Comparative Determination of Xenobiotics Adsorption

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Abstract

Xenobiotic solutions of different concentrations were analyzed by TLC method before and after passing trough the column with adsorbent M and compared with adsorption on the active charcoal. The efficiency of adsorption on adsorbent M was higher, compared to active charcoal. The best adsorption, in the value 90 - 100%, have shown certain organochlorine and organophosphorus pesticides, that were dissolved in non-aqueous solvents. Efficiency of adsorbent M was also proven *in vivo,* when solutions of tested xenobiotics before adsorption have caused death of experimental animals, and after the adsorption on adsorbent M, all treated animals have survived and had just mild symptoms of poisoning.

KEY WORDS: pesticides, TLC, toxicity, adsorption, adsorbent M

Introduction

Xenobiotics represent biologically active substances that are harmful for humans and by their chemical structure and mechanism of action belong to wide range of different substances. Pesticides constitute a large group of xenobiotics and represent organic, mainly synthetic compounds targeted for elimination of different pests in human environment. A basic action of pesticides is selective on targeted animals, but it is not the only one, so in the extreme case can lead to contamination of environment and can cause different kinds of poisoning in humans. From the chemical aspect two principal and most frequently used groups are oranophosphorus and organochlorine pesticides (1, 2). Group of organophosphorus pesticides represents large number of synthetic compounds, including malathion and dichlorvos, that can be recognised as derivates of phosphoric acid, and throughout the world are used as insecticides. They act neurotoxically by binding and by phosphorylation of acetylcholinesterase in central, but as well in peripheral nervous system. Inhibition of the enzyme results in an action on muscarinic and nicotinic receptors, so their toxic actions in humans, and in mammals in general, cause nausea, vomiting, general prostration, visual impairment and breathing disorders, lacrimations, increased saliva production, bradicardia, hypotension or hypertension, convulsions, dizziness, confusion, comma and depression of breathing and heart action (3, 4). Repeated exposure to organophosphorus compounds can have cumulative effect on organism, although they are mainly metabolised and excreted out of the body. Treatment of poisoning with organophosphorus pesticides include reactivators of cholinesterase. Study on relative potential of cumulative hatard and dose-response relation of organophosphorus pesticides, and cumulative risk of their usage, have determined unique basis for comparison of toxic potential of individual organophosphates with the use of comparative measurements methods, choice of gender and species of laboratory animals for all exposure routes of interest for humans. Presented analysis is targeted on plasma, red blood cell and brain cholinesterase inhibition of laboratory animals with specific data on enzyme activity in chronic and subchronic toxicity with oral, dermal and inhalation routes of use (5). Organochlorine pesticides are large group of synthetic compounds that are used as insecticides. Their effect on animals can be rather toxic because of the long presence in the body, especially DDT that is extremely toxic for organisms in the food chain, and since 1970s is prohibited in agriculture in USA. Toxic actions of organochlorine insecticides are based on stimulation of central nervous system. These agents interfere with inactivation of the sodium channel in excitable membranes and cause rapid repetitive firing in most neurons (2). Symptoms of the acute poisoning with organochlorine insecticides in mammals are nausea, vomiting, parestesia, tremor, convulsions, coma and breathing disorders, with pronounced toxicity on liver, kidneys and heart. Treatment of poisoning with organochlorine pesticides is mainly symptomatic (1, 3).

Aim of the Study

The aim of this study was to investigate adsorption of pesticides from solutes on adsorbent M and determination of the adsorption *in vitro* by chemical method of chromatography on thin layer (TLC), parallel with biological method of toxicity follow-up *in vivo* on laboratory animals, and comparison of obtained results with the adsorption on the layer of active charcoal.

