Coordinate and Independent Effects of Cocaine, Alcohol, and Morphine on Accumulation of IgG Aggregates in the Rat Glomeruli (43673)

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Abstract. **Focal glomerulosclerosis is the predominant glomerular lesion in patients with drug addiction. Since mesangial expansion has been considered a precursor of glomerulosclerosis we investigated whether the use of these drugs can cause accumulation of macromolecules into mesangium which may contribute to the expansion of mesangium. The majority of drug addicts at times take drugs in groups and may thus be exposed to a variety of drugs (cocaine, alcohol, and heroin). Therefore, we studied the effect of cocaine, alcohol, and morphine alone or in combination on the accumulation of radiolabeled human immunoglobulin-G (IgG) aggregates (AHIgG^{125}I) into glomeruli/mesangium. Cocaine enhanced accumulation of AHIgG^{125}I at 8 hr. Glomerular levels of AHIgG^{125}I levels were also higher in morphine treated rats when compared with untreated animals. Alcohol did not alter the deposition of AHIgG^{125}I. But at an earlier time (4 hr) alcohol enhanced the effect of cocaine on accumulation of IgG aggregates into the mesangium. The combined effects of morphine and cocaine, or morphine and alcohol were not different than the effect of morphine alone. The enhanced accumulation of phlogogenic macromolecules into the mesangium may not only increase the quantity of mesangial matrix but may also alter the quality of matrix. This may be playing an important role in the development of glomerular injury.**

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Focal glomerular sclerosis (FGS) is the predominant renal lesion in patients with drug addiction (1). The majority of drug addicts besides injecting heroin also use other drugs from time to time. These other commonly used drugs are usually cocaine and alcohol. Since mesangial expansion has been considered a precursor of FGS, we asked whether cocaine and alcohol when used alone or in combination with morphine (active metabolite of heroin) can cause an expansion of mesangium.

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The glomerular mesangium is centrally located and continuously perfused with macromolecules circulating in the blood (2–4). The deposition of macromolecules into the mesangium is dependent upon the net balance between delivery and the exit of macromolecules (3). Since increased deposition of macromolecules into the mesangium can cause expansion of the mesangium, we asked whether cocaine and alcohol alone or in combination with morphine can enhance the accumulation of circulating macromolecules into the mesangium. To determine whether these drugs can alter the accumulation of circulating macromolecules into the mesangium, we used iodinated heat aggregated immunoglobulin-G complexes (AHIgG^{125}I) as model macromolecules. The aggregated IgG complexes have many of the properties of antigen-antibody complexes (5). Furthermore, patients with drug addiction also have circulating IgG complexes, as they administer heroin and cocaine by blood-contaminated needles under unsterile conditions (6).

These conditions may lead to the production of antigen-antibody complexes in the blood.

Materials and Methods

Various investigators (7–10), including us (11), have previously shown that at 4 hr and later times, intravenously administered human IgG aggregates were localized entirely within the glomerulus and were confined almost totally to the mesangial area. In the present study we measured accumulation of AHIgG^{125}I in the isolated glomeruli between 4 to 8 hr to study the effects of cocaine or alcohol alone or in combination with morphine.

Four groups of five rats each (male, Sprague-Dawley, 100 g, source—Taconic Laboratory animals, Germantown, NY; diet—Purina Rat Chow #5001) were administered intraperitoneally 0.2 ml of normal saline (control) or 0.2 ml of normal saline containing cocaine (30 mg/kg body wt) or alcohol (2 gm/kg body wt) or cocaine (30 mg/kg body wt) + alcohol (2 gm/kg body wt) half an hour before AHIgG^{125}I infusion. Control and experimental rats were given AHIgG^{125}I (specific activity: 100,000 cpm/μg) in a dose of 0.20 mg per gram body weight by tail vein injection. Phenergan was administered intraperitoneally to the animals 5 min before the AHIgG^{125}I in a dose of 0.005 mg/g body wt to reduce the chance of anaphylactic shock. One hundred microliters of blood was collected from tail vein at 0 hr and at 4 hr. Blood hematocrit was measured at 0 hr and upon termination of the experiment. Control and experimental rats were sacrificed at 4 hr by injecting a massive intracardiac dose of sodium pentobarbital. One milliliter of blood was collected from each animal at the time of sacrifice. Isolation of glomeruli from both kidneys of each animal was carried out. In addition to measuring AHIgG^{125}I uptake by the glomerular mesangium, we removed and measured uptake in their lungs, spleen, and liver. AHIgG^{125}I content of blood was also measured.

