Evaluation of neurotoxicity of artificial dura mater and dura mater containing a high concentration of dibutyltin in rats after intracranial implantation

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Objectives: A synthetic biodegradable artificial dura mater (DM) can provide various benefits including avoiding the risk of virus infection. The major component is poly-L-lactides. For synthesis of the DM, a catalyst containing dibutyltin (DBT) is used. To evaluate the safety of the artificial DM, the effects of the artificial DM and a DM containing 100 ppm DBT (DBT-DM) on the behaviors in rats after intracranial implantation were evaluated using behavior tests and quantification of neurotransmitters in brain regions.

Methods: A circular disk of cranial bone 5 mm in diameter was cut from each rat's skull. The artificial DM or DBT-DM was implanted on the surface of the brain of each rat (the DM and the DBT-DM groups). The control group underwent a sham operation. Four weeks after the implantation, the prepulse inhibition (PPI) test and open field test were performed as indexes for learning, activities, and adaptation. The neurotransmitters in discrete brain regions were determined by HPLC (high-performance liquid chromatography).

Results: The mean %PPI at PP80 in the DBT-DM group was significantly lower than that in the control, but there were no significant differences among the groups in any indexes in the open field test. The mean value of dopamine was significantly lower compared to the control in the cerebellum in the DBT-DM group and hypothalamus in the artificial DM group.

Conclusion: The behaviors tested may be affected by the implantation of the DBT-DM but not by that of the artificial DM.

Key words: artificial dura mater, prepulse inhibition test, open field test, dibutyltin, rat

Introduction

A synthetic biodegradable artificial dura mater (DM) can provide various benefits including avoiding disadvantages such as the risk of chronic inflammation and virus infection of a biomaterial. Repeat surgery to remove artificial DM is not needed for biodegradable materials.

The major components of the artificial DM are poly-L-lactides and catalysts including dibutyltin (DBT) for polymerization. Because DBT remains in the artificial DM, the brain tissue is directly exposed to DBT when the artificial DM is absorbed after implantation. Under normal circumstances, the penetration of DBT across the blood-brain barrier is quite low, and for this reason, a few studies evaluating the neurotoxic potential...
of DBT have been performed.5-7 Alam et al. reported the neurotoxicity of DBT at very high doses.5 They administered dibutyltin dilaurate to rats at 40 mg/kg or 80 mg/kg orally for three consecutive days and observed lower concentrations of dopamine (DA) and 5-hydroxytryptamine (5-HT) compared to that in the control. In their study, behavioral changes were also observed among the rats administered dibutyltin dilaurate at 20 mg/kg and higher. In an in vitro study, Jenkins et al. demonstrated that DBT dichloride at concentrations of 0.1 μM and higher inhibited neurite outgrowth and induced cell death in cultured PC12 cells.6 In another in vitro study, it was suggested that DBT dichloride at concentrations of more than 0.5 μM show neurotoxicity in the brain and cause cell death and inhibition of cell proliferation in cultured astrocyte cells.7

Tributyltin (TBT), which is a parent compound of DBT in the environment, is neurotoxic.3,4 Behavioral changes in F1 rats were reported after the administration of TBT to two generations.8 The alteration of dopamine metabolism in the midbrain of mice due to the subacute administration of TBT was reported.9 DBT was detected in the brain of rats that were administered TBT orally at a dose of 40 μg/g, and the observed neurotoxicity induced by the administration of TBT might be partly due to DBT.6

Based on the in vitro and in vivo toxicity of high doses of DBT and TBT, DBT most likely has neurotoxicity when the brain is exposed to it. Because of the possible neurotoxicity of DBT, evaluation of the safety of artificial DM in which DBT remains is required before clinical application. To evaluate the toxicity, an adequate model of actual clinical application must be established. If the implantation operation is performed correctly, an intracranial implant of artificial DM in experimental animals would be an excellent model of an actual clinical application. The alterations in neurotransmitters and their metabolites have been used as indexes of neurotoxicity at the relatively lower levels of exposures to various toxic compounds11,12 as well as TBT.9 Whether the concentrations of neurotransmitters are altered by implantation of artificial DM or not may be a good indicator of toxicity.

The objectives of the present study were to establish an animal model for the clinical implantation of artificial DM and to evaluate the safety of artificial biodegradable DM. Artificial DM or DM containing a high concentration (100 ppm) of DBT (DBT-DM) as a possible positive control was implanted intracranially into rats as a model of a clinical application. The neurotoxic effects on the rats were evaluated by using behavior tests (PPI and open field tests) and determining the neurotransmitter levels in discrete brain regions.

