Ann. Ist. Super. Sanità, vol. 29, n. 1 (1993), pp. 167-174

Neuropathological and behavioral toxicology of trimethyltin exposure

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Summary. - The most prominent neuropathological and behavioral changes induced by trimethyltin (TMT) in different mammalian species were reviewed. From the analysis of the reported literature it becomes evident that the neuropathological effects are selectively present in the limbic system structures. In particular, the granular neurons of the fascia dentata and the pyramidal cells of the Ammon's horn are involved, with a different pattern of severity and extension according to the various species studied and to the dosage-schedule used. The neurological damage produced by TMT to several limbic structures is related to overt behavioral changes. TMT acute exposure in adult rats produces a remarkable behavioral syndrome, consisting in tremors, spontaneous seizures, tail mutilation, vocalization, hyper-reactivity and intra-specific aggression. Impairments in learning and memory processes are also induced following acute treatment. Specific behavioral changes in various species reflect the different sensitivity and vulnerability to the chemical compounds. In addition, prenatal and postnatal exposure induce long-term behavioral and neurological effects on developing central nervous system.

Key words: trimethyltin, neuropathology, behavioral effects.

Riassunto *(Neuropatologia e tossicologia comportamentale dell' esposizione al trimetiltin)*. - Le più evidenti alterazioni neuropatologiche e comportamentali prodotte dal trimetiltin (TMT) sono state oggetto di una "review". Dall'analisi della letteratura che è stata presa in considerazione, appare chiaro che le strutture del sistema limbico sono selettivamente coinvolte. In particolare, i neuroni granulari della fascia dentata e le cellule piramidali del Corno di Ammone sono danneggiate con gravità ed estensione diverse nelle varie specie studiate e con modalità differenti a seconda del livello di dosaggio utilizzato. Il danno neurologico riscontrato nelle varie strutture del sistema limbico è correlabile con alterazioni comportamentali conclamate. L'intossicazione acuta con TMT determina nel ratto adulto una caratteristica sindrome comportamentale che consiste in: tremori, contrazioni spontanee, mutilazione della coda, vocalizzazione, iper-reattività e aggressività intra-specifica. Il trattamento acuto causa inoltre alterazioni nei processi di apprendimento e della memoria. Le modifiche comportamentali specifiche osservate nelle varie specie riflettono la diversa sensibilità e vulnerabilità al composto chimico. Infine, il trattamento pre- e post-natale influisce sullo sviluppo del sistema nervoso, provocando effetti neurologici e comportamentali a lungo termine.

Parole chiave: trimetiltin, neuropatologia, effetti comportamentali.

Introduction

The great interest in neurotoxic compounds that has developed in the last decades is owed to some important reasons: the need to understand the potential hazards of environmental exposure to chemical compounds of industrial and agricultural origin; the possibility of increasing the knowledge in basic neurological physiology and function, through the study of the pathological mechanisms of neurotoxicants. Moreover, investigations on experimental exposure to chemicals often contribute to create animal models useful for research on human diseases. Another issue of interest comes from the fact that the study of the relationships between neuropathological damages and behavioral changes enhances the understanding of behavioral expression of neurological function.

In the present paper we shall provide an overview on some aspects of the neuropathogenetic mechanisms and behavioral toxicity of the organotin compound, trimethyltin (TMT). This chemical represents the most neurotoxic form of tin [1] and has been used as stabilizer of plastic, as chemosterilasant, and as biocide for the control of fungus, bacteria and insects [2]. Although in the past years the commercial use of TMT has been limited, some studies have reported that inorganic [3] and organic [4] tin compounds can be methylated by biotic and abiotic pathways in estuarine ecosystems, thus resulting in TMT production.

It is well established that TMT is a potent neurotoxicant, producing extensive damage to the central nervous system. Two human accidental exposures that showed TMT to be extremely neurotoxic have been reported [5]. Several morphological [6-17], biochemical [15, 18-20]

and behavioral [21-29] studies have revealed extensive neuropathological effects in the limbic system structures, including the hippocampus, pyriform and enthorinal cortices, olfactory tubercle and amygdaloid nucleus, as a consequence of TMT exposure in rodents and nonhuman primates. Furthermore, placental and blood-brain barrier transfer of TMT has been demonstrated in rat [30], with fetal brain concentrations that are equal to those found in maternal brain tissue.

