APPEARANCE OF INTOXICATION IN RATS BY INTRAPERITONEAL ADMINISTRATION OF CLOQUINOL*

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The investigation was undertaken to study the neurological symptoms in rats caused by maintaining high plasma concentration of about 30 nmol/ml or more, of cloquinol. Cloquinol suspension which was prepared using polysorbate 80 was administered intraperitoneally to rats and plasma and tissue concentrations were determined. On administration of cloquinol of 100 and 200 mg/kg, the mean plasma concentrations of cloquinol reached maximum values of 30 and 58 nmol/ml, respectively, after 0.5—1 h and thereafter decreased rapidly. With 400 mg/kg, however, plasma concentration reached maximum value of about 75 nmol/ml and fell slowly. By single and repeated administration of the suspension, cloquinol was distributed in the liver and kidney at a high concentration, and also in the nervous system. In experiments on appearance of neurotoxicity in rats by repeated administration of the suspension, all of 10 rats administered intraperitoneally with 100 mg/kg/d did not develop any neurological symptoms for about 30 d. On the other hand, one of 10 and 7 of 13 rats administered with 200 and 400 mg/kg/d, respectively, developed ataxia in the hind legs or all legs on the 3rd to the 12th day after starting administration. Pathologically, a slight change of the peripheral nerve, central chromatolysis of the anterior horn neuron and severe neuronal degeneration of the Ammon's horn were observed in the rats with ataxia.

Keywords —cloquinol; rat; cloquinol suspension for injection; intraperitoneal administration; plasma concentration; tissue concentration; neurological symptoms; pathological examination

INTRODUCTION

Cloquinol (chinoform, 5-chloro-7-iodo-8-quinolinol) and its ferric chelate were isolated and identified from the green urine and feces of subacute myelo-optico-neuropathy (SMON) patients. Upon this opportunity, relationship between occurrence of SMON and cloquinol medication was surveyed epidemiologically and it was suggested strongly that SMON was caused by the medication. The suggestion has been supported by animal experiments with dogs, cats and a monkey, which showed neurological symptoms and pathological changes similar to SMON by repeated oral administration of cloquinol. In spite of many attempts of investigators, however, neurological symptoms had been scarcely caused in small animals such as rats and mice.

On the other hand, we consider from the previous studies, that the plasma concentration of cloquinol requires to be maintained around 30 nmol/ml for the occurrence of neurodegeneration. We also found that a large part of cloquinol was converted to cloquinol glucuronide during passage through the intestinal wall of rat and so that, in rat, it was difficult to maintain

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the high plasma concentration of clioquinol by oral administration.

In this paper, intraperitoneal administration of clioquinol was tested as an administration route other than oral one to cause the neurological symptoms in rats by maintaining high plasma concentration of clioquinol.

MATERIALS AND METHODS

Materials and Reagents — Clioquinol, kindly supplied by CIBA-GEIGY (Japan) Ltd. (Osaka), was recrystallized twice from ethanol. 5-Chloro-7-bromo-8-quinolinol used as an internal standard (I.S.) was the same as that used previously.\(^9\) Polysorbate 80 was obtained from Tokyo Kasei Kogyo Co. (Tokyo). Distilled water for injection was obtained from Hikari Seiyaku Co. (Tokyo). Alumina was obtained from Wako Pure Chemical Industries (Tokyo). All other solvents and reagents used were of reagent grade.

Experimental Animals — Eleven- to twelve-week-old male Wistar rats weighing 320–380 g (Japan Laboratory Animals Co., Tokyo) were used in all the experiments and kept in a well ventilated cage (Ikemoto Chemical Industrial Co., Tokyo) at 21±2°C. The rats were fed with a solid feed, MF made by Oriental Yeast Co. (Tokyo), and water was allowed ad libitum.

Preparation of Clioquinol Suspension for Injection — Suspension for injection containing clioquinol of 24, 48 or 96 mg per milliliter for intraperitoneal administration to rats was prepared by the following procedure. Clioquinol of 2.4, 4.8 or 9.6 g was added to about 5 ml of chloroform in a mortar and was ground manually with a pestle to become a fine powder, and was well ground with 4 ml of polysorbate 80 to become a creamy mixture. To remove chloroform in the mixture completely, the mortar was kept in a desiccator under reduced pressure at ambient temperature for over 20 min. A suspension was prepared by gradually adding distilled water for injection to the mixture while grounding and finally making up to 100 ml. About 1.8 ml each of this suspension was divided into ampule. The ampule was sealed by heating and the suspension autoclaved at 115°C for 30 min. The mean particle size of clioquinol in the suspension (Green diameter) was obtained by microscopy when 300 particles were sized and the mean size was 5.0 μm (range: 1.2–16.5 μm).

