

A Behavioral Teratology Study on the Micrencephalic Rat
Experimentally Induced by
the Treatment with N-methyl-N-nitrosourea

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Recently, documented evidence of behavioral disorder in humans who prenatally exposed to a variety of agents, including alcohol, psychotropic drugs and x-irradiation, strongly indicates the need for behavioral evaluation in the animal experiments, e.g. the safety assessment of new drugs or chemicals.

However, in experimental animals, reproducible and available testing method to evaluate behavioral teratogenicity has not been established, since a few evidences have so far specified relationships between developmental anomalies in the nervous system and behavioral responses in the postnatal period. Therefore, it has been pointed out that to characterize the behavioral disorders resulting from macroscopic or microscopic brain disorders makes an advantage to animal experiments on behavioral teratology.

In the present study, micrencephalic rats were obtained from dams treated prenatally with N-methyl-N-nitrosourea (MNU), one of the known agents having a teratogenic action selectively on the central nervous system, and neurobehavioral functions in the micrencephalic rats were examined in order to characterize the behavioral disorders in relation to the gross brain disorders.

1. The effect of MNU on the development of rat fetuses.

The effect of MNU was assessed on the fetal and postnatal development of rat offspring by giving the drug (2.5, 5 and 10 mg/kg/day) intraperitoneally to pregnant rats during the period of embryonic organogenesis (day 7 to day 17 of gestation). The results were as follows;

10 mg/kg of MNU caused total implantation losses in all dams. No significant increase in the intrauterine mortality was observed up to 5 mg/kg.

Fetuses exposed to 2.5 and 5 mg/kg showed a decrease in body weight. Most of these fetuses revealed flat head as external abnormality that resulted from the small cerebrum involving severe telencephalic hypoplasia (micrencephaly).

As for the neonates, none of neonates exposed to 5 mg/kg survived after birth. However, the postnatal viability was not altered by the exposure to 2.5 mg/kg. This indicates that when appropriate doses are chosen, neonates can survive over a long period though they have severe micrencephaly.

By the behavioral tests conducted during postweaning period, the pups exposed to 2.5 mg/kg revealed remarkable maze learning deficit and hypoactivity that possibly reflected an alteration in emotionality.

2. The effect of MNU on the brain development of rat fetuses.

As a preliminary study on behavioral teratogenic action of MNU, pregnant rats were once treated with 5 mg/kg of MNU on one of gestational days (GD) from 7 to 17, and stage-specific teratogenicity on the developing brain was evaluated in the fetuses.

The vulnerability of the developing fetal brain was confirmed from GD10 to GD17. The sensitive part showing hypoplastic change in the brain

was transferred, and overlapped each other, from mesencephalon, telencephalon, olfactory lobe and metencephalon in that order, with the advance of the day of treatment. Among these brain areas, the telencephalon was the most sensitive area and its vulnerability occurred from GD12 to GD15. GD13 was the period of maximum vulnerability of the telencephalon.

Furthermore, additional examination where the rats were given 2 mg/kg of MNU on GD13 indicated that the micrencephaly was dose-dependently induced in the fetuses without other malformations.

3. Behavioral disorders in the micrencephalic rat induced by the MNU-treatment.

On GD13, the maximum vulnerable stage of telencephalon, micrencephalic rats were induced by the treatment with 2 or 5 mg/kg of MNU and they were subjected to a variety of tests for neurobehavioral ontogeny (behavioral development tests) during the suckling period, and for emotionality, maze and active avoidance learning in the postweaning period, from 4 to 10 weeks of age.

By the tests for neurobehavioral ontogeny, micrencephalic pups exposed to 5 mg/kg showed retardation in ontogeny of spinal reflexes and postural reactions, early hyperreflexia, and dysmetric abnormality in the limbs. Furthermore, as a distinctively impaired behavior, paired pelvic limb-movement was observed in the pups while walking or swimming. A few pups exposed to 2 mg/kg showed similar impairment. However, most of 2 mg/kg pups did not show other behavioral abnormalities except for slight retardation in the neurobehavioral ontogeny.

