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Abstract

In order to know the organ distribution of Chinoform, I4C.Chinoform was injected into the tail vein of the mice, and radioactivity was measured in the chloroform soluble fractions in some organs and tissues containing non.conjugated Chinoform. The results obtained are as follows. 1. Uptake of Chinoform by the visceral organs was found to be in the following ascending order: fat tissue, kidney, spleen, liver, small intestine, (blood), muscle and eye, and marked uptake by the fat tissue and kidney was observed. 2. The presence of radioactive Chinoform in the chloroform soluble fraction of the central nervous system was recognized and it was almost in the same degree of specific radioactivity as that of blood. 3. A higher uptake in the chloroform soluble fraction of the sciatic nerve than that of central nervous system was recognized, and the value of the former was about 3 to 8 times as high as that of the latter. 4. The presence of Chinoform in the chroloform soluble fraction of the bile, although it increased after incu bation of the bile with j9-glucuro. nidase was observed. High radiogctivity of chinoform in the total fraction of the bile suggests a possible presence of “liver-intestine-circulation” of the drug.

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METABOLISM OF ^{14}C-IODOCHLOROHYDROXYQUINOLINE (CHINOFORM) IN MICE

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5-Chloro-7-iodo-8-hydroxyquinoline (Chinoform) is a well-known intestinal antiseptic in the world-wide use. BERGGREN, L. *et al.* (1) drew attention by reporting that optic atrophy has followed long-term medication with Chinoform. It is often stated that Chinoform is not absorbed in the gastrointestinal tract. But urinary excretion of the glucuronic and sulfuric acid conjugates of Chinoform given orally to rabbit (2) and man (3～5) has been reported.

In Japan, so-called subacute myelo-optico-neuropathy with abdominal symptoms (SMON) was first reported by TAKASAKI (6) in 1961 in Mie Prefecture, central part of Japan. Since then, the number of patients of SMON has been found increasing in many places throughout Japan, and the number of patients reported during 1967 to 1968 reached 4, 280 (7). The clinical symptoms of SMON show signs of lateral corticospinal tracts and of posterior column disturbances (8) ensuing from abdominal symptoms, and 89.5 per cent of patients show sensory disturbances and 60.8 per cent motor disturbances and 23.5 per cent are accompanied by visual disturbances (7).

Recently, it is suspected that long term administration of Chinoform may be one of the causal factors of this syndrome (9). In the present study the distribution in the visceral and nervous tissues and bile-excretion of Chinoform in mice were investigated. The results are briefly described here.

MATERIALS AND METHODS

Materials:

ddN-Strain female mice weighing about 20 grams were used. Animals were kept in a stock on *ad libitum* diet. The day before the experiment, animals were administered *ad libitum* 1 per cent iodine solution, orally as thyroid blocking agent.

Substance: Chinoform -2, 3, 4, -^{14}C (specific activity: 1.63 mCi/mmole) was purchased from Daiichi Pure Chemicals Co., Ltd. Purity analyses by radio-thinlayer chromatography on silica gel in methanol: benzene: acetone: acetic acid [4:14:1:1] and benzene: methanol: acetic acid [45:8:9] showed that radioactive, ^{14}C-Chinoform has 99 per cent purity. The radioactive Chinoform was emulsified in carboxylmethyl cellulose suspension and served as materials for injection. Each mouse was injected 0.3 mCi Chinoform into tail vein.

Methods:

Mice were sacrificed one hour after the injection by means of blood drawing from orbital vein. Organs and tissues were separated respectively, and their wet weight was measured. Each sample was homogenized with 3 volumes of water in a teflon homogenizer for 5 minutes, thereafter, 3 ml chloroform was added, rehomogenized and centrifuged. Then chloroform soluble layer containing non-conjugated Chinoform (3) was separated by centrifugation, transferred to a counting vial and dried by blowing air. The dried material thus obtained was dissolved in 0.5 ml of methyl alcohol-hyamine solution, added 15 ml of toluene containing PPO and POPOP, and radioactivity was measured in a Packard liquid scintillation spectrometer.

The radioactivity was expressed as dpm by external standardization method.

RESULTS

Table 1 shows the distribution of radioactivity (dpm/gr, wet weight) in the chloroform soluble fractions of various organs. Between two mice, there is no significant difference in the distribution in visceral and nervous organs.

