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Maternal Exposure of Mouse to Low-Dose of Trichloroethane is Associated with Increased Birth Weight and Early Neonatal Neurobehavioral Abnormalities

Mohamed A. Al-Griw[1], Massaud S. Maamar[1], Naser M. Salama[1], Lubna N. Algadi[1], Abdul Hakim S. Elnfati[1], Emad M. Bennour[2]

[1]Division of Developmental Biology, Zoology Department, Faculty of Science, University of Tripoli, Tripoli, Libya
[2]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Email address

e.bennour@uot.edu.ly (E. M. Bennour)

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Abstract

Maternal exposure to environmental chemicals can adversely affect fetal health. This study aims to identify, *in-vivo*, the risk of maternal exposure to trichloroethane (TCE) on the birth weight and the neurobehavioral performance of newborns. Groups of female albino mice (F0 generation) were injected intraperitoneally twice weekly for three weeks with TCE (100 and 400 µg/kg BW). Animals were followed up for signs of toxicity and mortality. Neonate's motor behavior including large movement (crawling, pivoting, righting) and small movement (tremor) were assessed. No toxicity adverse signs or mortality were observed in the animals (F0 generation) treated with TCE. The results showed that TCE exposure led to a significant increase in the F1 mouse body weight compared to controls. The results also showed that tremor of neonates of dams exposed to TCE (100µg/kg and 400µg/kg BW) were significantly increased when assessed on postnatal day-1 (PND-1). These findings provide support to a role of the environmental toxicant, TCE, in abnormalities in birth weight and neonatal neurobehavior.

Keywords

Environmental Toxicant, Trichloroethene, Motor Behavior, Tremor, Mouse Neonate

1. Introduction

It is increasingly clear that different classes of chemicals present in the workplace and ambient environment play a role in the etiology of many neuropathies. Early exposure to environmental toxicants, such as trichloroethane (TCE), adversely affect CNS vitality later in life [1-5]. TCE, a widely used industrial solvent, especially in metal degreasing process, is a ubiquitous environmental pollutant [6]. TCE is well absorbed following oral and dermal exposure and inhalation. Blood levels approach steady state after approximately two hours of inhalation [7-8]. TCE is rapidly distributed throughout the body [8]. The highest concentrations are found in fatty tissues [7]. Most of the absorbed TCE is rapidly eliminated from the body by being unchanged in the expired air [8]. A small amount is metabolized in the liver to trichloroethanol and trichloroacetic acid, which are excreted in the urine [7-8].

During the fetal life, little changes in the intrauterine environment is critical for development, and may lead to alterations in CNS histology and function, and consequently in behavior [9].

It has been reported that the CNS is the most vulnerable target for TCE following exposure [7]. Most of the effects of TCE are thought to be produced by the parent compound, primarily by interfering with the function of mitochondrial and cellular membranes. Although produced only in low quantities, the metabolites trichloroethanol and trichloroacetic acid are known to have effects on the nervous system and liver, respectively, and may contribute to some observed effects on

these targets [7, 10].

It was also been found that the environmental exposure to TCE is associated with several types of neurological deficit [1], whereas occupational exposure is linked to the changes in neurobehavioral performance [11]. Deficits in neurobehavioral performance tests have been widely reported in humans and animals with acute exposure. Neurodevelopmental effects and neurochemical evidence of gliosis have also been reported in inhalation animal studies of longer duration [9]. Gross CNS depression was also observed following exposure (by inhalation or gavage) to high levels. Deficits in neurobehavioral performance tests have been widely documented in many humans and animals with acute exposure [7-8, 12].

There have been a vast number of animal toxicological studies carried out to evaluate the neurotoxic effect of TCE on pregnant animals and mature animals [12]. However, to the best of authors′ knowledge, little experimental information is currently available about how the *in utero* exposure to TCE affects the neurobehavior activities of newborns shortly postnatally. Thus, the purpose of this study was to evaluate the impact of *in utero* exposure of low-dose of TCE on the neurobehavior of mouse neonates.

