FDA U.S. FOOD & DRUG ADMINISTRATION

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Division of Biochemical Toxicology

Frederick A. Beland, Director

Disclaimer: *The information in these materials is not a formal dissemination of information by FDA and does not represent agency position or policy.*

DIVISION OF BIOCHEMICAL TOXICOLOGY

STAFF

- **Government Positions - # full time employees**
  - ✓ Research Scientists, Staff Fellows, & Visiting Scientists: 27
  - ✓ Support Scientists: 11
  - ✓ Administrative: 2
- **ORISE Post Docs and Graduate Students: 10**
- **Total = 50 staff members**

DIVISION OF BIOCHEMICAL TOXICOLOGY

OUTREACH

- **Collaborations with:**
  - ✓ Divisions of Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Microbiology, Neurotoxicology, and Systems Biology, and the Office of Scientific Coordination
  - ✓ CBER, CDER, CDRH, CFSAN, CTP, and CVM
  - ✓ NIEHS/NTP, NCI, EPA, CDC, and various universities.
- **Global leadership/outreach:**
  - ✓ IARC, WHO, EFSA, OECD, and FSCJ

DIVISION OF BIOCHEMICAL TOXICOLOGY

MISSION

- **Mission:** To conduct fundamental and applied research designed to define the biological mechanisms of action underlying the toxicity of FDA-regulated products.

- **Goals:** To characterize the toxicities and carcinogenic risks associated with chemicals, specifically those of interest to FDA.

- **Strategies:** Bioassays, mechanistic studies, and computational modeling

MAJOR ACCOMPLISHMENTS DURING THE LAST 5 YEARS

- **Bioassays and mechanistic studies on:**
  - ✓ Bisphenol A (CFSAN and CDRH)
  - ✓ Melamine/cyanuric acid (CFSAN and CVM)
  - ✓ Furan (CFSAN)
  - ✓ *Aloe vera* (CFSAN)
  - ✓ Acrylamide/glycidamide (CFSAN)
  - ✓ Pyrrolizidine alkaloids (CFSAN)
  - ✓ Triclosan (CDER)

REPRESENTATIVE CURRENT PROJECT #1 ARSENIC

(in collaboration with CFSAN)

- Average arsenic content in drinking water in the U.S. is 2 ppb, with some areas having concentrations >1000 ppb
- EPA maximum contaminant level and WHO guideline value for inorganic arsenic in drinking water is 10 ppb
- Estimated mean daily exposure to inorganic arsenic in U.S.
  - ✓ Adults, 0.08 - 0.20 µg/kg bw/day
  - ✓ Children, ages 1 - 6, 0.11 - 0.32 µg/kg bw/day
  - ✓ Children, < 1 year old, 0.24 - 1.19 µg/kg bw/day

PREVIOUS BIOASSAYS WITH INORGANIC ARSENIC (iAs)

- Adult (post weaning) exposure (mice and rats)
- "Transplacental only" exposure
  - ✓ C3H and CD1 mice treated with 42.5 or 85 ppm iAs
- "Whole life" exposure
  - ✓ CD1 mice treated with 0.05, 0.5, 5, 6, 12, or 24 ppm iAs
- Tumor target tissues: lung, liver, adrenal cortex, uterus/ovary

ARSENIC: PHARMACOKINETICS SUMMARY

- iAs is readily absorbed from the GI tract (DMA^{V} also)
- Metabolism is dominated by DMA^{V}
- Non-linear kinetics at doses > 50 µg/kg bw
- DMA^{V} can be reduced to DMA^{III}, which can react with sulfhydryls in proteins
- Very poor lactational transfer of arsenic species

ARSENIC: OUTSTANDING ISSUES AND DATA GAPS

- Inorganic arsenic (iAs)
  - ✓ Lack of a dose-response for lung tumors
  - ✓ Lack of tumors at additional sites (liver, adrenal cortex, ovary)
  - ✓ Poor lactational transfer

- Dimethylarsinic acid (DMA^V)
  - ✓ No experiments conducted with perinatal exposure
  - ✓ Poor lactational transfer

PROPOSED ARSENIC BIOASSAY

- **Conduct a "whole life" exposure bioassay**
  - ✓ Dams and sires before and during breeding, and dams during pregnancy; drinking water
  - ✓ Pups - Postnatal days 1 - 21; gavage
  - ✓ Weaning - 2 years; drinking water
  - ✓ iAs and DMA^V

REPRESENTATIVE CURRENT PROJECT #2

PEGYLATED BIOPHARMACEUTICALS

(in collaboration with CDER/CBER)

PEGylation is the process of both covalently and non-covalently binding a PEG polymer to another molecule, normally a drug or therapeutic protein/peptide.