**Materials and Methods**

Male Wistar rats, 9 weeks of age, were purchased from Japan Clea (Tokyo). Rats were divided into three groups (n = 10 per group): the control, artificial DM, and DBT-DM groups.

The implanted membranes were artificial DM (poly-L-lactides, molecular weight =5,000, tin concentration <10 ppm, Nacalai Tesque, Kyoto) and DBT-DM (custom-made, 300 μm thick, poly-L-lactides with DBT concentration 100 ppm as tin, Kawasumi Laboratories).

Under anesthesia by pentobarbital, the head of each rat was fixed with a stereotaxic instrument (SR-6R, Narishige, Tokyo). A special auxiliary bar with a round tip was used to secure fixation of the rat's ears. A circular disk of bone 5 mm in diameter was cut from the cranium of each anesthetized rat using a system composed of a drill and micromotor (Osada Success 40M2, Osaka Medical, Tokyo) with a bone trephine bar, 6 mm in diameter (BTB-60, Hasegawa Medical, Tokyo). A circular sheet of the DM or the DBT-DM approximately 4 mm in diameter was implanted on the surface of the brain. After implantation, the bone disk was returned to the skull. The control group underwent the sham surgical operation without the DM implantation.

Rats were housed two to a cage and maintained on commercial rodent chow for 4 weeks after the implantation. The food and water intake was recorded daily for each cage. The mean daily food or water intake per rat was calculated from the consumption of food or water per cage. The body weight of each rat was recorded every day. After 4 weeks, the PPI test and the open field test were carried out on two consecutive days.
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PPI test
The PPI test was performed by a method described in previous studies.\textsuperscript{13-15} A Startle Response System SR-LAB ABS System (San Diego Instruments, San Diego, CA, USA) composed of a startle chamber with a floor equipped with an electric sensor and a speaker mounted 24 cm above the floor to present an acoustic noise burst was used. Each rat was placed in a cylindrical holder in the chamber and allowed to acclimate for 5 minutes before the test session. In the PPI test session, there were three types of prepulses in the session: a burst of 70 dB (PP70 dB), one of 75 dB (PP75 dB), and one of 80 dB (PP80 dB). The acoustic stimulation without prepulse was also given in the session, i.e., the four types of acoustic stimulation given to each rat in a pseudo-random order were: a startle pulse of a burst of 120 dB with (P alone), combined trials of PP 70 dB followed with a pulse of 120 dB (PP70&P), PP75 dB followed with a pulse of 120 dB (PP75&P), and PP80 dB followed with a pulse of 120 dB (PP80&P). The numbers of acoustic stimulations in a test session were: 11 for P alone, PP70&P, and PP75&P, 10 for PP80&P. There were also 10 times of no acoustic stimulations. The startle response was measured by an electric sensor. The mean value of responses for respective stimulations in each session was calculated. The percent prepulse inhibition (%PPI) of a startle response was calculated by the following formulae.\textsuperscript{15}
\[
\%\text{PPI at PP70} = (1 - \text{PP70&P/P alone}) \times 100
\]
\[
\%\text{PPI at PP75} = (1 - \text{PP75&P/P alone}) \times 100
\]
\[
\%\text{PPI at PP80} = (1 - \text{PP80&P/P alone}) \times 100
\]

Open field test
On the next morning after the PPI test, each rat was placed in the center of a square white box (width, 1.0 m; height, 0.5 m) for the open field test described in previous studies.\textsuperscript{13-15} The locomotor behavior of each rat was recorded by using Image Open Field 2.15r (O’hara, Tokyo), and the total locomotor distance and locomotor distance for every 5 minutes were calculated. During the 30-minute observation, the following behaviors were recorded as indexes of activity, adaptation, and emotion: the time of the first grooming for more than 3 seconds and the number of instances of wall rearing, center rearing, face washing, body washing, defecation, and urination.

