EXPERIMENTAL STUDY

An acute toxic neuropathy caused by organo-phosphate poisoning in hens

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Abstract

Objective: **Organophosphorus compounds can induce an acute toxic peripheral neuropathy. In hens, the acute peripheral neuropathy was induced by poisoning with organo-phosphorus compound (OPC) — tri-ortho-cresyl phosphate (TOCP).**
Methods: **In the course of an acute TOCP-induced toxic neuropathy in hens the activity of following enzymes was analysed: asparaginase, glutaminase, glutamat-dehydrogenase, AMP and adenosine deaminases and 5'nucleotidase; ALT (SGPT), AST (SGOT) and proteins levels were estimated.**
Results: **A decrease in activity of all analysed enzymes was observed; the amount of proteins was increased.**
Conclusion: **The biochemical changes display the slowing or stoppage in axonal transport of proteins. The disturbance of axoplasmic flow and the axonal demyelination may be considered as an attribute of peripheral neuropathy.** ***(Tab. 1, Fig. 1, Ref. 32.)***
Key words: organo-phosphorus compound's (OPC) toxicity, tri-ortho-cresyl phosphate (TOCP), toxic neuropathy, enzymes and TOCP.

Many organo-phosphorus compounds (OPC), neurotoxic demyelinating agents, cause a specific secondary type of demyelination in the nervous tissue. A lesion of neuronal soma, axis cylinder and myelin sheath in the central and peripheral nervous system has been demonstrated. Toxicity of tri-ortho-cresyl phosphate (TOCP), a causative agent of an accidental flaccid motor paralysis with ataxia in humans, was reported in the USA (1930) and in Morocco (1958–1959) (1, 2). The first clinical and morphological picture was demonstrated by Harris (1930) and Smith and Lillie (1931) in humans, later by Glees and Janzik (1965) in animals (2, 3, 4).

After the World War II and also after the Gulf War, where OPC were used as chemical weapons, discussions about the influence of OPC were held (5, 6).

Subsequently, the toxic influence of TOCP on the nervous system in experimental animals has been subsequently described, most frequently in rats and in the most susceptible animals – hens (3, 7, 8, 9). Other OPC, such as alkylphosphates, diisopropylfluorophosphonate (DFP) or bis-/isopropylamino/-fluorophosphine oxide (mipafox), are neurotoxic, too. Recently, all these compounds were used as pesticides and their neurotoxic demyelinating influence was demonstrated later. In birds, the ascendent and descendent long spinal tracts were affected, but the most striking damage was observed in the peripheral nervous system in both myelinated and unmyelinated fibers. The distal type of OPC toxic neuropathy with demyelination of the axon, proliferation of Schwann's cells and connective tissue, and infiltration of macrophages has also been reported. Cavanagh (1964) describes a parallel between the alterations of peripheral nerves and typical Wallerian degeneration and interprets this phenomenon as a disease of the whole neuron, a process of retrogression (so called "dying back" phenomenon) (9, 10).

The aim of our study was to induce the acute OPC neuropathy in hens and to demonstrate several biochemical changes during the development of the distal toxic neuropathy.

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Material and methods

In the experiment, twelve-week old hens were used; 0.2 ml of TOCP per kg of body weight was administrated via a transesophageal tube over 4 days. The first signs of the disease were diarrhea and ocular signs. In three weeks after administration the symptoms of a flaccid paralysis with ataxia developed (Fig. 1). Experimental animals (n=18) and a control group of healthy animals (n=13) were killed using decapitation in certain time intervals after TOCP administration. The sciatic nerve was prepared, cleaned and after measuring the weigh it was homogenized in re-distilled water; the homogenate was used for biochemical analysis.

The specific activities of asparaginase (L-asparagine-amidohydrolase, E.C.3.5.1.1.) and glutaminase (L-glutamine-amidohydrolase, E.C.3.5.1.2.) using the method described by Turský and Valovičová (1964), AMP-deaminase (5'-AMP-aminohydrolase, E.C.3.5.4.6.) using the method of Lee and Wang (1968), and adenosin (ADO) deaminase (5'-adenosin-aminohydrolase, E.C.3.5.4.4.) using the method of Mališeva et al (1964) were evaluated; according to Conway (1957), the amount of the product (ammonia) per mg protein in homogenate was determined (11–14). The enzymatic activity of glutamate dehydrogenase (GLDH, L-glutamate-NAD+ (NADP)-oxidoreductase, E.C.1.4.1.3.), alanin aminotransferase (ALAT, L-alanin-2: oxoglutaramine aminotransferase, E.C.2.6.2.2.) and aspartate aminotransferase (ASPAT, L-aspartate-2-oxoglutarate-aminotransferase, E.C.2.6.1.1.) were evaluated using the tests of Biochemica Boehringer GmbH and expressed in international units (in cat/mg of protein homogenate).

The enzymatic activity of 5'-nucleotidase (5'-ribonucleotid phosphorylase, E.C.3.1.3.5.) was evaluated using the method described by Pecháň (1969) (15). The protein content (16) and water content were analysed simultaneously.

The results were calculated using the t-test.

Results

In acute toxic neuropathy the biochemical changes may be divided into two types: early changes in the 1st week after TOCP administration and changes in the period of clinical manifestation during the 3rd and 4th weeks after intoxication. On the 8th day after TOCP administration a gradual decrease in the enzyme activity was observed, subsequently followed by uniform and marked decrease in activity of all analysed enzymes and by a substantial increase in the protein concentration in peripheral nerve tissue (Tab. 1).