Because of the specific neurotoxicity of TMT, we believe that the comprehension of its pathogenic mechanisms may contribute to extend the knowledge in the behavioral and neurological functions of the limbic system.

Neuropathology

Rodents

The results of an experimental investigation on the neurotoxicity of TMT were published in 1979 by Brown *et al.* [6], who described neuropathological and behavioral changes in rats, receiving either a single or repeated doses of the compound. These authors determined that the oral LD50 of TMT chloride in arachis oil was 12.6 mg/kg. Following a single dose of 10.0 mg/kg or four weekly doses of 4.0 mg/kg of TMT, rat brains revealed evidence of neuronal changes. In the single-dose experiment the maximal severity of neuronal damage was observed at 21 days following the TMT administration, with extensive cell injuries in the pyriform cortex, amygdaloid nucleus and neocortex. In the hippocampal formation, the pyramidal cell band h1-5 and the fascia dentata were consistently involved. In the repeated dose experiment, the pyriform cortex, the amygdaloid nucleus and pyramidal neurons of h1-5 were extensively damaged, while the fascia dentata appeared less severely affected. A comparison of the neuropathological toxicity of single or repeated TMT dosages showed similar effects with the exception of the early and remarkable involvement of the fascia dentata in the acute experiment. As reported by the authors, the neuropathogenetic sequelae of neuronal damage appears to begin with a loss or dispersal of Nissl substance within unshrunken cytoplasm, whereas the nucleus is normal. Subsequently, a condensation and clumping of nuclear chromatin is observed within the nucleus, while the cytoplasm begins to shrink. This stage of degeneration is rapidly followed by shrinkage of the nucleus which becomes fragmented, while the cytoplasm appears more shrunken and highly eosinophilic. The eventual step of this pathogenetic sequence is represented by the neuronal dissolution and removal by phagocytosis. The authors suggested that the cumulative concentration of TMT may produce a continuous recruitment of damaged neurons, thus presumably explaining the presence of cell changes at different stages of the pathogenetic sequelae, as observed in longer-surviving animals (up to 35 days). In addition, the same authors were able to determine the distribution of TMT in rat blood and brain after various dosing schedules, using ^{113}Sn-trimethyltin chloride and counting the radioactivity. Their results showed that approximately 70% of the injected TMT was present in the blood and that the affinity of the compound for rat hemoglobin was high (3.8×10^5 M^{-1}), thus helping to explain the cumulative and persistent effects of TMT.

The ultrastructural cytopathologic and cytochemical effects of TMT neurotoxicity were investigated by Bouldin *et al.* [8]. The study was performed on acutely intoxicated adult rats (oral dose of 5.0 mg TMT/kg/day for one, two, three, four or five days), and chronically treated adult (1.0 mg/kg/day for 14-16 days) and neonatal rats (1.0 mg/kg on alternate days from the 3rd through the 29th day of life). The results of the acute experiment showed neuronal necrosis that preferentially involved the hippocampus and pyriform cortex. Multifocal collections of dense-cored vesicles and tubules and membrane-delimited vacuoles in the cytoplasm of the perikaryon and proximal dendrite were observed. Ultrastructural cytochemical examination revealed that the vesicles and tubules had acid phosphatase activity analogous to Golgi-associated endoplasmic reticulum. Increased electron density of the cytoplasm and conspicuous intranuclear masses were evident as result of neural necrosis. The damages observed in adult and neonatal chronically treated rats were comparable with those obtained in the acute dose experiment.

