Current Topics in Pharmacology
Vol. 17, *1*, 2013

Original Communication

Pharmacokinetics of FITC-labeled immunoglobulin G in normal mice and mice with cisplatin-induced acute kidney injury

Saori Mimaki, Junya Nagai, Ryoko Yumoto and Mikihisa Takano*

Department of Pharmaceutics and Therapeutics, Graduate School of Biomedical & Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan

ABSTRACT

The pharmacokinetics of fluorescein isothiocyanate (FITC)-labeled human immunoglobulin G (FITC-hIgG) were investigated in mice treated with or without cisplatin, cis-diamminedichloroplatinum (II) (CDDP). When FITC-hIgG was intravenously injected into normal mice, FITC-hIgG disappeared from the plasma with a half-life of 7.4 days, which was similar to the previously reported half-life of mouse IgG in mice. Next, we examined the effect of CDDP administration on the disappearance of plasma FITC-hIgG in mice. Treatment with CDDP (5 mg/kg) followed by FITC-hIgG injection enhanced the levels of blood urea nitrogen and kidney injury molecule-1 (Kim-1) mRNA expression in the kidney, showing induction of acute kidney injury. The plasma concentration-time profile of FITC-hIgG in mice treated with CDDP was similar to that in normal mice. On the other hand, the accumulation of FITC-hIgG in the kidney was significantly enhanced by CDDP administration. The renal mRNA level of neonatal Fc receptor (FcRn) was not affected by CDDP administration, whereas those of megalin and cubilin, endocytic receptors abundantly expressed in the renal proximal tubule, were increased. These observations suggest that acute kidney injury might have little effect on the plasma level of IgG, while renal accumulation of IgG might be increased during CDDP-induced acute kidney injury.

*Corresponding author: takanom@hiroshima-u.ac.jp

KEYWORDS: immunoglobulin G, cisplatin, acute kidney injury, FcRn, endocytosis

INTRODUCTION

Immunoglobulin G (IgG), composed of two light chains (~25 kDa each) and two heavy chains (~50 kDa each), is a monomeric immunoglobulin with a molecular weight of approximately 150 kDa. IgG is the dominant antibody in the secondary humoral immune response. The Y-shaped molecule consists of domain-like structures, in which there are two identical antigen-binding fragments (Fab) and a single non-antigen binding crystallizable fragment (Fc). IgG is the second most abundant protein in human serum, normal levels being in the range of 7-15 mg/mL (next to albumin with normal serum levels of 35-50 mg/mL) [1, 2]. Under normal conditions, the plasma half-life of IgG is considerably longer than those of other immunoglobulins. The half-life of IgG is 6-9 days in mice and 22-23 days in humans, while that of IgA (160 kDa) is 1.4 days in mice [3].

It has been reported that the neonatal Fc receptor (FcRn) is responsible for control of the plasma half-life of serum IgG [4, 5]. FcRn was initially identified in the neonatal rat small intestine, where it is responsible for the uptake of maternal IgG in the colostrum [6]. FcRn binds the Fc domain of IgG at acidic pH (below 6.5), exhibiting weak or no binding at neutral pH [7]. In vascular endothelial cells and circulating monocytes, it is suggested that the IgG, which is internalized via

fluid-phase endocytosis in the cells, binds to FcRn in an acidic endosomal compartment, and then FcRn is recycled back into the circulation, resulting in escape from lysosomal degradation. The serum half-life of mouse IgG_1 in FcRn-deficient mice has been shown to be considerably shorter (1.4 days) than that in wild type mice (9 days) [3]. At present, the vascular endothelium is assumed to be the main site where FcRn protects IgG from catabolism since FcRn expressed in the vascular endothelium is in extensive contact with the bloodstream, though this has not been fully substantiated yet.