Determinations of neurotransmitters and their metabolites
After being weighed, each rat was decapitated, and the brain was removed and weighed after observation of the cranium and the surface of the brain. The implanted DM or DBT-DM was sampled. Each brain was dissected on ice into its seven different regions: the cerebrum, cerebellum, medulla oblongata, midbrain, corpus striatum, hypothalamus, and hippocampus according to the method of Glowinski and Iversen.\textsuperscript{16} All tissue sampling was conducted at midafternoon (2:00-4:00) to avoid possible diurnal alterations in neurotransmitter levels.\textsuperscript{17} Brain samples were immediately soaked in ice-cold 0.05 M perchloric acid (Wako, Osaka) with 0.1% cysteine (Nacalai Tesque, Kyoto) in vials. The ratio of extraction solvent was approximately 1:4 (tissue weight/solvent volume). After weighing, each sample was homogenized, centrifuged, and filtered through a 0.2 μm pore-size filter (Millipore, Bedford, MA, USA). The filtrate was stored at -80°C until analysis.

The concentrations of catecholamines (norepinephrine [NE], DA), the DA metabolites dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA]), indoleamine 5-HT and a 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in each sample extract were determined simultaneously by high-performance liquid chromatography (HPLC) with electrochemical detection according to the previous study.\textsuperscript{18} The analysis system consisted of a GL Science ED623 Electrochemical Detector (GL Science, Tokyo), Hitachi L-6250 pump (Hitachi, Tokyo), GL Science DG660 degasser (GL Science), and a Sugai U620V #50 column heater with a temperature controller (Sugai Chemie, Wakayama). A reversed phase column, Inertsil ODS-3, 4.6 × 150 mm, particle size 5 μm (GL Science) was used for chromatography. The mobile phase composed of 9.6 g/l citric acid, 100 mg/l sodium octane sulfate, 40 mg/l EDTA (ethylendiaminetetraacetic acid) and 15% methanol. Samples were eluted at 35°C for 40 minutes at a flow of 0.75 ml/minute.

A calibration standard (100 ng/ml) containing NE bitartate, DA hydrochloric acid, DOPAC, HVA, 5-HT creatinine sulfate, and 5-HIAA dicyclohexylammonium (Sigma) in 0.05 M perchloric acid with 0.1% cysteine was employed. Detection limits were 5 ng/ml for 5-HT, 2.5 ng/ml for NE, DOPAC, HIAA and HVA, and 1.25 ng/ml for DA.

Statistical analysis
The mean values of indexes of behavioral tests, and the mean values (μg/g wet tissue) of the neurotransmitters, and their metabolites in brain regions, were calculated for all the groups. In addition, the ratios of DOPAC/DA, HVA/DA, and 5-HIAA/5-HT were also calculated. The data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's PLSD (protected least
significant difference) test using StatView J-5.0 software (SAS Institute, Cary, NC, USA). The level of significance was P < 0.05.

**Results**

The mean daily food intake was approximately 30 g per rat over the observation period. There was no significant difference in daily food intake among the groups on any day during the observation period. A significant difference in the mean daily water intake per rat between the group implanted with the artificial DM and the group implanted with the DBT-DM was observed on day 23 after the operation (the mean value ± standard error was 70.0 ± 3.4 ml for the control, 76.2 ± 4.3 ml for the DM group, and 58.0 ± 5.1 ml for the DBT-DM group). There were no significant differences in daily water intake on any other day during the observation period.

There were no significant differences in the body weight among the groups over the observation period. The mean body weight ± standard error for each group on the day of the PPI test was 494.8 ± 5.9 g for the control, 496.8 ± 10.8 g for the artificial DM group, and

![Figure 1. Reduction of prepulse inhibition in rats intracranially implanted with artificial dura mater (DM) or DM containing dibutyltin (DBT-DM).](image)

The control and artificial DM groups, n = 10 rats, each; the DBT-DM group, n = 9 rats.

Each bar represents the mean value, and error bars represent standard errors. P = 0.403 by ANOVA for %PPI at PP70, P = 0.125 by ANOVA for %PPI at PP75, P = 0.037 by ANOVA for %PPI at PP80

*P < 0.05 compared with the control, #P < 0.05 compared with the DM group by Fisher’s PLSD (protected least significant difference) test.

![Figure 2. Locomotor activity of rats implanted intracranially with artificial DM or DBT-DM in open field tests.](image)

The control and DM groups, n = 10 rats, each; the DBT-DM group, n = 9 rats.
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504.2 ± 12.3 g for the DBT-DM group.

