests; Mutagens; neoplastic cell; Nerve Degeneration; new technology; next generation; Nuclear; Outcome; Pharmacologic Substance; Phase; Phenotype; Plastics; Positioning Attribute; Production; Proteins; Radiation; repaired; Research; Research Personnel; response; Sepharose; Series; small molecule; Source; Surface; Technology; Therapeutic; tool; Tumor Cell Line;

Project Information

5R44ES017180-03

Project Number: 5R44ES017180-03
Title: SYSTEM FOR COMPREHENSIVE TRACKING AND ANALYSIS OF C. ELEGANS BEHAVIORS

Contact PI / Project Leader: GLASER, JACOB R
Awardee Organization: MICROBRIGHTFIELD, INC.

Abstract Text:

DESCRIPTION (provided by applicant): We propose a commercial product composed of a computerized microscope system that allows investigators to perform automated, quantitative analyses of locomotory and other complex behaviors of freely moving C. elegans nematodes. Using the soil nematode, C. elegans, as a practical and efficient model in toxicology studies requires creation of automated technologies to evaluate growth, size, reproduction, feeding, and movement under different experimental conditions. Currently, most methods to assess C. elegans phenotypic behavior are manual and laborious to perform which precludes medium-throughput studies. We believe that introduction of this robust commercial software solution will significantly contribute to further replacement and reduction of conventional toxicology tests by alternative in vivo toxicology assays using C. elegans. PUBLIC HEALTH RELEVANCE: The proposed project will increase the throughput of studies involving quantitative behavioral analysis using the model organism Caenorhabditis elegans (C. elegans). This will allow researchers to automatically perform complex and laborious tasks which are presently performed manually. The overall effect of this project will significantly improve the productivity and effectiveness of research in such diverse fields as toxicology, environmental monitoring, ecology and neuroscience research.

Public Health Relevance Statement::

Narrative The proposed project will increase the throughput of studies involving quantitative behavioral analysis using the model organism Caenorhabditis elegans (C. elegans). This will allow researchers to automatically perform complex and laborious tasks which are presently performed manually. The overall effect of this project will significantly improve the productivity and effectiveness of research in such diverse fields as toxicology, environmental monitoring, ecology and neuroscience research.

NIH Spending Category:

Bioengineering; Prevention

Project Terms:

Agar; Animal Model; base; Behavior; Behavioral; Biological Assay; Caenorhabditis elegans; Characteristics; charge coupled device camera; Chemical Stimulation; Collaborations; Complex; Computer software; computerized; Data; Detection; Development; early experience; Ecology; effectiveness research; Environmental Monitoring; experience; feeding; Frequencies (time pattern); Goals; Growth; Head; Hydra Polyps; Image; Image Analysis; improved; in vivo; innovation; Life; Lighting; Liquid substance; Locomotion; Manuals; Marketing; Measurement; Methods; micromanipulator; Microscope; Modeling; Monitor; Morphology; Movement; National Institute of Environmental Health Sciences; National Toxicology Program; Nematoda; Neurologic; Neurosciences Research; Organism; Phase; Positioning Attribute; Process; Productivity; prototype; public health relevance; Reproduction; Research Design; Research Methodology; Research Personnel; research study; Resolution; Shapes; Soil; Solutions; Specimen; Staging; Stimulus; Stream; Swimming; System; Technology; Testing; Time; tool; Toxic effect; toxicant; Toxicology; Translating; Validation; Withdrawal; Writing;

Project Information

5R44ES014312-03

Project Number: 5R44ES014312-03
Title: VALIDATION OF AN IN VITRO HUMAN AIRWAY MODEL

Contact PI / Project Leader: HAYDEN, PATRICK J
Awardee Organization: MATTEK CORPORATION

Abstract Text:

DESCRIPTION (provided by applicant): Hazard assessment, including evaluation of acute inhalation toxicity potential, is a mandatory international regulatory requirement for chemicals utilized in international commerce. Acute inhalation toxicity or irritation potential is an important consideration in establishing procedures for the safe handling, packaging and labeling and transport of chemicals and chemical mixtures, and in formulating responses to emergency exposure situations. Recently enacted legislation including the European Union (EU) Registration, Labeling and Authorization of Chemicals (REACH) program, and the US EPA High production Volume (HPV) Chemical Challenge will dramatically increase the need for inhalation toxicity information. The goal of the present grant proposal is to validate the EpiAirway in vitro human airway model for prediction of in vivo human inhalation toxicity hazard potential following Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and European Center for Validation of Alternative Methods (ECVAM) guidelines. Phase I experiments produced several prediction models that will be further tested in the current Phase II project. One hundred chemicals that have available in vivo human or animal inhalation toxicity data and established immediately Dangerous to Life or health (IDLH) concentrations established by NIOSH will be utilized in the Phase II validation project. Interlaboratory transferability of the method will also be evaluated in 4 laboratories using a subset of 30 chemicals chosen from the original 100 tested during the Phase II study. The study data will then be submitted for independent statistical analysis and the final results and report will be submitted to regulatory agencies (i.e. ICCVAM) in support of regulatory acceptance. The technology to be validated in the current Phase II proposal will address a critical barrier to implementation of worldwide requirements for inhalation toxicity testing of chemicals, and a technical capacity that is urgently needed but that does not presently exist. The methodology developed will provide a transformative technology that will facilitate the paradigm shift from in vivo rodent to in vitro human inhalation toxicology testing envisioned in the resent National Research Council Report "Toxicity Testing in the 21st Century: A Vision and a Strategy". PUBLIC HEALTH RELEVANCE: Hazard assessment, including evaluation of acute inhalation toxicity potential, is a mandatory international regulatory requirement for chemicals utilized in international commerce. Acute inhalation toxicity or irritation potential is an important consideration in establishing procedures for the safe handling, packaging and labeling and transport of chemicals and chemical mixtures, and in formulating responses to emergency exposure situations. The technology to be validated in the current Phase II proposal will address a critical barrier to implementation of worldwide requirements for inhalation toxicity testing of chemicals, and provide a technical capability that is urgently needed but that does not presently exist.

Project Terms:

Acute; Address; Animals; Applications Grants; Authorization documentation; Biological Assay; Breathing; Chemicals; Commerce; consumer product; Contracts; Data; Data Set; Emergency Situation; Equation; European; European Union; Evaluation; Goals; Government; Guidelines; hazard; Hazard Assessment; Hazardous Chemicals; Health; Human; In Vitro; in vivo; Industry; Inhalation Toxicology; Institutes; Interagency Coordinating Committee on the Validation of Alternative Methods; International; irritation; Label; Laboratories; Letters; Life; Manufacturer Name; Methodology; Methods; Modeling; National Research Council (U.S.); Occupations; Phase; phase 2 study; Procedures; Production; programs; Protocols documentation; public health relevance; Regulation; Reporting; Research; research study; respiratory; response; Rodent; Safety; Services; Statutes and Laws; success; Technology; Testing; Toxic effect; Toxicity Tests; Validation; validation studies; Vision;

Project Information

1R43ES019824-01A1

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Project Number: 1R43ES019824-01A1
Title: MODEL OF THE HUMAN TESTIS FOR REPRODUCTIVE TOXICOLOGY

Contact PI / Project Leader: JOHN, CONSTANCE M
Awardee Organization: MANDALMED, INC.