In the kidneys, FcRn is expressed in glomerular epithelial cells (podocytes) and the brush-border of proximal tubular cells [8]. Akilesh *et al.* [9] suggested that FcRn expressed in podocytes could transcytose IgG around the slit diaphragm into the urinary space in order to prevent the glomerular filter from becoming clogged. In addition, the involvement of FcRn in the transepithelial transport of IgG has been postulated in renal proximal tubular cells [10]. Furthermore, Sarav *et al.* [11] suggested that FcRn might be responsible for urinary excretion of IgG rather than its reabsorption in renal proximal tubular cells. In contrast, we recently reported that megalin/cubilin, two multiligand endocytic receptors [12], might be involved in the uptake of FITC-labeled human IgG (FITC-hIgG) in the opossum kidney (OK) epithelial cell line [13].

Cisplatin, cis-diamminedichloroplatinum (II) (CDDP), is one of the most effective drugs against a variety of solid tumors including head and neck, testicular, ovarian, bladder, esophageal, and lung cancers. However, the clinical use of the drug is limited because of its severe nephrotoxicity [14]. CDDP nephrotoxicity is mainly characterized by tubular damage, while the glomerulus exhibits no obvious morphologic changes. Damage to the tubules causes impaired reabsorption of low-molecular weight proteins and other solutes, leading to proteinuria, glucosuria, amino aciduria, hypomagnesemia and hypokalemia [15-17]. In addition, CDDP administration often causes a reduced glomerular filtration rate, which may subsequently occur through the tubuloglomerular feedback mechanism.

In this study, we investigated whether or not CDDP-induced acute kidney injury affects the plasma disappearance and renal accumulation of FITC-hIgG in mice. Our results showed that acute kidney injury had little significant effect on the plasma elimination of FITC-hIgG, while the accumulation in the kidney was significantly increased.

MATERIALS AND METHODS

Materials

FITC-labeled IgG from human serum (FITC-hIgG) and CDDP were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in the experiments were commercial products of the highest purity available.

Pharmacokinetic study

Experiments with animals were performed in accordance with the Guide for Animal Experimentation, Hiroshima University, and the Committee of Research Facilities for Laboratory Animal Sciences, Graduate School of Biomedical & Health Sciences, Hiroshima University. Male ddy mice weighing 26 to 36 g were given FITC-hIgG at a bolus dose of 50 mg/kg via a tail vein. In the human γ-globulin injection study, excess human γ-globulin was administered at 2 days (100 mg/body), 3 days (20 mg/body), 4 days (20 mg/body), and 5 days (20 mg/body) after the injection of FITC-hIgG. In the CDDP injection study, CDDP (1 mg/mL in saline) was administered at a bolus dose of 5 mg/kg by intraperitoneal injection at day 0 (the day on which FITC-hIgG was injected), 7 days and 14 days after the bolus injection of FITC-hIgG. Blood samples were withdrawn through a tail vein at the stated times. Plasma was separated immediately by centrifugation, and was kept at -30 °C until the assay. At 15 days after FITC-hIgG injection, mice were sacrificed by exsanguination under deep anesthesia with diethyl ether.

Measurement of plasma biochemical parameters

Blood urea nitrogen (BUN), a marker of renal function, was measured using a commercially available kit (BUN assay kit from Wako Pure Chemical Industries, Ltd., Osaka, Japan). Hepatic functional parameters (aspartate aminotransferase, AST; alanine aminotransferase, ALT) were measured using a Wako kit (transaminase CII-test Wako).

Measurement of FITC-hIgG in plasma and kidney samples

For plasma samples, the plasma was solubilized in a loading buffer; 2% sodium dodecyl sulfate (SDS), 62.5 mM Tris, 7% glycerol. The samples (5.1 μL) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5% polyacrylamide gels. After the SDS-PAGE, the fluorescence intensity of bands on the gels was quantified with a fluoroimage analyzer (FLA-2000; Fuji Photo Film, Tokyo, Japan) [18]. For kidney samples, at 3 days after the injection of FITC-hIgG (50 mg/kg) without or with CDDP (5 mg/kg), the kidneys were excised and weighed. Then, the tissue was homogenized with 4 volumes of phosphate buffered saline (PBS) (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mM ethylenediaminetetraacetic acid (EDTA), and was centrifuged at 3,000 rpm for 10 min. Fluorescence intensity in the supernatant was measured by using a Hitachi fluorescence spectrophotometer F-2700 (Tokyo, Japan) at an excitation wavelength of 500 nm and an emission wavelength of 520 nm. When the kidney homogenate samples were precipitated with 5% trichloroacetic acid, the supernatant had little fluorescence. Therefore, the fluorescence in the kidney homogenate was assumed to be protein-bound fluorescein, most probably the intact form of FITC-hIgG.