The surfaces of the brains and implanted DM were observed. No serious damage to the surface of the cerebral cortex of the rats was observed in any group. The DM was at least partly caught between the skull and regenerated bone in 5 rats in the artificial DM group and 7 rats in the DBT-DM group. The membrane was completely covered by the regenerated bone in 3 rats in each group. However, in only 1 rat in the DBT-DM group the membrane was extruded from the cranium by the regenerated bone, which excluded it from the analyses. The mean weight ± standard error of sampled membranes was 1.17 ± 0.27 mg for the artificial DM and 0.93 ± 0.21 mg for the DBT-DM.

The results of the PPI test are illustrated in Figure 1. The mean %PPI value of PP80 in the DBT-DM group was significantly lower than that in the control or the artificial DM group. Although the mean %PPI value at PP70 or PP75 in the DBT-DM group was lower, the differences among the groups were not statistically significant.

The results for locomotor activity in the open field test are illustrated in Figure 2(A), showing the results of the open field test for locomotor distance every 5 of 30 minutes, and 2(B) showing the total locomotor distance for 30 minutes. There were no significant differences in total and 5-minute locomotor distances among the groups.

The mean numbers of typical behaviors in the groups in the open field test are illustrated in Figure 3(A) and the mean of times of the first grooming in the groups are illustrated in Figure 3(B). There were no significant differences among the groups in the numbers of typical behaviors or the times of the first groomings.

The concentrations of NE in discrete brain regions of rats implanted with an artificial DM or DBT-DM are shown in Table 1. There were no significant differences in NE concentrations in any brain region among the groups revealed by ANOVA.

The concentrations of DA and its metabolites in discrete brain regions of rats implanted an artificial DM or DBT-DM are shown in Table 2. The mean DA values were significantly lower than that of the control in the hypothalamus in the DM group and the cerebellum in the DBT-DM group. There were no significant differences in DA metabolite concentrations in any other brain region among the groups.

The concentrations of 5-HT and its metabolite 5-HIAA in the seven discrete brain regions of rats implanted with

(A) Numbers of typical behaviors
(B) Time of first grooming

(A) The mean numbers of typical behaviors of rats in the open field during the 30-minute test period and standard errors are indicated. WR, wall rearing; CR, center rearing; FW, face washing; BW, body washing. There were no significant differences among the groups in any of the behaviors as revealed by ANOVA.

(B) The mean values of the time of the first grooming in the open field test and standard errors are shown. There were no significant differences among the groups revealed by ANOVA.

Figure 3. (A) Numbers of typical behaviors and (B) time of first grooming of rats intracranially implanted with artificial DM or DBT-DM in the open field test.

The control and artificial DM groups, n = 10 rats, each; the DBT-DM group, n = 9 rats. Each bar represents the mean value, and error bars represent standard errors.
Table 1. The concentrations of norepinephrine (NE) in discrete brain regions of rats implanted with an artificial dura mater (DM) or DM containing a high amount of dibutyltin (DBT-DM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Medulla</th>
<th>Midbrain</th>
<th>Striatum</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.295 ± 0.013</td>
<td>0.219 ± 0.012</td>
<td>0.444 ± 0.018</td>
<td>0.461 ± 0.023</td>
<td>0.446 ± 0.045</td>
<td>2.629 ± 0.146</td>
<td>0.547 ± 0.050</td>
</tr>
<tr>
<td>DM</td>
<td>0.271 ± 0.012</td>
<td>0.222 ± 0.015</td>
<td>0.387 ± 0.021</td>
<td>0.434 ± 0.026</td>
<td>0.435 ± 0.036</td>
<td>2.599 ± 0.130</td>
<td>0.542 ± 0.052</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.291 ± 0.020</td>
<td>0.231 ± 0.020</td>
<td>0.388 ± 0.044</td>
<td>0.457 ± 0.023</td>
<td>0.370 ± 0.021</td>
<td>2.455 ± 0.168</td>
<td>0.407 ± 0.025</td>
</tr>
</tbody>
</table>

Mean values (μg/g) ± standard errors are indicated (the control and the artificial DM group, n = 10, each; the DBT-DM group, n = 9).

