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ABSTRACT

The goal of this initiative was to demonstrate the feasibility of deploying enzymatic tools within the vascular space for the purposes of detecting and disarming chemical and biological agents. The research succeeded in utilizing the red blood cell membrane surface as a platform on which to deploy an enzymatic defense system. The initial emphasis was changed from detection to protection because the molecular tools suitable for protection were immediately available. The project demonstrated that the intravascular platform provided by the red cell membrane and the strategy of enzymatic dismantling of a chemical threat agent were successful to a striking degree. The success included protection against a > 20 fold lethal dose of paraoxone that was rendered totally innocuous with this strategy in less than 1 minute. Moreover, covalent attachment to the red cell surface provided such levels of protection from more than 3 days, in contrast to the rapid disappearance (within 3 hours) of protectant molecular tools that were not so immobilized. The detection aspect of the proposed project was placed second in priority to the protection aspect, but was in principle demonstrated by the protection experiments. We still needed the additional step of generating a signal for the cleaved chemical agent which could be amplified by the principles utilized for enzyme coupled immunoassays to generate detectable gustatory or olfactory signals in urine or plasma. The strategy proved totally unobtrusive and strikingly effective in dismantling threat agents. More time was required for further development of the detection strategies.

FINAL TECHNICAL REPORT

DOD/SPAWAR N65236-98-1-5410

Grant No.: N65236-98-1-5410

Principal Investigator: Dr. Mark W. Bitensky.

Institution: Department of Biomedical Engineering, Boston University.

Grant Title: "Use of an Erythrocyte Platform to Deploy Technology for Infection Detection".

Award Period: 12 March 1998 - 30 September 2001.

Objective: Our goal was to develop an approach that would enable early detection of biological or chemical agents.

1. Our focus was entirely upon the individual at risk, and no attention was given to scrutiny of the environment. The idea was to create technologies which would rapidly and unobtrusively signal the presence of threat agents within the targeted population.
2. A closely related objective was to fully preserve the physical and mental unencumbered functionality of the at risk war fighter. Emphasis was given to all head, trunk and extremity functions indispensable in combat.
3. The protected individual would not wear or carry equipment or items that could encumber full and free movement.

Approach:

1. The elements for detection and protection (DPEs) were to be designed in a manner that facilitated ready deployment *within the vascular space.*
2. Within the universe of possible choices two competing concepts emerged. The threat agents could either be sequestered, as for example with a high affinity antibody, or cleaved as with a hydrolytic enzyme.
3. With large enzyme turnover numbers (*i.e.*, high catalytic constants), the quantitative advantage inherent in the enzyme/antibody comparison is simply overwhelming. While each molecule of threat agent would require its own combining molecule, with optimized processing features, hundreds or even thousands of threat agents could be inactivated by a single enzyme molecule.
4. The decision was taken to utilize the power of enzymatic catalysis to disable threat agents. Our goal was best accomplished by the deployment of enzymatic DPEs within the vascular space. It then became necessary to deploy our DPEs so that they would function within the vascular space.
5. Two concepts were considered to retain our DPEs within the vascular space. In the first, we proposed the construction of a star dendrimer, roughly spherical in shape, with a diameter below 2.5 μm to which multiple DPEs could be covalently attached. In this approach, the decorated dendrimers carrying enzymes developed against selected threat agents could be prepared and stockpiled for use in response to anticipated threat attacks.
6. A second approach involved attaching DPEs to the red cell surface, *i.e.* exploiting the vast red cell membrane surface as a deployment platform.

7. The required linkage strategies could then be developed to permit deployment by intravenous injection. This approach might be well suited for Special Forces who require high levels of protection for limited missions. There was, nevertheless, a considerable degree of flexibility with regard to the identification of selected populations at risk, and as well, selected protection modalities.
8. A second aspect of this approach would be the addition of yet another element in the detection/protection system. In this format, the new element was a binding function as provided by an antibody whose purpose would be to concentrate threat agents in immediate proximity to the hydrolytic enzyme. This feature was intended to increase the efficiency of the enzymatic function by increasing substrate concentration in immediate proximity to the catalytic function of the enzyme. This is a delicately balanced strategy. Unless the on/off rates for the concentration device and proximity to the enzyme function are carefully tuned, introduction of the binding/concentrating function might compete with the enzymatic function rather than supplement it. The design and implementation of this concept therefore requires great care.
9. A condition of the proposed deployments was to achieve a degree of immunological stealthing which would permit introduction of the protection/detection agents using selection criteria and/or approaches that minimize or prevent provocation of the host immune system. It is essential to acknowledge that without immunological stealthing the proposed strategies could be used (and would work) for short periods of time, but would then be precluded from continued deployment by the danger of potentially hazardous immune responses.

Accomplishments: Our most compelling results were accomplished with the lethal chemical agent paraoxone. Although extremely toxic this chemical agent has low volatility and can be handled safely in an ordinary molecular biochemistry/biophysics lab without high level safeguards that would be essential for more volatile agents.

Impressive levels of protection: The intravenous injection of the enzyme paraoxonase prior to the intraperitoneal injection of paraoxone protected the laboratory rat against > 20 lethal doses of paraoxone.

Furthermore, when paraoxonase was attached to the rat red cell with simple biotin/avidin linkage strategy, extremely high (*e.g.*, protection against 20 lethal doses) protection levels were readily achieved and persisted for > 3 days. Without attachment to the red cell surface, paraoxonase was cleared from the rat circulation within 3 hours, and paraoxone challenge after that period revealed that enzymatic protection was no longer present.

Conclusions: The foregoing experiments demonstrated that an intravascular, red cell surface-mounted enzymatic strategy provided striking levels of protection against an otherwise lethal chemical agent. It is likely that more prolonged periods of protection and higher levels of protection could be achieved with modest adjustments in the efficacy of the protective enzymes and their linkage chemistries and advances in enzyme stealthing in order to reduce or entirely abolish the considerable problems of autoimmunity that are likely to develop in association with deployment of intravascular enzymes.

Significance: The intravascular deployment of enzymes for the purposes of detection and protection was found to afford very striking degrees of protection against a simple but lethal chemical agent. The findings clearly demonstrate the compelling power of this approach when deployed either in a detection or protection mode. Considering the totally non-obtrusive, non-encumbering nature of this approach, as well as the very compelling levels of protection achieved, the approach deserves further study and development. Were it possible to effectively address the problems associated with autoimmune rejection of the protective proteins, it would offer a powerful, wide range of applications fro the protection of subjects at risk.

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"Modified RBC that has surface molecules that neutralize chemical agents."

Award Information: Principal Investigator remains Research Professor and Director of the Visual and Circulatory Biophysics Laboratory.