Real-time PCR

Total RNA was extracted from a portion of kidney by an RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and was reverse transcribed with oligo(dT) primers using a ReverTra Dash RT-PCR kit (TOYOBO Co., LTD.). Real-time PCR was performed using SYBR Green Realtime PCR master Mix (TOYOBO Co., LTD., Osaka, Japan) as described previously [19]. The primer pairs of mouse Kim-1, FcRn, megalin, cubilin and GAPDH were as follows: Kim-1: forward 5'-AAACCAGAGATTCCCACACG-3', reverse 5'-GTCGTGGGTCTTCCTGTAGC-3'; FcRn: forward 5'-CAGCCTCTCACTGTGGACCTAGA-3', reverse 5'-TCGCCGCTGAGAGAAAGC-3'; megalin: forward 5'-AGGCCACCAGTTCACTTGCT-3', reverse 5'-AGGACACGCCCATTCTCTTG-3'; cubilin: forward 5'-GGGATCCTCTCAGGGAC ACA-3', reverse 5'-TGCTGGCCGATTCTAA ATCAA-3'; GAPDH: forward 5'-CGTGCCGC CTGGAGAAACCTG-3', reverse 5'-AGAGTGG GAGTTGCTGTTGAAGTCG-3'. The ratio for the mRNA was examined against GAPDH, and the fold-increase value for control mice was expressed as mean ± SE.

Data analysis

The plasma concentration-time curve for FITC-hIgG in mice was fitted to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$ for plasma concentration C at time t, with the aid of KaleidaGraph™ software (Synergy Software, PA, USA). The beta phase half-life was calculated as $t_{1/2\beta} = 0.693/\beta$. Statistically significant differences were determined by means of Student's *t*-test, or one way analysis of variance (ANOVA) with the Tukey-Kramer's test for post hoc analysis. A *p* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

First, we evaluated the validity of quantitative measurement of intact FITC-hIgG with a fluoroimage analyzer following separation by SDS-PAGE. After standard solutions of authentic FITC-hIgG at different concentrations ranging from 0 to 400 μg/mL have been subjected to SDS-PAGE, the gels were scanned with a fluoroimage analyzer, and then the fluorescence bands on the gels were quantified. Figure 1A shows a typical scanned image of a gel loaded with authentic FITC-hIgG. When the fluorescence intensity values were plotted against the FITC-hIgG concentrations, the fluorescence intensity linearly increased with increasing concentration of the FITC-hIgG solution loaded onto the gel (Fig. 1B). The correlation coefficient (r = 0.9968) was significant at the 1% level of confidence. Thus, the present method was applicable to the quantification of intact FITC-hIgG in samples in which the FITC-hIgG concentration was higher than 10 μg/mL.

By using this quantification method, we next examined the plasma concentration-time profile of FITC-hIgG in normal mice. Plasma samples were collected at 3, 24, 48, 72, 168, 264 and 360 hours after a bolus injection of FITC-hIgG into normal mice, and then the plasma samples were subjected

