Parathion effects on protein synthesis in the seminiferous tubules of mice

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Abstract

Agropesticides impart high risks of exposure for animals including humans. This work analysed the effect of parathion upon protein synthesis in seminiferous tubules cultured in vitro from adult male CF1 mice. They were incubated with parathion solutions (0.8, 0.4, 0.04, 0.004, 0.0004 mM) for 5 h. One hour after ending incubation tritiated leucine was added to each sample. In addition, groups treated with 0.8 mM parathion were incubated for 5 h, and thereafter transferred to a parathion-free medium; tritiated leucine incorporation was measured 1, 2, or 3 h afterwards. Results of protein synthesis denote an inhibitory dose-dependent effect of parathion. This is reversed after pesticide-free incubation. Testicular protein synthesis inhibition induced by parathion may compromise male fertility in view of the fact that, in spermatogenesis, proliferation (mitosis, meiosis) and cell differentiation occurs.

Keywords: Parathion; Spermatogenesis; Toxicology; Organophosphorate; Pesticides

1. Introduction

Modern human life has incorporated into our environment a great variety of xenobiotics, such as organophosphorates, which may have adverse effects on the health of humans and wildlife. The wide use of nonlethal chronic contaminants has shown multiple effects on the endocrine, immune, and reproductive systems, with reduction of fertility, presence of developmental alterations, and decreased viability of newborns (Hanazato, 2001). Epidemiological analysis on the massive agricultural and house uses of pesticides has resulted in numerous publications. However, information related to potential effects on the reproductive system of man or other animals is scarce.

Parathion is an organophosphorate agropesticide. It presents nucleophylic properties and the capability for methylation and phosphorylation of organic macromolecules (Tapia, 1994) such as DNA and RNA (Rodriguez and Bustos-Obregon, 2000). Parathion is transformed by oxydative reactions to paraoxon (the active molecule) that involve the enzymatic P-450 complex (Butler and Murray, 1997; Kim et al., 2000).

Organophosphoric pesticides act by inhibiting acetylcholinesterase, and their effects are due to acute cholinergic activation (Valenzuela, 1990; Wallace, 1994). Other researchers have evaluated different aspects such as interaction with DNA or RNA (Bartoli et al., 1991; Antunes-Madeira et al., 1994; Vijayaraghavan and Nagarajan, 1994), thus elucidating mechanisms of action of these pesticides practically unknown up to now. According to Carlson et al. (2000), in neuroblastoma cells, parathion and paraoxon at concentrations of 1 mM induce multifactorial toxic effects, altering mitochondria and activating respiratory enzymes. Cao et al. (1999) postulated that organophosphoric compounds modulate metabolic activity in different cell types, inducing irreversible changes through activation of muscarinic receptors. Both are important issues for

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reproduction since in the Sertoli cells intracellular pathways for muscarinic and cholinergic receptors have been described (Palmero et al., 1999). Since any of these receptors could be related to protein synthesis, it may be postulated that toxic effects of parathion could be mediated by altering testicular protein synthesis.

2. Material and methods

Testes from young adult CF1 mice were used (five animals for each experimental setup and their controls). Animals were kept at 18–22 °C, with a period of light/darkness of 12/12 h, and fed commercial pellet and water ad libitum. After sacrifice, the testes were removed, weighed, and decapsulated. Seminiferous tubules were manually dissected using fine needles under a stereoscopic microscope and placed in the incubation medium. The seminiferous tubules were dissociated.

2.1. Toxic assays

Seminiferous tubules were incubated in Ham F-10 medium (1 mL) with parathion (99.2%; Lab. Chemical Co.) at concentrations of 0.8, 0.4, 0.04, 0.004, and 0.0004 mM and one control with the same conditions but without parathion. According to Delaunois et al. (1992, 1995), 0.4 mM is the minimal concentration that damages the alveolocapillar basal lamina. Therefore a range of concentrations around 0.4 mM was used. Incubation was done in 2-mL culture dishes kept in bath at 35 °C, 95% humidity, and 5% CO_2, with 60 cycles/min of shaking for 4 h (Rodriguez and Bustos-Obregön, 2000). After that period 2 µL of tritiated leucine (H^3L) was added and incubation proceeded for an additional hour. Tubules were washed in Ham F-10 and frozen at −20 °C until biochemical procedures were initiated. The biochemical procedures for H^3L uptake have a high repeatability and low variation coefficients (<5%).

2.2. Reversibility of the toxic effects

Three other groups (A, B, and C) were similarly incubated for 5 h using 0.8 mM parathion. Then fresh medium (without parathion) replaced the incubation medium and H^3L was added. Incubation was for 1 (A), 2 (B), or 3 (C) hours. Thereafter tubules were frozen at −20 °C until protein extraction and determination of the total proteins, and their corresponding controls were used to test leucine uptake after 5 h of incubation in the absence of parathion.

2.3. Standard histology

A last group was incubated for 5 h in 0.8 mM parathion for routine histological analysis (hematoxylin–eosin). The tubules were fixed in alcoholic bouin and embedded in paraffin for routine histological sections.

