Kidney International, Vol. 53 (1998), pp. 1647–1653

Clusterin protects against oxidative stress *in vitro* through aggregative and nonaggregative properties

GARY B. SCHWOCHAU, KARL A. NATH, and MARK E. ROSENBERG

Departments of Medicine, University of Minnesota, Minneapolis, and Mayo Clinic/Foundation, Rochester, Minnesota, USA

Clusterin protects against oxidative stress *in vitro* through aggregative and nonaggregative properties. Perturbations of cell interactions, an early event in acute renal injury, have important pathophysiologic consequences. We hypothesized that promotion of cell interactions protects cells from injury. To test this hypothesis, a single cell suspension of LLC-PK_1 cells (porcine proximal tubular cell line) treated with albumin (control) was compared to cells aggregated with fibrinogen or purified human clusterin (aggregation graded 0 to 4). Following aggregation, the cells were injured with 1.5 mM hydrogen peroxide (H_2O_2) for three hours. Cell aggregation induced by clusterin but not fibrinogen protected against oxidant injury by H_2O_2. Complete abrogation of cytotoxicity occurred at a clusterin concentration of 2.5 μg/ml, which resulted in an aggregation score of 1. In the absence of aggregation, clusterin at concentrations of 20 and 50 μg/ml, but not lower doses, partially protected against injury induced by H_2O_2. Cell aggregation induced by both clusterin and fibrinogen partially protected against endogenously generated oxidant stress induced by incubating LLC-PK_1 cells with aminotriazole and 1-chloro-2,4-dinitrobenzene (CDNB). In conclusion, clusterin protects against models of oxidant stress *in vitro*, whether generated by exogenously administered hydrogen peroxide, or from endogenously produced peroxide, and such protective effects can accrue from aggregative and nonaggregative properties of clusterin.

Clusterin, a heterodimeric glycoprotein, is widely and prominently induced in injured tissue, particularly when such injury involves the kidney and the brain [1]. While the function of clusterin in such states of injury remain uncertain, a number of studies point to cytoprotective actions of clusterin induced in such settings [2–6].

Clusterin induces aggregation of some but not all cells *in vitro* and also is a potent cell adhesion molecule [7–9]. Prolonged incubation of cells with clusterin results in the formation of junctional contacts between cells and expression of cell adhesion molecules [8, 9]. Clusterin may also be involved in more complex cell interactions. For example, when vascular smooth muscle cells are grown in culture they generate morphologically differentiated nodules. Clusterin is differentially expressed in these nodules with a time course, suggesting a role in nodule formation [10, 11]. Addition of anti-clusterin antibodies but not control antibodies inhibits nodule formation [11]. Furthermore, clusterin is expressed during ontogeny at sites where critical cell interactions are taking place, such as the branching bronchial tree and in the ureteric bud prior to mesenchymal condensation [12, 13].

Abnormalities in cell interactions occur following renal injury leading to cell rounding, retraction and detachment; decreased binding of cells to matrix components is also recognized as well as aberrant cell-cell attachments [14–20]. Loss of intercellular contact may also induce apoptosis [21]. The loss of cell-cell and cell-substratum interactions incurs detachment of tubular epithelial cells and in turn, denudation of the epithelium and tubular back leak of filtrate. Thus, even mild injury can lead to profound nephron dysfunction mediated in part through alterations in cell attachment. Loss of normal cell interactions from their usual cellular contacts and substratum lead to variable effects on cellular viability. On the one hand, cells so removed may be otherwise intact, as evidenced by the presence of viable tubular epithelial cells recoverable from the urine in patients with acute renal failure [22]. On the other hand, the loss of these contacts may lead to cell death; for example, MDCK cells undergo apoptosis if they are prevented from attaching to culture plates [23]. The term "anoikis" has been used to describe this phenomenon. Similarly, human endothelial cells undergo apoptosis when cell-matrix interactions are inhibited [24]. In the setting of renal injury, promoting cell-cell contacts may enhance cell survival by allowing the exchange of critical nutrients or other factors between cells, or by providing the correct intracellular signals to prevent apoptosis. Promoting cell-substratum contacts would prevent sloughing of cells. In addition, maintenance of cell proximity would allow the normal junctional contacts to become re-established once the phase of injury subsides and the processes of repair and regeneration are initiated.

The prominent induction of clusterin in tubules following injury coupled with the capacity of this protein to promote cell interactions led us to hypothesize that the induction of clusterin in states of injury provides an adaptive response that attenuates injury that otherwise occurs. We thus tested whether clusterin could protect renal tubular epithelial cells from injury induced by a recognized pathway of renal injury, namely, oxidative stress. We studied two forms of oxidative stress: one was induced by the extracellular application of reagent hydrogen peroxide. The other was induced by a combined chemical manipulation that impaired intracellular scavenging mechanisms for hydrogen peroxide, a model based on the application of aminotriazole, an inhibitor of catalase, and

Key words: cytotoxicity, cell interactions, oxidant injury, cytoprotection, anoikas, fibrinogen, glycoprotein.

Received for publication October 28, 1997
and in revised form December 31, 1997
Accepted for publication January 2, 1998

1-chloro-2,4-dinitrobenzene (CDNB), a consumer of cellular glutathione [25].