Discussion

The pathogenesis of TOCP neuropathy is not clear. Generally, an inhibition of cholinesterase was presumed, but Adams (1965) did not confirm this hypothesis due to a long time interval observed between the decrease in cholinesterase activity and the appearance of motor disturbance and disability observed in experimental TOCP neuropathy. However, the decrease in cholinesterase activity is considered as a marker of OPC poisoning (1, 17).

Fig. 1. The flaccid paralysis in TOCP poisoned hen.

Aldridge (1954) proved that some metabolites are responsible for a direct toxic influence of TOCP (18). Eto et al (1962) showed that a direct neurotoxic effect could be caused by a dose of cyclic phosphate equivalent to 1/50 of TOCP dose after the loss of cresol (19). Although, the model of TOCP neuropathy is very frequent and popular in investigation of both neuronal degeneration and demyelination processes, biochemical data correlating demyelination and Wallerian degeneration in this model are not comparable.

A great attention was paid to investigation of lipid metabolism. Minimal changes in the concentration of lipidic phosphorus, changes after 32P "uptake" or after the decrease of lipogenesis from ^{14}C-acetate and only mild changes in some phospholipids, cholesterol esters and phosphatidylcholine were found (17, 20, 21, 22).

Porcellati (1971) supposed that the degradation of lipids is not very active in TOCP toxic neuropathy (17). Less attention was paid to and only a little is known about the metabolism of proteins. After TOCP poisoning, an increase in serine, threonine and glutamate activity and a decrease in acidic proteinase activity were described in the peripheral nerve of hens; an increase in ^{14}C-lysine and ^{14}C-leucine incorporation into peripheral nerve and spinal ganglia proteins was found, too (17, 23, 24, 25).

The decrease in cholinesterase activity in OPC poisoning was well known for more than fifty years. Clouet and Waelsch (1963) concluded, that OPC have an important influence on protein metabolism and cholinesterase enzyme system; a decrease in one step of proteosynthesis should be considered (25).

A progress in understanding of the influence of OPC was made by data from several enzymes' action, namely neuropathy target esterase (NTE). This enzyme is an integral protein of neurone membrane in vertebrates. Recent evidence suggests that NTE plays an important role in neural development, possibly by signal pathways between neurones and glial cells (12, 26, 27, 28).

The presumed mode of action of OPC is irreversible phosphorylation of axoplasmic proteins and also acetylcholinesterase,

Tab. 1. The specific enzyme activity in sciatic nerve of TOCP poisoned hens.

Enzyme activity nkat/mg Protein	Control (day 0) n=13 x±SD	TOCP (3rd day) n=4 x±SD	TOCP (8thd day) n=4 x±SD	TOCP (15thd day) n=4 x±SD	TOCP (30thd day) n=6 x±SD
Asparaginase	171±26	155±73	65±27**	37±12**	72±14***
Glutaminase	4025±440	3127±411	3604±264	1712±156**	1851±295***
GLDH	2887±394	2121±546	1374±746**	639±339***	667±186***
AMP-deaminase	1845±369	1756±187	2026±191	1404±147*	1345±158***
ADO-deaminase	734±85	723±180	272±35**	213±74***	251±68***
5'-nucleotidase	668±75	343±72**	549±246	234±42***	329±75***
ASPAT	967±102	860±88	612±17**	321±10***	315±57***
ALAT	73±8	47±10*	72±0.4	18±0.7***	45±0.8***
Protein	13.5±4.5	22.3±2.8	25±2.6	53.7±1.3***	30.0±4.5*

* p<0.05, ** p<0.01, *** p<0.001, x±SD — mean value and standard deviation

and possible block of neural transmission. The covalent reaction between OPC and NTE induces neuropathy. This event leads to calcium entry, elevation of calpain activity and presumably to Wallerian type of degeneration by "chemical transsection" of neuronal axon with the slowing or stoppage of axoplasmic flow (1, 9).

In the last century, an axonal transport along the axons of the nervous system was postulated, but was demonstrated in 1948 by Weiss and Hiscoe for the first time. An axonal transport has been investigated in many species by various techniques (23, 29, 30, 31). Bradley and Williams (1973) suggested that the possible pathogenetic mechanism of peripheral neuropathy could be a collapse of the supply of essential macromolecules from cell bodies to axons (32). The disturbances in retrograde and anterograde axonal transport or the slowing/stoppage of the slow component of axonal transport with axonal atrophy are associated with the development of peripheral neuropathy. In clinical investigation in humans, the slowing of nerve conduction velocity is considered as a sign of neuropathy. Probably, the simultaneous decrease in the enzyme activity in general and the increase in nerve protein content (with a lower biological value of proteins with enzyme characteristics) could be considered as an explanation. These events are presumably the evidence of slowing or stoppage of axoplasmatic flow and could be considered as a possible mechanism of the development of peripheral toxic neuropathy.

We suggest that in OPC toxic neuropathy in hens a typical biochemical evidence of the pathogenesis of peripheral neuropathy is present.

The increase in protein concentration and their accumulation in the distal part of peripheral nerves are the background of slowing or stoppage of axoplasmatic protein transport.

The decrease in activity of all mentioned enzymes may be the consequence of the decomposition or space deconformation of the protein transport and the decrease of their biological values.

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