2. Materials and Methods

2.1. Animals, Housing and Treatments

A total of twenty four female albino mice), with an age range of 3-4 weeks and weight range of 18–21g, were used in this study. All efforts were made to minimize the pain during animal handling and experimentation and to reduce the number of animals used. Mice were kept under a constant light-dark cycle (dark period from 7:00 pm to 7:00 am) at 24 ± 1ºC and 55 ± 5% relative humidity. Food and drinking water were available ad libitum.

Animals were divided into four groups, of six mice each, and were treated with either 100 or 400 µg/kg of TCE or used as vehicle or sham control. TCE (Baxter International) was suspended in corn oil and administered intraperitoneally at a defined time (10:00 am) every 3rd day. The doses were calculated and delivered in 80-100 µl of corn oil basing on the individual body weight [6, 13]. Vehicle controls were received only the dose of corn oil equivalent to their individual body weight whereas sham controls were not received any treatment.

Two weeks following the end of exposure period, female mice (F0 generation) were mated with fertility confirmed control males (1 male: 2 females ratio). Mating was confirmed by the presence of vaginal plug. Once the plug was observed, the mated female was separated from the male and individually caged. The day the vaginal plug was observed was defined as the day zero of gestation (GD0). The pregnant dams were observed daily to measure the body weight, to further confirm pregnancy, and to notice any adverse clinical signs or abnormal behavior that may result from TCE toxicity. The dams were allowed to deliver naturally and the delivery day was designed as postnatal day 0 (PND0).

2.2. Determination of Newborn Motor Activity

Motor activities of neonate mice were evaluated as previously described [9, 14]. In brief, a subset of pups (F1 generation) from each litter was used. The tare function of an analytical balance was applied to determine the newborn's motor activity. An electric balance (Analytical balance, RADWAG) was used to evaluate the absolute value obtained from the range of fluctuations between weighing values resulting from the newborn movement (crawling, pivoting, righting or tremor) on PND-1 from 10:00 to 11:00 am. The unstable weighing values obtained by the movement of the newborn in the plastic dish on the pan of the balance were recorded manually. The absolute value was defined as the activity of a newborn, and the total activity of a newborn was the sum of the absolute values for five minutes. The plastic dish was changed for each determination of newborn activity. The possibility of reflection was checked using a fixed weight (1-4g) on the balance for three minutes before the measurement of newborn activity in order to ensure that the weights of newborns on the balance did not reflect drift in the value of the balance. The measuring room was maintained under the same experimental conditions as the animal room. The large movement (crawling, pivoting and righting) was defined as a motor activity as it showed an absolute value of 0.0002 or more while the small movement (tremor) of newborn was defined as a motor activity as it showed an absolute value of 0.0001.

2.3. Statistics

Data were expressed as means ± SEM from six female mice of each group using SPSS software, version 20. A computerized Kolmogorov-Smirnov test was used to determine whether the data fitted a normal distribution. One-way ANOVA test followed by Tukey's post hoc comparisons were used to make multiple comparison between treatment groups. Student's t-tests were used to make comparisons between two groups. Mann-Whitney U-test was used for nonparametric samples. Statistical significance was assigned at $P \leq 0.05$.

3. Results

3.1. Effect of TCE on Animal Survival

There were no adverse changes in general condition, delivery process nor the letter number of F0 TCE-treated dams. In addition, no mortality has been recorded among F1 mice in all experimental groups along the course of the experiment, illustrating that the LD50 of this compound will be greater than 400µg/kg BW.

3.2. Effect of TCE on F1 Mouse Body Weight

The results of this study showed that early exposure to low-dose TCE had no effect on the overall body weight in F0

mice as statistical analysis indicated no differences between all groups (data not shown).

