Organophosphorus Pattern of Delayed Neuropathy
I. Structure/Activity Relationships

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INTRODUCTION

Toxic distal axonapathy (dying-back degeneration) is one of the most hazardous side effects of exposure to some organophosphorus (OP esters) insecticides. This effect is characterized pathologically by distal axonal degeneration, secondary irreversible demyelination of both the central and peripheral nervous axons and by a time-delay period of 8-14 days before the onset of clinical pathologic signs and symptomologies after exposure to the neurotoxic agent[1,2,3]. Some organophosphorus compounds were found to be neurotoxic agents in many animal species including man, hen, cat and sheep[1,4,5,6]. Tri-ortho-cresyl phosphate (TOCP) is one of the well known neurotoxic organophosphate compounds[7,8]. More than ten outbreaks of TOCP poisoning have been reported. Ginger paralysis, Jacke paralysis, Jamaica ginger polyneuritis or TOCP poisoning were some of the names given to a condition which afflicted a very large number of people throughout Southern states of the U.S.A. during the winter and early spring months of 1930. More than 10,000 people were stricken with a flacid paralysis of the lower limbs about 10 days after drinking a contaminated extract of Jamaica ginger . This great outbreak of paralysis first drew attention to the importance of OP compounds with respect to their toxic effects on the nervous system. Smith, et al.[4] identified the causative agent as TOCP. They also demonstrated the ability of TOCP to induce the same delayed neuropathic effect in laboratory animals. Smith and Lillie[7] gave the first histopathological description of the nervous system of TOCP-poisoned animals indlucing man, monkeys, cats, dogs and chickens. They indicated that the multiple neurosis of the paralysis induced by TOCP in these animals is essentially a degeneration of the myelin sheaths of the peripheral nerves, with a variable amount of relatively moderate central degenerative changes affecting the anterior horn cells throughout the spinal cord, but more in the lumber and cervical regions.

Renewed interest has been shown in the paralysis induced by organophosphorus compounds since Bidstrup and Hunter[9,10] reported two cases of paralysis in man following acute accidental poisoning by the insecticide mipafox. Moreover, Barnes and Denz[11] demonstrated that mipafox, TOCP and DFP produce delayed neuropathy in hens, and Marchi staining of tissue showed demyelination in the peripheral nerves and particularly in the spinal cord. The distribution of the lesions was the same in hens poisoned by each of the three compounds. In the spinal cord the fasciculus gracilis in the posterior columns, the spincocerebellar tract in the later columns, and long association fibers in the anterior columns were involved. In the same study, when given to rats and rabbits, mipafox produced general weakness but no permanent paralysis, but some demyelination was found in the peripheral nerves and in the fasciculus gracilis. Cavanagh[1]

studied the changes in the chicken nervous system following poisoning with TOCP and found degeneration of axis cylinders and myelin sheaths in both the peripheral nerves and long tracts of the spinal cord. The generation of the lesion appeared to affect the distal extremities of the axons. Long fibers of large diameter were particularly selected. Further histopathological investigation into the injury in chickens following poisoning by DFP with particular reference to whether myelin degeneration is the primary damage sustained by the nervous system was continued by Fenton[12]. He reported that the distribution of the damage to the nervous system following DFP poisoning confirmed the previous findings of Barnes and Denz[11] and that distribution of the damage could be explained on the basis of a degeneration of the long nerve fibers resulting from depression in the metabolism of the neuron. The neurotoxic effects of EPN has been reported, however, the incidence of clinical effects was also somewhat irregular[13]. Feeding of EPN up to 600 ppm and TOCP at 2500 ppm gave rise to definite clinical signs of paralysis in all tested chickens with histopathological evidence for myelin degeneration in all cases[14]. Moreover, Hine, et al.[15], studied the relationship between the chemical structure and neurotoxicity of some substituted phenyl phosphates including TOCP analogues. They conlcuded that the aromatic phosphates containing one or more orthotolyl groups and tri-orthotolyl borate produced delayed neuropathy in hens.

