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Studies on Metal-Catalyzed Hydrolyses of Clioquinol Conjugates in the Rabbit Body Following Intravenous Injection of Cu(II)- or Fe(III)-Gluconic Acid Complex System

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Metal-catalyzed hydrolyses of clioquinol-glucuronide (CG) and clioquinol-sulfate (CS) in the rabbit body were studied following intravenous injection of Cu(II)- or Fe(III)-gluconic acid complex system 6 h after the oral administration of clioquinol (C). Molar fractions of C, CG and CS remained nearly constant through 24 h after administration of C at doses of 90—300 mg/kg weight in rabbits which were not injected with Cu(II) or Fe(III). Therefore, molar fractions of C, CG and CS after the injection of Cu(II) or Fe(III) were monitored to clarify the metabolic changes of C. In rabbits injected with 2.5 mg of Cu(II)/kg weight, C rapidly increased and CG and CS decreased after the injection of Cu(II). In rabbits injected with 20 mg of Fe(III)/kg weight, C and CS slowly increased and CG decreased until 18 h after the injection of Fe(III). The patterns of the changes in molar fractions of C, CG and CS are consistent with the catalytic features of Cu(II) and Fe(III) seen in previous studies, and therefore, the changes in molar fractions of C, CG and CS observed in this study were considered to have been brought about as a result of metal-catalyzed hydrolyses of CG and CS or metal-catalyzed hydrolysis of CG in the rabbit body.

Keywords——SMON; clioquinol; clioquinol-glucuronide; clioquinol-sulfate; metabolism; metal-catalyzed hydrolysis; Cu(II); Fe(III); rabbit

Recently, there has been much interest in the biological roles of metals, for example in enzymatic, metabolic, pharmacological and toxicological processes. In subacute myelo-optico-neuropathy (SMON)[1] too, metal ions are considered to be involved in the toxic action of clioquinol (5-chloro-7-iodo-8-hydroxyquinoline: C), and thus it is important to elucidate the effects of metal ions on the metabolism and toxicity of C.

Clioquinol-glucuronide (CG) and clioquinol-sulfate (CS), which are the major metabolites of C, and analogous compounds such as 8-hydroxyquinoline-β-D-glucoside and 8-hydroxyquinoline-sulfate were reported to be hydrolyzed rapidly by some metal ions.[2-10] These results are considered to be due to the easy interaction of such compounds with metal ions through the 8-hydroxyquinoline structure; C and 8-hydroxyquinoline are known to form stable complexes with many metal ions. Therefore, CG and CS may be hydrolyzed by metal ions *in vivo*, as well as *in vitro*. Further, it was proposed that metal-catalyzed hydrolysis of CS occurs in the rat body, since the stability of CS to enzymatic hydrolysis in the rat body was less than that *in vitro*.[9] However, there has been no report on the interaction between metals and the metabolites of C or on metals-catalyzed hydrolyses of CG and CS *in vivo*.

In this paper, metals-catalyzed hydrolyses of CG and CS were investigated in order to obtain further insight into the effects of metals on the metabolism and stability of CG and CS *in vivo* in the rabbit body.

Cu(II) and Fe(III) were chosen as metals since the former had the largest catalytic activity for the hydrolyses of CG and CS among metals previously examined[2,8-10] and the latter was considered to play an important part in the toxic action of C.[11-13] Both Cu(II) and

Fe(III) were intravenously injected as their gluconic acid (GA) complex system.

Experimental

Materials——8-Hydroxyquinoline derivatives were obtained by courtesy of the Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo. Reagents for determination of C, CG and CS were used according to the previous paper.[14,15] Oriental Yeast RC-4 (pellet type) was given to rabbits. The other reagents were all commercial products.

Instruments——C, CG and CS were analyzed by using a Shimadzu GC-3BE gas chromatograph (GC) or a high performance liquid chromatograph (HPLC) composed of a Kyowa KLC-2290 ultraviolet (UV) monitor, a Kyowa KSU-16H minimicropump, a Pyrex column and a Toa Denpa EPR-10B recorder. Amounts of metals were determined by a Hitachi 170-10 atomic absorption spectrophotometer (AAS).