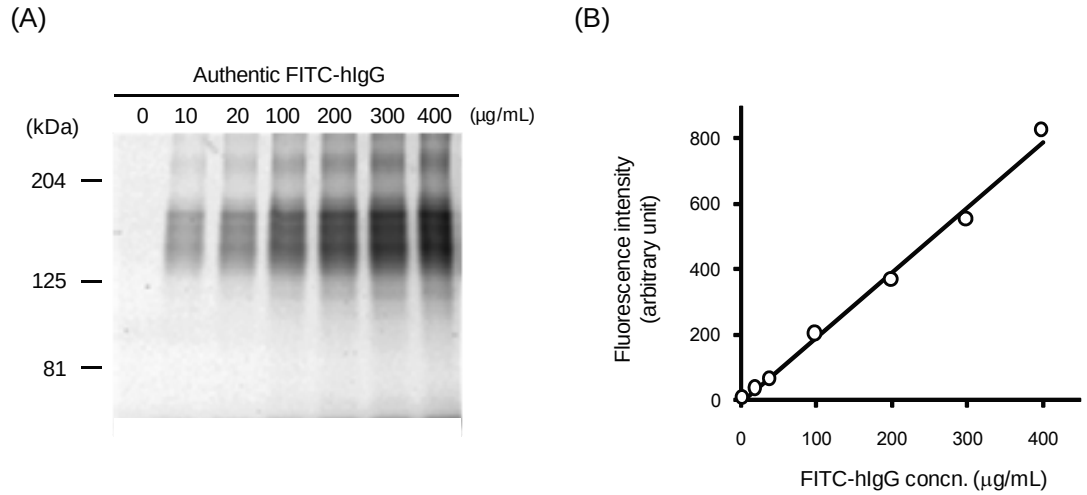

Figure 1. Quantification of fluorescein isothiocyanate-labeled human IgG (FITC-hIgG) with a fluoroimage analyzer following separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (A) A typical fluoroimage of a gel loaded with authentic FITC-hIgG solutions of seven different concentrations (0, 10, 20, 100, 200, 300 and 400 μg/mL). (B) The fluorescence intensities of the bands detected in Fig. 1A were plotted against the concentrations of FITC-hIgG.

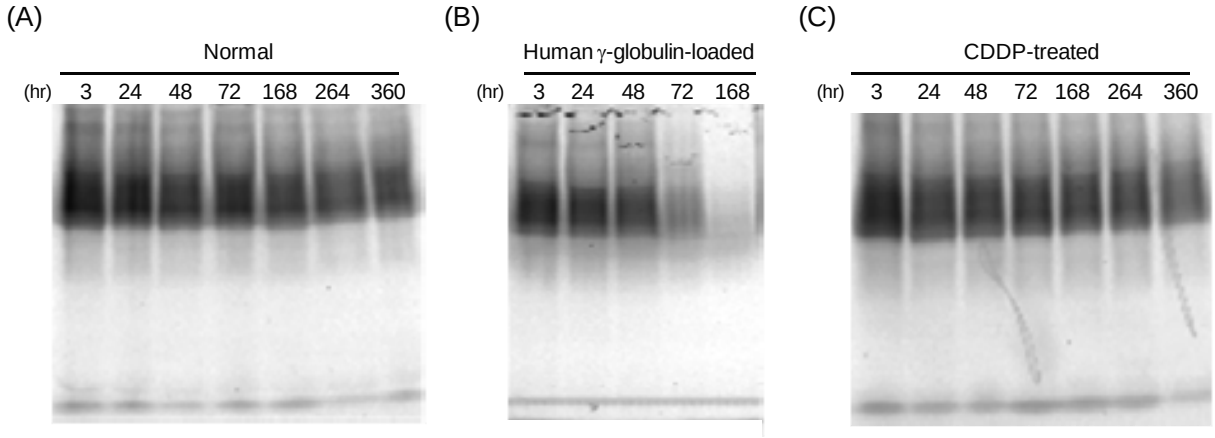

Figure 2. Detection of FITC-hIgG in plasma samples from mice injected with FITC-hIgG. Fluoroimages of gels loaded with plasma samples taken at the designated time points (3, 24, 48, 72, 168, 264 and 360 hours after FITC-hIgG injection) from normal (A), excess human γ-globulin-loaded (B), and cis-diamminedichloroplatinum (II) (CDDP)-treated (C) mice.

to SDS-PAGE for quantification of FITC-hIgG. Figure 2A shows a typical scanned image of a gel loaded with plasma samples taken from normal mice at the stated times. After FITC-hIgG in the plasma samples had been quantified, the plasma concentration of FITC-hIgG at each sampling time was plotted against time (Fig. 3, open circles). The plasma concentration of FITC-hIgG decreased biexponentially with time. The plasma half-life of FITC-hIgG in the beta-phase in normal mice was estimated to be 7.4 days, which was similar to that of mouse IgG in normal mice reported previously (6-9 days) [3, 4].