The results showed also that TCE exposure at a dose of 100μg/kg had no effect on the F1 mice body weight compared to controls (P=0.735), making 100μg/kg the lowest-observed-adverse-effect level (LOAEL) in this experiment (Figure 1). On the other hand, TCE exposure at a dose of 400μg/kg had significantly increased (P=0.043) the F1 mice body weight compared to controls and 100μg/kg TCE-treated F1 mice (Figure 1). Furthermore, no significant changes were observed in the brain weight of all mice groups (data not shown).

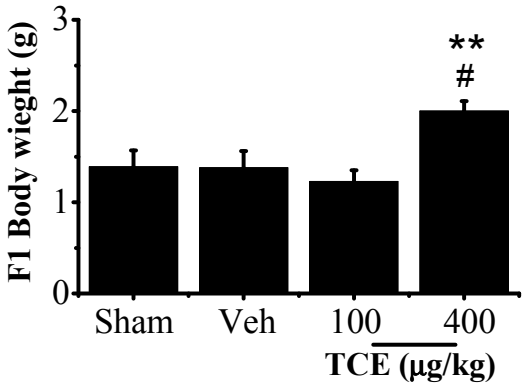

Fig. 1. *Effect of TCE exposure on F1 mice body weight. The body weight of pups were monitored and compared to controls at PND-1. Data represent mean ± SEM (n=6). #Significantly different (P ≤ 0.05) from controls. *Significantly different (P ≤ 0.05) from 100 μg/kg TCE-treated group.*

3.3. Effect of TCE on F1 Mouse Neurobehavioral Performance

The effects of TCE exposure on neurobehavior of F1 neonate mice at an early life stage have also been studied. For that, the large movements (crawling, pivoting and righting) as well as small movements (tremor) of neonate F1 mice have been monitored.

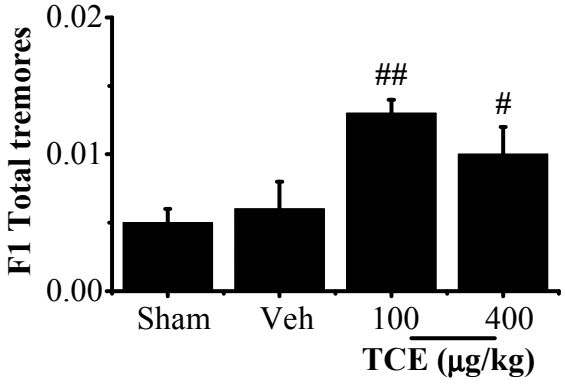

Fig. 2. *Total tremors of PND-1 newborn mice from TCE-treated or control dams. Data represent mean ± SEM (n=6). #Significantly different (P ≤ 0.05) from controls.*

The results of this study revealed no significant change in the total activities of F1 neonate mice of TCE-treated dams comparing to controls (data not shown). However, the total absolute values of 0.001 (tremors) for five minutes in F1 mice neonates of TCE-treated groups were largely increased compared to those in the control groups (Figure 2). In this context and surprisingly, the low dose of TCE (100μg/kg) had induced a higher frequency of tremors in the neonates than the high dose (400μg/kg) (Figure 2).

Taken together, the present study clearly demonstrated the increased tremors in neonates prenatally exposed to TCE, while no significant changes were observed in large movements, such as crawling, righting or pivoting, in the TCE-treated F1 neonate mice.

4. Discussion

It has been documented that the perinatal exposure to TCE affects the development of the brain, and adversely affects their functions [2, 6, 15-17]. It has been also reported that TCE exposure can cause neurobehavioral deficits [18-19]. Despite of the vast number of animal neurotoxicological investigations regarding neurotoxicity of TCE that have been carried out on pregnant and mature animals, there is a paucity of research on animal toxicological effects on newborn animals. Thus, the current study aimed to assess neurotoxic effects of TCE exposure on neonate mice using the previously developed methodology [9, 14].