METHODS

Experimental protocols

Cell culture. Porcine renal epithelial (LLC-PK_1) cells were obtained from the American Type Culture Collection (Rockville, MD, USA) at passage number 196. All experiments were performed between passage numbers 196 and 206. Cells were grown at 37°C in 95% air and 5% carbon dioxide in Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co., St. Louis, MO, USA) with 10% fetal bovine serum (Gibco BRL), L-glutamine (330 μg/ml), Hepes (5.96 mg/ml), sodium bicarbonate (3.7 mg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml).

Clusterin purification. Clusterin was purified from fresh human serum by immunoaffinity chromatography as described previously [9]. The column contained the mouse anti-human clusterin monoclonal antibody G7 (gift of Brendan Murphy, Melbourne, Australia). After the sample was applied to the column, the column was sequentially washed with: 20 ml of 10 mM phosphate, 500 mM sodium chloride, 10 mM EDTA, pH 7.4; 20 ml phosphate buffered saline (PBS; Sigma) containing 0.5% Triton X-100 (Research Organics, Cleveland, OH, USA); and finally 30 ml PBS. Clusterin was eluted with a 0.2 M glycine-hydrochloride, 0.5 M NaCl solution (pH 2.8) and neutralized with 1 ml of 2 mM Tris-HCl buffer (pH 7.0). The clusterin sample was further purified in a protein A column to remove any immunoglobulin. Purity was documented by SDS-PAGE and concentration determined by the Coomassie blue dye method of Bradford. using BSA (fraction V powder; Sigma) as a standard [26].

Hydrogen peroxide injury after aggregation. The following were added to different wells of a 96 U-shaped well polyprostyrene microtiter plate (Costar Corp., Cambridge, MA, USA): clusterin (various concentrations), bovine serum albumin (50 μg/ml BSA; Sigma) as a negative control, and fibrinogen (50 μg/ml; Sigma) as a positive control. A single cell suspension was made from LLC-PK_1 cells that were grown to near confluence in a 75 cm^2 flask. Cells were detached using 0.25% trypsin in HBSS with 0.2 g/l EDTA (Hyclone Laboratories, Logan, UT, USA), resuspended in full media and then washed twice with PBS. The cell suspension was added to each well to a final concentration of 4×10^5 cells/ml in a volume of 100 μl. Six wells were used for each experimental group. The plate was mounted at a 45° angle and rotated at 2 rpm in an incubator at 37°C in 95% air and 5% carbon dioxide in the incubator. After 60 to 90 minutes of rotation, the degree of aggregation was quantitated on a scale of 0 to 4 using the method of Fritz and Burdzy [7, 27]. No aggregation was scored as 0 and a tightly packed aggregate of cells was scored as 4. The continuum between these values was denoted 1, 2, or 3 (Fig. 1). To induce injury, hydrogen peroxide (1.5 mM) was added and the cytotoxicity was quantitated three hours later using percent specific ^{51}Cr or LDH release.

Hydrogen peroxide injury without aggregation. LLC-PK_1 cells were injured using the above protocol except the cell suspension was added to 96 well flat-bottom plates (Costar Corp., Cambridge, MA, USA), which for unclear reasons is associated with no cell aggregation. Injury was measured as percent specific ^{51}Cr release.

Aminotriazole/CDNB injury after aggregation. This is a model of intracellularly generated oxidant stress via inhibition of catalase by aminotriazole, and glutathione depletion by CDNB [25]. LLC-PK_1 cells were prepared as described above. After aggregation, cells were exposed to 50 mM aminotriazole in combination with 1 mM CDNB for six hours. Cytotoxicity was determined as percent specific ^{51}Cr release.

Measurement of cytotoxicity

^{51}Cr release assay. Prior to the generation of a single cell suspension, the LLC-PK_1 cells were labeled overnight with ^{51}Cr (250 to 500 mmCi/mg Cr; Amersham Corp., Arlington Heights, IL, USA). Following injury, the supernatant was aspirated from each well, combined with two 100 μl PBS washings, and centrifuged at 1800 rpm (GPR Centrifuge; Beckman Instruments Inc., Palo Alto, CA, USA) for 10 minutes. The supernatant, representing cytosolic release of ^{51}Cr, was separated from the cell pellet, representing detached cells. The cell pellet and the remaining attached cells on the plate were solubilized with sodium hydroxide (1 M). The supernatant and solubilized cell fractions were counted separately in a gamma counter (Gamma 7000; Beckman). The mean spontaneous ^{51}Cr release was determined by averaging the supernatant ^{51}Cr release for the PBS uninjured group and dividing by total counts in the wells. The percent specific ^{51}Cr release was calculated as:

$$\text{Percent specific } {}^{51}\text{Cr release} = \frac{\text{Cell supernate} - \text{Average spontaneous } {}^{51}\text{Cr release}}{\text{Solubilized cells} + (\text{Cell supernate} - \text{Average spontaneous } {}^{51}\text{Cr release})}$$