Abstract Text:

DESCRIPTION (provided by applicant): More than 87,000 chemicals have been developed and distributed over the past 50 years. The vast majority of these have not been tested for potential toxic effects in humans or animals. Most systematic toxicology research is conducted in animals and is very expensive. Although animal studies contribute important information, they may not closely approximate the exposure or the exposure effects in humans. There is an unmet need for cost-effective bioassay systems that mimic and assess human exposures and their reproductive effects for systematic, prospective investigation of potentially toxic substances. The overall goal of the research is to develop a functional in vitro human cell-based testis model of spermatogenesis as a clinically relevant product for reproductive toxicology. Development of the first generation product is enabled by our capability to isolate and propagate primary Sertoli cells from adult human donors (hSertCs) and to manipulate primordial germ cells (PGCs), human embryonic stem cells (hESCs), and induced pluripotent stem cells (iPSCs) in vitro to produce haploid cells. The hSertCs have been available to researchers worldwide through a partnership with Lonza (Walkersville, MD) since May 2009, and have been used to create a model of the blood-testis barrier (BTB) in coated inserts of transwell plates. By silencing and overexpressing genes that encode germ-cell-specific cytoplasmic RNA-binding proteins, we have been able to modulate human germ-cell formation and developmental progression, to promote later stages of meiosis and development of haploid gametes. In Phase I, we aim to establish a model using primary human Sertoli, Leydig, and peritubular myoid cells to form a functional (BTB) in a closed hollow fiber system mimicking physiologic shear stress conditions, and to perform analytical and toxicological studies of the cells. There are three Specific Aims for Phase I. 1. Develop an in vitro human Sertoli cell-based, 3-dimensional (3-D) model of the testis. 2. Develop methods to detect and quantify the cells within the testis model. 3. Demonstrate the effect of reproductive toxins on the viability of human testicular cells. In Phase II, we intend to drive the differentiation of induced pluripotent stem cells (iPSCs) and spermatogonial stem cells (SSCs) to the haploid state within the model, and test the effect of toxins on this process. From this effort, we expect to identify reducible, reliable, and relevant endpoints for reproductive toxicant testing, and then to establish outcome markers and assays enabling for F.D.A. approval of the model in conjunction with other toxicology testing required for all New Drug Applications. The need for more relevant and improved reproductive toxicology testing represents a significant commercial opportunity, especially in the pharmaceutical industry. The bioassay product would be provided to industry on a fee-for-service basis initially. This effort potentially could lead to the capability to replicate normal human spermatogenesis in vitro and generate de novo mature sperm for severely infertile, azoospermic men, including cancer survivors. PUBLIC HEALTH RELEVANCE: More than 87,000 chemicals have been developed and distributed discarded over the past 50 years. The vast majority of these have not been tested for potential toxic effects in humans or animals. There is an unmet need for more cost-effective bioassay systems that will mimic and assess human exposures and their reproductive effects for systematic, prospective investigation into potentially toxic substances. The overall goal of the research is to develop a functional in vitro human cell-based testis model of spermatogenesis as a clinically relevant product for reproductive toxicology. Better identification of reproductive toxins could help protect developing fetuses from unwanted toxic exposures, reduce the incidence of male infertility, potentially lower rates of testis cancer, and create safer drugs for the marketplace. Also, part of our interest in developing a testis model is develop the capability to replicate normal human spermatogenesis in vitro to potentially generate de novo mature sperm for severely infertile, azoospermic men, including cancer survivors.

Public Health Relevance Statement::

More than 87,000 chemicals have been developed and distributed discarded over the past 50 years. The vast majority of these have not been tested for potential toxic effects in humans or animals. There is an unmet need for more cost-effective bioassay systems that will mimic and assess human exposures and their reproductive effects for systematic, prospective investigation into potentially toxic substances. The overall goal of the research is to develop a functional in vitro human cell-based testis model of spermatogenesis as a clinically relevant product for reproductive toxicology. Better identification of reproductive toxins could help protect developing fetuses from unwanted toxic exposures, reduce the incidence of male infertility, potentially lower rates of testis cancer, and create safer drugs for the marketplace. Also, part of our interest in developing a testis model is develop the capability to replicate normal human spermatogenesis in vitro to potentially generate de novo mature sperm for severely infertile, azoospermic men, including cancer survivors.

NIH Spending Category:

Cancer; Contraception/Reproduction; Stem Cell Research; Urologic Diseases

Project Terms:

3-Dimensional; Adult; Animals; aromatic hydrocarbon receptor; Aromatic Polycyclic Hydrocarbons; Asbestos; Asthma; Automobile Driving; base; BAX gene; Biological Assay; bisphenol A; Blood-Testis Barrier; Cadmium; Cancer Survivor; cell type; Cells; Chemical Exposure; Chemicals; Clinical Trials; clinically relevant; cost; cost effective; Data; Development; developmental disease/disorder; drug development; Drug Industry; Environment; Environmental Exposure; exposed human population; Fee-for-Service Plans; Fertility; Fertility Study; Fetus; Fiber; General Population; Generations; Genes; Germ Cells; Goals; Growth; Haploid Cells; Haploidy; Human; human disease; human embryonic stem cell; Human Experimentation; improved; In Vitro; in vitro Model; in vivo; Incidence; induced pluripotent stem cell; Industry; Infertility; interest; Investigation; Lead; lead exposure; leydig interstitial cell; male; Male Infertility; Malignant neoplasm of testis; Malignant Neoplasms; Marketing; Mediating; Meiosis; men; Mercury; Methods; Microfluidics; Modeling; novel; Outcome; Overdose; overexpression; Pathway interactions; Pesticides; Pharmaceutical Preparations; Phase; phase 2 study; Physiological; Poisons; polycarbonate plastic; Process; prospective; Reference Standards; Reporting; Reproduction; reproductive; Research; Research Personnel; RNA-Binding Proteins; Seminal fluid; sertoli cell; shear stress; sperm cell; Spermatogenesis; Staging; Stem cells; Structure of primordial sex cell; System; Temperature; Testing; Testis; Toxic effect; toxicant; Toxicant exposure; Toxicity Tests; Toxicology; Toxin; Vinyl Chloride;

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Project Information

5R44ES015002-03

Project Number: 5R44ES015002-03
Title: RECONSTRUCTED SKIN MICRONUCLEUS GENOTOXICITY ASSAY

Contact PI / Project Leader: KALUZHNY, YULIA
Awardee Organization: MATTEK CORPORATION

Abstract Text:

DESCRIPTION (provided by applicant): As part of a standard toxicological evaluation, all new products must be tested to insure they are not genotoxic. Animal tests are available but European legislation that takes effect in 2009 will ban animal testing. This legislation will affect almost all large US multinational personal care, cosmetic, and pharmaceutical companies. Non-animal, in vitro tests exist; however, they lack specificity and result in an unacceptably high rate of false positives. This leads to a large number of materials being excluded from further development even though are safe. The long term goal of the proposal is to validate an in vitro test method to accurately determine human skin genotoxicity. Phase 1 research made significant progress in developing a reconstructed skin micronucleus (RSMN) assay for genotoxicity testing. A standardized protocol, a pre-screen cytotoxicity assay, and a prediction model (based on statistically significant increases in micronuclei in dividing cells) were defined and tested. A previously published method was improved to increase the sensitivity of the assay for detecting genotoxins requiring metabolic activation. In addition, long term reproducibility studies utilizing tissue from multiple donors showed highly reproducible results. Phase 2 will further build on Phase 1 results to optimize the assay method, automate scoring, expand the database of materials tested, demonstrate interlaboratory reproducibility, and adapt the assay to a high throughput format. These studies will lay the groundwork for formal validation and regulatory acceptance of the assay. PUBLIC HEALTH RELEVANCE: Current genotoxicity test methods rely on animals or in vitro tests. However, the in vitro methods give an unacceptable percentage of false positive test results and animal testing will be banned for US based multinational companies due to pending legislation. This project will develop an in vitro assay which accurately predicts human genotoxicity.

Public Health Relevance Statement::

Current genotoxicity test methods rely on animals or in vitro tests. However, the in vitro methods give an unacceptable percentage of false positive test results and animal testing will be banned for US based multinational companies due to pending legislation. This project will develop an in vitro assay which accurately predicts human genotoxicity.

NIH Spending Category:

Cancer; Emerging Infectious Diseases; Genetics; Infectious Diseases

Project Terms:

Affect; Amendment; Animal Testing; Animals; Bacterial Genes; base; Biological Assay; Carcinogens; Cell Count; Cell Culture Techniques; Cells; Chromosome abnormality; Cosmetics; cytotoxicity; Data; Databases; Development; Discrimination (Psychology); Europe; European; Evaluation; Gene Mutation; genotoxicity; Goals; Human; improved; In Vitro; in vitro Assay; in vitro testing; in vivo; Laboratories; Life; Lymphoma; Mammalian Cell; Manufacturer Name; Materials Testing; Metabolic; Metabolic Activation; Methods; Methyl Methanesulfonate; micronucleus; Micronucleus Tests; Microsomes; Mitomycins; Modeling; Mus; Mutagenicity Tests; Mutagens; Pharmaceutical Preparations; Pharmacologic Substance; Phase; Positive Test Result; Protocols documentation; public health relevance; Publishing; Rattus; Relative (related person); Reproducibility; Research; Robotics; Rodent; Safety; Salmonella; Self Care; Sensitivity and Specificity; Skin; Specificity; Statutes and Laws; System; Test Result; Testing; Time; Tissues; Topical application; Validation;

Project Information

1R41ES018022-01

Project **10** of **19**

Project Number: 1R41ES018022-01
Title: TEAMCHIP FOR HIGH-THROUGHPUT, PREDICTIVE HUMAN METABOLISM AND TOXICOLOGY
Contact PI / Project Leader: LEE, MOO-YEAL
Awardee Organization: SOLIDUS BIOSCIENCES, INC.