As for the tests conducted after weaning, neophilic hyperreactivity of the 5 mg/kg pups and slight hypoactivity of the 2 mg/kg were observed by

the test for emotionality. Maze learning ability was impaired remarkably in the 5 mg/kg pups and slightly in the 2 mg/kg. By the active avoidance tasks, abnormal high response was occurred in the 5 mg/kg pups at all through sessions and at early in the 2 mg/kg.

These results indicate that the MNU-treatment could induce specific type of behavioral disorders in the rat offspring but the type and magnitude of the bahavioral teratogenic effect probably depend on the dose of MNU.

4. Early neurobehavioral disorders in the micrencephalic rats.

Micrencephalic neonatal pups were obtained from pregnant rats once treated with 5 mg/kg of MNU or 40 mg/kg of methylazoxymethanol (MAM), as a reference compound, on GD12, 13, 14 or 15. They were reared by their own mothers and were subjected to various neurobehavioral tests during the suckling period, days 0 to 22 after birth. The brain weights in the MNU- and MAM-treated pups on postnatal day 22 were significantly less than those in the control pups. These micrencephalic pups were retarded in neurobehavioral ontogeny. By several tests, each of them showed an impaired performance such as paired limb movement, clumsy locomotion or hyperreflexive reaction. These behavioral disorders appeared different according to the day of treatment, without any substantial difference between the test compounds, MNU and MAM. The findings suggest that the different neurobehavioral characteristics in the micrencephalic pups may reflect their different brain disorders induced by the test compounds given on the different period of the treatment.

5. Degree of micrencephaly and disorders of the integrated behavior.

Microcephalic offspring were obtained from rats treated with MNU at a

dose of 1, 2, 3, 4 or 5 mg/kg on GD13. Brain weights of weanlings in the MNU treated groups were significantly reduced to approximately 95%, 85%, 80%, 70% and 55% of the controls, respectively, in the 1 to 5 mg/kg groups. Open-field test (for emotionality), wheel cage activity test (for spontaneous activity levels), Biel type water T-maze test (for maze learning ability) and shuttle-box avoidance test (for avoidance learning ability) were subsequently performed to determine the behavioral alterations in these offspring at 4, 5-6, 6-7 and 7-8 weeks of age, respectively. In the open-field test, the scores of ambulation and rearing significantly increased in all treated groups and the latent period significantly decreased in the groups treated with 3 mg/kg or more. Moreover, a significant increase of urination and a significant decrease of grooming and uncoordinated movement of the pelvic limbs were observed in the 5 mg/kg group. Spontaneous motor activity in the wheel cage also increased in all treated groups. In the water T-maze test and shuttle-box avoidance test, however, alteration in learning ability was detected only in the 5 mg/kg group. These findings suggest that hyperactivity is the fundamental disorder in these microcephalic rats and alteration of emotionality occurs dose-dependently. Threshold level on the learning deficit is higher than that on any other disorders.

6. Disorders of the integrated behavior in the micrencephalic rats.

To determine the stage specific effect of MNU on the integrated behavior, micrencephalic rats were obtained from dams once treated with 1, 3 or 5 mg/kg of MNU on GD12, 13, 14 or 15 and their emotionality, maze and active avoidance learning, and spontaneous locomotor activity levels were examined during the postweaning period, from 4 to 10 weeks of age.

The brain disorders appeared different according to the day of

treatment, and GD15-treatment caused the least effect.

As for the behavioral disorders, by the test for emotionality, a hyperreactive response was noticed in the GD13-treated pups and, in contrast, both GD12- and GD15-treated pups showed marked hyporeactivity. However, spontaneous locomotor activity in the GD15-treated pups was equivalent to the control levels, though other GD12- and GD13-treated pups revealed corresponding response to their emotionality. The period of maximum susceptibility of maze learning was GD15 and that of avoidance learning was GD13. This result seems that localization of lesions in the central nervous system corresponding to the maze learning deficit differs from that to the active avoidance learning. No dose-related change was examined in any behavioral test in the GD14-treated pups.

Generally, the above findings suggest that the type and magnitude of behavioral teratogenic effect of MNU depend on the stage and dose of the treatment, even in the sort period of organogenesis, from GD12 to GD15. Furthermore, the tests for neurobehavioral ontogeny, learning and activity would be useful to evaluate behavioral teratogenicity.