LDH assay. Following injury, the entire 100 μl aliquot was placed in a 1.5 ml microcentrifuge tube. The sample was centrifuged at 2000 rpm for 10 minutes and the supernatant was carefully removed. The cell pellet was lysed with 0.1% Triton X-100 (Research Organics, Inc., Cleveland, OH, USA) for 10 minutes at room temperature. LDH was quantitated by kinetic absorption at 340 nm (Beckman DU 64 Spectrophotometer; Beckman) after the addition of B-Nicotinamide adenine dinucleotide and Tris lactic acid buffer to 75 μl sample aliquot. Measurements were obtained every 15 seconds and the slope was tabulated from 0.5 to 2.5 minutes. Percent specific LDH release was calculated as:

$$\text{Percent specific LDH release} = \frac{\text{Cell supernate} - \text{Average spontaneous LDH release}}{\text{Cell lysate} + (\text{Cell supernate} - \text{Average spontaneous LDH release})}$$

Statistical analysis

Statistical significance was defined as $P < 0.05$. The results were recorded as mean ± SEM. The significance of the differences was analyzed by ANOVA, followed by the Student-Newman-Keuls test for multiple group comparisons.

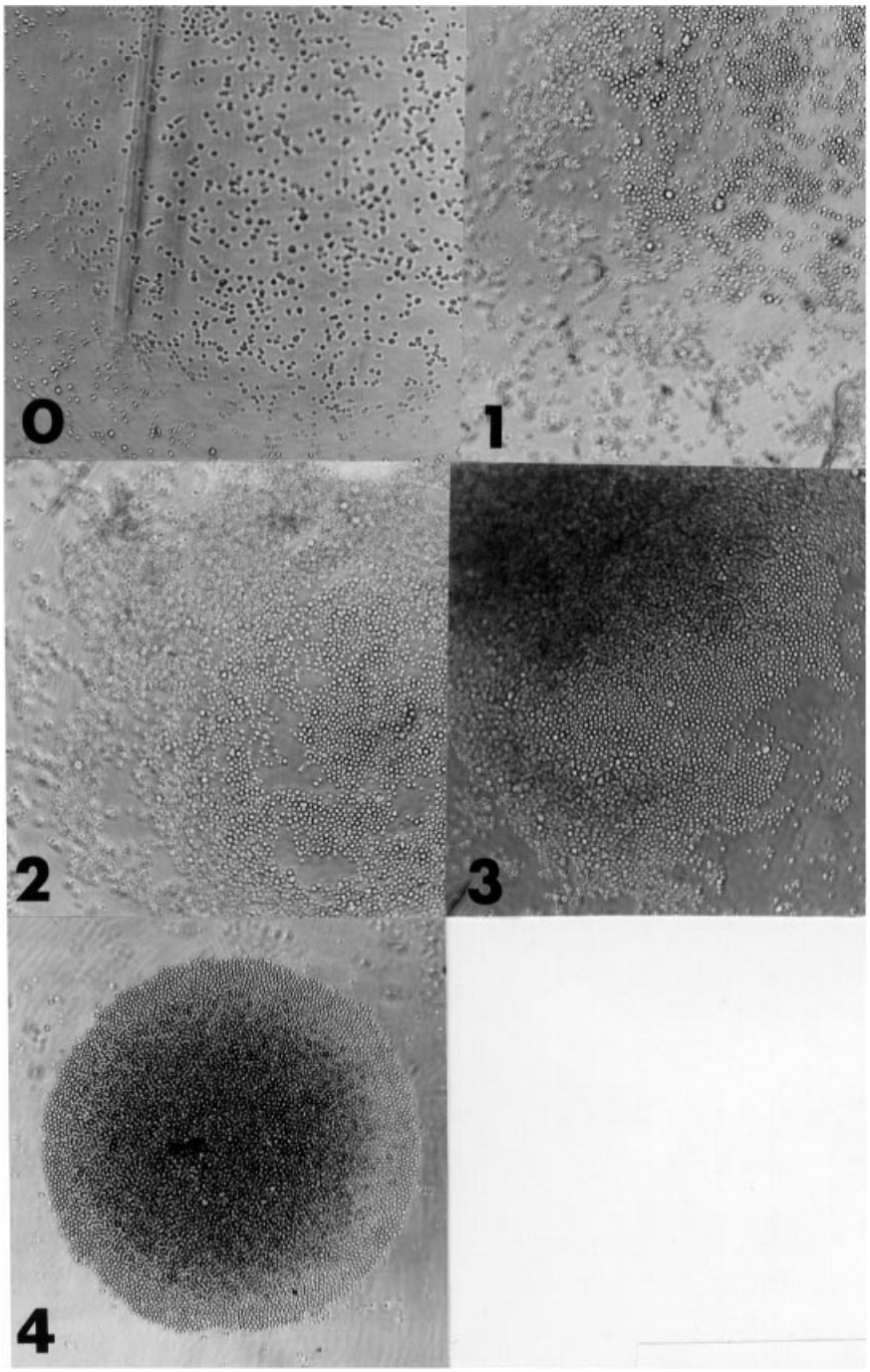

Fig. 1. Phase contrast photographs of a cell suspension of LLC-PK$_1$ cells illustrating the grading system used for defining the promotion of cell interactions by clusterin or fibrinogen. A scale of 0 to 4 was used.