Abstract Text:

DESCRIPTION (provided by applicant): Solidus Biosciences, Inc. in partnership with Rensselaer Polytechnic Institute is proposing to address a critical need in chemical safety technology through its proprietary "Transfected Enzyme and Metabolism Chip" (or TeamChip) for high-throughput analysis of systematic drug candidate and chemical metabolism and toxicology. The TeamChip is being developed to mimic the first-pass metabolism of the human liver and to predict enzyme-specific hepatotoxicity. The effects of metabolism on other cell types will also be demonstrated. Thus, the reactivity of target compounds with individual human metabolic enzymes or combinations of enzymes in the human liver or other organ types can be assessed and quantified at speeds commensurate with predictive human toxicity assessment of early stage drug candidates and environmental chemicals. The specific aims of this Phase I STTR project are to: 1. Develop efficient methods for transfecting genes into THLE-2 human liver epithelial cell lines and Beas-2B human lung epithelial cell lines encapsulated in 3D alginate matrices as small as 30 nL. 2. Construct recombinant adenoviruses that carry genes for metabolic enzymes from a human liver cDNA library (e.g., CYP450 isoforms including CYP1A2 and CYP3A4) and demonstrate gene transfection on monolayers of THLE-2 and Beas-2B cells using fluorogenic substrates in a 96-well plate. 3. Demonstrate 3D cellular microarrays containing metabolic enzyme-expressing THLE-2 and Beas-2B cells and identify metabolic genes whose differential expression affects the cellular response to chemicals as proof of concept. In vitro technologies that can be used to quickly assess large numbers of compounds for toxicity remain limited. A critical component of safety evaluation is metabolism and toxicology of chemicals (e.g., drug candidates and environmental chemical toxicants), which reflects the susceptibility of chemicals to be metabolized by human metabolic enzymes and the toxicity of parent compounds and their metabolites. Current approaches to chemical safety assessment are costly, time consuming, and use large amounts of compound and large numbers of animals. Thus, there is great potential and opportunity to apply the TeamChip as a safety assessment tool that can be used to evaluate whether and how specific metabolic enzymes contribute to the toxicity of drug candidates and chemical toxicants. This capability may also be used to predict differences among individuals in drug and chemical metabolism and toxicity. 1 PUBLIC HEALTH RELEVANCE: The drug discovery process is an investment-intensive, high-risk endeavor that results in low yields of effective and safe drugs; a problem that is confounded by the significant lack of information that exists in predicting the metabolic fate of drug candidates, in general, and in predicting the reactivity of drug candidates in the human body. The proposed Phase I STTR project for the development of Solidus Bioscience's TeamChip technology has significant relevance to public health by providing pharmaceutical researchers with the information needed to predict the in vivo metabolism of drug candidates, and thus help to decide which compounds are brought forward for lead optimization and the ultimate development of better and safer drugs. Furthermore, this research is relevant to the prioritization of industrial and environmental chemicals in terms of their safety and use.

Public Health Relevance Statement::

Project Narrative The drug discovery process is an investment-intensive, high-risk endeavor that results in low yields of effective and safe drugs; a problem that is confounded by the significant lack of information that exists in predicting the metabolic fate of drug candidates, in general, and in predicting the reactivity of drug candidates in the human body. The proposed Phase I STTR project for the development of Solidus Bioscience's TeamChip technology has significant relevance to public health by providing pharmaceutical researchers with the information needed to predict the in vivo metabolism of drug candidates, and thus help to decide which compounds are brought forward for lead optimization and the ultimate development of better and safer drugs. Furthermore, this research is relevant to the prioritization of industrial and environmental chemicals in terms of their safety and use. 1

NIH Spending Category:

Biotechnology; Digestive Diseases; Liver Disease

Project Terms:

Acetaminophen; Address; Adenoviruses; Affect; Alginates; Animals; base; Biological Assay; Catalysis; cDNA Library; Cell Line; cell type; Cells; Chemicals; Clinical Trials; Cyclophosphamide; CYP1A2 gene; CYP3A4 gene; Cytochrome P450; Data; Data Analyses; design; Development; drug candidate; drug development; drug discovery; Drug or Chemical; Encapsulated; environmental chemical; Environmental Pollutants; Enzymes; Epithelial Cells; Evaluation; Exposure to; flexibility; Fluorogenic Substrate; Generations; Genes; Glass; Green Fluorescent Proteins; Hepatocyte; Hepatotoxicity; high risk; high throughput analysis; Human; Human body; In Vitro; in vivo; Individual; Institutes; Investments; Lead; Libraries; Liver; Lung; Measurement; Metabolic; Metabolism; Methods; monolayer; Organ; Parents; Patients; Pharmaceutical Preparations; Pharmacologic Substance; Phase; Population; Predisposition; Process; programs; Protein Isoforms; Protocols documentation; public health medicine (field); public health relevance; Recombinants; red fluorescent protein; Reporter Genes; Research; Research Personnel; response; Safety; Screening procedure; Simulate; Slide; Small Business Technology Transfer Research; Speed (motion); Staging; Subgroup; System; Technology; Testing; Therapeutic; Time; tool; Toxic effect; toxicant; Toxicology; Transfection; Treatment Protocols; Viral; Xenobiotic Metabolism; Xenobiotics;

Project Information

5R44ES017366-04

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DESCRIPTION | DETAILS | RESULTS | HISTORY | SUBPROJECTS | SIMILAR PROJECTS BETA | NEARBY PROJECTS BETA | LINKS BETA | NEWS AND MORE BETA

Project Number: 5R44ES017366-04 **Former Number:** 2R44GM083366-02
Contact PI / Project Leader: LI, CHUNQI
Title: PHASE 2 SBIR: ZEBRAFISH CYTOCHROME P450 ASSAYS FOR ASSESSING DRUG METABOLISM AND
Awardee Organization: PHYLONIX PHARMACEUTICALS, INC.

Abstract Text:

DESCRIPTION (provided by applicant): Cytochrome P450 (CYP) enzymes catalyze the majority of known drug metabolism and the bulk of these reactions occur in the liver. As a consequence, many clinically relevant drug-drug interactions are associated with inhibition and/or induction of a specific CYP enzyme. Modifications of CYP activities can have profound effects on therapeutic efficacy and can lead to life-threatening toxicity. In order to provide an early warning system for potential serious side effects, detection of specific CYPs responsible for drug metabolism and drug-drug interactions is a goal of all pre-clinical studies. An ideal CYP assay should be rapid, robust, reliable, reproducible, and amenable to automation in multiwell plate formats. This research will develop a microplate format high throughput whole zebrafish CYP functional assay for assessing drug metabolism and drug safety. Based on genetic and physiological similarity to humans, zebrafish show promise as an efficient, predictive animal model for assessing drug metabolism and drug safety. PUBLIC HEALTH RELEVANCE: Project Narrative The zebrafish assay will facilitate detection of specific cytochromes responsible for drug metabolism and drug-drug interactions, and provide an early warning system for potential serious side effects,

Public Health Relevance Statement::

Project Narrative The zebrafish assay will facilitate detection of specific cytochromes responsible for drug metabolism and drug-drug interactions, and provide an early warning system for potential serious side effects,

Project Terms:

Adverse effects; Affect; Animal Model; Animals; Automation; base; Biological Assay; Biological Availability; clinically relevant; Collection; Complex; cost; CYP2C9 gene; CYP2D6 gene; CYP3A4 gene; Cytochrome P450; Cytochromes; Detection; drug candidate; drug discovery; Drug Interactions; drug metabolism; drug use screening; Enzymes; Evaluation; Exhibits; Family; Family member; FDA approved; Fullerenes; Genetic; Goals; Government; Hepatic; Hepatocyte; Human; In Vitro; in vivo; inhibitor/antagonist; Lead; Libraries; Life; Liquid substance; Liver; Mammals; Metabolic Clearance Rate; Metabolism; Modeling; Modification; mouse model; Mus; Persons; Pharmaceutical Preparations; Pharmacologic Substance; Phase; Physiological; Preclinical Drug Evaluation; preclinical study; Preclinical Testing; Process; public health relevance; Rattus; Reaction; Reliance; Research; Safety; Screening procedure; Small Business Innovation Research Grant; success; System; Technology; Testing; Therapeutic Agents; Toxic effect; Transgenic Mice; Transgenic Organisms; Treatment Efficacy; Zebrafish;

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Project Information

1R43ES020638-01

Project Number: 1R43ES020638-01
Title: FUNCTIONALIZING NON-USEABLE CRYOPRESERVED HUMAN HEPATOCYTES INTO USEABLE HEPATIC

Contact PI / Project Leader: MCCLELLAND, RANDALL
Awardee Organization: SCIKON INNOVATION, INC.