In a later investigation [11], the progression of pathological changes in the central nervous system of rats was studied, after acute TMT exposure. Animals were treated with a single dose of 6.0 mg TMT/kg and then sacrificed at 8 h, 3, 15, 30 or 60 days following dosage. The microscopic examination of the hippocampus in the 3 day-surviving rats, revealed an earlier but transient involvement of the fascia dentata, with neuronal necrosis among the granular cells, followed by a resumption of its normal appearance by day 60, thus suggesting that most of the toxic activity had subside in the hippocampus. The Ammon's horn pyramidal neurons were more severely and extensively damaged at later periods (15-30 days) after TMT exposure, following a pattern of decreased toxic damage from CA_3 toward CA_1 subfield. By day 60 the result of the neuropathological effect in the Ammon's horn was a severe thinning of some areas and the absence of the septal portion of CA_3, while the changes in the granular neurons of the fascia dentata were limited. Furthermore, neurotoxic injuries similar to those observed in the hippocampus were evident in neurons of the pyriform and entorhinal cortices, and of the olfactory tubercle. Brain stem neurons were also mildly affected, being the damage confined to chromatolytic changes.

A comparative analysis of the neuropathological effects of TMT exposure in different strains of rats has been carried out [10] with the purpose to provide the morphological evidence of strain differences in the pathogenetic mechanisms of TMT intoxication. Two strains of rats, Long Evans (LE) and Sprague Dawley (SD) were treated with 7.5 mg TMT Cl/kg and then sacrificed at the appearance of neurological or behavioral signs (3 days for LE rats and 5 days for SD rats). A significant difference in sensitivity was observed between the two strains studied, showing LE rats more severe damage of the hippocampal neurons in a shorter period of time (3 days *vs* 5 days) than SD rats. Moreover, cell necrosis, neuronal swelling in the fascia dentata and CA_{3c}, scattered neuronal death and vacuolation in the $CA_{1,2}$ neurons were observed in LE rats, while SD rats showed only milder changes in the fascia dentata and CA_{3c} neurons. Chromatolytic changes in the olfactory tubercle, pyriform and entorhinal cortices, and brain stem were evident in both strains of rats.

Numerous investigations have been performed to assess the neuropathological effects of TMT exposure in mice [9-11, 31, 32]. In an acute toxicity study [9], BALB/c mice received a single dose (IP) of 3.0 mg TMT/kg. In all the animals severe progressive neurological and behavioral impairment appeared within 24 h following treatment, thus advising their sacrifice at 48 h post-administration. Microscopic examination revealed extensive neuronal degeneration and necrosis in the fascia dentata of the hippocampal formation. The necrotic neurons appeared shrunken with pyknotic nuclei and highly eosinophilic cytoplasm. In same areas a severe neuronal loss and spongiosis (vacuolation) were observed. Many of the surviving neurons showed eccentrically located nuclei and increasing granulation of cytoplasm. In contrast, damage to the pyramidal cells in the Ammon's horn were unremarkable, with some neurons showing scattered intracellular vacuolation. Furthermore, neuronal changes and necrosis could be identified in the neocortex, pyriform cortex, amygdaloid nucleus and brain stem.

A strain comparison [10] between mice, BALB/c and C_{57}, receiving a single dose of 3 mg TMT/kg did not reveal any significant difference either in the extension or severity of lesions produced by the compound.

From the reports mentioned above it appears that species differences are more prominent than strain differences. The overall pathological effects of TMT in mice are much faster and more severe than those induced in rats. While in mice TMT at the dosage of 3 mg/kg determines rapid and severe neuromorphological and behavioral changes, in rats milder and slower effects are displayed with higher doses of TMT (6.0-10.0 mg/kg). In an attempt to explain the species differences of sensitivity, Aldridge *et al.* [33] hypothesized that mice have a lower hemoglobin/TMT binding capacity than rats. The higher and faster blood/tissue transfer of TMT may account for the more acute toxic effect after exposure in mice. This hypothesis has been strengthened by more recent results [7] which showed that TMT do not bind to hamster and gerbil hemoglobin.

