P1016
Metallothionein induction, acetylcholine esterase activity and antioxidantive response as biomarkers of thallium toxicity in *Tubifex tubifex* (Oligochaeta, Tubificidae)

G.A. Kılıç*, V. Kılıç

Biology, Anadolu University, Eskişehir, Turkey

Purpose: Metallothioneins (MTs) are cysteine-rich proteins involved in heavy metal homeostasis/detoxification and radical scavenging. MTs have been widely evaluated as biomarkers of metal contamination for different animal species including the pollution resistant aquatic oligochaeta *Tubifex tubifex*. However no reports are available about the role of MT in thallium (TI) intoxicated Tubifex worms in correlation with other defences. The present study aims to investigate the effects of sublethal TI concentrations on MT induction, acetylcholine esterase (AChE) activity and antioxidantive response in *T. tubifex*. *Methods*: The study was consisted of a control and six experimental groups. Experimental groups were exposed to sublethal doses of TI for 7 and 15 days at 0.0025, 0.005, and 0.01 mg/L^{-1} concentrations. The amount of MT was determined spectrophotometrically according to the method of Viarengo et al. (1997). Activities of antioxidant enzymes catalase (CAT) and glutathione peroxidase were measured. Total glutathione (GSH) level and acetylcholine esterase (AChE) activity were determined. *Results*: MT levels were significantly increased in all of the experimental groups when compared to control at the end of 7 and 15 days. However, longer exposure to TI caused a decrease of the MT content. GST activity and total GSH concentrations were observed to be significantly decreased. CAT activity was found to be increased in experimental groups. AChE was inhibited. High induction of MT and GSH depletion due TI intoxication is an evidence that MT may act as a secondary defender during TI toxicity and may be a useful biomarker of metal toxicity.

doi:10.1016/j.toxlet.2011.05.250

P1017
IGFBP-5 as a biomarker of de-differentiation in hepatocytes

E.M. Large[1,*], C.J. Henderson[1], D.J. Flint[2], M.H. Grant[1]

[1] *Bioengineering Unit, University of Strathclyde, Glasgow, UK*, [2] *Sipbs, University of Strathclyde, Glasgow, UK*

Hepatocyte based in vitro systems are currently widely used to address a variety of pharmacological and toxicological issues. Such systems remain a vital part of the development of new drugs. Currently in vitro hepatocyte systems are seen as preferable over in vivo studies, on ethical and economical grounds. However, to date there is still no ideal primary hepatocyte culture system. Current systems are limited in use due to the rapid de-differentiation of the cells in culture. The cytochromes P450 (CYP) enzyme function is a major loss seen after 48–72 h in culture. CYP play a major role in the metabolism of a large range of xenobiotics. Insulin-like growth factor binding protein-5 (IGFBP-5) has been associated with de-differentiation processes, this work examined whether IGFBP-5 was a biomarker of de-differentiation in primary rat hepatocytes. To investigate Phase I CYP mediated biotransformations in cultured rat hepatocytes over a period of 9 days in culture, CYP mediated hydroxylation of testosterone was measured. GSTP1, often referred to as a marker of de-differentiation, was also measured. GSTP1 is not present in normal rat liver in vivo and in freshly isolated cells; however expression is induced within 24 h in culture. GSTP1 expression significantly increased in cultured cells over time. Also, hydroxylation of testosterone reduced over time and levels of metabolites were undetectable after 5 d in culture. IGFBP-5 levels increased with time to 2.95 ± 0.05 ng/ml at 9 d, suggesting that it could be a possible marker of de-differentiation in primary rat hepatocytes in culture.

In vitro techniques, specifically hepatocyte based in vitro systems are currently widely used to address a variety of pharmacological and toxicological issues. Hepatocyte based cultured systems remain a vital part of the developmental stages of potentially new drugs. Currently in vitro hepatocyte systems are seen as advantageous over whole animal studies, which are no longer ethically, economically or scientifically acceptable. However, there is still a lack of an ideal primary hepatocyte culture system despite years of research in to developing one. Current hepatocyte culture systems are limited in use due to the rapid de-differentiation of the cells in culture. The cytochromes P450 (CYP) function is a major loss seen after just 48–72 h in culture. CYP play a major role in the metabolism of a large range of xenobiotics. Also, freshly isolated hepatocytes on a collagen film exhibit a flattened morphology, depolarise and lose many surface characteristics of normal hepatocytes in vivo. To investigate Phase I CYP mediated biotransformations in cultured rat hepatocytes over a period of 9 days in culture, CYP mediated hydroxylation of testosterone were undertaken.

doi:10.1016/j.toxlet.2011.05.251

P1018
Reference values of cholinesterases activity in whole blood and plasma in southern Brazil

S.B. Nerilo, F.A. Martins, G.H.O. Rocha, P. Nishiyama, M. Machinski Jr.*

Health Basic Sciences, University of Maringa (UEM), Maringá, Brazil

Purpose: The activities of cholinesterases in the blood are used as exposure biomarkers to organophosphate and/or carbamates insecticides. Its determinations are recommended in pre-exposure due to population variability. The aim of this study was to establish reference values for cholinesterase in whole blood (ChEs) and butyrylcholinesterase (BuChE) in subjects not exposed occupationally to cholinesterase inhibitors. *Methods*: Cross sectional study of 179 blood donors from different genders of Maringá, Brazil, from October/2009 to October/2010. A structured questionnaire was applied. To check the healths of donors were performed some biochemical and hematological parameters. The enzymatic activities were determined by the method of Ellman et al. modified of Harlin & Ross. *Results of the study*: The values of the activities of ChEs were significantly different between genders. The average value of the male group was 6.3 mmol/mL/min (±0.7) for whole blood and 2.4 mmol/mL/min (±0.4) for BuChE and for females, the average value of whole blood was 5.6 mmol/mL/min (±0.7) and 2.0 mmol/mL/min (±0.4) for BuChE. These results can be used as reference levels for the cholinesterases activity in the population of southern Brazil.

doi:10.1016/j.toxlet.2011.05.252