Treatment of Animals——Male Japanese white rabbits (weighing about 2.5 kg) were used in the experiments after acclimation for over a week. During experiments, rabbits were kept in a restraint cage and allowed only water *ad libitum*. A suspension of C in 5 ml of 0.5% sodium carboxymethylcellulose was orally administered to each rabbit with a stomach tube. i) Control group; six rabbits numbered from 1 to 6 were administered 90, 160, 200, 200, 300 and 300 mg of C/kg weight, respectively. ii) Metal-treated group; three rabbits were intravenously injected with 2.5 mg of Cu(II)/kg weight (about 1 ml of 0.13 M $CuCl_2$–0.63 M GA, pH 7.2) or 20 mg of Fe(III)/kg weight (about 2 ml of 0.5 M $FeCl_3$–0.63 M GA, pH 7.2) into the ear vein at 6 h after the administration of 200 mg of C/kg weight. Blood samples (5 ml each) were collected in heparinized tubes at intervals up to 16 h after the injection of metal solution and were centrifuged immediately to obtain plasma. Disodium ethylenediaminetetraacetate was immediately added to the plasma samples to avoid metal-catalyzed hydrolyses of CG and CS during storage. The plasma samples were preserved in a refrigerator.

Determination of C, CG and CS——C, CG and CS in the plasma samples were determined by the HPLC method[15] or the GC method.[14]

Determination of Cu and Fe——Cu and Fe in the plasma samples, which were diluted 5 times or more with physiological saline, were determined by AAS.

Results

Control Group

Results obtained in the control group are shown in Figs. 1 and 2. There were large individual variations in the changes of concentrations of C, CG and CS in plasma with passage of time. However, the molar fractions of C, CG and CS (f_C, f_{CG} and f_{CS}, respectively) remained

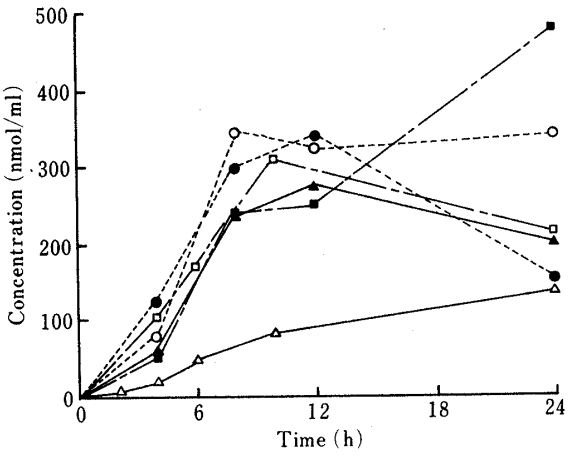

Fig. 1. Time Courses of Total Plasma Concentrations of Clioquinol and Its Metabolites (CG and CS) after the Oral Administration of Clioquinol in Rabbits

C doses (mg/kg weight) and rabbit numbers were as follows: △—△, 90 (No. 1); □---□, 160 (No. 2); ●---●, 200 (No. 3); ▲—▲, 200 (No. 4); ■---■, 300 (No. 5); ○---○, 300 (No. 6).

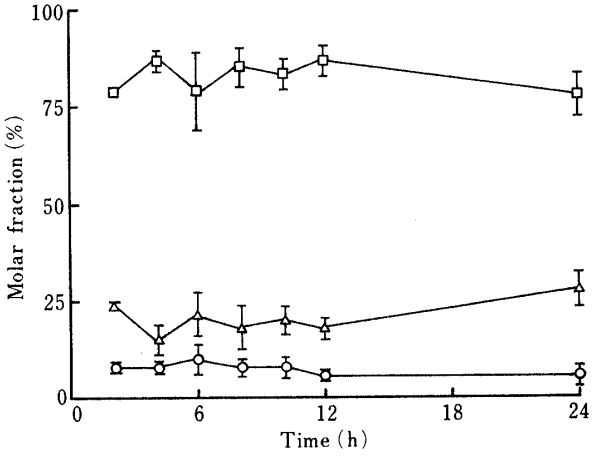

Fig. 2. Time Courses of Molar Fractions of C, CG and CS in Plasma after the Oral Administration of Clioquinol in Rabbits

C dose; 90—300 mg/kg weight.
○—○, C; □—□ CG; △—△, CS.
Each point represents the mean ± S.D. obtained for 4 rabbits (numbers 1, 4, 5 and 6).