Intraperitoneal injected volume per injection was 4.2 ml per kg of body weight.

Blood Sample Collection — A polyethylene cannula was inserted into a femoral artery of rat without anesthesia. The clioquinol suspension was administered intraperitoneally to rat at single dose of 100, 200 or 400 mg/kg, and blood sample (ca. 0.3 ml) was collected in heparinized tubes through the cannula at 15 and 30 min, and 1, 3, 5, 7 and 24 h after administration. Plasma was separated from blood by centrifugation with a KN-70 centrifuge (Kubota Seisakusho, Co., Tokyo) at 3000 rpm for 10 min and stored at −20°C until analysis.

Tissue Sample Collection — The clioquinol suspension was administered intraperitoneally to rats at single dose of 100 and 400 mg/kg, or repeated dose of 400 mg/kg/d. Blood sample was collected by heart puncture at an appropriate time after administration. Immediately, rats were sacrificed by bleeding and liver, kidney, lung, muscle, cerebrum, spinal cord (cervical, thoracic and lumbar cord) and sciatic nerve were removed. The liver, kidney and lung were rinsed with normal saline solution and blotted with filter paper to remove excess moisture. Plasma was separated from blood in the same manner as described above. Each tissue and plasma samples were stored at −20°C until analysis.

Determination of Clioquinol Concentration — Clioquinol Concentration in Plasma: Clioquinol concentration in plasma was determined according to the gas chromatographic-mass spectrometric (GC-MS) method described in the previous paper.\(^9\)

Clioquinol Concentration in Liver, Kidney, Lung and Muscle: To 0.1–0.2 g of tissue in centrifuge tube was added 4 ml of benzene–pyridine (9:1, v/v) mixture containing a known amount of the I.S. The mixture was homogenized using a
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homogenizer (Chemitron, Ikemoto Chemical Industrial Co.) on an ice bath. The homogenate was shaken for 5 min and centrifuged at 3000 rpm for 5 min. The upper organic phase was washed twice with 2 ml of 0.1 M acetate buffer (pH 5). Then 0.2—0.5 ml of the organic phase was transferred into another centrifuge tube and evaporated to dryness on a rotary vacuum evaporator. The residue was analyzed by the GC-MS method described in the previous paper.9)

Clioquinol Concentration in Nervous Tissue: Extraction of clioquinol from nervous tissue was performed according to a minor modification of the method of Tamura et al.7a)

To 0.05—0.1 g of nervous tissue was added 4 ml of benzene-pyridine (7:3, v/v) mixture containing a known amount of the IS. The mixture was homogenized in the same manner as described above. The homogenate was sonicated for 5 min, shaken for 5 min, and centrifuged at 3000 rpm for 5 min. The upper organic phase was washed once with 2 ml of 0.1 M acetate buffer (pH 5). The organic phase was transferred into another centrifuge tube containing 100 mg of alumina. To completely adsorb clioquinol on the alumina, the tube was allowed to stand for 15 min and rotated slowly several times during the period, and then the organic phase was aspirated off. The alumina in the tube was washed with 1 ml of acetone by rotating slowly several times the tube and the solvent was aspirated off. Furthermore, the alumina was washed with 1 ml of 0.1 M acetic acid in methanol and 2 ml of methanol successively, in the same manner as described above. Then, the alumina was added to 1 ml of saturated solution of NaF. Extraction was repeated twice with 3 ml of benzene each time. The combined organic phase was kept overnight with 0.2 g of anhydrous sodium sulfate. The organic phase was evaporated to dryness on the rotary evaporator. The residue was analyzed by the GC-MS method described in the previous paper.9)

Toxicity Test of Clioquinol — Fourty-three rats were divided into 4 groups of A, B, C and control. Group A, B and C consisted of 10, 10 and 13 rats, respectively, which were administered intraperitoneally repeated with clioquinol at a dose of 100, 200 and 400 mg/kg/d, respectively. Control consisted of 10 rats, which were administered with 4% polysorbate 80 aqueous solution (4.2 ml/kg of injected volume each). Rats were individually isolated and housed in stainless steel cages. The administration was rested at least once a week, and occasionally when general status of rat was observed to become worse.