Nonhuman primates

Induction of distinct behavioral and neurological changes by trimethyltin have been reported in nonhuman primates [33, 7, 13, 16]. Brown *et al.* [7] assessed the TMT blood concentration in marmosets (*Callithrix jacchus*) after oral dosing with 3.0 mg/kg. Their results showed that, since the compound does not bind to hemoglobin, blood concentrations were low. In addition, the distribution of TMT throughout the brain was uniform, suggesting that the selective damage is not due to any focal concentration of the chemical. The neuropathological examination in the shorter-surviving animals (up to 13 days following TMT exposure) revealed neuronal degeneration in many cells of the fascia dentata of the hippocampal formation, and less extensive damage in the amygdaloid nucleus, cortex (entorhinal, frontal, temporal, parietal and visual) and h2 of the hippocampus, caudate nucleus, putamen, Purkinje cells, subiculum, presubiculum and septum. In h3-5 and h2 of the hippocampus, empty spaces or remnants of neuronal cells were evident. In two longer-surviving marmosets (35 and 45 days), cells with naked nuclei (cell "ghosts") were visible principally in the fascia dentata and h3-5.

In a subsequent study [13] the effects of acute TMT intoxication were investigated in the cynomolgus monkey (*Macaca fascicularis*). Although the experimental dosages ranged from 0.75 to 4.0 mg TMT/kg (i.v.), the lowest concentration capable of producing neuropathological changes was determined to be 1.10 mg/kg. At this level of TMT exposure, pyramidal neurons of Ammon's horn (areas CA_3 and CA_4) in the hippocampal formation, cells of the amygdala, scattered medullary neurons, Purkinje cells and rare neurons in dorsal and ventral columns of the spinal cord showed histopathological alterations. In more severely affected animals extensive degeneration and necrotic damage involved the pyramidal neurons in CA_4 and CA_3 of the Ammon's horn. In contrast, in the fascia dentata only sporadic pyknotic granular cells were recognized.

Behavioral toxicology

It is well documented that neurological damage produced by TMT to several limbic structures is related to overt behavioral changes. Several reports [6, 22] have provided evidence that acute exposure to TMT in adult rats produces a remarkable neurobehavioral syndrome consisting of tremors, spontaneous seizures, tail mutilation, vocalization, hyper-reactivity and intra-

specific aggression. However, these effects subside within a few days (5-10 days following treatment) to reveal a range of more persistent long-term behavioral deficits, with a pattern reminiscent of that observed after hippocampal ablation or de-afferentiation [34, 35]. Swartzwelder *et al.* [24] reported that rats, at 40 days following a single oral dose of 7.0 mg/kg of TMT, were hyperactive in a 2 min test of open field activity. These results are in agreement with those described by Ruppert *et al.* [23]. These authors measured the activity in a "figure eight" maze over a 23 h period, in rats orally receiving 7.0 mg TMT/kg, 48-51 days after dosing. The animals were hyperactive during all periods in the 23 h test. In addition, TMT altered the spatial pattern of activity, that increased in the "figure eight" portion of the maze, while it remained unchanged in the blind alleys. Hagan *et al.* [36] confirmed these data, describing in rats, orally treated with 7.0 mg TMT/kg, a marked increase of locomotor activity in a novel environment.

Alteration of radial-maze learning has been reported by Walsh *et al.* [37]. Rats previously trained in an automated eight arm radial-maze exhibited a persistent impairment of maze performances, characterized by decreased selection accuracy, following 6.0 mg/kg oral treatment with TMT; the same animals appeared unable to perform the short-term working memory components of the task (i.e. remembering which arms were chosen during a session), but retained the reference memory aspects of the task (i.e. leave central arena, enter arm etc.). In addition, they utilized a different spatial pattern of responding within the maze, shifting their selection to more distant arms if compared with controls that generally preferred to select the arm immediately adjacent to that one they have just exited. These data are in agreement with findings by Swartzwelder *et al.* [25]. These authors reported that TMT orally treated rats (7.0 mg/kg) made significantly more errors on each of 12 Hebb-Williams maze problems than controls. Furthermore, the treated animals exhibited a characteristic pattern of perseverative behavior while running in the maze. Johnson *et al.* [38] assessed the effects of TMT exposure upon spontaneous and learned alternation, using a "T" maze task. They found that TMT orally treated rats (7.0 mg/kg) made fewer reinforced spontaneous alternations and ran through the "T" maze more rapidly than the controls. On the contrary, no significant differences between control and treatment group in the rate of learned alternation were observed, thus suggesting that the "T" maze is insensitive to the spatial learning deficits associated with TMT exposure. Walsh *et al.* [37] reported that a single oral administration of TMT (5.0, 6.0 or 7.0 mg/kg), 21 days prior to passive avoidance conditioning, produced an impairment of retention in animals tested at 24 h after training. TMT treated rats displayed shorter step-through latencies and freezing durations during the retention test than controls. The three different TMT dosages were equally effective in disrupting retention performances.