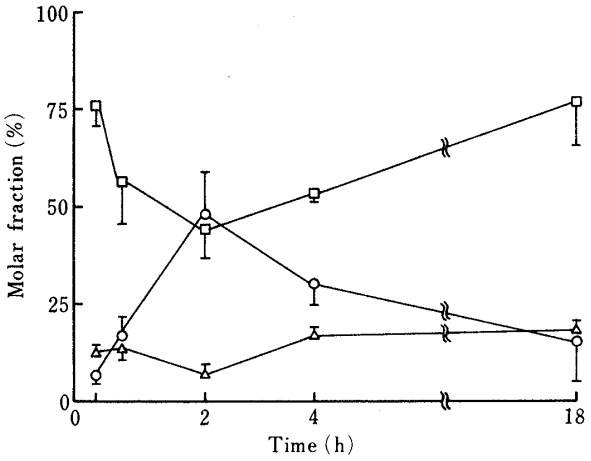

Fig. 3. Time Courses of Molar Fractions of C, CG and CS in Plasma after the Intravenous Injection of Cu(II)–GA Complex System

○—○, C; □—□, CG; △—△, CS.
Each point represents the mean ± S.D. obtained for 3 rabbits.

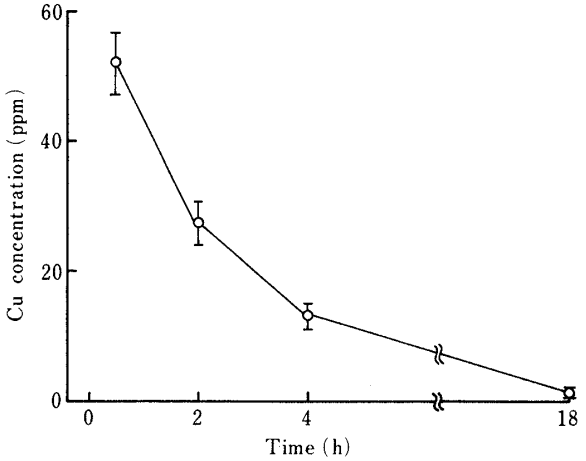

Fig. 4. Time Course of Cu in Plasma after the Intravenous Injection of Cu(II)–GA Complex System

Each point represents the mean ± S.D. obtained for 3 rabbits.

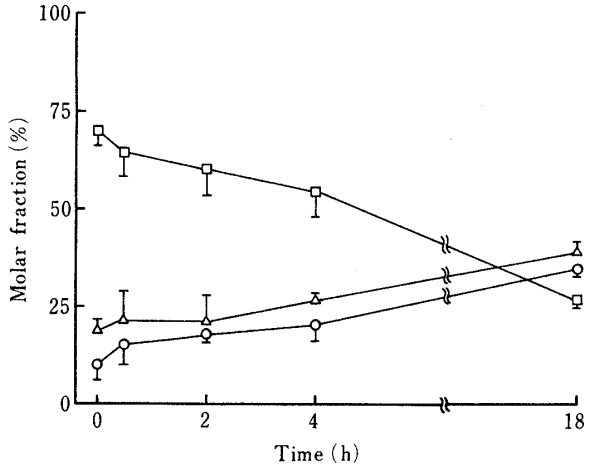

Fig. 5. Time Courses of Molar Fractions of C, CG and CS in Plasma after the Intravenous Injection of Fe(III)–GA Complex System

○—○, C; □—□, CG; △—△, CS.
Each point represents the mean ± S.D. obtained for 3 rabbits.

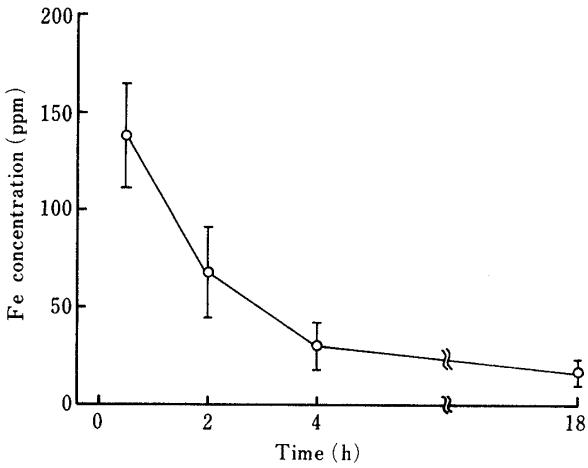

Fig. 6. Time Course of Fe in Plasma after the Intravenous Injection of Fe(III)–GA Complex System