Table 2. The concentrations of dopamine (DA) and DA metabolites in discrete brain regions of rats implanted with an artificial DM or DBT-DM

<table>
<thead>
<tr>
<th>Neurochemicals</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Medulla</th>
<th>Midbrain</th>
<th>Striatum</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.903 ± 0.040</td>
<td>0.011 ± 0.001</td>
<td>0.039 ± 0.005</td>
<td>0.480 ± 0.069</td>
<td>0.537 ± 0.123</td>
<td>0.558 ± 0.045</td>
<td>0.135 ± 0.042</td>
</tr>
<tr>
<td>DM</td>
<td>0.930 ± 0.029</td>
<td>0.008 ± 0.001</td>
<td>0.039 ± 0.001</td>
<td>0.363 ± 0.057</td>
<td>0.575 ± 0.119</td>
<td>0.382 ± 0.042</td>
<td>0.131 ± 0.035</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.923 ± 0.034</td>
<td>0.007 ± 0.001*</td>
<td>0.033 ± 0.005</td>
<td>0.438 ± 0.084</td>
<td>0.392 ± 0.079</td>
<td>0.441 ± 0.060</td>
<td>0.122 ± 0.033</td>
</tr>
<tr>
<td>DOPAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.131 ± 0.009</td>
<td>0.023 ± 0.005</td>
<td>0.018 ± 0.002</td>
<td>0.133 ± 0.025</td>
<td>0.145 ± 0.034</td>
<td>0.123 ± 0.009</td>
<td>0.165 ± 0.051</td>
</tr>
<tr>
<td>DM</td>
<td>0.134 ± 0.007</td>
<td>0.038 ± 0.016</td>
<td>0.016 ± 0.001</td>
<td>0.086 ± 0.010</td>
<td>0.196 ± 0.073</td>
<td>0.132 ± 0.018</td>
<td>0.139 ± 0.038</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.133 ± 0.009</td>
<td>0.033 ± 0.011</td>
<td>0.014 ± 0.002</td>
<td>0.113 ± 0.015</td>
<td>0.096 ± 0.015</td>
<td>0.123 ± 0.017</td>
<td>0.191 ± 0.037</td>
</tr>
<tr>
<td>HVA</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.083 ± 0.004</td>
<td>0.018 ± 0.003</td>
<td>0.014 ± 0.002</td>
<td>0.054 ± 0.009</td>
<td>0.063 ± 0.011</td>
<td>0.078 ± 0.010</td>
<td>0.083 ± 0.011</td>
</tr>
<tr>
<td>DM</td>
<td>0.086 ± 0.006</td>
<td>0.015 ± 0.002</td>
<td>0.017 ± 0.003</td>
<td>0.041 ± 0.003</td>
<td>0.088 ± 0.026</td>
<td>0.103 ± 0.010</td>
<td>0.100 ± 0.018</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.087 ± 0.006</td>
<td>0.015 ± 0.002</td>
<td>0.014 ± 0.003</td>
<td>0.052 ± 0.006</td>
<td>0.054 ± 0.008</td>
<td>0.089 ± 0.008</td>
<td>0.083 ± 0.016</td>
</tr>
</tbody>
</table>

Mean values (μg/g) ± standard errors are indicated (the control and the artificial DM group, n = 10, each; the DBT-DM group, n = 9).

Table 3. The concentrations of serotonin (5-HT) and 5-HT metabolite, 5-HIAA in discrete brain regions of rats implanted with an artificial DM or DBT-DM

<table>
<thead>
<tr>
<th>Neurochemicals</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Medulla</th>
<th>Midbrain</th>
<th>Striatum</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.541 ± 0.089</td>
<td>0.064 ± 0.013</td>
<td>0.590 ± 0.029</td>
<td>0.552 ± 0.034</td>
<td>0.700 ± 0.052</td>
<td>1.199 ± 0.079</td>
<td>0.135 ± 0.042</td>
</tr>
<tr>
<td>DM</td>
<td>0.415 ± 0.061</td>
<td>0.057 ± 0.006</td>
<td>0.479 ± 0.001</td>
<td>0.607 ± 0.049</td>
<td>0.642 ± 0.018</td>
<td>1.145 ± 0.104</td>
<td>0.131 ± 0.035</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.458 ± 0.068</td>
<td>0.061 ± 0.008</td>
<td>0.553 ± 0.059</td>
<td>0.500 ± 0.018</td>
<td>0.738 ± 0.083</td>
<td>1.190 ± 0.078</td>
<td>0.122 ± 0.033</td>
</tr>
<tr>
<td>5-HIAA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.294 ± 0.027</td>
<td>0.115 ± 0.010</td>
<td>0.375 ± 0.047</td>
<td>0.459 ± 0.017</td>
<td>0.469 ± 0.018</td>
<td>0.648 ± 0.090</td>
<td>0.416 ± 0.049</td>
</tr>
<tr>
<td>DM</td>
<td>0.289 ± 0.031</td>
<td>0.109 ± 0.015</td>
<td>0.418 ± 0.033</td>
<td>0.442 ± 0.027</td>
<td>0.526 ± 0.059</td>
<td>0.733 ± 0.108</td>
<td>0.429 ± 0.050</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.293 ± 0.043</td>
<td>0.115 ± 0.013</td>
<td>0.307 ± 0.052</td>
<td>0.460 ± 0.028</td>
<td>0.494 ± 0.015</td>
<td>0.650 ± 0.085</td>
<td>0.306 ± 0.042</td>
</tr>
</tbody>
</table>

