Inhibition of Monocyte Esterase Activity by Organophosphate Insecticides

By Martin J. Lee and Henry C. Waters III

Organophosphate insecticides, such as Vapona, Naled, and Rabon, are highly potent inhibitors of an enzyme found in human monocytes. The enzyme, a specific monocyte esterase, could be inhibited by Vapona in blood samples via airborne contamination at levels easily achieved from commercial slow-release insecticide strips. Fifty percent inhibition (IC₅₀)—as measured on the Hemalog D (Technicon Corp.)—occurred at solution concentrations of 0.22, 1.5, and 2.6 × 10⁻⁶ g/liter for Vapona, Rabon, and Naled, respectively. Parathion (a thiophosphate) and Baygon (a carbamate) were less potent, with IC₅₀ values of 3.7 × 10⁻⁵ and 1.4 × 10⁻⁴ g/liter, respectively. Dursban (another thiophosphate) and Carbaryl (a carbamate) showed only marginal inhibition. Eserine, malathion, nicotine and pyrethrum had no inhibitory effect up to 0.5 g/liter. The occurrence of this effect in vivo has not yet been shown, nor is it clear what the implications of such an effect would be. The inhibition of this enzyme by airborne contaminants, however, may interfere with the proper functioning of the Hemalog D.

A large body of evidence has accumulated suggesting that some common household insecticides have very little effect on humans at the levels routinely employed for the control of insect pests. Among these, cholinergic agents usually exhibit effects on human red cell and other esterases in vivo only after administration of relatively high doses. We report here on the complete inhibition in vitro of an enzyme in human monocytes by organophosphate-related insecticides in the air at levels attainable from commercial slow-release insecticide strips.

The several classes of human leukocytes contain enzymes which may be utilized for the specific staining of these cell types. Esterase activities have been observed in monocytes, neutrophils, and lymphocytes. The neutrophil enzyme is not fluoride sensitive and it has maximum activity against α-substituted aliphatic esters and very low activity against unsubstituted aliphatic esters. The monocyte enzyme is fluoride sensitive and has maximum activity toward unsubstituted aliphatic acids. Substrate specificity is determined primarily by the acid portion of the ester. Increasing chain length results in increased monocyte esterase activity but decreased substrate solubility. Butyrates appear optimal for balancing these parameters to achieve maximal cytochemical staining rates. Ansley and Ornstein have developed a convenient staining reaction for this enzyme employing α-naphthyl butyrate as substrate and hexazonium pararosaniline as the chromogenic coupling agent.

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MATERIALS AND METHODS

Monocyte Esterase Chemistry

Monocytes in human whole blood samples were stained for their specific esterase by a modification of the Ansley and Ornstein procedure: A 0.9% saline-0.5% EDTA solution (0.23 ml) was mixed with a sample of human whole blood (0.41 ml). An aliquot (0.26 ml) of the diluted blood was then mixed with a pH 6.3 acetate-buffered, 5% formalin-0.92% saponin reagent (0.16 ml). The formalin was added to fix and preserve the white cells, while the saponin served to lyse red cells. This fixative-lysis reagent also contained 0.058% eserine to inhibit serum pseudocholinesterase.

Separately, a substrate/dye-coupling reagent was prepared as follows: 1.0% pararosaniline hydrochloride in 0.84 N H₂SO₄ (0.42 ml) was mixed with 1.0% sodium nitrite (0.42 ml). To this mixture were added 0.33% α-naphthyl butyrate in diethylene glycol (0.16 ml), and 7.0% sodium cacodylate (1.40 ml). This procedure generated a diazonium salt coupler which reacted with α-naphthol as it was liberated in the esterase reaction. The coupler-substrate mixture was combined with the suspension of fixed white cells described above and heated to 40°C for 4 min. Then 1.0% acetic acid (0.8 ml) was added to the mixture to stop the reaction. This procedure yielded a suspension of unstained red cell ghosts and fixed white cells, with reddish-brown monocytes.

Determination of Esterase Activity

The above procedure has been automated via continuous-flow technology and, as such, forms the monocyte channel of the Hemalog D (Technicon Corp.) system. This system combines the esterase chemistry in one channel with separate channels for peroxidase and basophil chemistry in a technology for performing automated differential white blood cell counts.11,12 Use of the Hemalog D system has allowed us to obtain quantitative and precise data on the degree of monocyte staining of large numbers of monocytes in many blood samples.

In order to test the effect of potential airborne inhibitors, open tubes of human whole blood were exposed to the air for 2-3 hr in rooms containing Shell No-Pest Strips (containing Vapona, which is 2,2'-dichlorovinyl dimethyl phosphate, DDVP; molecular weight 220.9).

RESULTS

Tests in four different rooms employing three different Shell No-Pest Strips and more than 300 whole blood samples established that the presence of these slow-release insecticide strips caused a loss of monocyte esterase activity. To test this hypothesis, these experiments were repeated in a desiccator jar equilibrated with a Shell No-Pest Strip. Five minutes of such exposure was more than sufficient to inhibit monocyte esterase activity completely. These results, and numerous subsequent confirmatory experiments, led us to conclude that DDVP was diffusing into the air (within the desiccator) and then into the blood samples, and that the concentration achieved was a very potent inhibitor of human monocyte esterase.