RESULTS

Promotion of cell interactions by clusterin protected against cell injury

No cell aggregation was seen when the cells were incubated with PBS or BSA (50 μg/ml; Fig. 2). In contrast, incubation with the known cell aggregation molecule fibrinogen (50 μg/ml), resulted in an aggregation score of 3. Incubation with clusterin (50 μg/ml) resulted in an aggregation score of 4. Hydrogen peroxide induced significant cytotoxicity in the PBS and BSA groups ($P < 0.05$ vs. respective controls). Incubation with fibrinogen did not protect against H_2O_2 induced injury despite an aggregation score of 3. Clusterin incubation significantly decreased cytotoxicity under control conditions ($P < 0.05$ vs. other controls) and completely protected against H_2O_2 injury. Thus, at equal concentrations clusterin not only more effectively aggregated cells, but also protected cells from exogenous oxidant injury.

Effect of increasing concentrations of clusterin on cytotoxicity

This study was performed to determine if protection from cell injury was dependent on the concentration of clusterin and/or the

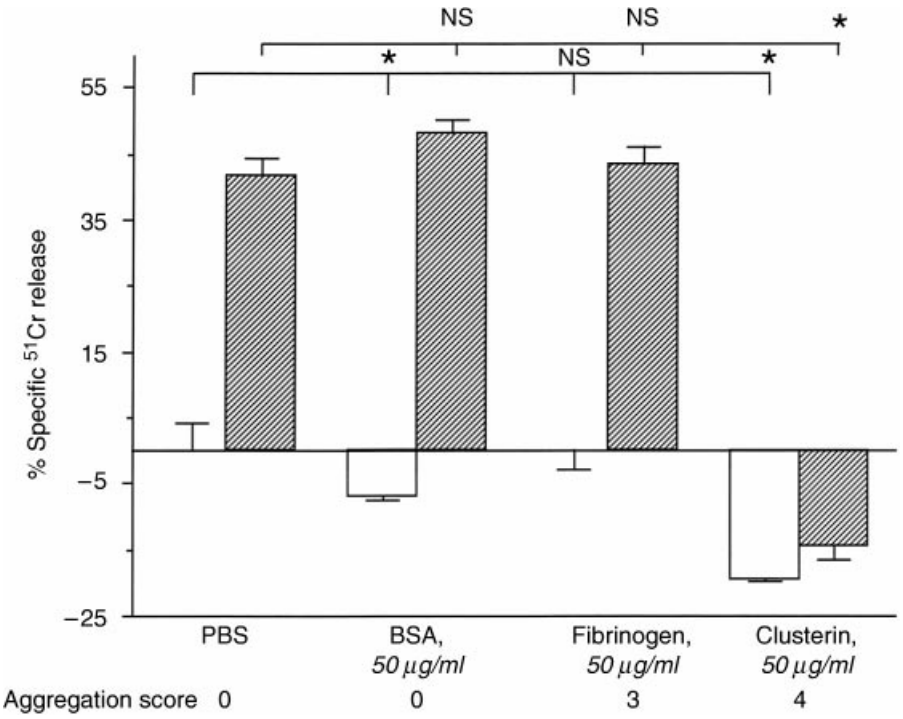

Fig. 2. Promotion of cell interaction by clusterin protects against cell injury induced by H_2O_2. The aggregation score was measured after one hour using a scale of 0 to 4. Cytotoxicity was measured by ^{51}Cr release three hours after addition of H_2O_2. Clusterin-induced cell aggregation was protective under both control and H_2O_2 injury conditions. (NS, not significant; $^*P < 0.05$). Number of observations = 6 each group. Symbols are: (□) control; (▨) H_2O_2 injury.

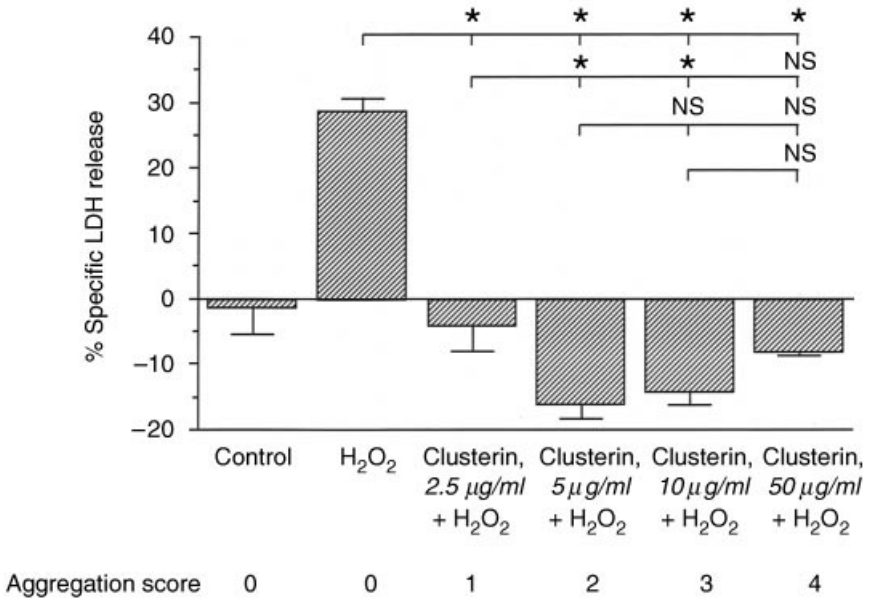

Fig. 3. Effect of increasing concentrations of clusterin on cytotoxicity. The aggregation score was measured after one hour using a scale of 0 to 4. Cytotoxicity was measured by LDH release three hours after addition of H_2O_2. Clusterin induced cell aggregation was protective at all dosages tested. (NS, not significant; $^*P < 0.05$). Number of observations = 6 each group.