The effects of TMT acute intoxication on Partial (PRF) or Continuous (CRF) Reinforcement responding were explored by Nation *et al.* [39] in a straight alley maze. The rat acquisition phase of training, lasting 40 trials (4 trials/day), was followed by 20 trials of extinction training (4 trials/day). Analyses performed on total speed revealed that subjects, orally treated with 3.0 mg TMT/kg, performed at lower levels during acquisition than controls, regardless of the schedule condition. The rate of resistance to extinction was also significantly reduced in treated subjects in comparison with that exhibited by controls, regardless of the training schedule used during acquisition. In TMT exposed rats PRF training showed a greater persistence during extinction than CRF training.

In a more recent investigation [29] the consequences of TMT administration in rats on Differential Reinforcement of Low Rate Responding (DRL) were studied. The animals were trained to respond under a schedule of reinforcement in which only the responses separated by a 10 to 14 s period of no responding produced a feed pellet (DRL 10 to 14 s). At a single dose of 7.5 or 10.0 mg TMT/kg (IP), the percentage of total responses, spaced 10 to 14 s apart, decreased over the first 8 to 12 days following TMT treatment. Rats receiving 7.5 mg TMT/kg gradually returned to control value within the next 2 to 3 weeks, while rats receiving 10.0 mg TMT/kg never recovered.

McMillan *et al.* [40] examined the effects of a single dose of TMT on eating, drinking, lever pressing for food pellets and running in the activity wheel. The authors observed that doses of 6.0 or 9.0 mg TMT /kg (IV) in rats induced a decrease in lever pressing and running lasting for several days, followed by marked increase in all the measures considered. Rats receiving 9.0 mg TMT/kg died 6 days post TMT administration, whereas rats receiving 6.0 mg/kg continue to show increases in behavior for 12 to 16 days, following TMT exposure.

Numerous investigations have reported that mice are much more sensitive to the effects of TMT than rats. Wenger *et al.* [26] studied in adult BALB/c mice the TMT intoxication influence on spontaneous motor activity (SMA) and responding under a multiple fixed-ratio 30, fixed interval 600 s (mult FR 30 FI 600) schedule of reinforcement. Following doses of 4.5 or 6.0 mg TMT/kg (IP), the cumulative 48 h lethality was 10% at 4.5 mg/kg, and 100% at 5.0 and 6.0 mg/kg. No deaths were observed within the first 48 h with 3.0 mg TMT/kg, although mice developed whole body tremors. This non lethal dose produced behavioral changes that are typically not observed in rats acutely exposed with doses as low as 10.0 mg TMT/kg. The SMA of mice receiving 3.0 mg TMT/kg was reduced to 70% during the first 24 h period following TMT treatment and a partial recovery of total activity was observed during the second 24 h period. The reduction in SMA was accompanied by a change in the

normal circadian cycle of activity. In addition, responding under the mult FR 30 FI 600 schedule was severely disrupted: when tested 3 h following TMT administration, the animals performed at a decreased rate of responding in both components, decrease which became higher during the next 48 h. The rate of FI 600 responding was also altered with an increase in responding, observed in the early portion of the FI. In two later reports [27, 28], TMT behavioral effects in two strains of mice, C57BL/6H and BALB/c, were compared. At 3.0 mg TMT/kg (IP), SMA initially decreased in both strains. However, the decrease was of smaller magnitude in C57BL mice, then a large increase followed. SMA did not return to control levels for approximately 1 week. A SMA increase was observed in the BALB/c strain at 5 days following TMT dosage, with a return to control values by day 6. At 3.0 mg TMT/kg C57BL mice exhibited severe whole body tremors and were hypersensitive to external stimuli. The whole body tremors were not as marked as in the BALB/c strain. Moreover, C57BL mice displayed a significant change in the temporal pattern of fixed interval (FI 30) responding, at 51 h after 1.0 mg TMT/kg dose. In contrast, no considerable change in the BALB/c strain was evident at this dose.