We therefore sought to quantitate the percentage inhibition as a function of DDVP concentration. A series of saline (0.9%)-EDTA (0.5%) diluents were made up, each containing a different concentration of DDVP. Replicate human whole blood samples from individual donors were manually diluted with this diluent, incubated for 15 min at room temperature, and then run on the Hemalog D.

Representative X-Y scattergrams for serial dilutions of Vapona are shown in Fig. 1. A concentration of $5 \times 10^{-9}$ g/liter produced only slight inhibition; $5 \times 10^{-8}$ g/liter produced significant inhibition; and $5 \times 10^{-7}$ g/liter greatly inhibited esterase activity. Values for percentage inhibition, defined as the ratio of the inhibited modal value to that of the control sample, correlated with the
calculated ratio of the fraction of cells above the esterase absorption voltage threshold to that of the control sample.

A plot of DDVP concentration in the diluted blood versus percentage inhibition is shown in Fig. 2. Fifty percent inhibition ($I_{50}$) values estimated from these curves are given in Table 1. The $I_{50}$ concentration for DDVP was $2.2 \times 10^{-7}$ g/liter of diluted blood. This level is comparable to household air concentrations routinely obtained in normal usage of Vapona commercial slow-release insecticide strips.$^{3,7}$

Rabon [2-chloro-1-(2',4',5'-trichlorophenyl) vinyl dimethyl phosphate] and Naled (1,2-dibromo-2,2 dichloroethyl dimethyl phosphate), organophosphate
insecticides related in structure to DDVP, were somewhat less active, with an $I_{50}$ of monocyte esterase at 1.5 and $2.6 \times 10^{-6}$ g/liter of diluted blood, respectively. These two substances and Vapona are the active agents in animal flea collars.

Parathion (diethyl p-nitrophenyl phosphorothionate), a broad-spectrum thiophosphate agricultural insecticide, was an inhibitor of monocyte esterase with an $I_{50}$ value of $3.7 \times 10^{-5}$ g/liter diluted blood. Dursban (diethyl 3,5,6-trichloro-2-pyridyl phosphorothionate), another thiophosphate insecticide, was almost inactive, its $I_{50}$ value being $1.8 \times 10^{-2}$ g/liter diluted blood. Baygon (orthoisoisoproxyphenyl methylcarbamate) was a weak monocyte esterase inhibitor, with an $I_{50}$ of $1.4 \times 10^{-4}$ g/liter. Carbaryl (1-naphthyl N-methyl carbamate) was almost ineffective, even at a concentration of 0.5 g/liter. Eserine (an alkaloid carbamate), malathion [S-1,2-bis(ethoxycarbonyl)ethyl-O,O-dimethylphosphorodithionate], nicotine, piperonyl butoxide, and Pyrethrum (Official 1972 Standard, Pyrethrum Marketing Board, Kenya, East Africa) showed no inhibitory effect against monocyte esterase at concentrations of 0.05–1.5 g/liter of diluted blood.

**DISCUSSION**

It is clear that a group of inhibitors exists for human monocyte esterase. All compounds found to date are also inhibitors of acetylcholinesterase. The most potent of the group of substances are organophosphate insecticides—the most

<table>
<thead>
<tr>
<th>Substance</th>
<th>$I_{50}$ (g/liter)*</th>
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<tbody>
<tr>
<td>Vapona</td>
<td>$2.2 \times 10^{-7}$</td>
</tr>
<tr>
<td>Rabon</td>
<td>$1.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Naled</td>
<td>$3.6 \times 10^{-6}$</td>
</tr>
<tr>
<td>Parathion</td>
<td>$3.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>Baygon</td>
<td>$1.4 \times 10^{-4}$</td>
</tr>
<tr>
<td>Dursban</td>
<td>$1.8 \times 10^{-2}$</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Est. $&gt; 5 \times 10^{-2}$</td>
</tr>
<tr>
<td>Malathion, eserine, nicotine, pyrethrum, and piperonyl butoxide</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

*Expressed as concentration per unit volume of diluted blood prior to sampling on the Hemalog D.
powerful single substance being DDVP. Thiophosphates and methyl carbamate insecticides are less potent inhibitors. Within each chemical class, considerable variation exists as to the potency of individual substances. Eserine (an alkaloid carbamate and a powerful cholinesterase inhibitor) and pyrethrins (not inhibitors of cholinesterases) do not inhibit human monocyte esterase.

These experiments have shown dramatically the effect that Vapona (and therefore Shell No-Pest Strips) can have on monocyte esterase activity and, in fact, on Hemalog D performance. It is clear that a Hemalog D cannot be operated in rooms containing these slow-release strips. Furthermore, the storage of reagents and vacutainers, and the collection of blood samples must be done in Vapona-free atmospheres. The interference caused by Vapona vapors is especially troublesome in that these fumes are adsorbed by plastic tubing in the instrument, requiring retubing of the chemical manifolds to restore complete function. The question of long-term inhibition of monocyte esterase in vivo remains unanswered at this time. Further experiments are required to define and characterize the effects of Vapona on human monocytes, as well as to understand the biologic and physiologic significance of these observations.

ACKNOWLEDGMENT

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REFERENCES

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