To determine the effect of various agents on IgG kinetics on later time periods (i.e., at 8 hr) seven groups of five to 15 rats each were administered either vehicle (control) or experimental agent. Control and experimental rats were administered intraperitoneally either 0.2 ml of normal saline (control) or 0.2 ml of normal saline containing morphine (30 mg/kg body wt) or cocaine (30 mg/kg body wt) or alcohol (2 gm/kg body wt) or cocaine (30 mg/kg body wt) + alcohol (2 gm/kg body wt) or cocaine (30 mg/kg body wt) + morphine (30 mg/kg body wt) or morphine (30 mg/kg body wt) + alcohol (2 gm/kg body wt) half an hour before AHIgG^{125}I infusion. Control and experimental rats were infused AHIgG^{125}I by tail vein in a dose of 0.20 mg/g body wt. Phenergan (0.005 mg/g body wt) was administered intraperitoneally 5 min before the AHIgG^{125}I infusion. One hundred microliters of blood was collected from tail vein at 0 hr and at 8 hr.

To evaluate the effect of these agents on the rats blood pressure, diastolic blood pressure was measured by the tail cuff pressure method before and after administration of various agents in some of the rats. At the end of 8 hr animals were sacrificed and the above mentioned protocol for measurement of the uptake of AHIgG^{125}I by mesangium, liver, and spleen was carried out.

Preparation and Iodination of Aggregated Human IgG (AHIgG^{125}I). Iodinated human IgG was prepared according to the method of McConahey and Dixon (12). Twenty-five milligrams of concentrated IgG (165 mg/ml) was added to a beaker containing 20 ml of a 0.15 *M* sodium phosphate buffer solution at pH 7.0. While continuously stirring, 5 mCi of ^{125}I was added after which chloramine-T (30 ml of 0.2 mg/ml solution) was added in a drop-wise manner. Sodium metabisulphite was added after 5 min to stop the reaction. This solution was then dialyzed in 0.1 *M* phosphate buffer, pH 7.0, with several changes over a period of 18 hr to remove any nonprotein-bound iodine.

To prepare radiolabeled aggregated human IgG, 0.2 mCi of labeled protein was added to 100 ml of a 2% IgG solution in 0.15 *M* saline buffer with 0.01 *M* sodium phosphate. This mixture was heated to 63°C for 20 min with continuous stirring. After the solution cooled to room temperature 40 ml of 2.18 *M* sodium sulfate solution was added and stirred for an additional 30 min. To pellet the protein the mixture was centrifuged at 3000 g, 4°C for 30 min. The pellet was resuspended in a small quantity of PBS and dialyzed against this buffer for 24 hr with at least three bath changes. The protein solution was recentrifuged at 5000 g to remove insoluble protein aggregates. Amount of protein was determined by the method of Lowry *et al.* (13). The free ^{125}I of the final AHIgG^{125}I preparation was <1%. The AHIgG^{125}I was stored at 4°C and used within one week of preparation.

The following experimental agents were used: morphine stocked in a concentration of 10^{-2} *M* in normal saline (NIDA); cocaine stocked in a concentration of 10^{-2} in normal saline and (Sigma); ethyl alcohol was stocked as absolute alcohol (Aaper Alcohol and Chemical Co., Shelbyville, KY) and used at a dose of 200 mg/100 g of body wt; ^{125}I (NEN Products, Boston, MA); human IgG stocked in a concentration of 165 mg/ml (Armour Pharmaceutical Co., Kankakee, IL).

Glomerular Isolation. After bilateral nephrectomy the rat kidneys were decapsulated and placed into clean petri dishes in normal saline. Both kidneys from each animal were pooled for isolation of glomeruli. Glomeruli were sliced in half with a razor blade. The cortex was separated out and minced with a razor blade. The minced kidney cortex was passed through

a 150 μm pore sieve. Glomeruli were collected in a 50 ml conical tube by washing with normal saline on the bottom of this sieve and the top of the next sieve of pore size 106 μm. Tubes were centrifuged at 1000 rpm for 5 min and the supernatant discarded. All steps were carried out at 4°C. The purity of each preparation of glomeruli was evaluated by light microscopy and was 95% or higher. The glomeruli were washed four times with normal saline and counted for ^{125}I. Glomerular protein content was measured by the method of Lowry *et al.* (13).

Preparation of Lung, Liver, Spleen, and Blood for Uptake of AHIgG^{125}I. Whole lung, liver, and spleen were obtained from each animal at the time of sacrifice. These tissues were washed twice in cold saline, dried in a ventilated oven overnight at 85°C, and weighed. Samples of these tissues were dissolved in 1.0 *N* sodium hydroxide containing 0.2 *N* sodium deoxycholate with heating at 95°C for 1 hr. The dissolved tissue was cooled and counted for ^{125}I and data expressed as AHIgG^{125}I per gram of protein content.