- ✓ Improved drug solubility
- ✓ Extended circulating half-life
- ✓ Increased drug stability
- ✓ Enhanced protection from proteolytic degradation
- ✓ Reduced dosage frequency, without diminished efficacy and with potentially reduced toxicity

PEGYLATED BIOPHARMACEUTICALS
CONCERNS AND DATA NEEDS

- Several PEGylated biopharmaceuticals have caused PEG accumulation and cellular vacuolization in various tissues, including the choroid plexus, in pre-clinical studies
- There is concern that PEG accumulation and the formation of these vacuoles may lead to adverse outcomes for PEGylated biopharmaceuticals used chronically and/or in pediatric populations
  - ✓ The tissue levels of PEG over time
  - ✓ Long-term effects of PEG on some tissues, especially the choroid plexus and kidney

PEGYLATED BIOPHARMACEUTICALS
EXPERIMENTAL DESIGN

- Assess the toxicities resulting from weekly repeated subcutaneous or intravenous injections of high-molecular-weight PEGs (20, 40, and 60 kDa) for 24 weeks to Sprague-Dawley rats
- Evaluate the toxicokinetic profile of high-molecular-weight PEGs given as a single subcutaneous or intravenous dose to Sprague-Dawley rats
- Evaluate the bioaccumulation of high-molecular-weight PEGs in organs/tissues of Sprague-Dawley rats upon repeat subcutaneous or intravenous injection of the test articles for 24 weeks

REPRESENTATIVE CURRENT PROJECT #3

NATTOKINASE/LUMBROKINASE

(in collaboration with CFSAN)

- Serine proteases
  - ✓ Nattokinase: produced by *Bacillus subtilis*
  - ✓ Lumbrokinase: powdered earthworms, mainly *Lumbricus rubellus* and *Eisenia fetida*

- Taken as a dietary supplement for their claimed support of cardiovascular and circulation health

NATTOKINASE/LUMBROKINASE
AIMS AND STUDY DESIGN

- To assess the effects of nattokinase and lumbrokinase on the risk of bleeding.
  - ✓ Individually
  - ✓ In combination with aspirin

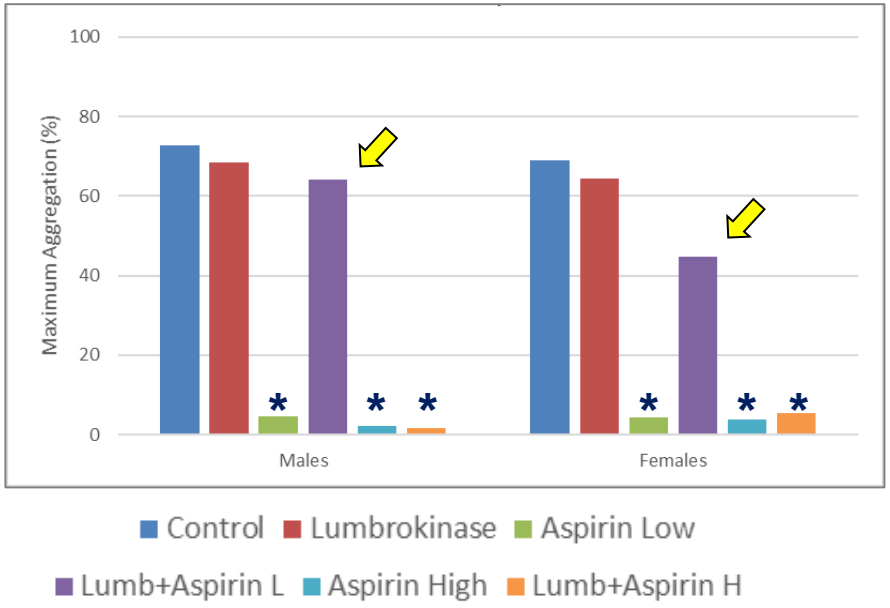
- Sprague-Dawley rats; 28-day exposure by gavage.
  - ✓ Nattokinase (1000 mg/kg bw/day)
  - ✓ Lumbrokinase (1000 mg/kg bw/day)
  - ✓ Aspirin (10 and 100 mg/kg bw/day)