2.4. Proteins

Proteins were extracted by the orcinol method (Schneider, 1957). The frozen tubules were homogenized in Tris buffer and then used for the following procedures. (1) *Total protein determination* by the colorimetric method of Bradford (Kruger, 1994) using Coomassie brillant blue G-250 C.I. 42655 (Lab. Sigma Co.) and a spectrophotometer UV –120–20 and. (2) *H^3L uptake* quantified by liquid scintillation (Schram, 1963) using a Beckman LS 100c counter. Uptake of the isotope by newly synthesized testicular proteins was expressed as counts per minute per microgram of proteins.

2.5. Statistics

Results are expressed as specific activity (cpm/µg). Statistical analysis was done by Student *t* test ($P<0.05$). These procedures have very low coefficients of variation (3–5%) and a narrow variance.

3. Results

Specific activity of H^3L uptake in the testis is shown in Fig. 1. Protein synthesis decreases with parathion concentrations of 0.8, 0.4, and 0.04 mM, though this effect is statistically significant only at 0.8 mM ($P\leqslant 0.05$). Concentrations lower than 0.04 mM (0.004 and 0.0004 mM) have no noticeable effect. Moreover it was observed that the seminiferous epithelium

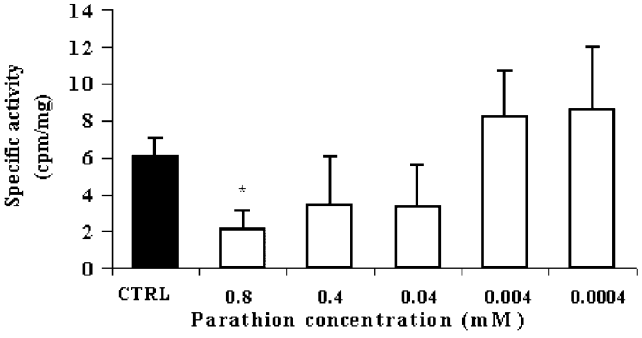

Fig. 1. Effects of decreasing concentration of parathion (mM) on the H^3L uptake (cpm/µg) in mouse seminiferous tubules cultured in vitro (0.8, 0.4, 0.04, 0.004, 0.0004 mM and control). Each group was done in triplicate (mean ± SD; $*P\leqslant 0.05$).

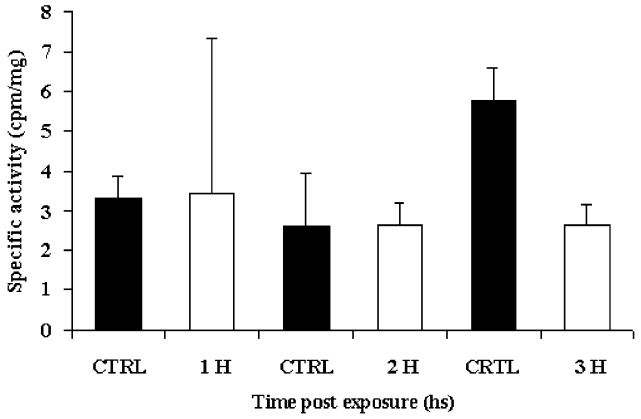

Fig. 2. Reversibility of the toxic effect of a fixed concentration of parathion (0.8 mM; 5 h) on the H^3L uptake (cpm/μg) in mouse seminiferous tubules cultured in vitro. After 5 h the seminiferous tubules were reincubated in a parathion-free medium for 1, 2, or 3 additional hours. Each group was done in triplicate (mean ± SD, $P > 0.05$).

responded with increased protein synthesis (neoformed proteins), with values above those of the controls, though not significantly ($P > 0.05$). Reversibility of the effects of parathion (0.8 mM) for 5 h are shown in Fig. 2, where it is seen that the inhibitory effect decreases gradually with time after withdrawal of the toxic. The controls (free of parathion) at 1, 2, and 3 h after 5 h of incubation do not differ significantly (Student t test, $P > 0.05$). Under the present conditions of incubation and the doses of parathion used, the histological features were unaltered.

4. Discussion

In the seminiferous epithelium, the level of genic expression is higher than that in other tissues and takes place during mitosis, meiosis, and cell differentiation, mainly in germ cells rather than Sertoli cells (Schlecht et al., 2004).

The change in the level of cell synthetic activity is assumed to be associated with a rise in effective incorporation of ^{3}H-leucine into proteins following an increase in its intracellular level and with an increase in the protein synthesis rate (Varaksina and Varaksin, 2001).

According to Stern et al. (1983), during spermatogenesis there is an active protein synthesis involving 95% of all germ cell lines and 5% specific for cell stages.

The present study demonstrates an inhibitory effect of parathion on testicular protein synthesis which is dose related. It is also shown that the toxic effect is reversible after withdrawal of the pesticide with regard to leucine incorporation. However, other effects, not directly depending on protein synthesis, cannot be excluded.