degree of cell aggregation. As shown in Figure 3, clusterin exhibited dose dependent aggregation. A dose of 2.5 μg/ml was associated with an aggregation score of 1, 5 μg/ml with a score of 2, 10 μg/ml a score of 3 and 50 μg/ml a score of 4. H_2O_2 (1.5 mM) resulted in significant injury to control cells. Clusterin induced cell aggregation protected against cell injury at each concentration ($P < 0.05$). Complete abrogation of cytotoxicity occurred at a clusterin concentration of 2.5 μg/ml, which resulted in an aggregation score of 1. Clusterin concentrations between 0 and 2.5 μg/ml showed no aggregation or protection (data not shown). These results, using a different cytotoxicity assay (LDH release), confirm the findings in Figure 2 that clusterin induced aggregation is cytoprotective and demonstrate maximal protection may occur at lower doses, associated with sub-maximal degrees of aggregation.

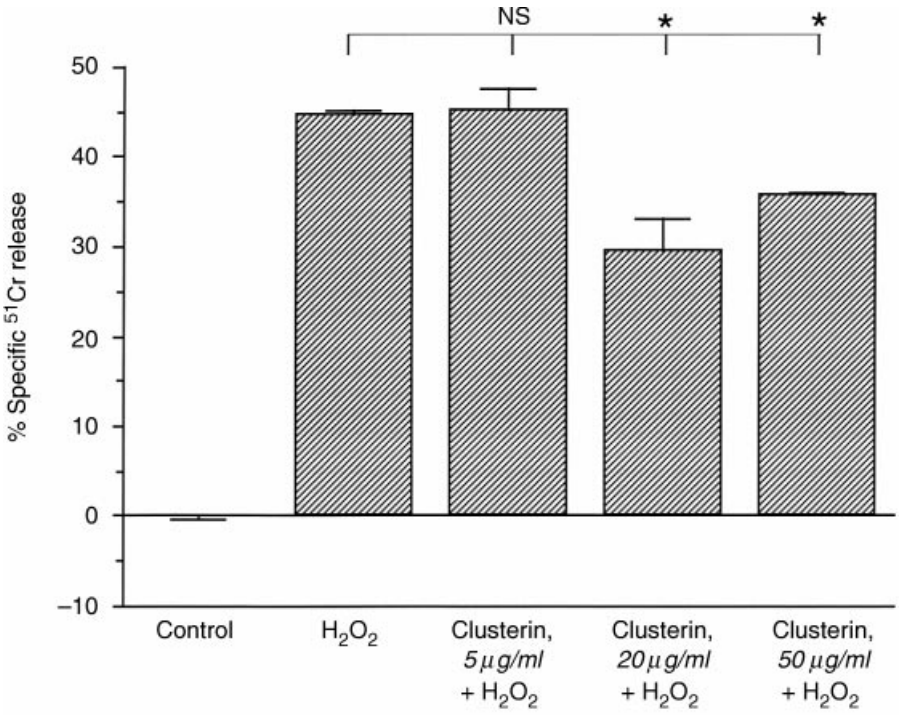

Fig. 4. Effect of increasing concentrations of clusterin, in the absence of aggregation, on cytotoxicity. The percent specific ^{51}Cr release was measured after injury in flat bottom plates, which inhibited aggregation. Despite the absence of aggregation, clusterin concentrations of 20 and 50 μg/ml decreased cytotoxicity. (NS, not significant; $^{*}P < 0.05$). Number of observations = 6 each group.

Effect of increasing concentrations of clusterin, in the absence of aggregation, on cytotoxicity

To determine if clusterin or the resultant promotion of cell interactions was responsible for the cytoprotection seen, a dose response study was performed in flat bottom plates, which completely inhibited clusterin-induced cell aggregation. As shown in Figure 4, H_2O_2 (1.5 mM) resulted in significant injury to control cells. Clusterin at doses of 20 and 50 μg/ml, but not lower doses, protected against cell injury compared to the control group ($P < 0.05$). The degree of protection was not as marked compared to the experiments when cells were able to aggregate (Figs. 2 and 3). These data suggest a partially protective effect of clusterin itself, although a more profound protective effect was seen when clusterin aggregated cells.

Effect of BSA, fibrinogen, and clusterin on oxidant stress induced by CDNB and aminotriazole

To determine if promotion of cell interactions by clusterin was protective in another model of oxidant injury, LLC-PK_1 cells were exposed to internally generated oxidant stress. Aminotriazole together with CDNB compromised the cells ability to metabolize oxidant species by inhibition of catalase and depleting glutathione content. As shown in Figure 5, the vehicle control did not cause injury. In contrast, the aminotriazole/CDNB combination injured the cells, but not as severely as seen with exogenously administered H_2O_2. No aggregation or protection was seen with the addition of 50 μg/ml BSA. In this model both fibrinogen and clusterin protected against injury despite differences in the degree of cell aggregation.

