Changes in Lipid Peroxide Concentrations in Plasma and Tissues by Repeated Administration of Clioquinol to Neonatal Rats

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The changes in lipid peroxide concentrations in plasma and tissues after subcutaneous administration of clioquinol to clioquinol-sensitive (S-rats) and -resistant neonatal rats (R-rats) were investigated. When a fixed dose of 150 mg/kg/d of clioquinol was given to R-rats for 14 d after birth, no significant difference in lipid peroxide concentrations in plasma, liver, kidney, brain and spinal cord at 5, 10 and 15 d was observed between clioquinol-treated and untreated rats. However, with increasing doses of clioquinol to R-rats every 5 d (150 — 300 — 600 mg/kg/d), the lipid peroxide concentrations at 15 d were higher in plasma, brain and spinal cord of clioquinol-treated rats than in those of untreated rats. These results suggested that repeated administrations of large doses of clioquinol to rats increased the lipid peroxides in nerve tissues. With S-rats at 5 d after birth, the lipid peroxide concentrations in liver were approximately twice those in R-rats regardless of the clioquinol administration.

Keywords — clioquinol; lipid peroxide; neonatal rat; clioquinol sensitive rat; clioquinol resistant rat; rat tissue; rat plasma; subcutaneous administration

Introduction

From results of etiological research and many studies on the neurotoxicity of clioquinol (5-chloro-7-iodo-8-quinolinol, chinoform), it has been considered as the causative agent of subacute myelo-optico-neuropathy (SMON). From in vitro experiments, lipid peroxidation initiated by clioquinol-ferric chelate, as a possible mechanism of development of clioquinol-intoxication, has been speculated to be the direct cause for the degradation of nerve tissues. However, whether lipid peroxidation was induced by administration of clioquinol still remains uncertain. Recently, three types of neonatal rats, highly sensitive, intermediately sensitive, and resistant to clioquinol, have been identified. In the present work, we examined the changes of lipid peroxide concentrations in plasma and tissues after repeated administration of clioquinol to neonatal rats with different sensitivity to clioquinol.

Chemicals and Reagents — Clioquinol was obtained from Sigma Chemical Co. (U.S.A.) and recrystallized twice from ethanol. 2-Thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS) and 1,1,3,3-tetraethoxypropane were obtained from Wako Pure Chemical Industries (Osaka). The other solvents and reagents used in this study were obtained from regular commercial sources.

Animals — Adult male and female rats of the Wistar strain, sensitive (S-rats) and resistant (R-rats) to clioquinol, were prepared by one of the authors, Hori, who used the pedigree method of breeding. The rats were classified as of sensitive, intermediate and resistant to toxicity of clioquinol after a subcutaneous administration of 150 mg/kg dose of clioquinol once a day for 15 d after birth. Male and female rats of each group were housed together for mating. Water and food (NMF, Oriental Yeast Co., Tokyo) were freely available. The temperature of the animal quarters was controlled at 22—26 °C. The pups delivered were used from day 1 in
Observation of Behavior after Increasing Dose of Clioquinol — A clioquinol suspension prepared with 4% polysorbate 80 aqueous solution according to the method described previously was administered subcutaneously in the backs of S- and R-rats once a day from 1 d after birth for 14 d. In all experiments, the dosing volume was 4.2 μl/g body weight. For R-rats, clioquinol was given at a dose of 150 mg/kg/d from 1 d after birth to 5 d, 300 mg/kg/d from 6 to 10 d and 600 mg/kg/d from 11 to 14 d. For S-rats, clioquinol was given at a dose of 75 mg/kg/d from 1 d after birth to 5 d, 150 mg/kg/d from 6 to 10 d and 300 mg/kg/d from 11 to 14 d. The general status of the rats were evaluated during the examination period for 15 d. The body weights of rats were measured every day before the administration.

Drug Administration — The same uterine rats were divided into 2 groups. One was the clioquinol-treated group, to which the clioquinol suspension was administered subcutaneously as described above, and the other was the untreated group. For R-rats, either a fixed dose of 150 mg/kg/d or an increasing dose of clioquinol, which was 150 mg/kg/d from 1 to 5 d, 300 mg/kg/d from 6 to 10 d and 600 mg/kg/d from 11 to 14 d of clioquinol, was given once a day for 14 d after birth. These rats were sacrificed by drawing blood via cardiac puncture with a microsyringe at 5, 10 and 15 d after the administration. For S-rats, a fixed dose of 150 mg/kg/d of clioquinol was given for 4 d and then the rats were sacrificed by the same method as described above at 5 d after administration. The blood collected was transferred into capillary tubes (Drummond Microdispenser, Drumond Sci. Co., U.S.A.) sealed at one end by heating, and the plasma was separated by centrifugation at 6800 × g for 5 min. The liver, kidney, brain and spinal cord were also removed. All tissues, except for the spinal cord, were rinsed with normal saline solution and blotted with filter paper to remove excess moisture and all tissues were weighed. Plasma and tissue samples were kept at −80 °C until assay of lipid peroxides.