Recent studies suggest that FcRn plays a central role in the maintenance of the plasma level of IgG [5, 20]. On the other hand, Ober *et al.* [21] reported species diversity in IgG-FcRn interactions. According to this report, mouse FcRn binds IgG

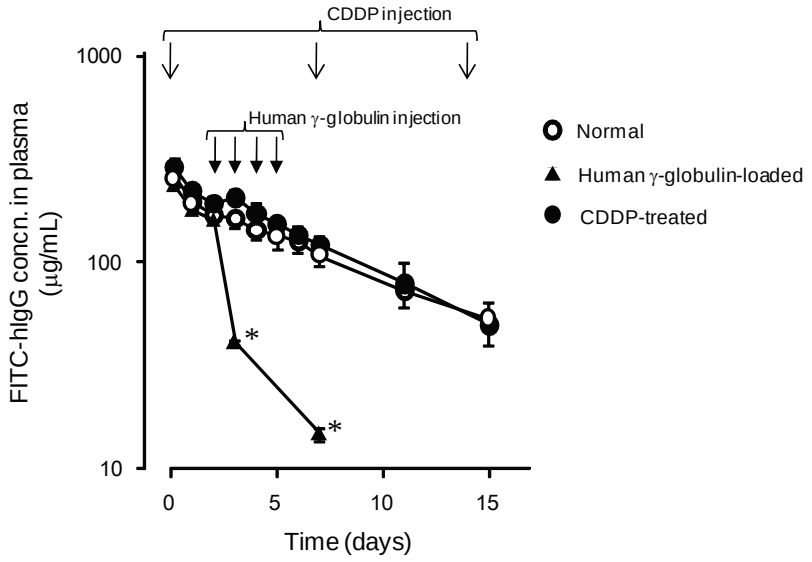

Figure 3. Plasma concentration-time profiles of FITC-hIgG in normal, excess human γ-globulin-loaded and CDDP-treated mice. At day 0, FITC-hIgG was administered at a bolus dose of 50 mg/kg via a tail vein of normal (open circles), human-γ-globulin-loaded (closed triangles), and CDDP-injected (closed circles) mice. Blood samples were taken at the designated time points (3, 24, 48, 72, 168, 264, and 360 hours after FITC-hIgG injection). Human γ-globulin was serially administered by means of intraperitoneal injection at day 2 (100 mg/body), day 3 (20 mg/body), day 4 (20 mg/body), and day 5 (20 mg/body). CDDP was given at a bolus dose of 5 mg/kg by means of intraperitoneal injection at days 0, 7 and 14. Values are expressed as means ± S.E. of the results for three to eleven mice. *$p < 0.05$, significantly different compared with the value for normal mice at each sampling time.

from various species including mouse and human, whereas human FcRn binds human IgG but not mouse IgG. The lack of affinity of human FcRn for mouse IgG is suggested to result in the shorter plasma half-life of mouse IgG, as compared to human IgG, in humans. In this study, as described above, the plasma half-life of FITC-hIgG was comparable to those of mouse and human IgG in mice, indicating that FITC-hIgG might have affinity to mouse FcRn.