Pathological Examination — Four rats which developed ataxia were sacrificed by perfusion of a fixative solution through the heart. Two rats received only 4% polysorbate 80 aqueous solution intraperitoneally were also sacrificed by the same method described above, and examined as control. The fixative consists of the initial solution of 4% paraformaldehyde in 0.05 M cacodylate buffer (pH 7.4) and the second solution of 2% paraformaldehyde and 2.5% glutaraldehyde in the same buffer. Brain, spinal cord and peripheral nerves including spinal ganglion were taken, and after sectioning, they were divided into the samples for histological and electron microscopic examinations. Histological examinations were carried out routinely and the sections were stained with Hematoxylin-eosin, Luxol Fast Blue for myelin stain, Bodian’s method for axon stain and GFA protein immunohistochemical method for astrogial stain. For electron microscopic studies, the samples were minced, post fixed with 1% OsO₄ solution, dehydrated in ethanol and embedded in Epoxy resin. After polymerization, the thin sections were stained with 1% toluidine blue, and the selected areas doubly stained with uranyl acetate and lead citrate were surveyed with a JEOL 100C electron microscope (JEOL, Tokyo).

RESULTS
Plasma Concentration of Clioquinol after Single Administration

The clioquinol suspension was administered intraperitoneally to rats at single dose of 100,
200 and 400 mg/kg. The mean concentration-time courses of clioquinol in plasma are shown in Fig. 1. Clioquinol, in these doses, was detected in the first blood sample withdrawn from each rat at 15 min after administration. At 100 and 200 mg/kg, plasma concentration in each rat reached maximum at 30 min and 1 h after administration and their mean were 30 and 58 nmol/ml, respectively. Thereafter the plasma concentrations of clioquinol were decreased rapidly and quite low at 24 h after administration. On the other hand, at 400 mg/kg dose, the maximal plasma concentration of clioquinol was observed at 3 h after the administration and the mean concentration was 75 nmol/ml. Thereafter plasma concentration of clioquinol decreased slowly and the mean concentration was about 20 nmol/ml at 24 h after administration.

**Tissue Concentration of Clioquinol after Single Administration**

The clioquinol suspension was administered intraperitoneally to rats at single dose of 100 and 400 mg/kg. Plasma and tissue concentrations of clioquinol at 0.5, 7 and 24 h after administration of a dose of 100 mg/kg are shown in Table I. Plasma and tissue concentrations of clioquinol were the highest at 0.5 h after administration. Clioquinol concentrations in the liver and kidney were higher than those in the other tissues. As for the nervous system, concentration of clioquinol was higher in the sciatic nerve than in the cerebrum and spinal cord at 0.5 h after administration, and the ratio of the mean concentration in the sciatic nerve versus that in the plasma was 1.4. Clioquinol concentration in each tissue was much decreased after 7 and 24 h.

Plasma and tissue concentrations of clioquinol at 2, 7 and 24 h after administration of a dose of 400 mg/kg are shown in Table II. Two hours after the administration, the clioquinol concentration was especially high in the liver and kidney. As for the nervous system, the concentration was the highest in the sciatic nerve, and the ratio of the mean concentration in the sciatic nerve.

![FIG. 1. Plasma Concentration-Time Courses of Clioquinol after Single Intraperitoneal Administration of Clioquinol (100, 200 and 400 mg/kg) to Rats](image)

(a): 100 mg/kg, (b): 200 mg/kg, (c): 400 mg/kg. Each points represents the mean of four rats and the vertical bar indicates the standard deviation.
nerve *versus* that in the plasma was 1.4. Evidently, clioquinol concentration in each tissue was maintained approximately the same level at 7 h and without much decline even at 24 h.