In the kidney and fat tissue of mice, uptake is most marked, 14, 326 dpm/gr., w. w. and 14, 692 dpm/gr., w. w. respectively. These values are about 6 to 7 times as much as much as radioactivity of blood (2, 384 dpm/gr., w. w.). The order of radioactivity in other visceral organs is as follows: spleen, liver, small intestine, muscle and eye. The level of radioactivity in these organs is similar to that of blood. The presence of radioactivity in various parts of the central nervous system was recognized, and the values of brain stem (2, 397 dpm/gr., w. w.), spinal cord (cervical, 2, 877 dpm/gr., w. w.; thoracic and lumbar, 3, 159 dpm/gr., w. w.) were higher than those of cerebrum (1, 469 dpm/gr., w. w.) and cerebellum (2, 103 dpm/gr., w. w.). A great deal of radioactivity was found in the sciatic nerve (10, 936 dpm/gr., w. w.). The value was about 3 to 5 times as high as that of central nervous system or 5 times as high as that of blood. This fact was also found in our previous experiment of rat administered ^{131}I-Chinoform (9, 10), and agrees with the experiments with rabbits (12) or rats (13), in which experimental neuritis of the sciatic nerve

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE CHLOROFORM SOLUBLE FRACTION OF VARIOUS ORGANS IN THE MICE AFTER INJECTION OF ^{14}C-CHINOFORM

organ	[dpm/gr. wet weight] mouse 1	mouse 2	mean
cerebrum	1,370	1,568	1,469
cerebellum	1,917	2,288	2,103
brain stem	2,285	2,508	2,397
cervical cord	3,001	2,752	2,877
thoracic & lumbar cord	2,929	3,388	3,159
sciatic nerve	11,379	10,493	10,936
eye	1,673	1,001	1,337
liver	2,983	2,565	2,774
kidney	11,817	16,835	14,326
spleen	4,713	4,591	4,652
small intestine	2,785	2,862	2,734
fat tissue	13,588	15,796	14,692
muscle	1,435	2,506	1,971
blood	2,875	1,892	2,384
bile	509,934*	17,543*	263,739*

* Radioactivity in the total fractions (chloroform soluble and insoluble fractions)

was induced by the administration of Chinoform, The data indicate that much higher specific radioactivity in the total fraction (Chloroform soluble and insoluble fraction) was found in the bile (263, 739 dpm/gr., w. w.). In the experiment, the total radioactivity of bile was about 110 times as high as that of blood. In the case of mouse 2, the ratio of radioactivity in the chloroform soluble fraction to insoluble fraction in the bile was 1 : 6 and that in the bile after incubation with β-glucuronidase (Fishman unit, 1000, for 45 min. at 37°C in phosphate buffer 0.15 M, pH 6.0) was 2 : 1. Therefore, it is considered that large portion of Chinoform in bile conjugated with glucuronic acid.

DISCUSSION

In the present experiment, radioactivity was measured with the chloroform soluble fractions obtained from various organs. The values show the distribution of most of free Chinoform, which can be combined with metals and exhibit high toxicity to the various organs. It is by now well known that administration of Chinoform substantially elevates the iodine content in the blood. LIEWENDAHL *et al.* (4) showed in their experiment administred ^{125}I-Chinoform orally, that iodine corresponding to 0.4 per

cent of Chinoform could be liberated from this agent within 24 hours in man. In our experiment, we used ^{14}C-Chinoform instead of ^{125}I-Chinoform and also administered iodine to mice before and during the experiment to prevent Chinoform binding to thyroxine and liberation of iodine from the Chinoform. Therefore, the effect of iodine liberation will be smallest. Our results suggest that some part of Chinoform is excreted into bile in the form of glucuronic acid conjugates, as the free Chinoform content in the bile increases after incubation with β-glucuronidase. This result agrees with the finding in our experiment described previously (10), in which ^{131}I-Chinoform was used as a tracer, and the presence of some free chinoform in the bile to be excreted into intestine suggests some possibilities of chinoform reabsorption. Therefore, a presence of a liver-intestine circulation of this drug is conceivably possible. A high level of specific radioactivity in the fat tissue indicates a possible accumulation of Chinoform in this tissue.

The organs measured in this experiment contain a small amount of blood. LAJTHA *et al.* (14) described that the maximum value of blood contained in the central nervous system, liver and muscle is, 0.9 per cent, 3.2 per cent and 0.9 per cent respectively by measurement of CO-Hb in these organs. And BOSSE *et al.* (15) reported that the quantity of blood contained in the organs was 1.98 ± 0.11 ($m+\sigma$) per cent in cerebrum, 2.7 ± 0.18 per cent in cerebellum, 3.02 ± 0.22 per cent in medulla oblongata, 2.45 ± 0.22 per cent in spinal cord, and 2.17 ± 0.24 per cent in sciatic nerve.

In the visceral organs and also in the central and peripheral nervous systems, uptake of ^{14}C-Chinoform was demonstrated by the presence of true radioactivity after subtracting blood radioactivity from measured radioactivity in studying the chronic intoxication of Chinoform. The problem, whether the ^{14}C-Chinoform can penetrate blood brain barrier or not, is yet to be reported. Chloroform soluble extract of contents of a small intestine containing 14.4% of total activity with a new mouse will be expressed on our next paper.

CONCLUSION

In order to know the organ distribution of Chinoform, ^{14}C-Chinoform was injected into the tail vein of the mice, and radioactivity was measured in the chloroform soluble fractions in some organs and tissues containing non-conjugated Chinoform.

The results obtained are as follows.

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