Determination of Lipid Peroxide Concentrations — Lipid peroxide concentrations in plasma were determined according to the method of Yagi. To 20 μl of plasma, 2 ml of 1/12 N H₂SO₄ and 0.3 ml of 10% phosphotungstic acid solution were added. The mixture was shaken for 5 min and centrifuged at 1680 × g for 5 min. The supernatant fluid was discarded. To the sediment, 4 ml of distilled water, 0.5 ml of acetic acid and 0.5 ml of 0.67% TBA solution were added, and the mixture was heated in a water bath at 95 °C for 60 min. After cooling, 5 ml of n-butanol was added to the mixture, and the mixture was shaken and centrifuged as above. Then, the reaction products of the lipid peroxides in the organic phase were assayed by setting the excitation and emission wavelengths of the spectrofluorophotometer (RF-540, Shimadzu Seisakusho, Kyoto) at 530 and 550 nm, respectively. 1,1,3,3-Tetraethoxypropane was used as an external standard.

Lipid peroxide concentrations in tissues were determined by a slight modification of the method of Ohkawa et al. Liver was homogenized with 39 volumes of ice-cold 1.15% KCl solution. Kidney, brain and spinal cord were homogenized with 29 volumes of the same solution. To 0.1 ml of each homogenate, 0.2 ml of 8.1% SDS solution, 1.5 ml of 20% acetate buffer (pH 3.5), 1.5 ml of 0.8% TBA solution and 0.7 ml of distilled water were added, and the mixture was heated as above. After cooling, 5 ml of a mixture of n-butanol and pyridine (15:1, v/v) was added to the mixture, and the mixture was shaken and centrifuged as above. Then, the reaction products of lipid peroxides in the organic phase were assayed by the fluorometric procedure described above.

Results and Discussion

Behavioral Change

In our previous study, ataxia was not induced in neonatal rats after a subcutaneous administration of a fixed dose of 150 mg/kg/d of clioquinol. Thus, increasing doses of clioquinol were administered in order to induce ataxia. Only one of ten R-rats developed ataxia at 11 d after administration, whereas out of eleven S-rats, one rat developed ataxia at 9 d, another at 10 d, and a third at 12 d after the administra-
of surviving R-rats increased from 6.1 ± 0.8 g at
1 d after birth to 22.2 ± 3.5 g at 15 d (mean ±
standard deviation, n = 10) and that of untre-
eted R-rats increased from 5.8 ± 0.5 to 27.3 ±
3.7 g in the same period. The body weight of
the clioquinol-treated S-rats was severely sup-
pressed and the body weight of surviving S-rats
increased from 6.0 ± 0.3 to 11.2 ± 2.1 g in the
same period. From these results, it was conclud-
ed that the increasing dose method of clioquinol
was effective in inducing ataxia in neonatal rats.

**Time Courses of Lipid Peroxide Concentra-
tions in Tissues and Plasma**

Because of the above results, R-rats were
used for the examination of the change of lipid
peroxide concentrations after long term admin-
istration of clioquinol.

Lipid peroxide concentrations at 5, 10 and 15
d in tissues and plasma of R-rats given a fixed
dose of 150 mg/kg/d of clioquinol and those of
untreated R-rats, and the concentrations at 15 d
in tissues and plasma given with 4% polysorbate

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**Fig. 2.** Time Courses of Lipid Peroxide Concentrations in Plasma and Tissues of Neonatal R-Rats Treated with a Fixed
Dose of Clioquinol (150 mg/kg/d), Untreated Neonatal R-Rats, and Neonatal R-Rats Treated with 4% Polysorbate 80 Aqueous Solution