DISCUSSION

The major finding of this study was the protective effects of clusterin against oxidant injury *in vitro*. This protection was specific for clusterin and in part dose-dependent. Cytoprotective effects were found at a concentration of clusterin which only minimally increased aggregation. In the complete absence of cell aggregation clusterin had a less prominent but statistically significant protective effect. The beneficial effect was not an artifact of the method used to quantitate cytotoxicity, as a beneficial effect of clusterin was present when cytotoxicity was measured by both ^{51}Cr and LDH release. The effect was not due to restricted access of H_2O_2 to critical cellular targets, since H_2O_2 is freely diffusible across cellular membranes; nor was the protective effect due to aggregation *per se*, since fibrinogen induced aggregation but offered no protection, while lower doses of clusterin, which evinced less cell aggregation as compared to higher doses, nonetheless, were as effective as the higher doses of clusterin in affording protection.

When cells were injured in the aminotriazole/CDNB model of internally generated oxidant stress both fibrinogen and clusterin protected against injury. The reasons for these findings may reside in the degree of cytotoxicity that was less in this model as compared to injury induced by hydrogen peroxide. Additionally, other damaging cellular effects of aminotriazole/CDNB may occur independent of increased cellular generation of hydrogen peroxide. Finally, protective responses elicited by these insults may differ. CDNB depletes cellular glutathione content, the latter representing a metabolite that facilitates degradation of H_2O_2 through the glutathione/glutathione peroxidase system. Aminotriazole effectively inhibits catalase, the latter representing the other major antioxidant system that scavenges H_2O_2. The combined treatment of cells with CDNB and aminotriazole cripples the major pathways by which cellular degradation of H_2O_2 occurs [25]. Increased cellular generation of H_2O_2 has been confirmed in this model, while the administration of scavengers of H_2O_2 such

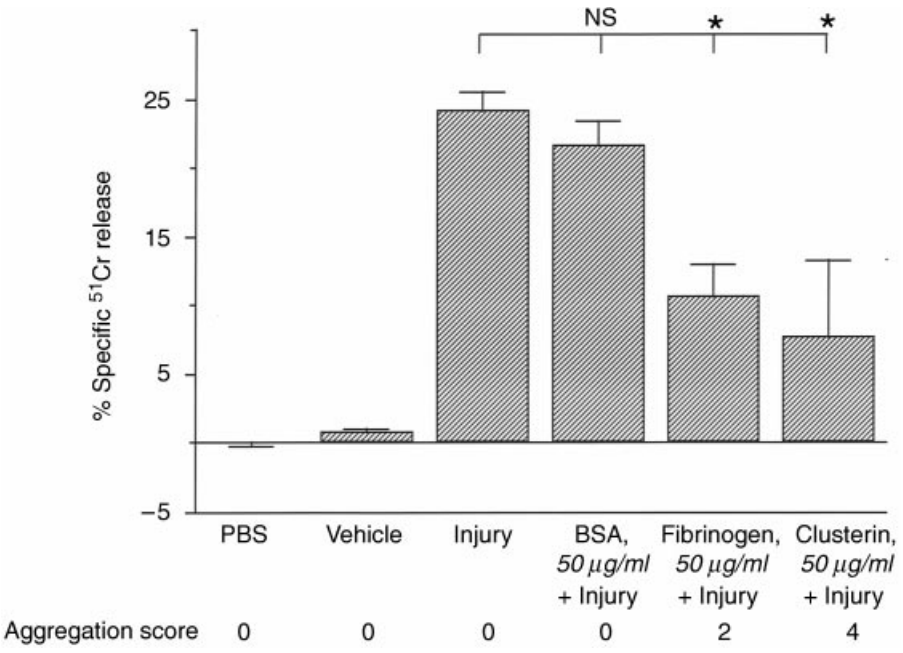

Fig. 5. Effect of bovine serum albumin (BSA), fibrinogen, and clusterin on oxidant stress induced by 1-chloro-1,4-dinitrobenzene (CDNB) and aminotriazole. The aggregation score was measured after one hour using a scale of 0 to 4. Cytotoxicity was measured by ^{51}Cr release three hours after LLC-PK_1 cells were injured with 50 mM aminotriazole (AT) and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). Both fibrinogen and clusterin protected against injury. (NS, not significant; $^*P < 0.05$). Number of observations = 6 each group.

as pyruvate effectively protect in this model and decrease generation of H_2O_2 [25]. However, quantitative differences in the concentration of H_2O_2 may exist between the models that could influence both the degree of cytotoxicity and the mechanism of cell death, since low doses of H_2O_2 cause apoptosis while higher doses results in necrosis [28, 29]. In addition, there are effects of CDNB and aminotriazole that may inflict cellular injury independent of H_2O_2. Thus, differences exist between this model and the model based upon the exogenous administration of H_2O_2. Notwithstanding these differences, clusterin clearly protected in both forms of oxidative stress whether instigated from reagent H_2O_2 exogenously applied or H_2O_2 endogenously generated as scavenging systems are compromised.

In prior studies, clusterin is strongly induced in models of acute and chronic oxidative injury in the kidney [30]. In the light of such induction by oxidant stress, we offer the speculation that expression of clusterin in diverse states of tissue injury may afford, at least in part, a protective response to oxidative injury incurred in such states. Indeed, it is increasingly recognized that antioxidant defense mechanisms are recruited in response to oxidative injury, and such mechanisms serve to limit the extent of injury that ensues [31].