NATTOKINASE/LUMBROKINASE ENDPOINTS

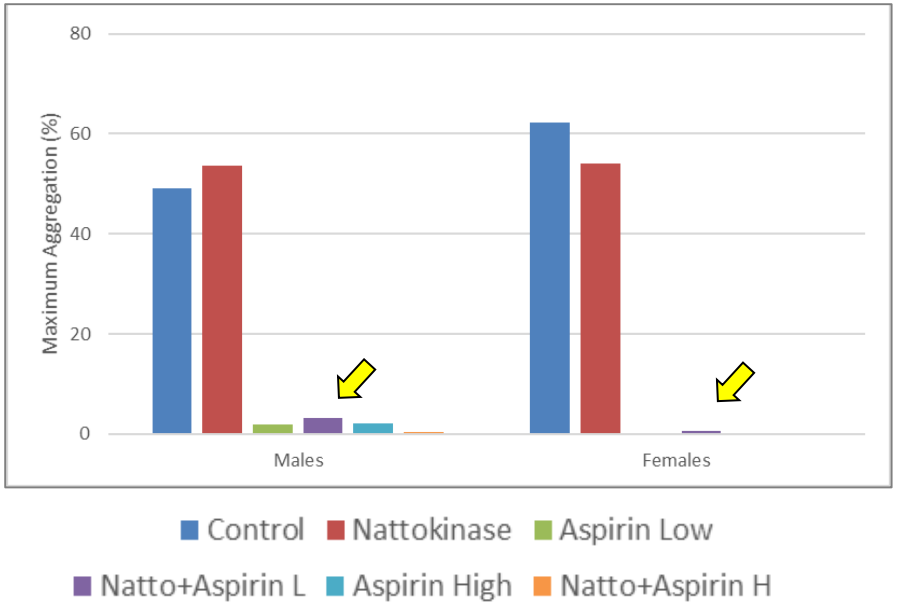
- Bleeding time
- Body weight, food and water consumption
- Blood parameters
  - ✓ Clinical chemistry
  - ✓ Hematology
  - ✓ Platelet aggregation
  - ✓ Whole blood hemostasis
  - ✓ Coagulation assays
  - ✓ Thrombin time
  - ✓ Fibrinolysis assays
- Histopathology
- Motor coordination and grip strength
- Serum salicylate levels
- pH of stomach and duodenum
- IgG leakage in brain

NATTOKINASE/LUMBROKINASE PLATELET AGGREGATION

Lumbrokinase



Nattokinase



Lumbrokinase decreased the **anti-platelet** aggregation effect of low aspirin in both males and females.

REPRESENTATIVE CURRENT PROJECT #4
OSELTAMIVIR PHARMACOKINETICS

(in collaboration with CDER)

- Compared to the general population, pregnant women have higher morbidity and mortality rates from influenza infections, with the severity increasing as the pregnancy progresses.

- Oseltamivir, a neuraminidase inhibitor, is an effective drug for the treatment and prophylaxis of influenza.