To further examine whether or not the plasma level of FITC-hIgG is regulated by FcRn in mice, the effect of administration of excess human γ-globulin on the plasma level of FITC-hIgG in mice was examined. If the plasma level of FITC-hIgG in mice is maintained through the FcRn-mediated recycling pathway, the injection of excess human IgG is expected to accelerate the elimination of FITC-hIgG from the plasma. This can be explained as follows: (1) If FITC-hIgG exhibits affinity to mouse FcRn, high-dose loading of human IgG competitively would inhibit the binding of FITC-hIgG to FcRn in endosomes. (2) FITC-hIgG not bound to FcRn in endosomes is transported to lysosomes to be degraded without being recycled back into the circulation. (3) The disappearance of FITC-hIgG from the plasma is enhanced. An early study showed acceleration of the elimination of ^{131}I-labeled mouse γ-globulin on serial injection of human γ-globulin into mice [22]. According to that report, in the present study, human γ-globulin was successively administered at 2 days (100 mg/body), 3 days (20 mg/body), 4 days (20 mg/body), and 5 days (20 mg/body) after the intravenous injection of FITC-hIgG. The fluorescence intensity of FITC-hIgG rapidly decreased after the initial injection of human γ-globulin at 2 days, and it was very faint at 7 days (168 hours) after FITC-hIgG injection (at 5 days after the initial injection of human γ-globulin) (Fig. 2B). The plasma half-life of FITC-hIgG in γ-globulin-loaded mice was estimated to be 0.6 days (Fig. 3, closed triangles), which was much shorter than that in normal mice described above (7.4 days) and was similar to that in FcRn-deficient mice (1.4 days) [3]. This observation indicates that the plasma level of

FITC-hIgG in mice may be modulated by mouse FcRn.

We next investigated the effect of CDDP administration on the disappearance of plasma FITC-hIgG in mice. After CDDP (5 mg/kg) has been injected 5 min before a bolus injection of FITC-hIgG, CDDP was further administered at the same dose once a week. At 3 days after the initial injection of CDDP, BUN and the renal mRNA level of kidney injury molecule-1 (Kim-1), a biomarker of nephrotoxicity [23], were higher in CDDP-treated mice than in control mice, indicating induction of acute kidney injury (Table 1 and 2). In contrast, the serum aspartate transaminase (AST) and alanine transaminase (ALT) levels, which are commonly used as biochemical indicators of hepatocellular injury, were not significantly elevated by CDDP administration (Table 1). After 3 days, the BUN level in CDDP-treated mice was maintained high during the experiments. Figure 2C shows a typical scanned image of a gel loaded with plasma samples taken from CDDP-treated mice at the stated times. The plasma concentration-time profile of FITC-hIgG in CDDP-treated mice is shown in Fig. 3 (closed circles). The plasma concentration of FITC-hIgG in CDDP-treated mice tended to be slightly higher than that in normal mice. However, there were no significant changes in the plasma level of FITC-hIgG between normal and CDDP-treated mice. The plasma half-life of FITC-hIgG in CDDP-treated mice was 6.8 days, which was similar to that in normal mice.

Recently, several transporters, such as organic cation transporter 2 (OCT2) and the mammalian copper transporter CTR1, were shown to play a role in the cellular uptake of CDDP [24-26]. However, such CDDP uptake transporters might not be abundantly expressed in the main tissue(s) that are involved in the systemic clearance of IgG because no significant effect of CDDP treatment on the plasma level of FITC-hIgG was observed in mice.

Furthermore, the fluorescence in the kidney was measured at 3 days after the injection of FITC-hIgG (50 mg/kg) without or with CDDP (5 mg/kg). As shown in Fig. 4, the fluorescence in the kidney from CDDP-treated mice was significantly higher than that from normal mice. We previously found that CDDP inhibited renal vacuolar H^+-ATPase activity [27], which is responsible for the acidification of intracellular compartments including endosomes and lysosomes. Therefore, the enhanced FITC-hIgG in the kidney of CDDP-treated mice might be, at least in part, due to increased retention following a decrease in lysosomal degradation of FITC-hIgG in renal tubular cells.

In addition, we investigated the effect of CDDP administration on mRNA expression levels of FcRn, megalin and cubilin in the kidney at day 3 after CDDP administration. FcRn mRNA level was not affected by the injection of CDDP (1.1-fold), while the mRNA levels of megalin and cubilin were increased by 2.3- and 2.5-fold, respectively (Table 2). It is likely that the change in megalin mRNA level is in accordance with our previous observation that CDDP administration induced an increase in the expression level of megalin protein in rat renal cortex [17]. Further studies will be needed to investigate how the changes in the expression level of these endocytic receptors are related to the enhanced accumulation of FITC-hIgG in the kidney of CDDP-treated mice.

