[Chem. Pharm. Bull. 24(9)2007—2013(1976)]

UDC 615.917.076.9 : 547.831.7.09

Serum Levels of 5-Chloro-7-iodo-8-quinolinol and Its Toxicity in Various Animals

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(Received September 4, 1975)

During long-term chronic intoxication studies, serum levels were measured in dogs and monkeys orally given 5-chloro-7-iodo-8-quinolinol (Clioquinol or Chinoform) (CF) with fixed or increasing doses. Typical neurotoxic symptoms and histological changes developed both in the adult and infant dogs, while the symptoms were poorly required in the monkeys which also showed neurohistological changes.

The minimum serum levels of CF at the beginning of intoxication were 6.0—22.6 μg/ml in the adult dogs administered with 100 mg/kg/day, 0.1—1.9 μg/ml in the infant dogs with 740 mg/kg/day, and 2.6—8.8 μg/ml in the monkeys with 1100 mg/kg/day.

The single dose experiments were also carried out in mice, rabbits and man. The maximum serum levels of CF reached for the corresponding dosages, (μg/ml)/(mg/kg) were; 7.2/100 in mice, 0.5—0.7/100 in the rabbits and 2.7—5.8/7—9 in man. The data indicates the relatively easy absorption of CF in man.

The relative molar abundance of CF, the glucuronide (CF-G) and the sulfate (CF-S) at maximum serum levels were CF-S≧CF-G>CF in mice, CF-G>CF-S>CF in rabbits, CF-G>CF-S>CF in monkeys, CF>CF-S>CF-G in dogs and CF>CF-G>CF-S in man.

The toxicity studies of 5-chloro-7-iodo-8-quinolinol (Clioquinol or Chinoform) (CF) have been carried out with various animals[2-4] since this drug was thought to be closely related with the problem of subacute myelo-optico neuropathy (SMON).[5] In these studies it was shown that the species differences exist in the neurotoxic dose-response to CF. The doses which developed neurologic symptoms by a long-term administration of CF were 60—150 mg/kg/day for mongrel dogs,[3d] 350—450 mg/kg/day for beagle dogs,[3d] 200—700 mg/kg/day for monkeys[6] and 600—3000 mg/kg/day for quail.[3c] These values were fairly higher than 20—30 mg/kg/day being estimated for SMON patients.

In this paper, CF levels in sera were measured in dogs and monkeys during long-term intoxication studies. The serum levels of CF and CF conjugates in healthy rabbits, mice and man were also measured after a single dose administration of CF.

Materials and Methods

The animals used were adult and infant mongrel dogs, cynomolgus monkeys, rabbits and mice.

The CF preparations used were Emaform (Tanabe) and Entero-vioform (Ciba-Geigy). CF powder was offered by Tanabe Pharmaceutical Co., Ltd.

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3) *a*) Y. Ikeda, M. Tobe, Y. Suzuki, K. Kobayashi, S. Suzuki, and Y. Kawasaki, *Reports of SMON Research Commission*, No. 3, 190 (1971); *b*) K. Ueda, M. Kawai, and H. Takahashi, *ibid.*, No. 9, 10 (1972); *c*) Y. Egashira, and S. Otaki, *ibid.*, No. 9, 38 (1972); *d*) S. Otsuki, M. Watanabe, J. Tateishi, A. Saito, and S. Kuroda, *ibid.*, No. 9, 49 (1972) ;*e*) Y. Okun, M. Takahashi, J. Osa, K. Okano, and H. Taniguchi, *ibid.*, No. 9, 136 (1972).
4) *a*) T. Tsubaki, *Ann. Reports of SMON Research Commission*, **1972**, 46 (1973); *b*) Y. Egashira, **1972**, 53 (1973); *c*) T. Takeuchi, *ibid.*, 110 (1973).
5) R. Kono, *Japan. J Med. Sci. Biol.*, **24**, 195 (1971).
6) J. Tateishi, *Medicine and Biology* (in Japanese), **83**, 313 (1971).