**Toxicity Test of Clioquinol**

In order to study neurotoxicity of clioquinol in rats, the clioquinol suspension was repeatedly administered intraperitoneally. The results are shown in Fig 2. Neurological symptoms were not observed in control group and group A during examination period of 28–30 d. A decrease of the body weight was not also seen in these groups during the administration. Ataxia was definitely noted in all legs of 1 of 10 rats of

| TABLE I. Plasma and Tissue Concentrations of Clioquinol after Single Intraperitoneal Administration of Clioquinol (100 mg/kg) to Rats |
|---|---|---|
| Plasma and tissue | Concentration (nmol/ml or g) |
| | 0.5 h Mean ±S.D. | 7 h Mean ±S.D. | 24 h Mean ±S.D. |
| Plasma | 23.6±15.7 | 2.9±0.6 | 0.4±0.3 |
| Liver | 135.4±65.5 | 149±3.0 | 9.1±6.2 |
| Kidney | 126.5±38.1 | 10.2±7.9 | 8.6±6.3 |
| Lung | 17.2±10.6 | 0.1±0.1 | Trace |
| Muscle | 6.2±3.3 | 0.9±0.3 | 0.3±0.2 |
| Cerebrum | 8.8±3.9 | 1.7±1.0 | Trace |
| Cervical cord | 11.5±5.0 | 1.8±0.6 | Trace |
| Thoracic cord | 10.9±4.1 | 2.5±1.2 | Trace |
| Lumbar cord | 10.8±4.3 | 2.1±0.4 | Trace |
| Sciatic nerve | 32.5±14.3 | 3.7±2.0 | Trace |

The data represent the mean ± standard deviation (S.D.) of four rats at each time.

| TABLE II. Plasma and Tissue Concentrations of Clioquinol after Single Intraperitoneal Administration of Clioquinol (400 mg/kg) to Rats |
|---|---|---|
| Plasma and tissue | Concentration (nmol/ml or g) |
| | 2 h Mean ±S.D. | 7 h Mean ±S.D. | 24 h Mean ±S.D. |
| Plasma | 53.3±13.2 | 50.1±14.1 | 14.6±4.7 |
| Liver | 363.9±96.9 | 501.1±313.4 | 125.4±59.0 |
| Kidney | 841.1±268.6 | 682.5±260.9 | 361.8±94.6 |
| Lung | 82.7±26.8 | 58.0±34.4 | 33.8±6.5 |
| Muscle | 9.2±3.6 | 6.4±1.7 | 3.3±1.6 |
| Cerebrum | 24.3±7.9 | 21.8±11.0 | 8.2±2.6 |
| Cervical cord | 37.3±12.7 | 21.8±10.9 | 14.4±4.6 |
| Thoracic cord | 33.7±9.8 | 17.7±10.0 | 13.4±4.9 |
| Lumbar cord | 35.3±10.5 | 22.9±10.9 | 18.5±6.2 |
| Sciatic nerve | 74.1±7.2 | 52.3±26.5 | 32.1±7.2 |

The data represent the mean ± standard deviation (S.D.) of four rats at each time.
group B on the 12th day after starting the administration. The body weight of group B decreased gradually. Ataxia was definitely noted in the hind legs or all legs of 7 of 13 rats of group C.

![Graph showing neurological symptoms and intraperitoneal Clioquinol dose](image)

**FIG. 2. Relationship between Appearance of Neurological Symptoms and Intraperitoneal Clioquinol Dose in Rats**

- : appearance of ataxia, ◦: death due to convulsion, ×: death due to general weakness, *: suspension of administration.

![Images of rats with ataxia symptoms](image)

**FIG. 3. A Rat on the 5th Day after Onset of Ataxia Involving All Legs**
on the 3rd to 12th day after starting the administration. The body weight of group C decreased more markedly than that of group B. Death due to convulsion was seen in 1 rat of group B and 4 rats of group C. The convulsion appeared within 30 min after the administration, and was soon followed by death. Death due to general weakness was also seen in 2 rats of group C. On the other hand, green feces was found in all of the group A, B and C from the second day of administration. Green urine was noted in 2 rats of group C during the administration period. A typical example of a rat with ataxia is shown in Fig. 3. In the early stage of onset of ataxia, rats showed an awkward hip-raising gait and marked instability. Ataxia progressed on hind legs or all legs. The rats raised their head and moved their necks freely, therefore the ataxia was not due to weakness.