Table 1. Blood urea nitrogen (BUN), aspartate transaminase (AST), and alanine transaminase (ALT) levels in control and CDDP-treated mice.

	BUN (mg/dL)			AST (IU/L)			ALT (IU/L)		
	day 3	day 7	day 15	day 3	day 7	day 15	day 3	day 7	day 15
Control	17.7 ± 2.5	14.5 ± 1.7	20.5 ± 3.6	50.6 ± 15.6	70.4 ± 25.1	30.3 ± 7.1	12.0 ± 1.7	14.4 ± 2.1	4.6 ± 2.9
CDDP-treated	42.1 ± 5.9*	30.0 ± 3.5*	51.1 ± 11.6*	90.7 ± 34.7	43.5 ± 3.6	47.5 ± 8.9	13.2 ± 1.4	13.0 ± 1.9	8.2 ± 0.8

CDDP was given to mice at a bolus dose of 5 mg/kg by means of intraperitoneal injection at days 0, 7 and 14. Values are expressed as means ± S.E. of the results for three to four mice. *$p < 0.05$, significantly different compared with the value for control mice at each sampling time.

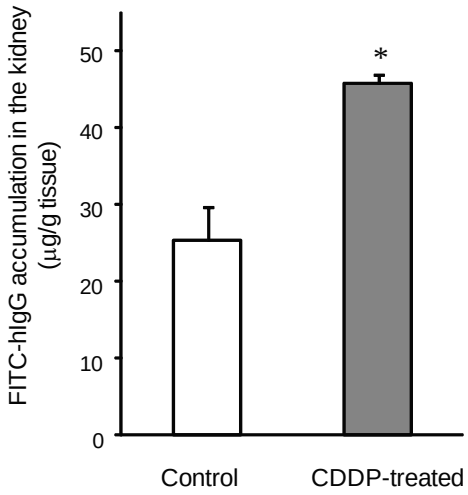

Figure 4. Renal accumulation of FITC-hIgG in control and CDDP-treated mice. At 3 days after the injection of FITC-hIgG (50 mg/kg) without or with CDDP (5 mg/kg), the fluorescence in the kidney were measured as described in Materials and Methods. Each column is expressed as means ± S.E. of the results for three mice. *$p < 0.05$, significantly different compared with the value for control mice.

Table 2. Changes in the expression levels of Kim-1, FcRn, megalin and cubilin mRNAs in kidney from CDDP-treated mice.

mRNA	Fold-change (ratio)
Kim-1	5.34 ± 1.31
FcRn	1.05 ± 0.05
Megalin	2.28 ± 0.59
Cubilin	2.46 ± 0.30

Values are expressed as the fold-change in expression ratio of target gene to GAPDH mRNA in kidneys from CDDP-treated mice (day 3 after CDDP injection at 5 mg/kg) compared with control mice by quantitative real-time PCR. The values are expressed as means ± S.E. of the results for three mice.

CONCLUSION

In conclusion, we showed that FITC-hIgG injected intravenously into mice disappeared from the plasma with a half-life of 7.4 days, which is consistent with that of mouse IgG in mice. In addition, acute kidney injury due to CDDP administration caused no significant change in the disappearance of plasma FITC-hIgG in mice, while CDDP administration increased the accumulation of FITC-hIgG in the kidney. These observations suggest that acute kidney injury might have little effect on the systemic clearance of IgG, while the renal handling of IgG might alter during acute kidney injury.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