Initial ^{125}I measurements were carried out on whole blood samples. An hour later, serum was separated, and measurement of ^{125}I counts was carried out on the serum samples. Subsequently, 2.0 ml of 10% TCA was added to precipitate proteins. The samples were centrifuged at 5000 g, and the supernatant was counted to determine free ^{125}I. Protein-bound counts were calculated and expressed as per gram of protein count.

Preparation of Gold Particles. Colloidal gold particles, 10–50 nm in diameter, were prepared as described previously (14). To achieve an equal number of gold particles in a given volume, a fixed amount of colloidal gold solution was mixed with IgG. After the addition of a fixed quantity of IgG, the mixture was stirred well and kept at 37°C for 30 min. The coated gold particles were then concentrated by centrifuging for 20 min at 17,000 rpm in a Sorvall RC 2B refrigerated superspeed centrifuge. The supernatant was poured off, and the pellet washed twice with Dulbecco's phosphate-buffered saline (D-PBS), pH 7.4 (GIBCO), and recentrifuged. The third pellet was resuspended in 5 ml of normal saline and served as IgG-coated gold particles.

Preparation of Renal Tissue for Transmission Electron Microscopic Studies. Control and morphine treated (two rats in each group) rats were administered IgG-coated gold particles by tail vein. Four hours later, all the rats were sacrificed and kidneys were removed. Renal cortices were cut as tiny pieces and fixed in a solution containing 1% glutaraldehyde and 0.2% tannic acid for 24 hr at room temperature, then post-fixed with 1% osmium tetraoxide (OsO_4) for 30 min on ice, and stained with 1% uranyl acetate in 10% ethanol for 30 min at room temperature. Sequential dehydration was carried out in ethanol. A gradual transfer from ethanol to Epon 812 (polysciences, Warrington, PA) was carried out. Subsequently, the tissue was transferred to embedding molds. Molds were kept in an oven for slow cure for 72 hr (48°C for the first 24 hr and 60°C for the next 48 hr). Thin sections were made with an LKB ultramicrotome and observed with a 1200 EX JEOL electron microscope, after being counterstained with uranyl acetate and lead citrate.

Statistical Analysis. Comparison of the accumulation of AHIgG^{125}I in control and experimental rats was carried out by unpaired *T*-test. Multiple group analyses was carried out by analysis of variance (ANOVA). To get q value Newman-Keuls multiple range test was used. Differences were considered significant at $P < 0.05$. All values are means ± SEM.

Results

Effect of Cocaine on Glomerular Accumulation of IgG Aggregates. At 4 hr, glomerular levels of AHIgG^{125}I in control and cocaine treated rats are shown in Table I. There was no difference in the deposition of AHIgG^{125}I between the control vs experimental animals. However, at 8 hr glomerular levels of AHIgG^{125}I were higher ($P < 0.05$) in cocaine-treated rats when compared with vehicle-treated rats. These results indicate that cocaine enhances deposition of IgG aggregates into the mesangium at the later time periods.

Effect of Alcohol on Glomerular Accumulation of IgG Aggregates. The effect of alcohol on glomerular levels of AHIgG^{125}I at 4 and 8 hr has been shown in Table I. Glomerular levels of AHIgG^{125}I were not significantly different at 4 hr (Table I) as well as at 8 hr

Table I. Effect of Cocaine, Alcohol, and Cocaine + Alcohol on Glomerular IgG Aggregate Kinetics[a]

	Duration	Control	Cocaine	Alcohol	Cocaine + alcohol
Accumulation of AHIgG^{125}I/g glomerular protein	4 hr	258,160 ± 11,671	219,872 ± 14,220	267,700 ± 27,434	353,649*† ± 35,834
	8 hr	63,630 ± 3,984	102,297* ± 24,299	69,777 ± 5,211	–

[a] Four groups of five rats each including control and experimental were administered intraperitoneally normal saline (control) or normal saline containing 30 mg/kg body wt of cocaine or 2 g/kg body wt of alcohol or cocaine (30 mg/kg body wt) + alcohol (2 g/kg body wt). Half an hour later all rats were given AHIgG^{125}I in a dose of 0.20 mg/g body wt by tail vein. All rats were sacrificed by groups at 4 hr, kidneys removed and glomeruli isolated. Results are means ± SEM (cpm/g glomerular protein).
*$P < 0.05$ compared with respective control; †$P < 0.01$ compared with cocaine alone.

between the alcohol-treated and vehicle-treated rats. These results indicate that alcohol does not affect deposition of macromolecules into the mesangium.