Tissue Concentration of Clioquinol after Repeated Administration

Plasma and tissue concentrations of clioquinol in rats with ataxia in all legs by repeated intraperitoneal administration of clioquinol are shown in Table III. Plasma and each tissue concentrations of clioquinol 24 h after the last administration to rat-1 and rat-2 tended to be higher than those after single administration as indicated in Table II. In these rats, very high concentrations of clioquinol were observed in the liver and kidney, and also the high concentrations were observed in the lung. As for the nervous system, concentration of clioquinol was higher in the sciatic nerve than in the cerebrum and spinal cord in these rats. In rat-3, whose administration was suspended for 3 d after the onset of the ataxia, clioquinol was still retained in plasma and each tissue. On the other hand, in rat-4 and rat-5, convulsion occurred within 30 min after administration and died soon thereafter. Clioquinol concentrations in the nervous system at the time of death were significantly higher than

<table>
<thead>
<tr>
<th>Plasma or tissue</th>
<th>Rat-1 a)</th>
<th>Rat-2 a)</th>
<th>Rat-3 b)</th>
<th>Rat-4 c)</th>
<th>Rat-5 d)</th>
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</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>40.4</td>
<td>23.0</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>756.6</td>
<td>381.8</td>
<td>89.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>1065.6</td>
<td>831.1</td>
<td>160.2</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Lung</td>
<td>156.8</td>
<td>112.6</td>
<td>28.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscle</td>
<td>16.8</td>
<td>9.8</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>20.6</td>
<td>8.7</td>
<td>3.8</td>
<td>106.3</td>
<td>70.5</td>
</tr>
<tr>
<td>Cervical cord</td>
<td>26.2</td>
<td>16.2</td>
<td>3.5</td>
<td>146.2</td>
<td>121.4</td>
</tr>
<tr>
<td>Thoracic cord</td>
<td>25.7</td>
<td>14.9</td>
<td>3.9</td>
<td>164.3</td>
<td>115.5</td>
</tr>
<tr>
<td>Lumbar cord</td>
<td>26.5</td>
<td>15.6</td>
<td>6.7</td>
<td>143.6</td>
<td>127.8</td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>74.2</td>
<td>92.9</td>
<td>18.7</td>
<td>310.7</td>
<td>193.9</td>
</tr>
</tbody>
</table>

Intraperitoneal dose: 400 mg/kg/d.
—: not determined.

a) On the 3rd day after starting administration, ataxia appeared in all legs. The rat was sacrificed at 24 h after administration on the 3rd day.
b) On the 3rd day after starting administration, ataxia appeared in all legs and then the administration was suspended. The rat was sacrificed on the 6th day.
c) On the 4th day after starting administration, ataxia appeared in all legs. The rat developed convulsion within 30 min after administration on the next day, and died soon.
d) On the 12th day after starting administration intermittently, ataxia appeared in all legs. The rat developed convulsion within 30 min after administration on the 16th day, and died soon thereafter.
those of the other rats.

Pathological Examination

Four rats who developed ataxia were sacrificed on the 1st, 5th, 14th and 24th day after the onset, and were examined pathologically. The long fiber tracts of the spinal cord and optic nerve were well preserved in all cases. In the rats sacrificed on the 14th and 24th day, the anterior horn neurons, however, showed the ballooning with dispersed Nissl granules (Fig. 4a), in contrast the neurons of the control rats for a month were well preserved (Fig. 4b). The neuronal bal-

FIG. 4. Changes of the Anterior Horn Cells
   a) Chromatolytic changes of the anterior horn cells of rat receiving 400 mg/kg/d Dose of Clioquinol, 14 d after onset of ataxia.
   b) Well preserved nissl granules in the control rat.
   Klüver-Barrera stain. × 200.

FIG. 5. Diffuse Neuronal Loss in Ammon’s Horn of Rat, Receiving 200 mg/kg/d Dose of Clioquinol, 24 d after Onset of Ataxia
   Hematoxylin-eosin stain. × 30.

