Available online at www.sciencedirect.com
ScienceDirect
Toxicology 233 (2007) 23–30
TOXICOLOGY
www.elsevier.com/locate/toxicol

Development of antidotes: Problems and strategies

Ladislaus Szinicz [a,*], Franz Worek [a], Horst Thiermann [a], Kai Kehe [a], Saskia Eckert [a,b], Peter Eyer [b]

[a] *Bundeswehr Institute of Pharmacology and Toxicology, Neuherbergstr. 11, 80937 Munich, Germany*
[b] *Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Goethestr. 33, 80336 Munich, Germany*

Received 29 June 2006; accepted 3 July 2006
Available online 14 July 2006

Abstract

Antidotes against chemical warfare agents are "orphan drugs" given that these poisonings are rare. Therefore, they are of limited interest to the pharmaceutical industry. For this reason, and recognizing the increasing threat of terrorist or asymmetrical use of chemical warfare agents, the responsibility for research into medical countermeasures against these weapons is of primary interest to armies. Accordingly, the research activities of the Bundeswehr Institute of Pharmacology and Toxicology are dedicated to improving diagnosis, prophylaxis and therapy of individuals who are exposed to a chemical agent. Here, antidote development and testing are a high priority in the research program, particularly with respect to organophosphorus (OP) nerve agents and sulphur mustard. The Institute has been coordinating research activities undertaken in house and in collaboration with external researchers. The research program aims to develop primarily *in vitro* models to minimize the sacrifice of animals, using strategies, which involve human material early in antidote testing. An animal model using isolated mouse diaphragm demonstrated the correlation between AChE activity and neuromuscular function. A similar relationship was found between erythrocyte AChE and neuromuscular function in patients with acute OP pesticide poisoning. *In vitro* rate constants of the various reactions that are involved in enzyme inhibition and reactivation using human material were used for prediction of what would happen *in vivo*. This prediction could be confirmed in a patient with acute OP pesticide poisoning. Finally, computer models are being established to estimate the therapeutic effect of an antidote in various human poisoning scenarios. This approach is necessary to compensate for the lack of human clinical pharmacodynamic studies that are usually required for drug regulatory approval, given the obvious ethical issues preventing human volunteer studies with these agents.

Keywords: Antidotes; Development strategies; Chemical agents; Organophosphates; Sulfur mustard

1. Introduction

Although the threat that chemical weapons will be used for military purposes is regarded to decrease in recent years, the risk of asymmetric and terrorist use is considered to have increased. Further, the target of such chemical attacks by terrorist groups includes not only the armed forces, but the civilian population as well (Gordon et al., 2005).

Acute poisoning with chemical weapons may induce severe toxicity and death, requiring immediate therapy. A life-saving component of poisoning therapy is the use of specific antidotes. But evidence supporting the efficacy of antidotes in acute poisoning with chemical weapons is lacking. Since these types of poisonings are generally

* Corresponding author. Tel.: +49 89 3168 2925; fax: +49 89 3168 2333.
E-mail address: LadislausSzinicz@Bundeswehr.org (L. Szinicz).

0300-483X/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2006.07.008

rare, there has been limited research interest by pharmaceutical companies to develop new antidotes, but also to confirm the efficacy of those that are currently available. Hence, such antidotes are considered "orphan" drugs, requiring assistance by government agencies.

For this reason, research divisions associated with armed forces have coordinated and sponsored research for improving medical countermeasures to chemical warfare agents. The research activities of the Bundeswehr Institute of Pharmacology and Toxicology aim to improve the diagnosis, prophylaxis and therapy of those who may be exposed to a chemical warfare agent. In particular, antidote development and testing has been a priority in our research program.

More specifically, the research activities of the Bundeswehr Institute of Pharmacology and Toxicology are dedicated to (a) protective and therapeutic countermeasures against organophosphorus (OP) nerve agents, and (b) exploration of the mechanisms of toxicity and treatment of tissue damage by sulphur mustard (SM). In the latter field skin and lung tissue are investigated and comparative studies with Lewisite and lung agents (phosgene) are performed.

The research program has developed techniques using primarily *in vitro* models, but also animal experiments, to demonstrate that specific antidotes would be effective in the circumstance of attack with a chemical warfare agent. This is necessary given the obvious ethical issues with conducting human volunteer studies using these agents. To achieve these outcomes, there has been collaboration with external organizations. This is useful for broadening the research projects possible, in addition to developing the skills and knowledge of researchers of the Institute. For obvious reasons, work with chemical warfare agents is limited to the Bundeswehr Institute and external projects are performed with model chemicals of similar action.

This paper is an overview of the research program coordinated by the Bundeswehr Institute of Pharmacology and Toxicology. We will describe the strategies used in our research program, in particular: (a) early involvement of human material in antidote development e.g. cells, tissue, organotypic *in vitro* models to limit the sacrifice of animals, (b) determination of species specific dose-effect relationships for nerve agent antidotes, (c) the application of using *in vitro* reaction rate constants of nerve agents and antidotes to predict the effect that would be observed in animals and humans, including the influence of dose, (d) comparison of laboratory data to the observations in patients who have been poisoned by similar chemicals, for example organophosphorus pesticides as a surrogate for nerve agents (validation), and finally (e) to establish computer models to predict the therapeutic effect of an antidote in various human poisoning scenarios.

2. Specific research topics

2.1. Sulphur mustard (SM)—use of human tissues in vitro

SM is a cytotoxic agent that was used as a weapon of mass destruction in World War I, but also recently in the Iraq–Iran war (1980–1988). Research in various countries since World War I have attempted to elucidate the mechanism of toxicity and development of a specific antidote. It is only in the last few years that basic research has provided some insights into the treatment of some special aspects of tissue damage by SM, e.g. blistering of the skin. This has been a focus of research at our Institute.

Cell cultures are an appropriate model to study the toxic effects of cytotoxic drugs like SM and for testing possible antidotes. The mechanism of toxicity of SM is dose-related (Papirmeister et al., 1985). At lower doses toxicity is mediated via DNA alkylation, while at higher doses toxicity is predominantly due to interactions with other cell constituents. This is particularly important when there is dermal exposure to liquid SM due to the effect of surface spreading. Specifically, the SM concentration is extremely high at the contact zone and then decreases as it spreads. Consequently, the mechanism of toxicity to cells in these zones will differ.

In addition, specific types of cells in the same zone of exposure may react differently, causing additional variability in the toxic effects of SM. For this reason, the effects of a range of concentrations of SM on mono-and co-cultures of various cell types have been studied. For example, keratinocyte-fibroblast submersed co-culture have been studied to assess the inflammatory response (cytokines), energy status, viability (apoptosis and necrosis) and production of the transcription factor NF-κB following SM exposure (Schneider et al., 2004). Here, different patterns of cytokine secretion were found in each mono-culture of the single cells, while the co-culture gave a different pattern (Kehe et al., 2000; Schneider et al., 2004).

In reality, exposure of the skin to SM is not homogenous, so models were established in our Institute to deal with these variables. An example is the development of an *in vitro* full thickness human skin equivalent, to study the consequences of liquid droplet exposure (Kehe et al., 1999). Primary human keratinocytes and fibroblasts were used to minimize artifacts caused by species differences. The model was validated by histological features