The determination of CF, CF-glucuronide (CF-G) and CF-sulfate (CF-S) was carried out by gas chromatography (GC).[7,8] High performance liquid chromatography (HPLC)[9] was used for the determination of CF-G in urine.

Results

Adult Dogs in Long-term Intoxication Studies

The experiments were performed with 6 mongrel dogs including 2 controls according to the schedule of CF administration in Fig. 1.

In the 1st stage with increasing doses, Emaform was given in capsules for 4 weeks, then in milk for 16 weeks. On the 4th and 9th week, blood samples were collected 24 hr after the drug adminstration. All the animals other than the controls developed toxic symptoms on the 6th or 7th week.

In the 2nd stage, after 11 weeks suspension of CF, a fixed dose of 200 mg CF/kg/day was given for around 3 months, then, after 8 weeks suspension of CF, the administration was repeated for 4 weeks. On days 1, 2, 3, 4, 5, 10, 12, 19, 26 and 30 after the 51 st week, blood samples were taken 4 and 24 hr after every administration.

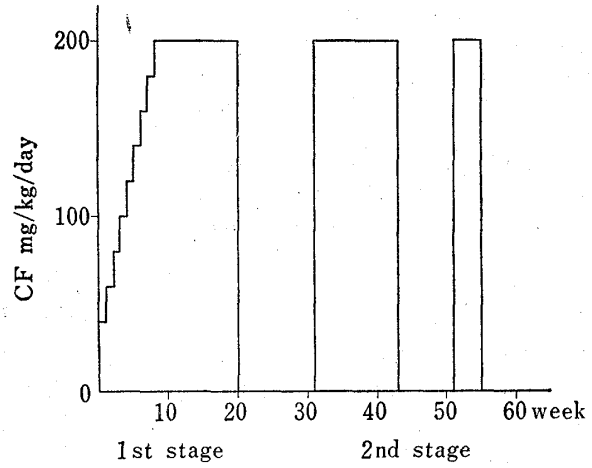

Fig. 1. History of CF Administration to Adult Dogs

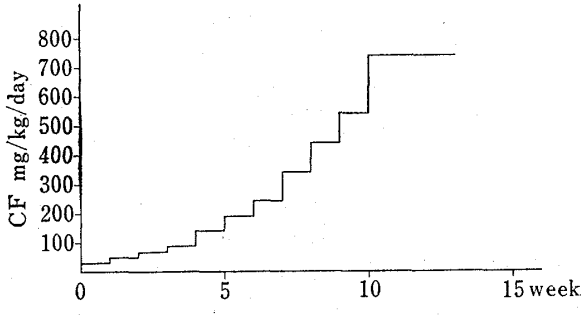

Fig. 2. History of CF Administration to Infant Dogs

Table I. Concentration (μg/ml) of Unconjugated Chinoform (CF) in Serum of Adult Dogs Orally Administerd CF for a Long Term

Stage of experiments[a]	First		Second	
Term of CF administration	4 th week	9 th week	52—56 th week	
Daily dose of CF in the week when bled	100 mg/kg	200 mg/kg	200 mg/kg	
Time of bleeding (hr after the last administration of CF)	24 hr	24 hr	4 hr	24 hr
Animal No. 2	22.6	1.1	3.9— 5.0	0—5.0
No. 3[b]	21.5	13.5		
No. 4	6.9	3.9	5.6—26.0	0.3—2.5
No. 6	6.0	18.5	2.0—14.1	0.3—1.0

a) See text and Fig. 1.
b) died on the 35th week

7) Z. Tamura, M. Yoshioka, T. Imanari, J. Fukaya, J. Kusaka, and K. Samejima, *Clin. Chim. Acta*, **47**, 13 (1973).
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9) C. Chen, K. Hayakawa, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **23**, 2174 (1975).

