Organophosphorus (OP) nerve agents are among the most deadly chemicals identified. The development and testing of medical countermeasures to OPs requires an appropriate animal model (Wales & Reeves, 2011). Mice are attractive candidates, however, wild type (WT) mice (unlike human and non-human primates) express high levels of serum carboxylesterase (sCaE) in the plasma. sCaE acts as a natural bioscavenger, rendering mice less sensitive to the effects of OP exposure as compared to humans (Duyssen & Lockridge, 2011). The goal of this project was to characterize a strain of genetically mutated mice to determine its suitability for use in OP research. Es-1 knockout (KO) mice were generated, and found to exhibit life spans, and reproductive / developmental health, similar to wild-type mice. It was predicted that these mice would be biologically and genetically identical to their wild-type counterparts (with the exception of the inability to produce sCaE and the presence of the Es-1 gene) and that they would display increased susceptibility to the OP nerve agents GB and GD. Together, these hypotheses suggest that Es-1 knockout mice will exhibit increased sensitivity to OP exposure as compared to wild-type mice making them a more predictive of human responses to OPs than other currently utilized animal models.

Materials and Methods

Approximately 50 µL of blood was collected from Es-1 homozygous intact (wild-type), heterozygous or homozygous Es-1 deficient mice (C57BL/6 background, sCaE knockouts produced by Oxgene Ltd.) via tail snip once mice reached an age of 3 weeks. Pups were also ear-tagged with unique numbers for identification purposes. Blood samples were collected into heparin-coated tubes and micro centrifuged to separate plasma. One (1) µL of plasma was pipetted into a 96-well plate, to which 99 µL of potassium phosphate (KPO2) buffer and 100 µL of para-nitrophenyl butyrate (pNPB) were added. All samples were prepared in triplicate. Samples were assayed for change in absorbance at OD405 every 6 seconds for 5 minutes using a SpectraMax plate reader equipped with SoftMax Pro version 5.4 (Molecular Devices). The sCaE activity, measured as the slope in mOD405 units per minute, of each sample was determined. Using known ranges of sCaE activity for wild-type, heterozygous, and homozygous knockout mice, the genotype of each mouse was inferred using the measured phenotype. Characterization was repeated throughout the course of the project as pups were being born and weaned. The breeding, weaning and care of the mouse population was carried out in accordance with USAMRICD protocol U978.

Introduction

Characterizing Es-1 knockout mice to aid in the development of an organophosphorus nerve agent bioscavenger

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Organophosphorus (OP) nerve agents are among the most deadly chemicals identified. The development and testing of medical countermeasures to OPs requires an appropriate animal model (Wales & Reeves, 2011). Mice are attractive candidates, however, wild type (WT) mice (unlike human and non-human primates) express high levels of serum carboxylesterase (sCaE) in the plasma. sCaE acts as a natural bioscavenger, rendering mice less sensitive to the effects of OP exposure as compared to humans (Duyssen & Lockridge, 2011). The goal of this project was to characterize a strain of genetically mutated mice to determine its suitability for use in OP research. Es-1 knockout (KO) mice were generated, and found to exhibit life spans, and reproductive / developmental health, similar to wild-type mice. It was predicted that these mice would be biologically and genetically identical to their wild-type counterparts (with the exception of the inability to produce sCaE and the presence of the Es-1 gene) and that they would display increased susceptibility to the OP nerve agents GB and GD. Together, these hypotheses suggest that Es-1 knockout mice will exhibit increased sensitivity to OP exposure as compared to wild-type mice making them a more predictive of human responses to OPs than other currently utilized animal models.

Materials and Methods (cont.)

Once the colony was brought to a sufficient size, three homozygous (knockout and wild-types) mice of each sex (a total of 12) were euthanized, their blood was isolated into heparin or EDTA tubes, and tissue from the liver, kidney, lung, heart and brain was collected. Blood samples were then used in basic metabolic panels (BMPs) to detect any possible differences that may have been present in the knockout mice due to the lack of the Es-1 gene. Mice aged 8 weeks, (males and females, knockouts and wild-types) were exposed to OP agents, GB, GD, or VX by subcutaneous injection using the Dixon- Massey method to determine an LD50.

Conclusions

Assays of plasma samples obtained from knockout samples showed little activity with the common substrate pNPB (Figure 1, Table 1) indicating that the absence of the Es-1 gene disables production of sCaE. Analysis of data collected from BMPs revealed minimal differences between wild types and knockouts, providing evidence for biological similarity between the two strains (Figure 2). These results suggest that sCaE knockouts are biologically identical (with the exception of the presence of sCaE) to wild-types and are suitable in vivo models for testing medical countermeasures to OPs. Affymetrix gene chip analysis will be conducted in the future to determine the relative gene expression levels in the two strains of mice. Although statistical significance has not yet been reached, preliminary data indicate that knockout exhibit roughly 4 and 10-fold higher sensitivity to GD and GB, respectively (Figure 3) compared to WT mice. Little difference in sensitivity was observed with VX, implying that sCaE affords little protection against this agent. Toxicity studies will continue until statistical significance is reached, culminating in the use of this animal model to determine the efficacy of candidate OP countermeasures. A sCaE KO /acetylcholinesterase knock-in mouse is in the early stages of production and is predicted to be an even more predictive model of human responses to OPs.

References