The more recent TOCP poisoning outbreak took place in Morocco in 1959 due to poisoning by TOCP formulated in lubricating oil that was sold as "olive oil". The lubricating oil was man-made and was formulated to withstand the very high temperatures pertaining to turbo-jet aircraft engines. More than 2000 paralyzed cases were reported during that outbreak[8]. The ability of DFP to induce delayed neuropathy in chicken led Davies, et al.[16], to test thirty-six alkyl organophosphorus compounds for neurotoxicity in the chicken. Seventeen substances were found to be neurotoxic, fifteen for the first time. All of those contained fluorine. On the other hand, a number of tri-(alkyl substituted phenyl) phosphates had been examined for neurotoxicty to chickens by Aldridge and Barnes[17]. They suggested that there was no correlation between chemical structure and neurotoxicity. Moreover, histopathological evidence supported a suggestion that tri-p-ethyl phenyl phosphate, which is able to induce delayed neurotoxic effect, acts in a different manner from TOCP and DFP[18]. Other studies on the metabolism and action of TOCP and its metabolites indicates that TOCP is easily metabolized in a major pathway that involves methyl hydroxylation and subsequent cyclization to yield a very bioactive cyclic phosphate, the active metabolite[19,20]. Demyelination of the spinal cord following administration of the metabolite occurred at doses considerably below those necessary to induce peripheral nerve degeneration[21]. In 1964, cats were used to study the peripheral nerve changes in TOCP poisoning in detail by Cavanagh[2]. Cats had been given TOCP by subcutaneous injection (SC) in doses from 0.01 to 0.75 ml per kg. Ataxia supervented from the thirteenth day. The changes found in the peripheral nerves showed that the damage is selective to the large diameter and long fibers, wherever the nerve cell bodies did not show morphological changes. Cavanagh suggested that the nerve changes may be the consequence of an interference with energy transference or storage within the nerve cell. Tributyl phosphorotruthiolate (DEF) and tributyl phosphortrithioate (Merphos, Folex), which were used as plant defoliants, were initially shown by Casida, et al.[22],

to induce clinical signs of ataxia in hens following a series of intraperitonial injections. A histopathological examination was made of the neurological disruption produced in hens by these two alkyl phosphorus esters[23]. It was concluded that DFP and Merphos produced central and peripheral nervous system lesions accompanied by clinical signs of ataxia similar to those seen following administration of TOCP. Twenty-two additional OP compounds have been given to adult hens to test their ability to induce delayed neuropathy[24]. Fourteen of these compounds were active as neurotoxic agents. Davies, et al., [25] examined twelve phosphrodiamidic fluorides for neurotoxicity in hens. All were active. The character of the functional disorder and the distribution of the histological lesions were identical with those seen after poisoning by DFP or TOCP. Moreover, they found tha N,N'-di-n-butyl phosphorodiamidic fluoride was extremely neurotoxic.

In 1971, a mysterious epidemic of paralysis struck several hundred water buffaloes in Egypt and eventually resulted in the death of about 1300 animals[26]. It was reported that those animals fed on plants in areas treated with the insecticide, leptophos [O-methyl O-4-bromo-2,5-dichlorophenyl) phenyl phosphonothioate]. As early as 1976, leptophos was reported to be a delayed neurotoxic compound in hens[27]. Additionally, leptophos was found to induce the delayed neurotoxic symptoms in roosters[28]. Moreover, some workers exposed to leptophos in a leptophos-producing plant in the USA developed the symptoms of delayed neurotoxicity[27]. Cyanofenphos [O-ethyl O-(4-cyanophenyl) phenylphosphonothioate] was reported for the first time as a delayed neurotoxicant in sheep and chickens[6,29]. The delayed neurotoxicity of O-alkyl-O-phenyl phenylphosphothioate analogues related to leptophos was demonstrated in the hen[30].