Table II. Time Course of Serum Concentration of CF and Its Conjugates in Adult Dogs

Species	Dose mg/kg	Metabolites	Range of CF levels (μg/ml) Time after administration (hr) 0.5	2	6	24
Adult dogs $n=3$	200	CF	1.7—4.4	3.3—9.7	0.6—2.2	0.2—3.5
		CF-G	0.3—2.6	1.3—2.4	0.2—4.0	0.2—1.7
		CF-S	1.3—2.3	2.8—5.0	1.2—8.4	0.2—1.8

Table III. Concentration (μg/ml) of CF and Its Conjugates in Serum of the Infant Dogs Orally Administered CF for a Long Term

Term of CF administration[a]			9th week[b]		11th week			13th week
Daily dose of CF in the week when bled			440 mg/kg		740 mg/kg			740 mg/kg
Time of bleeding (hr after the last administration of CF)			4 hr	24 hr	2 hr	5 hr	24 hr	24 hr
Aminal	I	CF	3.1	0.3	31.6	6.4	0.7	0.1
		CF-G	3.6	0.5	8.4	5.5	0.8	0.3
		CF-S	4.3	2.3	21.3	11.4	5.1	0.8
	II	CF	5.2	0.1	14.2	8.7	0.1	6.5
		CF-G	7.0	2.6	8.9	5.9	0.1	6.2
		CF-S	0.7	4.3	17.5	15.9	0.2	7.0
	III	CF	8.0	0.3	4.6	0.9	0.3	0.1
		CF-G	7.9	2.7	4.1	1.7	0.4	0.1
		CF-S	1.9	5.5	18.2	7.2	3.0	0.3
	IV	CF	6.0	0.2	21.4	5.5	1.9	7.3
		CF-G	5.6	0.3	5.6	2.6	0.3	2.7
		CF-S	1.9	8.6	16.0	2.6	2.5	4.8
	V	CF	4.3	1.1	27.8	3.5	0.2	0.5
		CF-G	8.2	1.9	10.4	2.3	0.1	0.5
		CF-S	9.2	8.3	30.0	2.8	1.6	2.6

a) See text and Fig. 2.
b) On the 5th week (140 mg/kg/day), the minimum (24 hr) contents of CF in sera of these amimal were less than 0.1 μg/ml.

The serum levels of CF in both stages are shown in Table I.

At the end of the 43rd week, the serum levels of CF and its conjugates were followed for 24 hr after CF administration (Table II).

Infant Dogs in Long-term Intoxication Studies

Five infant dogs born of a mongrel dog which had been administered CF repeatedly for a few weeks before and after the parturition were weaned after 45 days lactation and subjected to the toxicity study. The mother dog's milk contained 0.3—8.5 μg of CF, 0.4—7.1 μg of CF-G and 0.7—11.3 μg of CF-S in 1 ml.

Entero-vioform was administered to the puppies with increasing doses as shown in Fig. 2. Slight trouble in walking appeared in all the infant dogs on the 10th week, and the animals except one showed serious trouble on the 12th week. On the 5th, 9th, 11th, and 13th week, blood samples were taken to assay the serum levels of CF and its conjugates. The data obtained are shown in Table III.

Monkeys in Long-term Intoxication Studies

CF powder added with 10% CMC was adminstered to 11 monkeys in capsules as shown in Fig. 3 (1st stage). CF levels in sera at 24 hr after the administration were measured on the day 2 weeks after the beginning of CF treatment (Table IV).

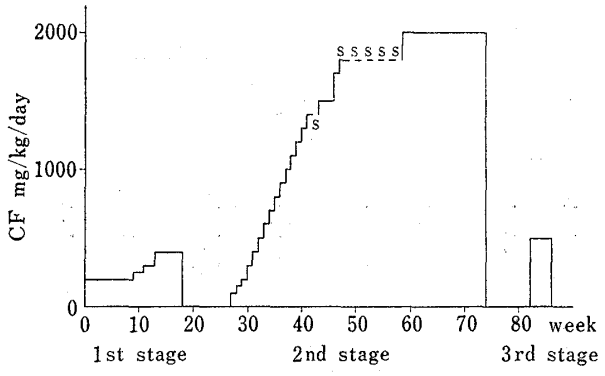

Fig. 3. History of CF Administration to Monkeys
s: suspension of CF administration

After 9 weeks suspension of CF, serum levels of CF were measured on the 27th week, and prolonged administration with increasing dose was resumed (2nd stage) in 9 of 11 monkeys used for the first stage. CF levels in sera 24 hr after CF administration were determined on the 39th week (Table IV).