Clusterin-induced cell aggregation may protect against injury in several different ways. Weinberg previously demonstrated decreased hypoxic injury when isolated proximal tubules were studied as a high density pellet versus a low density suspension [32]. This reduced susceptibility to injury was linked to a lower pH in the pellet, an observation that has been confirmed in many other settings [32, 33]. A similar mechanism is possible for the protective effect of clusterin-induced cell aggregation. Aggregation of cells likely alters the cellular microenvironment in other ways including changes in the availability of nutrients.

Other possible explanations include differences in the amount of cell membrane accessible to oxidant injury, or better maintenance of cell to cell contacts. The latter effect would facilitate communication between cells, sharing of antioxidant defense mechanisms, or stabilize intracellular structure through interaction with the cell cytoskeleton. Maintenance of cell-substratum contact may also prevent cell death by apoptosis [23].

A protective effect of clusterin has been demonstrated in several different settings. Clusterin has been localized to the immune deposits of a complement-mediated model of glomerulonephritis, passive Heymann nephritis [34]. In the isolated rat kidney model of this disease, depletion of clusterin leads to increased proteinuria, greater deposition of terminal complement components, and greater glomerular epithelial cell injury [2]. Overexpression of clusterin in a prostate cell line protects against cell death induced by tumor necrosis factor [3]. Consistent with this finding is the increased cell death observed in these cells when clusterin production is inhibited by antisense transfection [3]. Clusterin expression is increased in the brains of patients with Alzheimer's disease where it has been localized to amyloid-β deposits [35]. Clusterin interacts with amyloid-β 1-40 in a specific manner. Clusterin can prevent the neurotoxicity of amyloid- β 1-40 in primary cultures of rat mixed hippocampus [4]. Oxidized LDL, a pathogenic factor, but not normal LDL, induces clusterin mRNA in cultured hepatic cells [5, 36]. Clusterin inhibits LDL-induced lipid hydroperoxide formation and monocyte transmigration in co-cultures of human artery wall cells, suggesting a protective role in atherosclerosis [5]. The mechanism proposed for this effect is the removal of oxidation products by clusterin [5]. In addition, overexpression of clusterin protects L929 cells, a fibroblast cell line, from tumor necrosis factor-α mediated cytotoxicity [6].

In conclusion, clusterin protects against models of oxidant stress *in vitro*, whether generated by exogenously administered hydrogen peroxide, or from endogenously produced peroxide, and such protective effects can accrue from aggregative and nonaggregative properties of clusterin.

ACKNOWLEDGMENTS

This work was supported by US Public Health Service Grants RO-1 48452 (M.E.R), DK47060 (K.A.N.) and a National Research Service Award (G.B.S.); and the Baxter Extramural Grant Program. This work was done during the tenure of an Established Investigatorship from the American Heart Association (M.E.R.). Part of this work was presented and at the 1996 American Society of Nephrology meeting in New Orleans, LA. The anti-human clusterin antibody was a gift of Brendan Murphy, Melbourne, Australia.

Reprint requests to Mark E. Rosenberg, M.D., Division of Renal Diseases and Hypertension, Department of Medicine, University of Minnesota, Box 736 UMHC, 516 Delaware Street S.E., Minneapolis, Minnesota 55455, USA.
E-mail rosen001@maroon.tc.umn.edu