FIG. 6. Marked Proliferation of Hypertrophied Astrocytes in Degenerated Area of Ammon’s Horn from the Same Rat of Fig. 5
   GFA protein immunohistochemical stain. × 400.
looning with loss of Nissl granules was more prominently observed in the neurons of medul-

lary reticular formation and thalamus in these rats. In the brain, the degeneration of Ammon's horn was detected in the three rats sacrificed later on the 5th day after the onset of the ataxia. The neurons of Sommer's sektor were markedly degenerated and diminished in number diffusely (Fig. 5) which was well demonstrated in the rat sacrificed on the 24th day. This change was associated with markedly hypertrophic astrocytes (Fig. 6). No pathological changes were found in the two control rats. In the peripheral nerve, the spinal ganglion and proximal portion of the sciatic nerve were relatively well preserved, but in the rats sacrificed later on the 5th day, the distal part of the nerves running the ulnar and plantal parts showed a bubble-like disintegration of the myelin sheath (Fig. 7a). This type of myelin change could not be detectable in the control rats (Fig. 7b). Electron microscopically, the degeneration of myelin sheaths was more easily recognized at the node of Ranvier (Fig. 8). The axons, unmyelinated fibers and Schwann cells were free from any noticeable changes.

DISCUSSION

Brückner et al.\(^6\) and Tateishi et al.\(^6\) reported that rats did not develop the neurological symptoms by oral clioquinol administration of the large dose even for a long period. Jones et al.\(^5\) however, reported that rats had paralysis of the hind legs between 125 and 132 weeks after feeding them with a maize diet containing clioquinol. Thereafter, Murai et al.\(^4\) reinvestigated Jones et al.'s report and stated that feeding with its diet containing clioquinol did not lead to appearance of neurological symptoms. Thus, on the appearance of neurological symptoms in rats, a clear proof has not been made.

Consequently, we studied administration route of clioquinol other than an oral one to cause the neurological symptoms to rats. From preliminary examinations on preparation of clioquinol suspension, it was found that a fine suspension of clioquinol was successfully prepared by using polysorbate 80. Intraperitoneal administration of this suspension was found to be effec-
tive to increase the plasma level of clioquinol. With repeated clioquinol doses of more than 200 mg/kg/d, rats developed ataxia in the hind legs or all legs. These facts demonstrate that the intraperitoneal administration of the fine suspension is effective to cause the ataxia, since higher plasma concentration of clioquinol is maintained by the repeated administration.

Undissolved clioquinol in the abdominal cavity of rats at 7 h after administration was not seen at single dose of 100 and 200 mg/kg, whereas a fair amount remained at 400 mg/kg. From these findings, it is considered that plasma concentration of clioquinol with 400 mg/kg is maintained at higher level than that with 100 and 200 mg/kg, due to prolonged supply of clioquinol in case of 400 mg/kg.

However, repeated administration with 400 mg/kg/d increases the frequency of death by convulsion. In order to avoid the acute death and increase the frequency of the appearance of the ataxia, it may be necessary to make a further study on optimal dosage of clioquinol.

Some rats developed convulsion within 30 min after administration during the repeated administration and the rats died soon. The cause for the death is not clear. However, it seems that clioquinol directly disturbed the function of the nervous system, since the concentration of clioquinol in the nervous system at the time of death was abnormally high (Table III, rat-4 and rat-5).

In single and repeated administration of clioquinol, clioquinol was observed in the nervous system and its concentrations were always higher in the sciatic nerve than in the cerebrum and spinal cord. These results are in disagreement with the findings that the concentrations in the central nervous system were close to those in the peripheral nervous system after repeated oral administration to beagle dogs.

Pathologically, the degeneration of long fiber tracts of the spinal cord, which is the most characteristic findings of the SMON patients, was not observed in the rats. Instead, a slight change of the distal part of the peripheral nerves and the chromatolytic change of anterior horn cells could be produced experimentally. These changes may be responsible for the ataxia. Moreover, the involvement of Ammon’s horn was most striking findings in the rats. Puschner and Fankhauser in their experiment of clioquinol intoxication described the Ammon’s horn degeneration in mice. This findings was confirmed by Tateishi, but the mice showed convulsive seizure and the results were interpreted that the Ammon’s horn lesion could be secondarily induced by convulsion. In our experiments, however, the rats examined pathologically did not develop convulsion. Moreover, in a few cases of SMON patients, the Ammon’s horn was reported to be the target of clioquinol intoxication. Therefore, our experiments may provide a good experimental model of Ammon’s horn involvement in SMON. As for the reasons of intact long fiber tracts of the spinal cord, it is considered that administration period of clioquinol after onset of ataxia is too short to show the overt degeneration, and/or that the degeneration differs in species of animals.

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