After the treatment was stopped for 2 months, CF was administered to 4 of the same monkeys in a dose of 500 mg CF/kg/day for one month (3rd stage). CF levels in sera (4 and 24 hr) were determined on days 1, 2, 3, 4, 5, 10, 12, 19, 26 and 30 after the 82nd week (Table IV). On the last dose at the end of the 86th week, time course of the serum level was measured as in Table V.

Throughout the whole experiments mentioned above, the athletic injury was insignificant and the symptoms were difficult to recognize, however, 7 cases of histological change in spinal cords were observed.[10]

Table IV. Concentration (μg/ml) of Unconjugated CF in Serum of the Monkeys Orally Administered CF for a Long Term

Stage of experiment[a]	First	Second		Third	
Term of CF administration	2nd week	27th week	39th week	83—86th week	
Daily dose of CF in the week when bled	200 mg/kg	0 mg/kg	1100 mg/kg	500 mg/kg	
Time of bleeding (hr after the last administration of CF)	24 hr	0 hr	24 hr	4 hr	24 hr
10085	0.9	0	6.0		
10577	1.3	0	8.8		
10580	4.1	0	2.6	4.0—9.4	0.1—3.7
10583	1.5				
10587	0.9	0.1	3.5		
10638	0.7	0	4.5	1.2—6.0	0—2.1
10641	0.8	0	3.1	1.1—3.5	0—3.5
10642	1.3				
10643	0.4	0	2.6	1.5—4.4	0—2.2
10644	1.4	0	4.6		
10659	2.3	0.2	3.3		

a) See text and Fig. 3.

Table V. Time Course of Serum Concentrations of CF and Its Conjugates in Monkeys

Species	Dose mg/kg	Metabolites	Concentration (μg/ml) Time after administration (hr)			
			0.5	2	6	24
Monkeys $n=4$	500	CF	2.7—4.5	6.0— 6.5	1.0— 3.2	0.6—2.3
		CF-G	3.6—6.1	10.0—16.1	1.1—16.5	0.9—2.4
		CF-S	2.6—5.4	5.0—10.5	1.1— 7.9	0.5—3.3

10) Y. Egashira, *Ann. Reports of SMON Research Commission*, **1972**, 53 (1973).

Rabbits

Three female rabbits (Japanese white) weighing about 3 kg were used. Single dose of 100 mg CF/kg (Entero-vioform) was administered with a stomach tube as suspension. The animals were abstained from food throughout 24 hr of the experiment, however, free intake of water was allowed. Blood samples were taken from ear-vein 1, 2, 4, 6 and 24 hr after administration. The data of serum levels are presented in Table VI.

Table VI. Time Course of Serum Concentrations of CF and Its Conjugates in Rabbits

Species	Dose mg/kg	Metabolites	Concentration (μg/ml) Time after administration (hr)				
			1	2	4	6	24
Rabbits $n=3$	100	CF	0.3—0.7	0.5—0.6	0.3—0.5	0.2—0.4	0.2—0.4
		CF-G	1.1—5.5	2.1—2.8	1.5—1.7	1.2—1.8	0.8—1.3
		CF-S	0.7—1.5	0.8—1.7	0.7—1.0	0.6—0.9	0.3—0.6

Mice

Twenty four male mice of ddY-strain weighing about 30 g were used. Single dose of 100 mg CF/kg (CF powder containing 10% CMC) was administered with a syringe as suspension. The animals were abstained from food, but free intake of water was allowed throughout the experiment. Blood samples of each 3 mice taken from the carotid artery were pooled at 0.5, 1, 2, 4, 6, 8 and 24 hr after CF administration and submitted to the assay. The data are shown in Table VII.