Effect of Morphine on Mesangial Deposition of IgG Aggregates. The effect of morphine on deposition of AHIgG^{125}I into the mesangium has been shown in Table II. Glomerular levels of AHIgG^{125}I were higher ($P < 0.02$) in morphine-treated rats when compared with vehicle treated rats. These results further confirm our preliminary observations (11).

Effect of Combined Treatment of Cocaine and Alcohol on Accumulation of IgG Aggregates. The combined effect of treatment of cocaine and alcohol on accumulation of AHIgG^{125}I into the mesangium is shown in Table I. The combined treatment of cocaine and alcohol enhanced accumulation of IgG aggregates into the mesangium when compared with either control ($P < 0.05$) or cocaine alone ($P < 0.01$). These results suggest alcohol enhances the effect of cocaine on accumulation of IgG aggregates into the mesangium.

Effect of Combined Treatments of Morphine and Alcohol or Morphine and Cocaine. The effects of combined treatments of morphine and alcohol are shown in Table II. The combined treatment of morphine enhanced ($P < 0.02$) accumulation of AHIgG^{125}I into the mesangium when compared with vehicle-treated (control) rats. However, this combined effect of morphine and alcohol did not reach to significant level when compared to the effect of morphine alone (Table II). The combined effect of cocaine and morphine on the accumulation of IgG aggregates was not different when compared with morphine-treated rats.

Uptake of AHIgG^{125}I by Liver, Spleen, and Lungs. There was no significant difference in the uptake of IgG aggregates amongst the livers of animals treated under variable conditions. Similarly, the mean uptake of IgG aggregates in spleen and lungs in various groups were not different.

Blood Levels of AHIgG^{125}I at 0 and 8 hr. Blood levels of AHIgG^{125}I at the 0 hour and 8 hr in various groups have been shown in Figure 1. Blood levels of AHIgG^{125}I were comparable in between the two groups at 0 hr. Blood levels of AHIgG^{125}I declined to 50% at 8 hr.

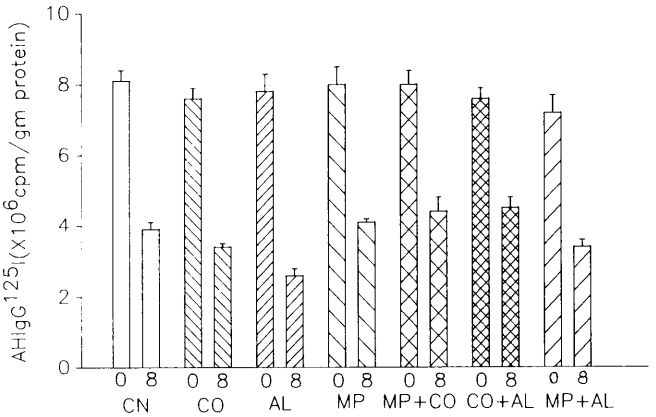

Figure 1. Blood levels of AHIgG^{125}I at 0 hr and 8 hr. Vehicle (CN), cocaine (CO), alcohol (AL), morphine (MP), MP + CO, CO + AL, or MP + AL treated rats were infused AHIgG^{125}I by tail vein. Blood samples were collected immediately (0 hr) and at 8 hr. Results represent means ± SEM.

Transmission Electron Microscopic Studies. IgG-coated gold particles were aggregated predominantly in mesangial enodosomes and lysosomes in control as well as in morphine-treated rats. Representative electron microphotograph showing aggregation of gold particles in glomerular mesangium of a morphine-treated rat is shown in Figure 2.

Discussion

The present study shows that cocaine-treated rats have increased accumulation of IgG aggregates in to glomeruli/mesangium at later time periods. Although alcohol alone did not alter deposition of IgG aggregates into the mesangium, rats treated with alcohol and cocaine together had a higher accumulation of IgG aggregates. Morphine-treated rats had increased accumulation of AHIgG^{125}I into the mesangium. Morphine in combination with cocaine and alcohol also increased accumulation of IgG complexes into glomeruli/mesangium. These results suggest that at times other drugs besides morphine, used either alone or in combination, may also result in increased accumulation of macromolecules into the mesangium.

We have recently demonstrated that morphine attenuates phagocytosis of IgG complexes by macrophages as well as mononuclear phagocyte system (11).