Each point represents the mean ± S.D. obtained for 3 rabbits.

nearly constant through 24 h after the administration of C and were not affected by the above mentioned individual differences, as shown in Fig. 2. f_C was 5—10%, f_{CG} was 70—80% and f_{CS} was 15—25% in the control group. Plasma levels of Cu and Fe also remained constant through 24 h after the administration of C and were 1.1—1.4 ppm and 1.4—1.8 ppm, respectively. These values are within the normal ranges in rabbits.

In subsequent studies, metal was intravenously injected at 6 h after the administration of C when it was expected that the concentrations of C, CG and CS in plasma would have increased enough to be determined accurately (Fig. 1). Metals-catalyzed hydrolyses of CG and CS in the rabbit body were investigated by monitoring f_C, f_{CG} and f_{CS} in the plasma.

Cu(II)-Treated Group

Results obtained in the Cu(II)-treated group are shown in Figs. 3 and 4. As shown in Fig.

3, a rapid increase of f_C and decreases of f_{CG} and f_{CS} occurred immediately after the injection of Cu(II). f_C, f_{CG} and f_{CS} amounted to 48, 44 and 8%, respectively, at 2 h after the injection of Cu(II). Subsequently, a decrease of f_C and increases of f_{CG} and f_{CS} were observed, and the levels of f_C, f_{CG} and f_{CS} recovered to the control levels at 18 h. Plasma concentration of Cu was 35 ppm at 30 min and then decreased with a half-life of about 2 h. At 18 h, Cu concentration had reached the control level.

Fe(III)-Treated Group

Results obtained in the Fe(III)-treated group are shown in Figs. 5 and 6. As shown in Fig. 5, f_C and f_{CS} slowly increased and f_{CG} decreased after the injection of Fe(III). By 18 h, f_C, f_{CG} and f_{CS} had reached 30, 25, and 40%, respectively. Plasma concentration of Fe was 140 ppm at 30 min and decreased with a half-life of about 2 h. However, even at 18 h Fe concentration amounted to 17 ppm, which is 10 times the control value.

Discussion

For the purpose of the present study, the metal ion or metal complex to be injected intravenously was required to possess the following properties; a) catalytic activity to hydrolyze CG and/or CS, b) the metal hydroxide does not precipitate in the physiological pH range, c) low toxicity. Gluconic acid complexes of some metal ions are stable in the physiological pH range[16] and are used in clinical therapy owing to their low toxicities[17] Furthermore, Cu(II) and Fe(III) complex systems have catalytic activity to hydrolyze CG and CS, as shown in the previous work.[10] Thus, Cu(II) and Fe(III) were selected for injection as their GA complex systems.

There were large individual differences in the changes of concentrations of C, CG and CS with time. However, f_C, f_{CG} and f_{CS} were nearly constant through 24 h after the administration of C and therefore, monitoring of the molar fraction values could be used to clarify metabolic changes of C.

A possible reason for changes in f_C, f_{CG} and f_{CS} might be that Cu(II) and Fe(III) injected into the rabbit body change the activities of enzymes which participate in the metabolism of C. In the Cu(II)-treated group, Cu(II) might inhibit enzymes participating in glucuronic acid