Mean values (μg/g) ± standard errors are indicated (the control and the artificial DM group, n = 10, each; the DBT-DM group, n = 9).
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Table 4. The DOPAC/DA or HVA/DA ratio in discrete brain regions of rats implanted an artificial DM or DBT-DM

<table>
<thead>
<tr>
<th>Neurochemicals</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Medulla</th>
<th>Midbrain</th>
<th>Striatum</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPAC/DA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.145 ± 0.006</td>
<td>2.169 ± 0.446</td>
<td>0.843 ± 0.407</td>
<td>0.272 ± 0.024</td>
<td>0.306 ± 0.042</td>
<td>0.234 ± 0.023</td>
<td>2.317 ± 1.154</td>
</tr>
<tr>
<td>DM</td>
<td>0.143 ± 0.006</td>
<td>3.900 ± 1.247</td>
<td>0.410 ± 0.032</td>
<td>0.257 ± 0.018</td>
<td>0.300 ± 0.036</td>
<td>0.396 ± 0.085</td>
<td>2.113 ± 1.028</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.143 ± 0.006</td>
<td>5.617 ± 2.608</td>
<td>0.783 ± 0.418</td>
<td>0.293 ± 0.030</td>
<td>0.268 ± 0.021</td>
<td>0.297 ± 0.030</td>
<td>4.466 ± 1.859</td>
</tr>
<tr>
<td>HVA/DA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.092 ± 0.004</td>
<td>2.022 ± 0.435</td>
<td>0.531 ± 0.161</td>
<td>0.116 ± 0.009</td>
<td>0.151 ± 0.025</td>
<td>0.153 ± 0.024</td>
<td>1.345 ± 0.492</td>
</tr>
<tr>
<td>DM</td>
<td>0.092 ± 0.006</td>
<td>2.175 ± 0.537</td>
<td>0.437 ± 0.067</td>
<td>0.135 ± 0.018</td>
<td>0.152 ± 0.016</td>
<td>0.307 ± 0.050*</td>
<td>1.182 ± 0.201</td>
</tr>
<tr>
<td>DBT-DM</td>
<td>0.094 ± 0.006</td>
<td>2.190 ± 0.197</td>
<td>0.779 ± 0.402</td>
<td>0.143 ± 0.016</td>
<td>0.156 ± 0.016</td>
<td>0.242 ± 0.050</td>
<td>1.345 ± 0.492</td>
</tr>
</tbody>
</table>

Mean values ± standard errors are indicated (the control and the artificial dura mater groups, n = 10, each; the DBT-DM group, n = 9). *P < 0.05 compared to the control by Fisher’s PLSD (protected least significant difference) test as a post hoc test.

An artificial DM or DBT-DM are shown in Table 3. There were no significant differences in 5-HT or 5-HIAA concentrations in any of the brain regions between the groups revealed by ANOVA.

The DOPAC/DA and HVA/DA ratios in discrete brain regions of rats implanted with an artificial DM or DBT-DM are shown in Table 4. The mean HVA/DA value was significantly higher in the hypothalamus of the DM group than that in the control.

Discussion

The development of biodegradable artificial DM offers various benefits such as avoiding repeat neurosurgery to remove the nondegradable artificial DM. However, such clinical application will directly expose a patient’s brain to DBT remaining in the artificial DM. Therefore, prior to any clinical application of artificial DM, its toxicity must be evaluated. DBT has strong cytotoxicity on various cells such as macrophages and PC12 cells. Administration of a high dose of DBT induced neurotoxicity in rats. Therefore, neurotoxicity was the focus of this study. Behavior tests have been used for the evaluation of neurotoxicity. Among them, PPI test and open field test could be adequate screening tests. In addition, alterations in neurotransmitters and their metabolites have been used as indexes of the neurotoxicity of various toxic compounds at relatively low levels of exposures including TBT. Alterations in the concentrations of neurotransmitters due to implantation of artificial DM may be good indicators of toxicity.