Materials and Methods

CHEMICAL METHOD OF THIN LAYER CHROMATOGRAPHY - TLC

Solutes of tested pesticides: malation, dichlorvos, linden, DDD and DDE as metabolites of DDT (p.a. purity), were prepared in different concentrations of non-aqueous solvent (isopropyl alcohol, benzene, cyclohexane, ethanol, acetone and chloroform). Semiquantitative chemical analysis of pesticides from solutes was performed with TLC method, with the use of Silica gel G-60 alufoliae F_{254} (Merck, Germany) in chromatographic system of acetonitrile (HPLC purity). Identification of obvious and clearly marked violet stains on florescent background was conducted with UV light (254 nm) whose intensity corresponded well with concentrations within standard curve for each individual pesticide. Testing of pesticides adsorption was performed by passing solutes of individual pesticide through grained adsorption layer (25 cm^3) of adsorbent M (purified mixture of solid, saturated carbohydrates, natural origin, white or gray transparent mass, crystal structure, m.p. 47^o - 65^oC, without odor, not soluble in water and ethanol, soluble in ether and volatile oils) and, parallel through the layer of active charcoal – *carbo medicinalis.*

BIOLOGICAL METHOD IN VIVO

Solutes of tested pesticides of different concentration prepared in the range of their multiple LD_{50} value, were administered subcutaneously, intraperitonealy or orally to laboratory animals (4). Determined dosage for each tested pesticide was administrated in equal volume to adult mice (Swiss-albino strain, both sexes, 25-30 g. of weight, groups of 6-10 animals), before and after the passing through adsorption layer 25 cm^3 of adsorbent M in one group, and through the layer of active charcoal in the second group of animals. Control groups of mice were given pure solvent. Acute toxicity and symptoms of poisoning during 24-48 hours of exposure was followed.

Results

MALATHION ANALYSIS

- TLC-method - Primary solute of malathion, prepared in concentration of 0,5%, 1%, 2%, 4%, and 10% in isopropyl alcohol and 57% in benzene was applied and passed through a layer of adsorbent M and parallel through active charcoal. Obtained eluate is a secondary solute of malathion and was used for analysis. Samples of primary and secondary solute of malathion (20 μl), underwent chromatography in acetonitrile and were identified under UV light. Intensity of violet areas of malathion corresponded to concentrations within standard curve for malathion with sensitivity of 5 μg. Obtained results of analysis are suggesting that adsorbent M efficiently removes malathion from primary solutes with efficiency of 80-100%, and active charcoal with efficiency of 50-70%.

- Biological method *in vivo* - Primary solute of malathion was applied orally to the group of 6 mice in the dosage of triple LD_{50} (LD50 orally, mice/rat 1,37/kg) and it caused death of all treated mice within couple of hours with severe presentation of poisoning – disturbed breathing, salivation, tremor, convulsion, cyanosis, coma and death. Percentage of mortality within the group was 100%. To the second group of mice in the same volume was administrated secondary solute of malation passed through the adsorbent M and did not lead to lethality. All animals have survived with the mild symptoms of poisoning (general prostration, difficulty in breathing). In the control group there were no dead animals.

ANALYSIS OF DICHLORVOS,
DDD, DDE AND LINDANE

- TLC-method - Primary solute of dichlorvos was prepared in the concentration 1,8% and 0,9% in isopropyl alcohol; DDD and DDE 1% and 3% in isopropyl alcohol; lindane 1% and 6% in ethanom abs. or chloroform. Results of the analysis of tested pesticides according to described procedure of TLC method are demonstrating that concentrations of primary solutes of pesticides are decreased for 80-100% after the adsorption on layer M, except for lindane dissolved in ethanol abs., whose adsorption was between 30-50%.

- Primary solute of each pesticide dichlorvos, DDD, DDE, lindane was administered to a group of mice in the multiple LD50 doses. Percentage of mortality in each group of animals was 90-100%. Secondary solute of each individual pesticide, after the passing through adsorbent M, was applied to the groups of mice in a same manner. Animals from these groups had mildly present symptoms of poisoning, they all survived and completely recovered, except for secondary solute of lindane whith mortality rate of 30%. Control groups did not have dead animals, except ethanol abs. with mortality rate of 25%. Quantity of adsorbed dichlorvos on adsorbent M was 80-98%, and it is comparable with active charcoal, for DDD and DDE were 90 - 100% and is higher in comparison to active charcoal.

- Preliminary data of new investigations are suggesting high efficiency of adsorbent M for aqueous and non-aqueous solution of some carbamate derivatives, iodine and natural colors from plants, which will be the goal of investigations in future.

Conclusion

Based on the results of this study it can be concluded that

- adsorbent M efficiently removes tested organophosphorus and organochlorine pesticides from non-aqueous solutions.
- adsorption through adsorbent M was higher, compared to active charcoal.

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