

ALTERNATIVE METHODS TO STUDY OP EFFECTS: CELL LINES OR BRAIN CAPILLARY

I Svensson, B Karlsson, L Johansson, A Göransson-Nyberg and G Cassel.
Department of Medical Countermeasures, FOI NBC Defence, Defence Research Agency,
S-90182 Umeå, Sweden.

Abstract

Organophosphates are compounds that are commonly used as pesticides and furthermore conceivable as chemical warfare agents. These compounds are also used for medical treatment in diseases like glaucoma and Alzheimer's. All of the promising leads to date are based on observations made from in vivo studies conducted in animals and humans, and therefore have a strong mechanistic foundation. A current basic shift in the ethical, scientific and commercial engagement for alternatives to animal experimentation have engaged scientists from academia and industry to the development of alternative, non-animal test methods for assessing the toxic effect of a chemical.

However, it remains to be demonstrated whether a single in vitro test, or several in vitro tests in combination, which model the critical steps in intoxication can replace animal experiments for predicting toxic effects in humans.

A presentation on various alternatives is given with emphasis on cell culture methods and toxicology. Some known limits of in vitro systems are discussed in comparison with the value of results from animal experiments and in the context of safety evaluations in toxicology.

We used different cell lines, SH-SY5Y (known to have cholinergic and adrenergic receptors) and P19 cells (known to have both cholinergic and GABA-ergic receptors), primary endothelial cells and capillaries from rat brain.

SH-SY5Y cells are cells taken from a biopsies metastatic tumor of neuroblastoma and subcloned from SHSY and SK-N-SK lines. After exposure to soman, the cells were checked for necrosis and apoptosis. By using the MTT method, we could

detect reduced capacity after treatment with soman, which indicate a tendency to both necrosis and apoptosis. Lactate dehydrogenase (LDH) was slightly increased after exposure of cells to 2-5 mM soman.

Cells in differentiated P19 cultures (embryonic carcinoma cells) synthesize acetylcholine but not catecholamines, suggesting that at least some of the neurons are cholinergic. The cells also carry high-affinity uptake sites for GABA but not for serotonin. In differentiated P19 cells we measured the GABA A receptor activity.

In endothelial cells from rat brain capillary and in the capillaries themselves, we study the inflammatory response (IL-1 β protein) and necrosis (LDH levels) after treatment with soman. Neither in endothelial cells nor in brain capillaries we could detect any effects of soman.

This paper shows a lot of difficulties in using in vitro methods for studies concerning OP intoxication. It has to be further discussed if in vitro tests with OP compounds can replace animal experiments for predicting toxic effects in humans.

Regardless of this, the future looks promising with continued development of our understanding of the chemical and biological aspects of intoxication with OP and, most importantly, with the application of genomics to this field in the future.

Correspondence: I. Svensson

Department of Medical Countermeasures
FOI NBC Defence
Defence Research Agency
S-90182 Umeå
Sweden

Received: 11. 10. 2001