Abstract Text:

DESCRIPTION (provided by applicant): The first isolated human hepatoctyte 2D culture, in 1981, provided baseline protocols to support freshly isolated and immediately cultured populations. With cryopreservation technology, human hepatocytes are commercially-available on demand, however, about 75% of the world stores of cryopreserved human hepatocytes are below industry quality control standards and deemed non-useable. Creating methods to transition available but non-usable cryopreserved human hepatocytes into serviceable cell products is a primary goal. Using stable isotope (13C) resolved metabolomics (SIRM) applied to 2D cultures has revealed a major discovery that is contrary to metabolic paradigms: human hepatocytes do not metabolize the two primary carbon sources in basal media recipes, glucose and glutamine. When compared to rat hepatocytes in the starved state, it was concluded that human hepatocytes are in a stressed state that is probably catalyzed by surgery, death and the liver procurement process. The stressed hepatocytes consume internal proteins and lipid stores to generate glucose. In addition, nonhuman extracellular matrices are standard 2D culture conditions. Functionalizing these cells is based on two hypotheses. The first hypothesis: pyruvate and propionate are energy sources other than glucose and glutamine that are one enzyme step from the Krebs cycle, and will decrease lipolysis and proteolysis permitting amino acid and lipid stores to be diverted to proteins and membrane synthesis for increased cell attachment and viability. The second hypothesis is that natural biomatrices encountered by human hepatocytes will increase attachment once proteolysis is inhibited and protein synthesis of extracellular matrices is optimized. Two products are created from this study, (1) supporting media and extracellular matrices capable of transitioning non-usable human hepatocytes into useable cryopreserved hepatocyte products, and (2) a validated 13C SIRM method for contract research services using suspensions or attached human hepatocytes presently non-useable by conventional assays. The two specific aims will: (1) determine if addition of TCA nutrients (2-13C-pyruvate and 3-13C-propionate) increase human hepatocyte viability and/or attachment then (2) using the optimal media determined in specific aim 1, use the new basal media formulation to test three mixtures of human liver derived extracellular matrix for their efficacy at increasing viability and attachment. PUBLIC HEALTH RELEVANCE: In this grant application entitled "FUNCTIONALIZING NON-USEABLE CRYOPRESERVED HUMAN HEPATOCYTES", our studies catalog readily available, but currently ineffective, human hepatocytes as significant opportunities to transform subpar cellular products into serviceable in vitro human liver toxicodynamic and biokinetic models. This would be achieved by validating inactive and active metabolomic pathways using stable isotope (13C) resolved metabolomics (SIRM) that can demarcate needed cell eco-system supplements in support of culture success, and ultimately be applied in biokinetic models of human acute and chronic systems biology. This proposal will help to provide an abundance of cell material while decreasing developmental costs and time to commercialization.

Public Health Relevance Statement::

In this grant application entitled "FUNCTIONALIZING NON-USEABLE CRYOPRESERVED HUMAN HEPATOCYTES", our studies catalog readily available, but currently ineffective, human hepatocytes as significant opportunities to transform subpar cellular products into serviceable in vitro human liver toxicodynamic and biokinetic models. This would be achieved by validating inactive and active metabolomic pathways using stable isotope (13C) resolved metabolomics (SIRM) that can demarcate needed cell eco-system supplements in support of culture success, and ultimately be applied in biokinetic models of human acute and chronic systems biology. This proposal will help to provide an abundance of cell material while decreasing developmental costs and time to commercialization.

NIH Spending Category:

Digestive Diseases; Liver Disease

Project Terms:

Acute; Amino Acids; Applications Grants; base; Biological Assay; Carbon; Cataloging; Catalogs; Cell Survival; Cell-Matrix Junction; Cells; Cessation of life; Chronic; Chronic Toxicity Tests; Citric Acid Cycle; Collagen Type I; commercialization; Computer software; cost; Cryopreservation; demographics; Development; Dose; Drug Formulations; Ecosystem; Energy-Generating Resources; Enzymes; Extracellular Matrix; Glucose; glucose metabolism; Glutamine; Goals; Health; Hepatic; Hepatocyte; Human; Image; improved; In Vitro; Industry; Label; Lipids; Lipolysis; Liver; matrigel; membrane synthesis; Metabolic; metabolomics; Methods; miniaturize; Modeling; Nutrient; Octanoic Acids; Operative Surgical Procedures; Pathology; Pathway interactions; Phase; Phenotype; Population; preconditioning; Procedures; Process; Production; Propionates; Protein Biosynthesis; Proteins; Proteolysis; Protocols documentation; Pyruvate; Quality Control; Rattus; Recipe; Research Contracts; research study; response; scale up; Science; Services; Solutions; Source; stable isotope; Standardization; Sterilization; Stress; success; Suspension substance; Suspensions; System; Systems Biology; Technology; Testing; Time; Tissue Donors; Tissue Procurements; Tissues; toxicant; Variant;

Project Information

1R43ES019807-01

Project **13** of **19**

Project Number: 1R43ES019807-01
Title: NOVEL HIGH THROUGHPUT PLATFORM FOR SCREENING CYTOCHROME P450 INDUCTION

Contact PI / Project Leader: MORANO, MARIA INES
Awardee Organization: ORIGINUS, INC.

Abstract Text:

DESCRIPTION (provided by applicant): Metabolism of administered drugs is determined by expression and activity of drug-metabolizing enzymes, such as cytochrome (CYP) P450 enzymes. These P450s are subject to inhibition and sometimes induction by xenobiotics, leading to possible pharmacokinetic drug-drug interactions between co-administrated drugs. Recognizing the problem, the FDA has issued a Draft Guidance for Industry Drug Interaction Studies, which advice at a minimum in vitro induction evaluation of CYP1A2 and CYP3A4. The importance of evaluating CYP2B6 induction has recently been recognized as well. The "gold standard" of P450 induction in vitro testing is the determination of enzymatic activity in freshly isolated human hepatocytes. However, availability and individual variability of human hepatocytes as well as inability to discriminate between P450 inhibition and induction in this system complicates the evaluation. The induction of P450s by xenobiotics is mediated by ligand-activated nuclear receptors, including PXR, AhR and CAR. Thus, reporter gene assays for nuclear receptors mediating induction of P450s are considered valid methods of drug evaluation in vitro. Many pharmaceutical industries and some CROs use PXR transactivation assays and very few evaluate AhR activity. There is no commercially available assay for CAR. However, combinations of several nuclear receptors and other transcription factors are needed to induce the expression of specific P450s by all known inducers. The goal of this proposal includes the design and implementation of a novel application of Originus' STEP (Surface Transfection and Expression Protocol) technology for safety testing of novel drug candidates. During Phase I, we will develop sophisticated STEP platforms for simultaneous evaluation of CYP1A2, CYP3A4 and CYP2B6 transcirptional regulation using reporter gene assays in human hepatic cell lines. Secreted alkaline phosphatase (SEAP) gene will be used as a reporter under the control of each human CYP upstream regulatory sequences. STEP co-transfection of the different CYP-SEAP reporters and single or relevant combinations of xenobiotic-activated receptors will be optimized to test SEAP response by a set of inducers at multiple time points in 96-well microplates. Milestones for Phase I are the production and beta-testing of the prototypes of STEP platforms (individually or combined) with appropriate assay robustness and reproducibility well-to-well, plate-to-plate and batch-to-batch. These CYP P450 induction platforms will be available for toxicological screening of drugs early in the drug discovery process by high throughput screening laboratories in industry and academia, or as simple assay kits for small laboratory use at a modest cost. During Phase II, we will extend the studies to evaluate other inducible CYP P450s, expand the spectrum of nuclear receptors tested, and to further develop cell-based assays of relevant allelic variants of the xenobiotic-activated receptors. In addition, we plan to develop STEP assay platforms for pathway profiling of CYP induction. PUBLIC HEALTH RELEVANCE: Drug safety is one of the major factors for compound attrition during clinical development. Simple assays for fast evaluation of metabolism and toxicology of compounds are urgently needed to minimize patient risks and improve the success rate of new molecular entities early in the drug discovery pipeline. The goal of this proposal is to use Originus' proprietary technology, STEP, to develop platforms for in vitro evaluation of drug candidates that may induce certain hepatic enzymes (cytochrome P450s) triggering drug-drug interactions and compromising the health of the patient.