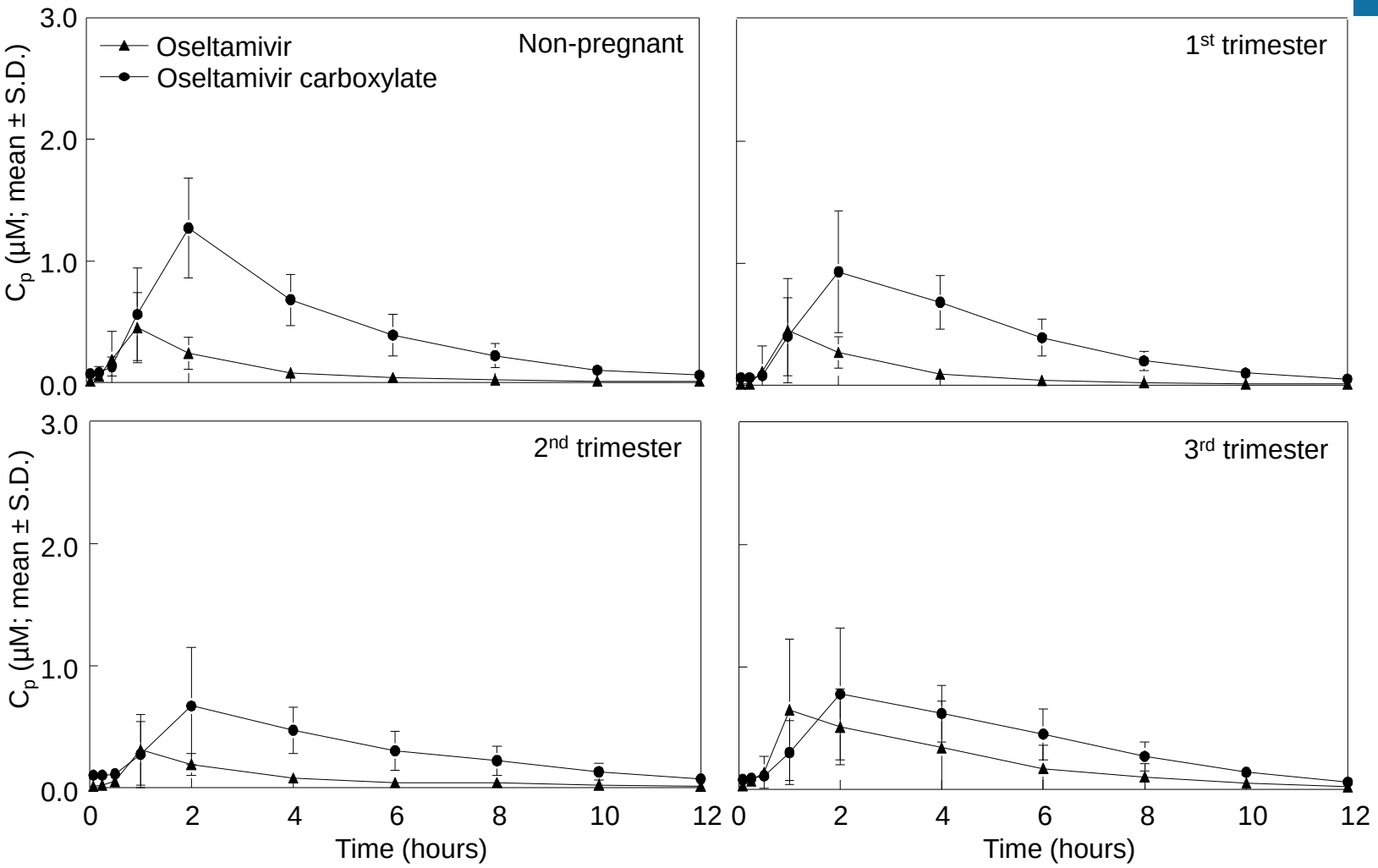
- When given to pregnant women, oseltamivir appears to reduce both the morbidity and mortality resulting from influenza; however, the appropriate dose to give to pregnant women is currently not known.

OSELTAMIVIR PHARMACOKINETICS OBJECTIVES

- To determine if rhesus monkeys are an appropriate model to construct a pharmacokinetic model that could be extrapolated to humans.

- To determine the pharmacokinetics of oseltamivir and its pharmacologically active metabolite oseltamivir carboxylate in non-pregnant rhesus monkeys and during the first, second, and third trimesters of pregnancy.

OSELTAMIVIR PHARMACOKINETICS



Pregnancy had only a modest effect upon the pharmacokinetic parameters of oseltamivir and oseltamivir carboxylate.

QUESTIONS, COMMENTS, AND/OR SUGGESTIONS