Table VII. Time Course of Serum Concentrations of CF and Its Conjugates in Mice

Species	Dose mg/kg	Metabolites	Concentration (μg/ml) Time after administration (hr)						
			0.5	1	2	4	6	8	24
Mice	100	CF	7.2	5.4	3.1	2.6	1.6	1.8	0.3
		CF-G	10.9	14.2	4.1	1.8	1.9	2.2	0.3
		CF-S	12.5	10.6	6.7	4.9	3.4	3.8	0.9

Man

Besides the study of the metabolic pattern in serum, the time course of serum levels was compared with that of urinary excretion in 4 men aged 25—51, weighing 54—68 kg. Entero-vioform (500 mg, calculated as CF) was orally administered before meal in the morning. Blood was withdrawn after CF administration and urine was collected for 24 hr at the same intervals except later period. The time course of the serum levels is presented in Table VIII and a typical feature is in Fig. 4.

Table VIII. Time Course of Serum Concentration of CF and Its Conjugates in Man

Species	Dose mg/kg	Metabolites	Concentration (μg/ml) Time after administration (hr)				
			2	4	6	8	24
Man $n=4$	7—9	CF	0.3—3.5	2.7—5.8	2.5—5.8	0.9—3.4	0.6—1.1
		CF-G	0.1—2.3	3.0—5.0	2.2—3.4	0.3—2.9	0.3—1.3
		CF-S	0.1—0.6	1.0—2.4	1.1—1.5	0.3—1.2	0.1—0.5

The glucuronide in urine samples was determined by HPLC, and CF and CF-S were assayed by GC. The majority of the metabolite in human urine was the glucuronide which comprise 95% or more; only trace amounts of CF-S (<3%) and CF (<1%) were detected. The rate of CF-G excretion in urine reached its peak at 4—6 hr, and the rise and fall of the urinary excretion were similar to those of serum level of CF-G (Fig. 4). As for other metabolites, Liewendahl, *et al.*[11] demonstrated the unknown metabolite in human urine and serum, and Urakubo, *et al.*[12] detected 5-Chloro-8-quinolinol in a dog urine. Our search for new metabolites showed negative results by the procedure of GC (FID) and HPLC, although 5-Chloro-8-quinolinol was detected in human urine which had been allowed to stand for several days at 4°.

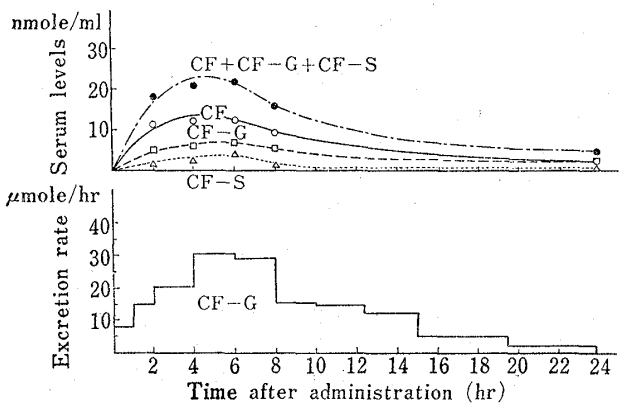

Fig. 4. Time Course of Serum Concentration of CF and Its Conjugates, and Excretion Rate of CF-G in Urine after Oral Administration of CF in Man Subject : T, ♂, 51yr.

CF concentrations in human red cells at 4—6 hr (see Table VIII) were also determined according to the same method as that for serum after the samples were washed with phosphate saline and hemolyzed in acetate buffer. The maximum concentrations of CF and CF-S in red cells were roughly estimated as 0.02 μg/ml and 0.04 μg/ml respectively; CF-G was not detected.

Discussion

All the adult mongrel dogs tested developed the symptoms at the doses of 140—160 CF/kg/day and the values were similar to those previously reported.[3d] As shown in Table I, considerable minimum serum levels (24 hr) of CF (6—22.6 μg/ml) were attained before the appearance of symptoms.

On the other hand, the infant dogs who had been continuously exposed to the drug showed no symptoms at the similar dose of 140 mg CF/kg/day, when the minimum serum levels of CF were quite low (<0.1 μg/ml). Their symptoms appeared at doses of 540—740 mg CF/kg/day, even when the minimum levels of CF did not arise so much (0.1—1.9 μg/ml), while the maximum levels (2 hr) were considerable (4.6—31.6 μg/ml) as shown in Table III.