Public Health Relevance Statement::

Drug safety is one of the major factors for compound attrition during clinical development. Simple assays for fast evaluation of metabolism and toxicology of compounds are urgently needed to minimize patient risks and improve the success rate of new molecular entities early in the drug discovery pipeline. The goal of this proposal is to use Originus' proprietary technology, STEP, to develop platforms for in vitro evaluation of drug candidates that may induce certain hepatic enzymes (cytochrome P450s) triggering drug-drug interactions and compromising the health of the patient.

NIH Spending Category:

Biotechnology; Genetics; Patient Safety

Project Terms:

Academia; Alkaline Phosphatase; Americas; Aryl Hydrocarbon Receptor; Award; base; Binding (Molecular Function); Biological Assay; Cell Line; Cells; Clinical; constitutive androstane receptor; cost; CYP1A1 gene; CYP1A2 gene; CYP2B6 gene; CYP3A4 gene; Cytochrome P450; Cytochromes; design; Development; DNA; DNA receptor; drug candidate; drug discovery; Drug Evaluation; Drug Industry; Drug Interactions; Drug Kinetics; drug metabolism; Elements; enzyme activity; Enzyme Induction; Enzymes; Evaluation; Family member; Genes; Goals; Gold; Health; Hepatic; Hepatocyte; high throughput screening; Human; human AHR protein; improved; In Vitro; in vitro Assay; in vitro testing; Individual; Industry; Laboratories; Lead; Ligands; Manufacturer Name; Mediating; Medicine; Metabolism; Methods; Molecular; novel; Nuclear; Nuclear Receptors; Pathway interactions; Patients; Pharmaceutical Preparations; Pharmacologic Substance; Phase; Plasmids; Population; Preclinical Drug Evaluation; pregnane X receptor; Process; Production; Productivity; Promotor (Genetics); Protocols documentation; prototype; receptor; Receptor Activation; Regulation; Reporter; Reporter Genes; Reproducibility; Research; response; Risk; Safety; safety testing; Screening procedure; Small Business Innovation Research Grant; success; Surface; Surveys; System; Technology; Testing; Time; Toxicology; Trans-Activation (Genetics); transcription factor; Transfection; United States Food and Drug Administration; Validation; Variant; Variation (Genetics); Work; Xenobiotics;

Project Information

1R43ES019037-01A1

Project **14** of **19**

Project Number: 1R43ES019037-01A1
Title: ADVANCED GST PROTEOMICS FOR EARLY STAGE ORGAN-SPECIFIC TOXICITY SCREENING

Contact PI / Project Leader: PATRIE, KEVIN M
Awardee Organization: OXFORD BIOMEDICAL RESEARCH, INC.

Abstract Text:

DESCRIPTION (provided by applicant): Current biomarkers employed in the detection of organ toxicity are often not sensitive enough to detect the early stages of acute organ damage, i.e. at a stage when reducing or eliminating exposure to a toxin could prevent progression of organ damage or a disease process. By the time current toxicity biomarkers are detected in biological fluids there is already significant, often irreversible, damage to organs resulting from prolonged exposure to toxic compounds. For example, the level of aminotransferases (ALT and AST) in blood is a widely accepted practice for detecting liver damage. However, these current toxicity biomarkers are typically not detectable in blood at low levels of liver damage and require a relatively long exposure to a toxin before they are detected. The cytosolic glutathione transferase (GST) family of enzymes offer a more reliable alternative as a biomarker for organ damage as they exhibit many of the required characteristics, i.e. tissue specific localization, release into the blood at low levels of toxicity (high sensitivity), and a high intracellular concentration. The GST protein family is comprised of several classes (e.g. A, M, P) with some classes containing multiple isoforms. The many GST isoforms exhibit marked differences in tissue distribution. For example, GSTA1-1 and A2-2 are the predominant GST enzymes found in the liver, whereas GSTA3-3 is mainly expressed in steroidogenic tissues and GSTA4-4 is expressed in all tissues that have been examined. Therefore, measuring the level of specific GST isoforms in blood would be a valuable indicator of damage to a particular organ or tissue. However, current available antibodies for GSTs are not capable of distinguishing among the different GSTA isoforms. Assays for specific GST isoforms would enable a higher degree of resolution and sensitivity to organ damage due to xenobiotic exposure. Recent identification of several additional cytosolic GST classes (S, O and Z) provides an additional opportunity to further expand the toxicology applications of GST assays with the development of immunoassays that are specific for specific isoforms associated with specific tissues. The goal of Phase I feasibility studies is to develop (a) highly specific antibodies capable of distinguishing between three GSTA isoforms, namely GSTA1-1/2-2, GSTA3-3 and GSTA4-4 and (b) ultrasensitive immunoassays for these biomarkers. In addition, in Phase I we will (c) employ animal models to validate these assays as a reliable way to detect organ-specific toxicity. The long-term goal (Phase II) involves development of a comprehensive GST Proteomics panel for high sensitivity organ-specific toxicology testing that has significant preclinical and clinical commercial applications. PUBLIC HEALTH RELEVANCE: There is a great need for more sensitive, minimally-invasive methods to detect the early stages of toxicity to specific organs from exposure to drugs, or as the result of environmental or occupational exposure. The goal of the proposed research is to develop a set of highly specific and ultrasensitive tests to monitor the levels of a family of proteins (the glutathione transferases) in blood, and to incorporate these tests into a comprehensive panel for high sensitivity organ-specific toxicology testing that has significant preclinical and clinical commercial applications.

Public Health Relevance Statement::

There is a great need for more sensitive, minimally-invasive methods to detect the early stages of toxicity to specific organs from exposure to drugs, or as the result of environmental or occupational exposure. The goal of the proposed research is to develop a set of highly specific and ultrasensitive tests to monitor the levels of a family of proteins (the glutathione transferases) in blood, and to incorporate these tests into a comprehensive panel for high sensitivity organ-specific toxicology testing that has significant preclinical and clinical commercial applications.

NIH Spending Category:

Biotechnology; Prevention

Project Terms:

Acute; Animal Model; Animals; Antibodies; Archives; Biological; Biological Assay; biomarker; Blood; Cells; Characteristics; Clinical; commercial application; cross reactivity; Detection; Development; Disease; Dose; Drug or chemical Tissue Distribution; Early Diagnosis; Environmental Exposure; Enzymes; Exhibits; Exposure to; falls; Family; Feasibility Studies; Glutathione S-Transferase; Goals; Half-Life; Hepatotoxicity; Human Development; Immunoassay; improved; Intervention; Isoenzymes; Liquid substance; Liver; Measures; meetings; member; Methods; minimally invasive; Modeling; Molecular Profiling; Monitor; Occupational Exposure; Organ; Pattern; Pharmaceutical Preparations; Pharmacologic Substance; Phase; Poisons; pre-clinical; prevent; Process; Protein Family; Protein Isoforms; Proteomics; Rattus; Reagent; Reporting; Research; Resolution; Screening procedure; Small Business Innovation Research Grant; Specificity; Specimen; Staging; Structure; Technology; Testing; Time; Tissues; Toxic effect; Toxicant exposure; Toxicity Tests; Toxicology; Toxin; Transaminases; Xenobiotics;

Project Information

1R43ES018016-01A1

Project **15** of **19**

Project Number: 1R43ES018016-01A1
Title: NOVEL USE OF CONFOCAL MICROSCOPY ON CULTURED PORCINE CORNEAS FOR PRE-CLINICAL TES

Contact PI / Project Leader: PIEHL, MICHELLE
Awardee Organization: MB RESEARCH LABORATORIES, INC.