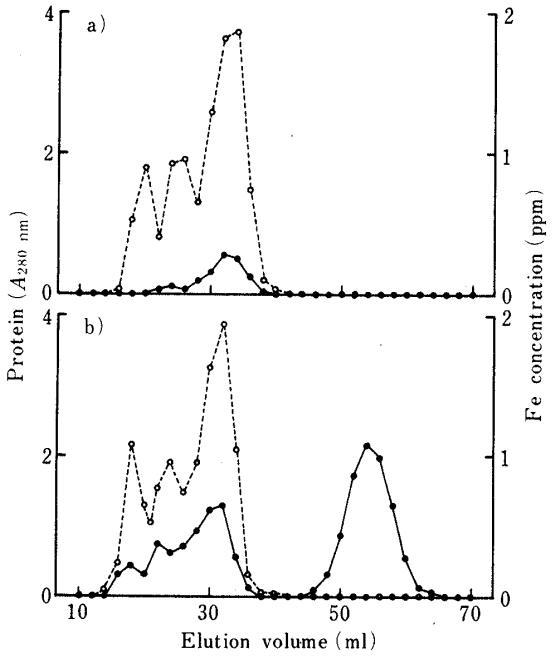

Fig. 7. Gel Filtration Profiles of Rabbit Plasma on a Sephadex G-150 Column

a) Plasma collected before the injection of Fe(III)–GA complex system.
b) Plasma collected at 18 h after the injection of Fe(III)–GA complex system.

Each plasma sample was charged on a Sephadex column (1.5 cm *i.d.* × 35 cm) and eluted with NaCl–0.05 M Tris HCl buffer (pH 7.8) at a flow rate of 3 ml/h. ○---○, $A_{280\,nm}$; ●—●, Fe concentration.

and sulfate conjugation and/or might activate β-glucuronidase and arylsulfatase. In the Fe(III)-treated group, Fe(III) might inhibit enzymes participating in glucuronic acid conjugation and/or might activate β-glucuronidase. Nevertheless, it has not been reported that Cu(II) and Fe(III) affect the activities of enzymes participating in the metabolism of C or other drugs which have phenolic OH. A more important point is that the changes of f_C, f_{CG} and f_{CS} observed in this study are explainable in terms of known catalytic properties of Cu(II) and Fe(III) as discussed below.

The increase of f_C after the injection of Cu(II)- or Fe(III)-GA complex system was rapid in the Cu(II)-treated group and slow in the Fe(III)-treated group. Catalytic activity of Cu(II) is known to be much higher than that of Fe(III) for hydrolyses of both CG and CS *in vitro*.[8-10] On the other hand, f_{CS} increased in the Fe(III)-treated group, although f_{CG} decreased. This result at least shows that the change in the amount of CS was smaller than that of CG after the injection of Fe(III). In an examination *in vitro*, Fe(III) catalyzed the hydrolysis of CG more efficiently than that of CS.[8-10] Thus, the patterns of changes of f_C, f_{CG} and f_{CS} observed in the Cu(II)- and Fe(III)-treated groups are consistent with the known catalytic features of Cu(II) and Fe(III) in hydrolyses of CG and CS *in vitro*.

Cu(II) is well known to have a strong affinity to albumin, a major plasma protein, so the injected Cu(II) would be expected to bind to albumin and lose its catalytic activity. Cu(II)-catalyzed hydrolyses of CG and CS were actually inhibited by albumin *in vitro*.[10] After the injection of Cu(II), increase of C and decrease of CG and CS were observed within 2 h, and this period corresponded to that when the concentration of Cu in plasma was higher than that of albumin in plasma. Hence, it is conceivable that changes of the molar fractions of C, CG and CS depend mainly on the concentration of non-bound Cu(II).

In the Fe(III)-treated group, since about 60% of Fe in plasma at 18 h was located in the lowest molecular weight fraction (Fig. 7), a part of the injected Fe(III) appears to remain in the plasma as complexes with GA, carboxylic acid, amino acid and so on. Such Fe(III) complexes were reported to catalyze the hydrolyses of CG and CS.[10] Therefore, the gradual and sustained increase of f_C in the Fe(III)-treated group can be understood in terms of the prolonged presence of the injected Fe(III) in the rabbit body as low molecular weight iron compounds.

Thus, it is reasonable to consider that the increase of f_C observed in this study was a result of metals-catalyzed hydrolyses of CG and CS or of CG alone.

Interestingly, a number of SMON patients had actually received metal preparations.[18] Of course, biological Cu, Fe, and Zn which have high catalytic activity to hydrolyze CG and/or CS are commonly bound to albumin, transferrin, hemoglobin, ferritin and other proteins, but the concentrations of free metal ions or metal compounds which easily release metal ions might increase in patients with diseases involving abnormal metal metabolism. In fact, the Fe(III) chelate of C was detected from urine of a SMON patient.[11] The present results suggest that CG and/or CS are hydrolyzed by metal ions in the body, when the concentrations of metals with catalytic activity are increased.

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