The monkeys, who were more resistant than the adult dogs, also showed lower serum levels of CF compared with the dose (200—1100 mg/kg/day) of CF as shown in Table IV.

On the contrary, the men (Table VIII) showed similar serum levels of CF to the monkeys (Table V) and the dogs (Table II) at the quite low dose compared with the others (7—9 mg/kg to 500 mg/kg and 200 mg/kg) although some influences of long-term administration to the serum levels in monkeys and dogs should be taken in considerations.

Supposing CF itself other than its conjugates has neuro-toxicity, these results will clearly demonstrate the cause of the species differences in the intoxication doses of CF; the toxic action of CF on nerve has already been demonstrated by tissue culture studies,[13–15] and absence of cellular toxicity of CF-G was suggested by another.[16]

11) K. Liewendahl, V. Kivikangas, and B.A. Lamberg, *Nucl. Med.*, **6**, 32 (1967).
12) G. Urakubo and Y. Kido, *Ann. Reports of SMON Research Commission*, **1972**, 64 (1973).
13) Y. Toyokura, H. Beppu,and J. Nakai, *Reports of SMON Research Commission*, No. **9**, 227 (1972).
14) T. Yonezawa, T. Saida, and Y. Sato, *Reports of SMON Research Commission*, No. **9**, 293 (1972).
15) T. Yonezawa, *Ann. Reports of SMON Research Commission*, **1973**, 92 (1974).
16) N. Yamanaka, T. Imanari, Z. Tamura and K. Yagi, *J. Biochem.*, **73**, 993 (1973).

The results also indicate that in CF-resistant animals, the metabolism and excretion of CF are relatively rapid, while the absorption is slow, to produce lower serum levels of CF. Actually rabbits and mice who are difficult to develop the symptoms[2,4] also showed lower serum level of CF as shown in Table VI and VII.

TABLE IX. Species Difference in Molar Fraction of CF and Its Conjugates in Serum at the Maximum Concentration

Species	Ref. Table	Dose mg/kg	Time (hr) after administration	Molar fraction (mean)		
				CF	CF-G	CF-S
Mice	VII	100	0.5—1	0.27	0.35	0.38
Rabbits	VI	100	1—2	0.14	0.60	0.26
Monkeys	V	500	2	0.24	0.50	0.27
Dogs (adult)	II	200	2	0.62	0.12	0.26
Dogs (infant)	III	740	2	0.52	0.12	0.36
Man	VIII	7—9	4—6	0.51	0.31	0.18

To compare the metabolic feature of CF in those animals described above, molar fractions of CF, CF-G and CF-S in serum at the maximum total concentration were calculated as in Table IX. From the table, conjugation abilities seem to exceed in the resistant animals while in dogs, who are most suitable animals to reproduce SMON, unconjugated CF is predominant as in man. Hence it is probable that the conjugation followed by the excretion will play an important role to suppress the serum level of CF.

Although the molar fractions of CF in dogs and man are similar, there exists the discrepancy in their intoxication doses as well as the doses required to maintain the similar serum levels of CF (Tables II and VIII). Moreover, the data obtained in this study (Tables I—VIII) demonstrate large individual and daily variations in the serum level of CF. These facts suggest the existence of labile factors which affect the absorption of the drug from intestines. While CF is scarcely soluble in water, it is fairly soluble in a solution of bile acids, and increased absorption of CF by bile has been demonstrated.[17] Bile will probably be one of the factors, but contribution of other factors such as hyperacidity and intestinal stasis[18] remains to be investigated.

Acknowledgement We are pleased to acknowledge the support of our work by a Grant-in-Aid for SMON Research from the Ministry of Welfare of Japan. We also wish to thank Ciba-Geigy Ltd. and Tanabe Pharmaceutical Co., Ltd. for their supplying samples.

17) M. Hanano, S. Awazu, Y. Fuwa, and M. Hayashi *Ann. Reports of SMON Research Commission*, **1973**, 118 (1974).
18) N.A. David, N.M. Phatak, and F.B. Zener, *Amer. J. Trop. Med.*, **24**, 29 (1944).