Abstract Text:

DESCRIPTION (provided by applicant): There is an important need for screening methods that can detect and distinguish the relative eye discomfort or "sting" potential of cosmetic, personal care, or pharmaceutical formulations early in their developmental stages. The only currently reliable method available is costly and sometimes painful human clinical eye testing, in which test substances are introduced into human eyes and test subjects report perceived discomfort or stinging. While this may be a critical final clinical test for an ocular product, products in development and formulation stages must also be screened in the same human clinical test, as there are no other available screening options. Available ocular irritation testing can indicate that a product or formulation is "non-irritating", which is a good start. However, current ocular irritation tests are not very predictive of eye sting. Laden (1973) has reported that formulations which had low irritancy often still caused stinging. Hence, the stinging potential of a formulation was often unrelated to irritancy. Van Abbe (1973) has also reported ocular irritancy to be a poor predictor of human reported eye stinging, pain, and discomfort. We hypothesize that this disconnect between measurable irritation and sting potential is due to lack of sensitivity in current irritation tests. Current tests use non-sensitive endpoints such as lack of corneal opacity (i.e. rabbit tests and BCOP) and substantial tissue-wide cell death (i.e. EpiOcular"). EpiOcular" (MatTek Corp, Ashland, MA) is an industry standard ocular irritation test with relatively high sensitivity. EpiOcular " tissues are exposed to test substances and then tissue viability is measured by reduction of a metabolic indicator. Extraction and subsequent measurement of the colorimetric indicator from the entire tissue is the endpoint for overall tissue irritation/cytotoxicity effects. Once the EpiOcular tissues reach 100% viability compared to the negative control tissues (no measurable tissue death), the tissues can no longer be indicative of potential sub-irritation cell death that may be an ocular sting indicator. The Porcine Cornea Confocal Assay, PorFocal, developed by MB Research, likely has the amplified sensitivity to potentially predict human eye sting due to measurement of individual cell death per tissue volume by confocal microscopy. The PorFocal uses waste porcine corneas from the meat industry to assay individual corneal cell death with high sensitivity due to a confocal microscopy endpoint. In PorFocal, test substances are placed directly onto living corneal tissue in culture; therefore solubility of the test substance is irrelevant. PorFocal cultured corneas are maintained in a living state for up to 7 days and are dosed daily with the test substance. This multiple-exposure dosing schedule allows for quantification of extremely mild ocular cell death with additive effects over time. These additive effects are then measured by quantification of individual stained dead cells within the corneal tissue by confocal microscopy. Corneal tissue is imaged in an "optical histological" manner where a series of image "slices" are acquired at increasing depths into the corneal tissue. The images can then be digitally reconstructed to exhibit the entire corneal tissue volume imaged (see Research Strategy). Therefore, extremely low amounts of corneal damage can be quantified because the endpoint is actual individual dead cell number per tissue volume. In Aim 1 seven test substances will be tested in both the EpiOcular " and the PorFocal to determine if the PorFocal assay is more sensitive than the EpiOcular ", the most sensitive in vitro ocular irritation assay (industry standard). This will test the hypothesis that sting is not detected in current in vitro assays due to lack of sensitivity. If PorFocal demonstrates higher sensitivity than the industry standard EpiOcular ", then it may have great potential to predict sting. In Aim 2, commercially available products that are known non-stingers or stingers will be tested in both EpiOcular " and PorFocal. The EpiOcular" will be used to establish whether "stingers" would be considered "non-irritants" by industry standards. If PorFocal can resolve stingers from non-irritants, this may allow for prospective culling of stinging product formulations before final human clinical eye sting testing. Upon further characterization of PorFocal, this test could significantly reduce human test subject pain and distress, and cost/time expenditure during product development and formulation. PUBLIC HEALTH RELEVANCE: This project will test the use of a cultured porcine cornea assay as a highly sensitive pre-clinical screen test to predict human eye-sting potential. A human eye sting screening test is needed for testing during product formulation and development phases, thus allowing for further culling of stinging product formulations before final human clinical eye sting testing. This would reduce overall product development cost and human test subject pain and distress while promoting robust product safety for public health.

Public Health Relevance Statement::

This project will test the use of a cultured porcine cornea assay as a highly sensitive pre-clinical screen test to predict human eye-sting potential. A human eye sting screening test is needed for testing during product formulation and development phases, thus allowing for further culling of stinging product formulations before final human clinical eye sting testing. This would reduce overall product development cost and human test subject pain and distress while promoting robust product safety for public health.

NIH Spending Category:

Eye Disease and Disorders of Vision

Project Terms:

base; Benzalkonium Chloride; Biological Assay; Cell Count; Cell Death; Cells; Cessation of life; Clinical; Confocal Microscopy; Cornea; Corneal Opacity; Cosmetics; cost; cytotoxicity; Data; Data Analyses; Development; Distress; Dose; Drug Formulations; Exhibits; Expenditure; Eye; Family suidae; Grant; Human; Image; Image Analysis; In Vitro; in vitro Assay; Individual; Industry; irritation; Laboratories; Life; Materials Testing; Measurable; Measurement; Measures; Meat; Metabolic; Methods; novel; Optics; Oryctolagus cuniculus; Pain; Pharmacologic Substance; Phase; pre-clinical; product development; prospective; Protocols documentation; public health medicine (field); Relative (related person); Reporting; Research; research clinical testing; Safety; Schedule; Screening procedure; Self Care; Series; Slice; Sodium Dodecyl Sulfate; Solubility; Staging; Staining method; Stains; Sting Injury; Sunscreening Agents; Testing; Time; TimeLine; Tissue Viability; Tissues; titanium dioxide; wasting; Writing;

Project Information

1R43ES017590-01

Project Number: 1R43ES017590-01
Title: HIGH-CONTENT ANALYSIS TOOLS FOR DEVELOPMENTAL TOXICITY SCREENS IN ZEBRAFISH

Contact PI / Project Leader: SENG, WEN L
Awardee Organization: PHYLONIX PHARMACEUTICALS, INC.

Abstract Text:

DESCRIPTION (provided by applicant): The zebrafish has become an important new animal model for drug screening, target identification, and validation of various disease models. Although progress has been made in adapting image based high- content screening systems, there are several challenges for automation. To date, the quantitative evaluation of zebrafish assays is performed semi-manually at best, which constitutes a significant bottleneck in terms of workflow and screening cost. In Phase I research, we will specialize software algorithms that will enable automated quantitative assessment of various toxicity endpoints, thus allowing the screening of a large number of potentially toxic compounds. PUBLIC HEALTH RELEVANCE: Use of zebrafish as an alternative animal model for drug screening can greatly accelerate the drug screening process, decrease costs, and provide more accurate results than cell-based assays.

Public Health Relevance Statement::

Project Narrative Use of zebrafish as an alternative animal model for drug screening can greatly accelerate the drug screening process, decrease costs, and provide more accurate results than cell-based assays.

NIH Spending Category:

Bioengineering; Biotechnology; Networking and Information Technology R&D

Project Terms:

Algorithms; alitretinoin; Anatomy; Animal Model; Animals; Area; Atlases; Automation; base; Biological Assay; Bladder; Cell Culture Techniques; Cells; Collection; Computer software; Contracts; cost; Culture Media; Data; Defect; Development; Devices; Dimethyl Sulfoxide; Disease model; Drug Industry; Edema; Environmental Pollution; Evaluation; Eye; Fertilization; Gastrointestinal tract structure; Government; Head; Healthcare; Heart; high throughput screening; Image; Image Analysis; instrumentation; Jaw; Joints; Length; Liver; Manuals; Measurable; Measurement; Measures; Modeling; notochord; Organ; Penicillins; Pericardial body location; Persons; Pharmaceutical Preparations; Phase; Poisons; Preclinical Drug Evaluation; Process; programs; public health relevance; Quantitative Evaluations; Reader; Reagent; Research; Safety; Sampling; Scientist; Screening procedure; Slide; software development; Staging; Standardization; Swimming; System; Tail; Teratogens; tool; Toxic effect; Tretinoin; United States Environmental Protection Agency; Validation; Valproic Acid; Variant; Width; Zebrafish;

Project Information

2R44ES018004-02A1

Project **17** of **19**

Project Number: 2R44ES018004-02A1
Title: NEW IN VITRO HUMAN LIVER TOXICITY BIOASSAY SYSTEM

Contact PI / Project Leader: SINGH, RAJ K.
Awardee Organization: VIVO BIOSCIENCES, INC.