REFERENCES

1. Weeke, B. 1968, Scand. J. Clin. Lab. Invest., 22, 107.
2. Kratochwil, N. A., Huber, W., Müller, F., Kansy, M. and Gerber, P. R. 2002, Biochem. Pharmacol., 64, 1355.
3. Roopenian, D. C., Christianson, G. J., Sproule, T. J., Brown, A. C., Akilesh, S., Jung, N., Petkova, S., Avanessian, L., Choi, E. Y., Shaffer, D. J., Eden, P. A. and Anderson, C. L. 2003, J. Immunol., 170, 3528.
4. Junghans, R. P. and Anderson, C. L. 1996, Proc. Natl. Acad. Sci. USA, 93, 5512.
5. Roopenian, D. C. and Akilesh, S. 2007, Nat. Rev. Immunol., 7, 715.
6. Simister, N. E. and Rees, A. R. 1985, Eur. J. Immunol., 15, 733.
7. Raghavan, M., Bonagura, V. R., Morrison, S. L. and Björkman, P. J. 1995, Biochemistry, 34, 14649.
8. Haymann, J. P., Levraud, J. P., Bouet, S., Kappes, V., Hagège, J., Nguyen, G., Xu, Y., Rondeau, E. and Sraer, J. D. 2000, J. Am. Soc. Nephrol., 11, 632.
9. Akilesh, S., Huber, T. B., Wu, H., Wang, G., Hartleben, B., Kopp, J. B., Miner, J. H., Roopenian, D. C., Unanue, E. R. and Shaw, A. S. 2008, Proc. Natl. Acad. Sci. USA, 105, 967.
10. Kobayashi, N., Suzuki, Y., Tsuge, T., Okumura, K., Ra, C. and Tomino, Y. 2002, Am. J. Physiol. Renal Physiol., 282, F358.
11. Sarav, M., Wang, Y., Hack, B. K., Chang, A., Jensen, M., Bao, L. and Quigg, R. J. 2009, J. Am. Soc. Nephrol., 20, 1941.
12. Christensen, E. I. and Birn, H. 2002, Nat. Rev. Mol. Cell Biol., 3, 256.
13. Nagai, J., Sato, K., Yumoto, R. and Takano, M. 2011, Drug Metab. Pharmacokinet., 26, 474.

14. Yao, X., Panichpisal, K., Kurtzman, N. and Nugent, K. 2007, Am. J. Med. Sci., 334, 115.
15. Daugaard, G., and Abildgaard, U. 1989, Cancer Chemother. Pharmacol., 25, 1.
16. Kim, Y. K., Byun, H. S., Kim, Y. H., Woo, J. S. and Lee, S. H. 1995, Toxicol. Appl. Pharmacol., 130, 19.
17. Takano, M., Nakanishi, N., Kitahara, Y., Sasaki, Y., Murakami, T. and Nagai, J. 2002, Kidney Int., 62, 1707.
18. Takano, M., Koyama, Y., Nishikawa, H., Murakami, T. and Yumoto, R. 2004, Eur. J. Pharmacol., 502, 149.
19. Sato, K., Nagai, J., Mitsui, N., Yumoto, R. and Takano, M. 2009, Life Sci., 85, 800.
20. Keizer, R. J., Huitema, A. D., Schellens, J. H. and Beijnen, J. H. 2010, Clin. Pharmacokinet., 49, 493.
21. Ober, R. J., Radu, C. G., Ghetie, V. and Ward, E. S. 2001, Int. Immunol., 13, 1551.
22. Fahey, J. L. and Robinson, A. G. 1963, J. Exp. Med., 118, 845.
23. Ichimura, T., Hung, C. C., Yang, S. A., Stevens, J. L. and Bonventre, J. V. 2004, Am. J. Physiol. Renal Physiol., 286, F552.
24. Yonezawa, A., Masuda, S., Nishihara, K., Yano, I., Katsura, T., Inui, K. 2005, Biochem. Pharmacol., 70, 1823.
25. Filipski, K. K., Mathijssen, R. H., Mikkelsen, T. S., Schinkel, A. H. and Sparreboom, A. 2009, Clin. Pharmacol. Ther., 86, 396.
26. Larson, C. A., Blair, B. G., Safaei, R. and Howell, S. B. 2009, Mol. Pharmacol., 75, 324.
27. Shiraishi, Y., Nagai, J., Murakami, T. and Takano, M. 2000, Life Sci., 67, 1047.