Investigations on the behavioral effects of TMT on nonhuman primates are relatively scarce [7, 13, 16]. Brown *et al.* [7] observed that adult marmosets (*Callithrix jacchus*) treated with an acute oral dose of TMT (3.0 mg/kg) showed fine tremor and frothy salivation, 24 h following treatment. These signs progressed within two days, with an increase of whole body tremors, ataxia, agitation, cries, shrieks, aggression and lost of appetite. The aggression took the form of challenging behavior at the front of the cage, with piloerection of the animal's coat. Most of the marmosets treated with 3.0 mg TMT/kg survived the acute effects and became normal by 7-10 days.

Reuhl *et al.* [13] reported that the clinical progression of TMT intoxication in cynomolgus monkey (*Macaca fascicularis*) was similar to that observed in marmosets. Adult cynomolgus monkeys were exposed to concentrations of TMT ranging from 0.75 to 4.0 mg TMT/kg (IV). Dosages as low as 1.10 mg TMT/kg induced a consistent pattern of clinical changes, emerging within 3 days after TMT exposure among animals surviving intoxication. Initially, the animals were lethargic and feed and water consumption was depressed. The state of lethargy lasted approximately 24 h and was followed by a period of apparent normality where general activity, appetite and behavior were comparable to the pre-treatment levels. At 72 h following TMT dose animals displayed gradual and progressive tremors beginning from the hand during movement, then involving feet and finally the whole body. In addition, hyper-reactivity was evident during this period, comparable to that previously reported in TMT intoxicated rodents [6, 22].

Developmental changes

Some authors have extended their investigations to the developmental toxicity of TMT, in an attempt to provide a better definition of the neuropathogenetic mechanisms of the compound. With this purpose, Chang [41] injected (IP) rat pups with TMT chloride at a dose of 6.0 mg/kg between PND 1-30. No lesions were observed in animals treated between PND 1-4, whereas increasing damage in the Ammon's horn was evident in pups exposed to TMT after PND 5. The severity and extent of damage progressed with treatment at later neonatal ages, with the entire Ammon's horn involvement at PND 13-15. After PND 20 the toxic effects of TMT appeared reduced and were comparable with those observed in adult rats. Ruppert *et al.* [42] found that, on PND 5, acute TMT exposure of rat pups interfered with ontogenetic processes and produced long-term neurobehavioral deficits. Following a single dose of 6.0 mg TMT/kg (IP), the size of the milk bands decreased in pups, 48-96 h after dosing, while in pups receiving 5.0 mg/kg (IP), milk bands were reduced, 96 h after dosing. At dosages of 5.0 or 6.0 mg/kg TMT, a growth decrease and performance impairment in rope descent test during the preweaning period were reported. As adults, motor activity in "figure-eight" maze increased in TMT exposed animals (6.0 mg TMT/kg). The startle response to acoustic stimulus (a 13 khz, 120 dB tone) was also affected by TMT when measured during 30-trial sessions. Amplitudes were decreased in rats receiving 5.0 or 6.0 mg/kg of TMT on postnatal days (PND) 12-13, and in rats receiving 4.0, 5.0 or 6.0 mg/kg of TMT on PND 18-19 and 20-21. Startle amplitude in adults was decreased at all dosages considered. The behavioral changes were accompanied by a decrease in adult brain weight. Miller and O'Callaghan [43] assessed TMT neurotoxicity throughout rat development using functional endpoints, as locomotor activity and learning ability. They observed that following 6.0 mg TMT/kg (IP) administration on PND 5, the ontogeny of locomotion was altered: TMT induced hypoactivity early in the development (PND 13) and hyperactivity by weaning (PND 21). This latter was still evident during adult age. In preweaning alleyway task, animals treated with 6.0 mg TMT/kg required more trials to exhibit a "homing" response and were unable to retain the response over a 30 min delay. In addition, TMT affected learning ability throughout development, as the acquisition of the passive avoidance response. The group exposed to 6.0 mg TMT/kg required almost twice as many days of training before they displayed correct avoidance if compared with controls. In addition, deficient radial arm maze performance was observed after 6.0 mg TMT/kg: differences were found in the accuracy of the task performance. The 6.0 mg TMT/kg dosed group always made more errors than controls, selecting fewer correct arms in the first eight choices. Although