Abstract Text:

DESCRIPTION (provided by applicant): The overall objective of this SBIR proposal is to develop and commercialize a human 3D or mini- liver bioassay system suitable for real-time analysis of metabolic functions and long-term drug toxicity profiles. At present, liver functions are mostly studied using HepG2 cell line and primary hepatocytes cultured onto collagen or polymer matrices. However, these 2D monolayer models allow only partial or short-term toxicity analysis due to rapid loss of metabolic functions. Interestingly, spheroid formation in 3D matrix/scaffold cultures is shown to improve host cell functions. In Phase I studies, we successfully demonstrated the feasibility of a new human bioscaffold- 3D HuBiogel (high-density gel) for supporting long-term survival and maintenance of primary liver cells. Moreover, integration of rotary bioreactor system allowed efficient production/analysis of viable mini-liver spheroids. We now propose to complete the development of new 3D-liver bioassay platform that exhibits improved metabolic functions, and thus will enable precise drug toxicity testing in vitro. Phase II specific goals are: 1. Establish an efficient hepatic cells cultivation protocol by optimizing 3D HuBiogel scaffolds and rotary bioreactor or perfusion culture strategies; 2. Test functionality of 3D-liver culture model by evaluating key biochemical and metabolism endpoints; and 3. Validate the practical utility of mini-liver assay system via real- time analysis of CYP450 induction and chemical/drug toxicity responses. Comparative control studies will include 3D culture scaffolds of Type-I collagen or Matrigel as well as fresh human liver-slice cultures. High throughput adaptability of 3D-liver bioassay will also be examined for toxicogenomics via CYP-microarrays. Thus, biologic relevance of new marketable 3D-liver bioassay system will be confirmed for improved drug toxicity analysis/prediction in humans. We are confident this advanced in vitro hepatotoxicity model will positively impact basic, preclinical and biomedical research arenas. Commercial Importance & Significance: No acceptable commercial liver toxicity assay currently exists which utilizes a human bio-scaffold culture system. VBI will develop a market-ready 3D-liver assay platform adaptable to HTS applications. Sale of 3D HuBiogel culture kits or in-house bioassay services would have significant world-wide market, as a research & diagnostic tool. PUBLIC HEALTH RELEVANCE: Current cell-based assay models for studying drug metabolism and toxicity are of limited utility because isolated liver cells die rapidly in their no-physiologic, 2-dimensional (2D) culture formats. In Phase I, we have shown that a novel human 3-dimensional (3D or high- density) biomatrix scaffold- HuBiogel" supports long-term cultivation, survival and functions of primary human liver cells. In Phase II, we will complete the development of new mini-liver bioassay platform by employing 3D HuBiogel scaffolds combined with rotary or perfusion culture techniques. High throughput utility of this robust 3D hepatotoxicity assay will greatly advance biochemical, metabolic and molecular analysis beyond 2D culture methods. Thus, our R&D effort provides a much needed in vitro tool for real-time analysis of chemical/drug metabolism and liver toxicity responses in humans. A successful outcome will offer new market-ready bioassay kits and services with world-wide demand in both basic and pharmaceutical research areas.

Public Health Relevance Statement::

Current cell-based assay models for studying drug metabolism and toxicity are of limited utility because isolated liver cells die rapidly in their no-physiologic, 2-dimensional (2D) culture formats. In Phase I, we have shown that a novel human 3-dimensional (3D or high- density) biomatrix scaffold- HuBiogel" supports long-term cultivation, survival and functions of primary human liver cells. In Phase II, we will complete the development of new mini-liver bioassay platform by employing 3D HuBiogel scaffolds combined with rotary or perfusion culture techniques. High throughput utility of this robust 3D hepatotoxicity assay will greatly advance biochemical, metabolic and molecular analysis beyond 2D culture methods. Thus, our R&D effort provides a much needed in vitro tool for real-time analysis of chemical/drug metabolism and liver toxicity responses in humans. A successful outcome will offer new market-ready bioassay kits and services with world-wide demand in both basic and pharmaceutical research areas.

Project Terms:

3-Dimensional; Acute; Adherent Culture; Agreement; Antineoplastic Agents; Area; base; Basic Science; Biochemical; Biological; Biological Assay; Biomedical Research; Bioreactors; Businesses; Cell Line; Cell physiology; Cell Survival; cell type; Cells; Chemicals; Chronic; Clinical; Collagen; Collagen Type I; comparative; Controlled Study; Culture Techniques; density; design; Development; Diagnostics Research; Dose; drug metabolism; Drug Monitoring; Drug toxicity; enzyme activity; Enzyme-Linked Immunosorbent Assay; Exhibits; fluorescence imaging; Future; Gel; gene induction; Goals; Growth; Hepatic; Hepatocyte; Hepatotoxicity; Housing; Human; human tissue; improved; In Vitro; indexing; Joint Ventures; Legal patent; Liver; liver function; Maintenance; Marketing; matrigel; Metabolic; Metabolism; Methods; Microscopy; Microspheres; Modeling; Molecular Analysis; Monitor; monolayer; novel; Outcome; Perfusion; Pharmacologic Substance; Pharmacotherapy; Phase; phase 1 study; Physiological; Polymers; pre-clinical research; Production; Progress Reports; Protocols documentation; Reaction Time; Relative (related person); Reporting; Reproducibility; Research; research and development; response; Reverse Transcriptase Polymerase Chain Reaction; Sales; scaffold; Screening procedure; Series; Services; Slice; Small Business Innovation Research Grant; Study models; System; Technology; Testing; Time; Tissue Sample; tool; Toxic effect; toxicant; Toxicity Tests; Toxicogenomics; tumor; two-dimensional;

Project Information

1R43ES017997-01

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DESCRIPTION | DETAILS | RESULTS | HISTORY | SUBPROJECTS | SIMILAR PROJECTS BETA | NEARBY PROJECTS BETA | LINKS BETA | NEWS AND MORE BETA

Project Number: 1R43ES017997-01
Title: A NOVEL ANALYTICAL ASSAY FOR PREDICTIVE EMBRYOTOXICITY USING HUMAN EMBRYONIC STEM

Contact PI / Project Leader: TOUMADJE, ARAZDORDI
Awardee Organization: GLOBALSTEM, INC.

Abstract Text:

DESCRIPTION (provided by applicant): New substances are routinely screened by pharmaceutical companies for their biological toxicity but failure to predict the toxicity of compounds to humans is a major financial cost for the pharmaceutical industry. In vivo multigeneration studies are expensive, laborious, and time consuming. Alternatively, in vitro cellular models can be completed in substantially less time and cost. The predictive potential of in vitro cellular model assays in drug screening is largely influenced by the cell types and the endpoint in the assays. Primary cells are limited in their proliferation capacity and the immortalized cell lines do not represent the in vivo counterparts. Here we propose to develop a novel, simple, low cost bead-based flow cytometric analytical assay with potentially high predictive in vitro embryotoxicity assessment using human embryonic stem cells. In order to assess the embryotoxic effects of chemicals, assays need to be developed to detect the early chemically-induced stress conditions. When placed in cytotoxic or genotoxic culture conditions, undifferentiated pluripotent ES cells respond by either activating apoptosis processes, or by inducing differentiation process. These survival processes protect hESC against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. Murine ES cells maintain their pluripotency and genomic stability longer than hESC under suboptimum conditions. This indicates that hES cells are a better choice than mES cells for a more sensitive in vitro toxicity system to environmental-induced stress conditions for spontaneous differentiation and apoptosis. The protein with a central role in controlling undifferentiated hESC to spontaneous apoptosis and differentiation under culture-induced stress condition is the p53 protein. P53 was shown to express strongly in both early and late apoptotic hESC. In Phase I of this proposal, we will develop an analytical bead-based flow cytometry immunoassay to capture and quantify the chemically-induced p53 accumulation in undifferentiated hESCs culture medium in response to known embryotoxic substances. The Relative Embryotixic Potency (REP) values of known embryotxic compounds with high-, medium-, and no-embryotoxicity effects will be calculated using p53 expression and compared with the published REP values for validation. In Phase II we will apply this technology to screen a broader spectrum of chemicals for embryotoxicty using undifferentiated hESCs and specific hESC-derived differentiated cells. Ultimately, the technique will be adapted to a high- throughput screening platform. PUBLIC HEALTH RELEVANCE: This proposal will develop a novel flow cytometric based assay to predict embryotoxic compounds using undifferentiated human embryonic stem cells.