Many non-phosphorus containing compounds were reported to induce nerve lesions in hens. Among these, 4-bromophenylacetylurea, 4-bromo-phenyl-isothiocyanate, acrylamide and sodium diethyl-di-thiocarbamate were able to induce the same "organophosphorus pattern" of degeneration[31,32].

Recently, it was concluded that neurotoxic compounds(i.e. DFP) induce a focal, distal but notterminal, axonal degeneration. This "chemical transection" of the axon then precipitates Wallerian degeneration of the more distal axon. Thus, the traditional hypothesis that dying-back neuropathies evolve from a retrograde axonal degeneration is not valid for organophosphorus neuropathy[33,34].

The insecticidal and mammalian acute toxicity of the organophosphorus compounds are generally accepted as due to the phosphorylation of acetylcholinesterase, AChE[35]. Delayed neurotoxicity, that is induced by some orgaonphosphorus esters, does not appear to be directly related to AChE or ChE inhibition[15].

Extensive studies have been done in order to find the relationship between the chemical structure and the neurotoxicity of organophosphorus esters[36,37,38,39,17]. In this report we will discuss briefly some evidence for chemical structure/neurotoxicity relationships of some groups of the organophosphorus esters.

A. Triphenyl Phosphates

The delayed neurotoxic effects of many triphenyl phosphate derivatives (I) were studied by many workers[15,17,22,36,37,38a]. In this class, all neurotoxic compounds, with the exception of tri-p-ethylphenyl and diphenyl-n-ethylphenyl

phosphates, contain at least one ortho-cresyl moiety.

TOCP and perhaps all of the ortho-cresyl phosphate derivatives do not exert a direct neurotoxic action but acts rather by way of the metabolites (II), i.e. tolyl saligenin cyclic phosphate, which are produced by in vivo metabolism of the parent molecules [19,20,22]. With tri-p-ethylphenyl and diphenyl-p-ethylphenyl phosphates, the neurotoxic syndrome arises from the keto metabolite (III) which is produced in vivo by the action of a soluble dehydrogenase[40,41]. Recognition of these metabolic facts shows that in a discussion on structure and neurotoxic action, considerable attention must be paid to lethal synthesis.

B. Phosphoro- and Phosphonofluoridates

Among the phosphoro and phosphonofluoridates, neurotoxic derivaties (IV) are relatively numerous. The neurotoxic effects of phosphoro and phosphonofluoridates were studied by many workers[16,19,24,38,42]. Methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl, 2-pentyl and cyclohexyl phosphophoro- or phosphono fluoridates were reported to be neurotoxic agents. The minimum ataxic dose in hens for this group of compounds ranged between 0.25 and 20.00 mg/kg. At least one alkoxy group attached to the phosphorus atom would appear to be prerequisite for neurotoxic activity.

A fluorine-containing structure, which is a common feature of many neurotoxic organophosphorus derivatives, led to a correlation of this property with the fluoride ion liberated by hydrolisis and the biochemical mechanism of neurotoxicity[43].

C. Phosphrodiamidic Fluorides

Davies, et al.,[25] studied the delayed neurotoxicity of phosphorodiamidic fluorides (V). All the tested alkyl or aryl derivatives of this group werefound to be neurotoxic agents. However, in the N,N'-diallyl phosphorodiamidic fluorides, the length of the alkyl chain appeared to be significant for the order of neurotoxicity. The dose required for the same degree of neuropathy is decreased by increasing the number of carbon atoms in the alkyl chain from one to four, although with alkyl derivatives longer than butyl, the required dose is increased again. The reverse is the case with the tri-phenyl phosphates. N,N'-diphenyl phosphorodiamidic fluoride was found to be more neurotoxic than the p-cresyl derivative and the latter was five times as neurotoxic as the N,N'-di-(O-cresyl) phosphoro-

diamidic fluoride. The dose required to induce ataxia in hens by the phosphorodiamidic fluoride derivatives was found to be 0.1 to 100 mg/kg.