The central nervous system has been recognized as the principal target of TCE, and several studies of this volatile solvent have demonstrated effects on animal behavior [7]. Recently, we have shown that low level chronic exposure to TCE at an early life stage triggered a significant neurobehavior abnormality of mature F0 mouse in later life [20].

In the current study, we have found that prenatal TCE exposure using a very low dose, comparable to that in humans, significantly increased body weight of F1 mice as well as impaired motor activity of F1 mice. The doses of TCE (100 and 400 μg/kg) used in the present study were below the previously considered a NOAEL (no-observed-adverse-effect level) dose [21-23]. Although the dosing window and dose levels in our experiment were not the same as in previous studies [21-22], our results on TCE-related abnormal behavior are consistent with those studies. Collectively, these data suggest that brains of neonate mice seem to be very sensitive to low-dose TCE exposure, even at environmentally relevant levels, and that developmental TCE exposure has long lasting effects on the nervous system, concluding that TCE could be neurotoxicant.

Body weight of newborns on PND-1 in the 400μg/kg TCE-treated group was comparable to that in 100μg/kg TCE-treated group and controls. Moreover, for the neurobehavioral changes of the newborns on PND-1, no significance difference in the total activities was detected between the TCE-treated groups and controls. However, the total absolute values of 0.0001 (tremors) for five minutes in mice of 100 μg/kg and 400 μg/kg TCE-treated groups were significantly increased compared to those in controls. In addition, there was significant difference in tremors in the 400

μg/kg TCE-treated group comparing to 100 μg/kg TCE-treated group.

The current study clearly demonstrated the increased frequency of tremors in neonates prenatally exposed to TCE, while no significant increase in large movements, such as crawling, righting or pivoting was detected.

An increase in the total activities was found in newborns exposed to TCE at a fairly high dose (750 mg/kg) has been reported [4, 24-25]. The disagreement between these findings and the present study may be attributed to the applied dose of TCE. However, one should consider that human exposure to environmental toxicants is often below toxic dose levels that could cause cell death [4, 24-25].

Tremors have been correlated to specific risk factors such as maternal pathologies, maternal use of drugs or substance abuse, low birth weight or prematurity, electrolyte abnormalities, sepsis or brain lesion, or perinatal complications including perinatal asphyxia [26-27]. Tremors have also been reported in two-thirds of low-risk term-born neonates during the first few days after birth [28]. Tremors associated with neurological signs are generally thought to be part of a more complex neurological pattern, and are often associated with brain lesions or other risk factors [29].

In the present study, the total activities or tremors frequency of newborns exposed prenatally to TCE was comparable to that in controls. The motor behavior of newborn is a regulatory behavior and is orchestrated and coordinated by the neocortex. It is considered that the increase in motor behavior was caused by neocortex anomalies [9, 14]. In other unpublished work, we identified neocortex layer anomalies in TCE-treated mice, suggesting that these anomalies might relate to the abnormal motor behavior of newborn. In addition, we found that the intraperitoneal dosing of TCE was related to brain degeneration. Taking these findings together, it would be reasonable to suggest that the increased tremors of newborns exposed prenatally to TCE may be related to the cerebral cortex damage induced by this compound. Through such the current neurobehavioral toxicity laboratory animal model, a quantitative correlation could be made possible to predict a value for TCE-exposure human risk assessment.

5. Conclusion

The present study revealed that a maternal exposure to low-dose of TCE might lead to early-stage motor hyperactivity in neonate mice. Although the mechanisms of the hyperactivity in newborns exposed prenatally to TCE are unclear, it is tempting to speculate that maternal exposure to TCE might underlie the recent increase in the number of children with neurobehavioral disorders including autism. As perspective, further neurobehavioral studies using this in-vivo model will be performed to explore the correlation between brain damage, including neocortex layer abnormalities, and the neurobehavioral abnormalities of newborns. However, it should be taken into account that the early stage of postnatal brain development in the mouse is congruent with the development of the brain in the human fetus in the later part of gestation.

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