preliminary, the histological results showed damage to the hippocampal formation consisting in pyramidal cell lost in CA_3 and CA_4 of Ammon's horn.

Few reports are present in literature on the effects of prenatal TMT exposure and all have been performed exclusively in rats. Paule *et al.* [44] found that pregnant rats treated on either gestational days (GDs) 7, 12 or 17 with a single dose of TMT at 5.0, 7.0 or 9.0 mg/kg (IP) showed a decrease in maternal weight at term, which persisted during lactation until PND 15. At all the dosages used, the term weights in dams exposed on GDs 7 and 12 were greater than those treated on GD 17. In addition pup toxicity, consisting in the decreased number of surviving animals and weight reduction as adult, was more evident when the highest dose of TMT (9.0 mg/kg) was administered later in gestation (GDs 12, 17). Neuropathological changes were only observed in pups dosed on GDs 12 or 17 and sacrificed on PND 1, and consisted of subtle degenerative lesions in the CA_3 and CA_4 regions of the Ammon's horn.

The effects of prenatal TMT administration on the development and learning in pup rats were investigate by Miyake *et al.* [45]. Pregnant rats were injected (IP) on GD 12 with a single TMT dose of 5.0 or 7.0 mg/kg. Significant differences between treated and control offspring rats were not observed in terms of body weight, pinna detachment, incisor eruption, eye opening, surface righting, cliff avoidance, pivoting, negative geotaxis and auditory startle. SMA and open field behavior were also not affected by TMT. In Sidman avoidance test, however, the avoidance rate of treated offspring rats was lower when compared to that performed by controls. These results suggest that prenatal TMT exposure is capable of disrupting learning acquisition, as reported in adult and postnatal TMT administration.

A proposed neuropathogenetic mechanism of TMT exposure

Based on the numerous neuropathological, neurobehavioral and biochemical investigations, Chang [12] proposed a working hypothesis on the pathogenetic mechanisms of TMT on the nervous system. The author suggested that TMT might act on the inhibitory system (e.g., GABAergic system, inhibitory basket cells and/or other interneurons) of the hippocampus determining a hyperexcitatory state of the granular neurons of the fascia dentata and, consequently, causing a hyper-stimulatory damage to CA_3 pyramidal neurons in the Ammon's horn. The detailed sequence of events can be described in term of a hyperexcitation involving a trisynaptic path and progressing from the pyriform/entorhinal cortex, dentate granule cells of the fascia dentata, CA_3 and $CA_{1,2}$ pyramidal neurons of the Ammon's horn. This hypothesis seems to be sustained by *in vivo* and *in vitro* electrophysiological results [22, 46] which have shown a rapid and marked reduction in the recurrent inhibition in the dentate gyrus of rats, following TMT treatment. The underlying mechanisms of the hyperexcitatory induction by TMT are still unidentified. The eventual neuronal necrosis in the entorhinal cortex may be explained with a long-term sustained hyperexcitatory state. At conclusion of his report the author emphasized that the proposed pathogenetic mechanisms only represented a postulation and a working hypothesis, which needed further investigation to be confirmed. Nevertheless, noteworthy improvements have yet to be achieved in this direction. Further data from other areas of investigation may be helpful in elucidating the composite and intriguing question of the neurological and behavioral toxicity of TMT.

Acknowledgements

This work was supported by grant 9103613, National Research Council (CNR) targeted project "Prevention and Control Disease Factors", Subproject "Disease Factors in the Maternal-Infantile Pathology".

Submitted on invitation.
Accepted on 25 September 1992.

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