- ✔️, clioquinol-treated rat; ✗, untreated rat; ✕, polysorbate 80 treated rat. (a) plasma, (b) liver,
(c) brain, (d) spinal cord, (e) kidney. Each bar represents the mean ± the standard deviation of 12-19 rats from 3-4 uTE-
rine groups. Data of spinal cord and kidney at 5 d represent the mean values of each pooled uterine group, each consisted of
3-5 rats.
80 aqueous solution only, which was used for the preparation of the clioquinol suspension, are shown in Fig. 2. There was no significant difference between lipid peroxide concentrations at 15 d in each tissue and plasma of treated R-rats with 4% polysorbate 80 aqueous solution and those of untreated R-rats (Student’s t-test; \( p > 0.05 \)). These results indicated that the polysorbate 80 aqueous solution did not affect lipid peroxide concentrations in plasma and tissues. The lipid peroxide concentrations in the liver, kidney, brain, spinal cord and plasma of the clioquinol-treated rats were not significantly different from those of the untreated rats. The lipid peroxide concentrations at 5, 10 and 15 d in tissues and plasma of R-rats given an increasing dose of clioquinol and those of untreated R-rats are shown in Fig. 3. The lipid peroxide concentrations in each tissue and plasma at 5 and 10 d of the clioquinol-treated rats were not significantly different from those of untreated rats. However, those in the brain, spinal cord and plasma at 15 d were significantly higher (\( p < 0.05 \)). From these results, it is suggested that lipid peroxide concentrations in nerve tissues were increased by repeated administration of large doses of clioquinol. It has been reported\(^4,7\) that clioquinol was accumulated in nerve tissues by repeated administration of clioquinol to dogs and rats. Furthermore, in animal experiments,\(^1,8\) it has been reported that clioquinol produced neural disturbance in dogs, cats, and monkey. The degradation of nerve tissues caused by the oxidation of lipid is considered to be a possible mechanism in the neurotoxicity of clioquinol.\(^2\) Therefore, the increase of lipid peroxide concentrations in the nerve tissues suggests that neural disturbance might begin to occur in R-rats at 15 d after the increasing dose, since ataxia appeared in R-rat on the 12th day after administration as described in the behavioral change section. On the other hand, it has been reported\(^1,6\) that liver and kidney were injured pathologically by chronic administrations of clioquinol to dogs. However, the lipid peroxide concentrations in rat tissues did not increase
during the administration as mentioned above. It seems that their tissues were not still injured by clioquinol.

Since all S-rats died within 7 d after administration of a fixed dose of 150 mg/kg of clioquinol, the same dose of clioquinol was administered to S-rats for 4 d after birth, and the lipid peroxide concentrations in plasma and tissues in S-rats at 5 d after administration were compared with those in R-rats (Fig. 4). Lipid peroxide concentrations in the brain, spinal cord, kidney, and plasma of the clioquinol-treated S-rats were not significantly different from those of the untreated S-rats, and the results with R-rats were also similar. The reason for this may be due to the short period of administration of clioquinol. On the other hand, the lipid peroxide concentrations in the liver of clioquinol-treated S-rats were approximately twice those of clioquinol-treated R-rats (Fig. 4). The concentrations in the liver of untreated S-rats were also approximately twice those of untreated R-rats. Furthermore, there was not a significant difference between the clioquinol-treated and untreated S-rats, and between clioquinol-treated and untreated R-rats. Therefore, this difference in the lipid peroxide concentrations in the liver between S- and R-rats may be due to genic background. It has been reported that increase in the lipid peroxide concentrations in the liver led to a decrease in the activity of drug metabolizing enzymes in liver microsomes of rats. In the previous paper, we reported that plasma concentrations of clioquinol in S-rats were increased after subcutaneous administration of clioquinol when compared to those in R-rats, and the difference in the elevation of plasma concentration may result from the difference in the metabolizing rate of clioquinol. Thus, it appeared that the difference of lipid peroxide concentrations in the liver in S- and R-rats might be due to the difference in the sensitivity to toxicity of clioquinol. In the present study, it was found that lipid peroxide concentrations in the nerve tissues of rats increased with repeated administration of clioquinol. Yagi, et al. reported that ferric chelate of clioquinol accelerated the lipid peroxidation of nerve tissues in in vitro experiments. Hayakawa, et al. also reported on the effect of several metal chelates of clioquinol on the accel-
eration of lipid peroxides in rabbit plasma. In addition, it is of interesting that metal compounds were prescribed for treatment of many SMON patients. Details in *in vivo* of the relationship between the changes in lipid peroxide concentrations and in metal concentrations in nerve tissues of cloquinol administration require further investigation.

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References