In the present study, as a model for neurosurgery, artificial DM or DBT-DM was implanted onto the surface of the brain of rats. DBT-DM was used as a possible positive neurotoxic material. The effects were evaluated 1 month after implantation to allow time for the degradation of the artificial DM in the cranium after surgery.

There were no significant differences in food and water intake or body weight during the observation period among the groups except for the water intake on day 23 after the implantation. These data indicate that the general condition of the rats did not differ significantly among the groups. In addition, no serious damage to the surface of the cerebral cortex was observed in the sampling period among the groups of rats implanted with the artificial DM or the DBT-DM. Except for 1 case in which the DBT-DM was extruded from the cranium, there were no technical problems due to the implantation procedure.

The mean value of %PPI at PP80 of the DBT-DM group was significantly lower than those of the control and DM groups. Although there were no significant differences, the mean values of %PPI at PP70 and %PPI at PP75 in the DBT-DM group were also lower than that of the respective control. Therefore, the functions of cognition and learning might be impaired in the DBT-DM implantation group. It was also suggested that PP80 was more sensitive to differences between the groups compared with PP70 and PP75.

There were no significant differences among the groups in the open field test. An open field test was used to measure various behavior responses such as locomotor activity and emotion in rodents. The total and 5-minute locomotor distances traveled were used as indexes of locomotor activity. The time of the first grooming for more than 3 seconds was used as the index of adjustment. The frequencies of wall rearing and center rearing events were counted as indexes...
of exploration. There were no differences in these indexes among the groups in the present study. Under the conditions of the current protocol, implantation of the DM and the DBT-DM did not affect the indexes of emotion and exploration in the open field test.

The neurotransmitters and their metabolites in discrete brain regions have been used as indexes of the neurotoxicity of DBT and TBT. In the current study, the mean DA value was significantly lower compared to the control hypothalamus in the DM group and in the cerebellum in the DBT-DM group. For the DBT-DM group, the difference was limited to the cerebellum, which is not involved in the main central neuronal pathways containing DA. In a previous study, administration of DBT at the very high doses of 40 mg/kg or 80 mg/kg for three consecutive days lowered the concentrations of DA and 5-HT compared with the control. The subacute administration of TBT at 125 ppm in their food also induced alterations in DA metabolism in the midbrain of mice. However, because the decrease in DA induced by the implantation of DBT-DM was limited to the cerebellum, it may not be a common toxic effect, which was suggested in previous studies.

A decrease in DA and an increase in HVA/DA in the DM group was observed only in the hypothalamus, which is involved in the main central neuronal pathways containing DA. Whether or not these alterations are related to toxicity of the artificial DM remains to be evaluated in future studies. The physical pressure produced by the implantation of DM intended for humans into the cranium of a rat may effect the alteration in the hypothalamus.

A limitation of the present study is due to the rapid regeneration of bone. The DM and the DBT-DM were sandwiched by regenerated bone and cut-out bone. Even if the DM sample was completely covered by bone, resorption of the sample occurred inside the cranium until it was completely covered by bone. However, to bridge the discrepancy between the model used in this study and actual surgery, it is necessary to create a condition in which each sample continues to dissolve on the surface of the brain. It may be useful to increase the diameter of the hole in the bone by 1 cm, as a larger hole in the cranium would allow implantation of a larger sample. Further studies to clarify this point are, therefore, warranted.

Evaluation of the safety of the artificial DM revealed no significant differences in behavioral tests between the control and DM groups. Alterations in neurotransmitters due to the artificial DM were limited to DA in the hypothalamus. This suggests that there are no serious drawbacks to the implantation of artificial DM under the current protocol. However, when the DM contained a high concentration of DBT, alterations in the PPI test results were observed. The PPI test may be useful for the evaluation of neurotoxicity in the animal model for clinical application of artificial DM. The toxic effects of DBT in artificial DM warrant further study.

Acknowledgement
This work was partially supported by Health and Labour Sciences Research Grants on Regulatory Science of Drug and Medical Devices from Japanese Ministry of Health, Labour and Welfare.

References


Neurotoxicity of artificial dura mater in rats