Public Health Relevance Statement::

This proposal will develop a novel flow cytometric based assay to predict embryotoxic compounds using undifferentiated human embryonic stem cells.

NIH Spending Category:

Biotechnology; Regenerative Medicine; Stem Cell Research; Stem Cell Research - Embryonic - Human

Project Terms:

Address; Adverse effects; Animal Model; Antibodies; Apoptosis; Apoptotic; base; Biological; Biological Assay; Cardiac Myocytes; Cell Culture Techniques; Cell Line; Cell membrane; Cell model; cell preparation; cell type; Cells; Chemicals; Clinical; Complex; Conditioned Culture Media; Contracts; cost; Culture Media; cytotoxic; cytotoxicity; design; Development; DNA; DNA Damage; Drug Delivery Systems; drug discovery; Drug Industry; Embryo; Embryonic Development; embryonic stem cell; Extravasation; Failure (biologic function); Financial cost; Flow Cytometry; Genome Stability; genotoxicity; high throughput screening; Human; Human Development; human embryonic stem cell; immortalized cell; Immunoassay; In Vitro; in vitro Model; in vivo; Masks; Measurable; Metabolism; Mus; Mutation; Neurons; novel; Oncogenic; Organ; Peptide Hydrolases; Pharmacologic Substance; Phase; pluripotency; pre-clinical; Preclinical Drug Evaluation; Process; protein expression; Protein p53; Proteins; Protocols documentation; public health relevance; Publishing; Relative (related person); Reporter; response; Role; Signal Transduction; skills; Staging; stem; Stress; System; Techniques; Technology; Testing; Time; Toxic effect; TP53 gene; Undifferentiated; Validation;

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Project Information

1R41ES020659-01

Project **19** of **19**

Project Number: 1R41ES020659-01
Title: AN IN VITRO ASSAY FOR SCREENING CHEMICALS INTERACTING WITH THE BLOOD-BRAIN BARRIE

Contact PI / Project Leader: UZGARE, AARTI RAJNEESH
Awardee Organization: IN VITRO ADMET LABORATORIES, LLC

Abstract Text:

DESCRIPTION (provided by applicant): The objective of our grant application is to develop an in vitro model of the blood-brain barrier and assays to examine permeability by using isolated brain microvessels. Unlike capillaries in other organs, brain capillaries establish a barrier that is largely impermeable to polar and larger chemicals, which are due to tight junctions, the absence of both fenestrae and pinocytosis. Although, the permeability of most chemicals to cross the blood-brain barrier is directly associated to its' oil/water partition coefficient, many lipophillic chemicals fail to enter the brain because of the expression of the multidrug transporters. In contrast, polar nutrients that would be predicted to be less permeable display higher uptake because of the expression of nutrient transporters. Many chemicals that are potentially toxic to the brain fail to cross the blood-brain barrier. Additionally, many drug candidates for treating mental illnesses and neurological diseases fail because of the blood-brain barrier. Consequently, a model to study the blood-brain barrier is needed for the new paradigm in which testing chemicals for toxicity will be accomplished with in vitro and in silico methods. Additionally, a model is needed to aid chemists in their attempts to design neurotrophic drugs. Current cell culture models fail to display the tightness and the many transporters found in vivo. In contrast, isolated brain microvessels retain all of the transporters and display a tight barrier, and have been used in the past 40 years in research studies on blood-brain barrier permeability. The major obstacles in using isolated brain microvessels have been the laborious procedure for their preparation and their short life span. A commercial source of brain microvessels will overcome these obstacles. The overall objective of our study is to develop cryopreserved BM as a model of the blood-brain barrier and optimize reagents and assays to measure permeability and transport that will either be conducted by our company or sold to others. To accomplish the objective, in Specific Aim 1 conditions will be standardized for preparing, cryopreserving viable bovine brain microvessels and assure consistency in different batches of microvessels sold. The functionality of the brain microvessels will be assessed by conducting assays to measure amino acid and sugar transport, multidrug transporters, and viability. Assays achieving Z-factors closest to 1.0 will indicate the best preparation conditions. In Specific Aim 2, assays will be developed to examine chemical toxicity of the blood-brain barrier by measuring non specific transport. Sensitivity and specificity will be assessed by computing receiver operating curves. In the phase 2 grant application, assays will be developed to measure multi drug efflux pumps and the luminal and abluminal transporters. By establishing a commercial source of reagents for assessing the blood-brain barrier, pharmaceutical companies and chemical testing laboratories will have a readily available model to screen test chemicals for permeability and toxicity. When considering the importance of the blood-brain barrier in neurological functions, these products will potentially have wide distribution and commercial success. PUBLIC HEALTH RELEVANCE: The lack of an effective model for measuring the effects of drugs and chemicals on the blood-brain barrier (BBB) has been an impediment to evaluating chemicals for toxicity to the brain as well as to the successful development of drugs to treat diseases of the brain18,29. The proposed model of the blood-brain barrier, isolated brain microvessels (BM) from cows, and assay for blood-brain barrier permeability, has the potential for enormous impact in the field of neurotoxicity testing and drug development. The innovation here is in taking a successful research model, freshly isolated BM, and converting it into a cryopreserved, marketable product as well as translating its use into assays that address the previously unmet need for evaluating BBB permeability.

Public Health Relevance Statement::

The lack of an effective model for measuring the effects of drugs and chemicals on the blood-brain barrier (BBB) has been an impediment to evaluating chemicals for toxicity to the brain as well as to the successful development of drugs to treat diseases of the brain18,29. The proposed model of the blood-brain barrier, isolated brain microvessels (BM) from cows, and assay for blood-brain barrier permeability, has the potential for enormous impact in the field of neurotoxicity testing and drug development. The innovation here is in taking a successful research model, freshly isolated BM, and converting it into a cryopreserved, marketable product as well as translating its use into assays that address the previously unmet need for evaluating BBB permeability.

NIH Spending Category:

Brain Disorders; Neurosciences; Prevention

Project Terms:

Address; Amino Acids; Amino Sugars; Applications Grants; Biological Assay; Blood; Blood - brain barrier anatomy; Blood capillaries; Brain; Buffers; capillary; Cattle; Cell Culture Techniques; cell growth; cell type; Chemicals; Computer Simulation; Contracts; cost effective; Cryopreservation; Culture Media; design; Development; dextran; Dextrans; Disease; drug candidate; Drug Design; drug development; Drug Efflux; Drug Industry; Drug Transport; efflux pump; Endothelial Cells; Fluorescein; Fluorescent Probes; Goals; Growth; In Vitro; in vitro Assay; in vitro Model; in vivo; in vivo Model; innovation; Label; Laboratories; Longevity; Measures; Mediating; Membrane; Mental disorders; Metabolic; Methods; Modeling; multi drug transporter; nervous system disorder; Nervous System Physiology; neurotoxicity; Nutrient; Oils; Organ; Partition Coefficient; Peptide Hydrolases; Performance; Permeability; Pharmaceutical Preparations; Pharmacologic Substance; Phase; Pinocytosis; Poisons; Preparation; Procedures; Property; Publishing; Reagent; Relative (related person); Research; research study; Screening procedure; Sensitivity and Specificity; Services; Source; success; Surface; Testing; Tight Junctions; Time; Toxic effect; Toxicity Tests; Translating; uptake; Validation; Water;