Mipafox (N,N'-di-isopropyl phosphorodiamidic fluroide), specific neurotoxic agent, is one of the members of this group.

D. Phosphates

Most of the widely used insecticides are phosphate or phosphorothioate derivatives. Few of these were found to be neurotoxic agents. All the reported neurotoxic active derivatives of thie group are with the general structure (VI) and it appears that one of the R moiety must be chloro-alkyl group and R is not methyl.

O-methyl-O-(2-chloroethyl)-O (2,2-dichloro-vinyl) phosphate was found to be neurotoxic in hens at a dose of 10 mg/kg. The O,O-bis (2-chloroethyl) analogue was also found to be neurotoxic in hens at 25 mg/kg[24]. On the other hand, Haloxan [di(2-chloroethyl) 4-methyl-coumarin-7-yl phosphate] and the 4-nitrophenyl and 2,3,5-trichlorophenyl derivatives of di(2-chlorethyl) phosphate were reported as neurotoxic phosphate analogues[24].

E. Alkyl Phosphonates

It was reported tha Agritox^{R} trichloronate (O-ethyl-O-(2,4,5-thichlorophenyl) ethyl phos-phonthioate] induced neuropathy in chickens[44]. This compound is used as an insecticide. Chem-ically, trichloronate is a member of the alkyl-phophonate group (VII). Recently, Francis, et al[45], studied the neurotoxicity of some alkylphosphono-thioate derivates in chickens. Eight out of eleven methylphosphonthioates and three of seven ethylphosphonothioates were overtly neurotoxic. They suggested that neurotoxic activity varied both with the number of halogen substituents on the phenyl group and with the identity of the 4-phenyl halogen. Moreover, they mentioned that the neurotoxic activity also varied with the alkyl group involved in the P-C and with the identity of the O-alkyl group.

F. Phenyl Phosphonates

EPN, leptophos and cyanofenphos are good examples for the neurotoxic insecticide members of the phenylphosphonate structure (VIII). In this group, the identity of O-alkyl moiety affects the ability of the compound to induce neuropathy. All of the tested O-methyl-O-(substituted phenyl) phenylphosphonates (or thioates) were found to be potent neurotoxic agents[30], desbromo derivative of leptophos [O-methyl-O-(2,5-dichlorophenyl) phenylphosphonothioate] being the most potent neurotoxic compound of this group[30,46]. Substitution for the methyl by ethyl group reduced neurotoxic activity (unpublished data).

It was suggested that neuropathy is a property of all of the phenylphosphonate derivatives. The data supporting those suggestions[5,47] were based on tests with a few derivatives, only five phenylphosphonates. A study with twenty phenylphosphonate derivatives indicated that eight of them were not neurotoxic while the other twelve were[30]. In that study, all the tested methyl halogen- or nitro-substituted phenyl phenylphosphonate derivatives were neurotoxic agents. Methyl unsubstituted phenyl and methyl 4-cresyl phenylphosphonates induced no neurotoxic symptoms even at a high dose as 500 mg/kg of hen body weight. Studies with additional three phenylphosphonate derivatives, Inezin^{R} (O-methyl-s-benzyl phenylphosphonothioate); O-methyl-O-(benzylidenylphenylhydrazone) phenylphosphonothioate which are recommended as fungicides and NIA-16388 [O-n-propyl-O-(2-propynyl) phenylphosphonate which is recommended as insecticide synergist, indicated that only one of them, NIA-16388, is neurotoxic in hens[48].

According to previous findings, it may be possible to predict, but not definitely, the neurotoxic activity of an organophosphorus compound based on its structure. However, it is difficult to find a common (definite) denominator that will give a clue to the structural conditions required for neurotoxic action.

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