According to Brinkworth and Handelsam (1997), testicular toxicants may affect any of the gonadal cells, either somatic (Sertoli, Leydig) or germ cells, but because of the close interdependence of all these cell types the damage will be extensive to all of them.

The toxic effect of the organophosphoric compounds corresponds a to strong cholinergic mechanism that inhibits irreversibly the acetylcholinesterase. Aside from this mechanism, it should be remembered that organophosphoric compounds strongly interact with macromolecules such as DNA, RNA, or proteins (Bartoli et al., 1991; Rodriguez et al., 2001). This could be another way of damaging male reproductive ability. It may even be speculated that spermatozoa could be transporting molecules of the pesticide into the fertilized egg.

Parathion alters the normal growth and cell association development of the stages of the cycle at early periods of spermatogenesis. However, with respect to morphology, this effect can be recognized at longer intervals with chronical exposures (Sobarzo and Bustos-Obregon, 2000).

Many authors have described changes in the sperm count and testicular function (Ray et al., 1992; Leone et al., 1988) after immediate exposure to organophosphoric pesticides and other reproductive alterations after longer periods (Dikshith et al., 1978). Many adverse effects may be due to the metabolic effects induced by the pesticides, i.e., protein synthesis alterations, enzymatic modifications, etc.

It has been recently demonstrated that, in addition to the classical inhibition of acetylcholinesterase, parathion induces an inhibition of nicotinic receptors, thus affecting intracellular metabolism (van der Beukel et al., 1996). Both mechanisms may be important in the testis, since many studies have identified cholinergic molecules, cholinesterases, and nicotinic receptors in the germinal cells of different animal species (Nelson, 1978; Egbunike, 1980; Cariello et al., 1986; Baccetti et al., 1995). The presence of these molecules in the Sertoli cells points to the role of the somatic component of the testis exposed to pesticides. These pesticides can also interfere with intercellular communication during spermatogenesis (Falugi et al., 1993) and with interaction of the sperm with the ovum (Ibañez et al., 1991). In addition the nicotinic receptor may be involved in the regulation of sperm motility.

The present work has tested in vitro the toxic effects of parathion using the same concentration of the agropesticide known to destabilize molecules involved in the structure and function of biological barriers such as the blood–alveolar barrier, as reported by Delaunois et al. (1992). Therefore, one may assume that the blood–testis barrier is not an efficient barrier for the pesticide. Organophosphoric pesticides also induce disorganization of the cytoskeleton by affecting

structural and functional proteins and other important macromolecules, including tubulins and membrane lipids (Antunes-Madeira et al., 1994).

No obvious changes in testicular histology and in the cell association could be detected in the presence of parathion (Sobarzo and Bustos-Obregon, 2000). Similar observations are reported in the present paper.

The reversibility of the toxic effects of parathion (and presumably of other xenobiotics) is due to the high detoxifying ability of the testis, mainly represented by the esterase family and cytochrome P-450 (Yoganathan et al., 1989; Kobayashi et al., 1991; Tohyama et al., 1994) and the systems for DNA repair present mainly in spermatogonia and spermatocytes (Dixon and Lee, 1980). Specific esterases hydrolize paraoxon (paraoxonases) (La Du et al., 1999; Rodrigo et al., 1999), which is the active metabolite of parathion. These esterases are amply represented in the testis (Von Deimling et al., 1985) where they will fulfil a detoxifying function that explains the reversibility of the protein synthesis inhibition.

Both parathion and paraoxon are inducers of apoptosis at concentrations similar (1 mM) to those used in this work, as demonstrated by Carlson et al. (2000) in neuroblastoma cultured cells. The mechanism could be by toxic alteration of mitochondrial membranes, thus activating enzymes of the caspase type. Additionally, organophosphoric compounds may modulate metabolic cell activity by binding irreversibly to different macromolecules, some of which could correspond to muscarinic receptors (Cao et al., 1999). This is an important point for the reproductive system, since the presence of the acetylcholinesterase and muscarinic receptors in male germ cells has been described. Chakraborty and Nelson (1974) described the presence and distribution of cholinesterases during spermatogenesis and sperm epidydimal maturation, indicating that the onset and maintenance of sperm motility could be related to the acetylcholinesterase–cholinesterase system. For all the above reasons, testes seem to be good targets for toxic damage elicited by organophosphoric agropesticides and deserve closer analysis.

Finally, it is possible to assume that the toxic effects of parathion occur by inhibiting acetylcholinesterase acting on muscarinic and nicotinic receptors present in both somatic and germinal cells in the seminiferous epithelium. In addition, it is known that organophosphoric compounds alter acetylcholine muscarinic and nicotinic receptors (mAChRs and nAChRs), giving rise to the so-called cholinergic syndrome (which comprises symptoms and signs attributable to inappropriate stimulation of mAChRs), and the protein synthesis may be affected (Sheridan et al., 2005). This is presumably explained by the fact that, normally, protein synthesis is stimulated by the mAChRs which depend on kinase-regulated phosphorylation (Jimenez et al., 2002).

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