Table II. Effect of Combined Treatment of Morphine and Alcohol or Morphine and Cocaine on Mesangial IgG Aggregate Kinetics at 8 hr[a]

	Control	Morphine	Morphine + alcohol	Morphine + cocaine
Accumulation of AHIgG^{125}I/g glomerular protein	63,630 ± 3,984	80,420* ± 3,700	116,890* ± 23,099	85,657 ± 17,279

[a] Four groups of rats (control—15 rats, morphine—8 rats, morphine + alcohol—10 rats, morphine + cocaine—10 rats) were administered intraperitoneally normal saline (control) or normal saline containing morphine (30 mg/kg body wt) or morphine (30 mg/kg body wt) + alcohol (2 g/kg body wt) or morphine (30 mg/kg body wt) + cocaine (30 mg/kg body wt). Half an hour later all the rats were infused equal amount of AHIgG^{125}I by tail vein. All the rats were sacrificed at 8 hr and glomeruli isolated. Results represent means ± SEM (cpm/g of glomerular protein).
* $P < 0.02$ compared with control.

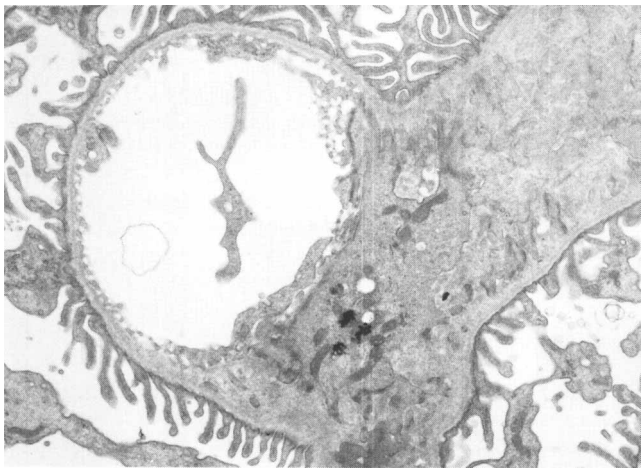

Figure 2. Electron micrograph showing aggregation of IgG-coated gold particles in lysosomes of a mesangial cell (Magnification ×10,000).

Other investigators have also demonstrated that morphine impairs function of the mononuclear phagocyte system in multiple ways, including impairment of the phagocytic process and modulation of respiratory burst activity (15, 16). A decreased uptake of circulating phlogogenic macromolecules by the mononuclear phagocyte system may lead to increased concentration of phlogogenic macromolecules in the circulation in patients with drug addiction. Haakanstad *et al.* (17) demonstrated a relationship between the generalized localization of immune complexes and blood levels in mice. Other investigators also demonstrated that blood concentration of macromolecules was an important determinant of the delivery of these macromolecules into the mesangium (3, 4).

The role of increased accumulation of macromolecules in the development and progression of glomerulosclerosis has been investigated by various workers (18–20). Keane and Raij (18) demonstrated that administration of puromycin aminonucleoside (PAN) as well as adriamycin resulted in the development of minimal change glomerulopathy in rats. However, PAN-induced nephrosis progressed subsequently to focal segmental sclerosis (FGS), whereas adriamycin-treated rats did not progress to FGS. Mauer *et al.* (11) demonstrated increased deposition of IgG aggregates into the mesangium of PAN rats when compared to control rats. Kean *et al.* (18) and Mauer *et al.* (10) suggested that increased mesangial deposition of macromolecules into the mesangium in PAN rats has contributed to the progression of FGS. Grond *et al.* (20) also elaborated the role of macromolecular deposition in the PAN model. They showed increased deposition of colloidal carbon particles in the glomeruli of the rats of PAN-induced nephrosis.

We propose that in patients with drug addiction, it is the recurrent or persistent delivery of phlogogenic macromolecules into the mesangium which may be acting as a determinant for initial expansion and subsequent development of sclerosis of the mesangium. Interaction of accumulated phlogogenic macromolecules with mesangial cells and macrophages may cause generation of lipid mediators of inflammation and reactive oxygen species (14, 21, 22). These substances may stimulate mesangial cell proliferation and matrix synthesis. Furthermore accumulated phlogogenic macromolecules into the mesangium may not only directly increase the quantity of mesangium but may also alter the quality of matrix. Growth response and matrix synthesis by mesangial cells have been demonstrated to be different when mesangial cells were grown on plastic substrate vs plastic substrate coated with collagen (23).

We conclude that cocaine and alcohol, when used together, increase deposition of IgG aggregates into the glomeruli/mesangium. Morphine also enhances deposition of macromolecules into glomeruli/mesangium. However, neither alcohol nor cocaine adds to the effect of morphine on the deposition of macromolecules into the mesangium.

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