28. ELECTROCHEMICAL NANOBIOSENSOR ALARM DEVICES FOR THE DETERMINATION OF ENDOCRINE DISRUPTOR AGENTS

Emmanuel Iwuoha
Nocolette Hendricks and Priscilla Baker
SensorLab, Department of Chemistry
University of Western Cape, Bellville
Cape Town
South Africa

The role of cytochrome P450 (CYP) enzyme systems in the detoxification of bioactive and hydrophobic xenobiotics, such as drugs, environmental pollutants, food supplements, steroids and endocrine disruptors, cannot be over-emphasized. In this study we present the development and amperometric transduction of cytochromal biosensor alarm device for the determination of endocrine disruptors. As a class II microsomal b-type heme enzyme, CYP3A4 requires the obligatory presence of electron transfer donor redox protein, NAD(P)H, and cytochrome b5 for its physiological reactivity. Optimal reconstitution assays preferably involves vesicle forming phospholipids, detergents and specialized reducing agents. Biosensor offers the possibility of observing direct electron transfer reaction of cytochrome P450-3A4 (CYP3A4) without the requirement of the enzyme's physiological redox partners (1,2). In this study, a nanobiosensor alarm device for the determination of 2,4-dichlorophenol (an endocrine disruptor and hepatocarcinogen) was developed with genetically engineered CYP3A4 imprinted on carbon electrode chips that was modified with polypyrrole-gold nanoparticles. The sensor amperometric signals resulted from the two-electron monooxygenation reaction between the ferri-heme CYP3A4 enzyme and the endocrine disruptor compound. The biosensor was interrogated electrochemically for its ability to detect and report the presence of the endocrine disruptor compound in real time. Accordingly, the response time, sensitivity, storage stability, dynamic linear range and detection limits of the device were evaluated. The biosensor alarm device had a detection limit of 43 ng/L for 2,4-dichlorophenol which is lower than the European Union limit of 300 ng/L for pesticide compounds in ground water; as well as the USA Environmental Protection Agency's drinking water equivalent level (DWEL) of 2000 ng/L (3,4). Chromatographic studies despite their tedious sample preparation and time-consuming pre-concentration steps report detection limits (10-500 ng/L) that do not compare with those of the CYP3A4 nanobiosensor alarm device.

Dr. Emmanuel Iwuoha

SensorLab, Department of Chemistry
University of Western Cape, Bellville
Cape Town, South Africa

29. DOSE RESPONOSE TOXIC EFFECTS OF DIFFERENT OXIMES *IN VIVO*: PATHOHYSTOLOGICAL EVALUATION

Vesna Jaćević
Prof. dr., National Poison Control Centre
Military Medical Academy
11000 Belgrade
Republic of Serbia

Dr. Kamil Kuča, Centre of Advanced Studies and Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic
Ana Vukajlović, National Poison Control Centre, Military Medical Academy, Belgrade, Republic of Serbia
Dr. Ivica Milosavljević, Department for Forensic Pathology, Military Medical Academy, Belgrade, Republic of Serbia
Dr. Lidija Zolotarevski, Department for Pathology, Military Medical Academy, Belgrade, Republic of Serbia

The acute toxicity of oximes is crucial for the assessment of a dose applied as a treatment for organophosphorus intoxications. This is why we decided to investigate which morphological lesions could be produced in Wistar rats after treatment with increasing doses of HI-6, Obidoxime, K027, K048, and K075. In the first part of this study, tested oximes were preliminarily tested in order to obtain their LD_{50} values. Survival rates were monitored 24 hours after application of each oxime. In separate experiment animals were sacrificed 7 days after single *im* application of 0.1 LD_{50} and 0.5 LD_{50} of each oxime, and hearts, diaphragms and musculus popliteus were obtained for pathohistological analysis. Tisue damage score (TDS) was based on an estimation scale from 0 (no damage) to 5 (strong damage, massive necrotic fields). In rats treated with of 0.1 LD_{50} of HI-6 and K027 microscopic findings were similar to those evaluated in the control groups, only. More intensive alterations, but still mild and reversible degenerative and vascular changes, were established in tissue samples after treatment with 0.1 LD_{50} of Obidoxime, K048 and K075, but their values were also similar to the control group. Acute lesions were developed in tissue samples within 7 days following treatment with 0.5 LD_{50} of all oximes. The most severe tissue alterations were found in rats treated with 0.5 LD_{50} of K048 and K075 ($p < 0.001$ vs. control and HI-6). These observations of the earliest tissues events are helping to guide of applications of novel development oximes.

Key Words/ Phrases: Oximes, Heart, Diaphragm, Musculus popliteus.