REFERENCES

1. Rosenberg ME, Silkensen JR: Clusterin: Physiologic and pathophysiologic considerations. *Int J Biochem Cell Biol* 27:633–645, 1995
2. Saunders JR, Aminian A, McRae JL, O'Farrell KA, Adam WR, Murphy BF: Clusterin depletion enhances immune glomerular injury in the isolated perfused kidney. *Kidney Int* 45:817–827, 1994
3. Sensibar JA, Sutkowski DM, Raffo A, Buttyan R, Griswold MD, Sylvester SR, Kozlowski JM, Lee C: Prevention of cell death induced by tumor necrosis factor a in LNCaP cells by overexpression of sulfated glycoprotein-2 (clusterin). *Cancer Res* 55:2431–2437, 1995
4. Boggs LN, Fuson KS, Baez M, Churgay L, McClure D, Becker G, May PC: Clusterin (apo J) protects in vitro amyloid-β (1–40) neurotoxcity. *J Neurochem* 67:1324–1327, 1996
5. Navab M, Hama-Levy S, Van Lenten BJ, Fonarow GC, Cardinez CJ, Castellani LW, Brennan M-L, Lusis AJ, Fogelman AM: Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. *J Clin Invest* 99:2005–2019, 1997
6. Humphreys D, Hochgrebe TT, Easterbrook-Smith SB, Tenniswood MP, Wilson MR: Effects of clusterin overexpression on TNF-α and TGFβ-mediated death of L929 cells. *Biochemistry* 36:15233–15243, 1997
7. Fritz IB, Burdzy K, Sétchell B, Blaschuk O: Ram rete testes fluid contains a protein (clusterin) which influences cell-cell interactions in vitro. *Biol Reprod* 28:1173–1188, 1983
8. Tung PS, Burdzy K, Wong K, Fritz IB: Competition between cell-substratum interactions and cell-cell interactions. *J Cell Physiol* 152:410–421, 1992
9. Silkensen JR, Skubitz KM, Skubitz APN, Chmielewski DH, Manivel JC, Dvergsten JA, Rosenberg ME: Clusterin promotes the aggregation and adhesion of renal porcine epithelial cells. *J Clin Invest* 96:2646–2653, 1995
10. Diemer V, Hoyle M, Baglioni C, Millis AJT: Expression of porcine complement cytolysis inhibitor mRNA in cultured aortic smooth muscle cells. Changes during differentiation *in vitro. J Biol Chem* 267:5257–5264, 1992
11. Thomas-Salgar S, Millis JT: Clusterin expression in differentiating smooth muscle cells. *J Biol Chem* 269:17879–17885, 1994
12. Harding MA, Chadwick LJ, Gattone VH II, Calvet JP: The SGP-2 gene is developmentally regulated in the mouse kidney and abnormally expressed in collecting duct cysts in polycystic kidney disease. *Dev Biol* 146:483–490, 1991
13. French LE, Chonn A, Ducrest D, Baumann B, Belin D, Wohlwend A, Kiss JZ, Sappino A-P, Tschopp J, Schifferli JA: Murine clusterin: Molecular cloning and mRNA localization of a gene associated with epithelial differentiation processes during embryogenesis. *J Cell Biol* 122:1119–1130, 1993
14. You Y-D, Hirsch DJ, Morgunov NS: Functional integrity of proximal tubule cells. Effects of hypoxia and ischemia. *J Am Soc Nephrol* 3:965–974, 1992
15. Bacallao R, Fine LG: Molecular events in the organization of renal tubular epithelium: From nephrogenesis to regeneration. *Am J Physiol* 257:F913–F924, 1989
16. Weinberg JM: The cell biology of ischemic renal injury. *Kidney Int* 39:476–500, 1991
17. Molitoris BA, Nelson WJ: Alterations in the establishment and maintenance of epithelial cell polarity as a basis for disease processes. *J Clin Invest* 85:3–9, 1990
18. Molitoris B: New insights into the cell biology of ischemic acute renal failure. *J Am Soc Nephrol* 1:1263–1270, 1991
19. Bonventre JV: Mechanisms of ischemic acute renal failure. *Kidney Int* 43:1160–1178, 1993
20. Goligorsky MS, Lieberthal W, Racusen L, Simon EE: Integrin receptors in renal tubular epithelium: New insights into pathophysiology of acute renal failure. *Am J Physiol* 264(Renal Fluid Electrolyte Physiol 33):F1–F8, 1993
21. Bates RC, Buret A, van Heldin DF, Horton MA, Burns GF: Apoptosis induced by inhibition of intercellular contact. *J Cell Biol* 125:403–415, 1994
22. Racusen LC, Fivush BA, Li Y-L, Solez K: Dissociation of tubular cell detachment and tubular cell death in clinical and experimental "acute tubular necrosis." *Lab Invest* 64:546–556, 1991
23. Frisch SM, Francis H: Disruption of epithelial cell-matrix interactions induces apoptosis. *J Cell Biol* 124:619–626, 1994
24. Meredith JE Jr, Fazeli B, Schwartz MA: The extracellular matrix as a cell survival factor. *Mol Biol Cell* 4:953–961, 1993
25. Nath KA, Enright H, Nutter LM, Fischereder MF, Zou JN, Hebbel RP: The effect of pyruvate on oxidant injury to isolated and cellular DNA. *Kidney Int* 45:166–176, 1994
26. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 72:248–254, 1976
27. Fritz IB, Burdzy K: Novel action of carnitine: Inhibition of aggregation of dispersed cells elicited by clusterin in vitro. *J Cell Physiol* 140:18–28, 1989
28. Ueda N, Shah SV: Endonuclease-induced DNA damage and cell death in oxidant injury to renal tubular epithelial cells. *J Clin Invest* 90:2593–2597, 1992
29. Gardner AM, Xu FH, Fady C, Jacoby FJ, Duffey DC, Tu Y, Lichtenstein A: Apoptotic vs. nonapoptotic cytotoxicity induced by hydrogen peroxide. *Fre Rad Biol Med* 22:73–83, 1997
30. Nath KA, Dvergsten J, Correa-Rotter R, Hostetter TH, Manivel JC, Rosenberg ME: Induction of clusterin in acute and chronic oxidative renal disease in the rat and its dissociation from cell injury. *Lab Invest* 71:209–218, 1994
31. Nath KA: The functional significance of induction of heme oxygenase by oxidant stress. *J Lab Clin Med* 123:461–463, 1994
32. Weinberg JM: Oxygen deprivation-induced injury to isolated rabbit kidney tubules. *J Clin Invest* 76:1193–1208, 1985
33. Weinberg JM, Venkatachalam MA: Relationships between oxidant and non-oxidant mechanisms in the pathogenesis of acute renal failure. *Miner Electrolyte Metab* 18:123–132, 1992
34. Eddy AA, Fritz IB: Localization of clusterin in the epimembranous deposits of passive Heymann nephritis. *Kidney Int* 39:247–252, 1991
35. Choi-Miura N-H, Oda T: Relationship between multifunctional protein clusterin and Alzheimer disease. *Neurobiol Aging* 17:717–722, 1996
36. Witztum JL: The oxidation hypothesis of atherosclerosis. *Lancet* 344:793–795, 1994