CMLS, Cell. mol. life sci. 53 (1997) 911–916
1420-682X/97/120911-06 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Advanced glycosylated end products activate the functions of cell adhesion molecules on lymphoid cells

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Received 25 July 1997; received after revision 25 August 1997; accepted 15 September 1997

Abstract. It has been proposed that advanced glycosylated end products (AGEs) are involved in the pathogenesis of vascular damages in both type 1 and type 2 diabetes. Furthermore, it has been assumed that AGEs cause an alteration of both expression and activity of cell adhesion molecules which are responsible for migration of circulating cells through the endothelial layer of the vessels. The effect of AGEs on the activity of cell adhesion molecules was studied in our experiments using the homotypic adhesion assay, specific monoclonal antibodies and lymphoid cell lines. It was shown that proteins glycosylated in vitro seemed to increase the percentage of homotypic aggregation of lymphoid cells. This effect was mediated via the interaction between LFA-1 and ICAM-1 which was demonstrated by the fact that specific monoclonal antibodies against these cell adhesion molecules could block the effect of the AGEs. The results obtained reveal that the advanced glycosylated end products activate the function of cell adhesion molecules on lymphoid cells. It can be speculated that the activation of cell adhesion molecules might enhance the direct cellular contacts between the lymphoid cells in the immune response. Moreover, the effect of AGEs might be responsible for an enhanced adhesion of monocytes to endothelial cells and their migration through the vessel wall.

Key words. Advanced glycosylated end products; cell adhesion molecules; lymphoid cells activation; integrins.

Vascular damage in patients with diabetes mellitus is the primary cause for morbidity and mortality [1]. Various factors are involved in the pathogenesis of this damage, namely, hypertension, biochemical alterations in plasma lipoproteins, increased vascular permeability, and so on. However, the principal metabolic abnormality is the increased level of glucose in the blood in both insulin-dependent and non-insulin-dependent diabetes mellitus. It has been shown that non-enzymatic glycosylation of plasma proteins accompanying the increased glucose levels occurs under physiological conditions in both experimental animals [2] and diabetic patients [3, 4]. The non-enzymatic glycosylation alters the structure and biological properties of a number of proteins such as serum albumin, collagen, cell membrane components, enzymes, haemoglobin, immunoglobulins, and nucleic acids (for review see [5]). The late products of non-enzymatic glycosylation are termed advanced glycosylated end products (AGEs) which are supposed to be related to the pathogenesis of diabetes mellitus.

The pathogenesis of vascular damage includes interactions between different types of cell adhesion molecules which facilitate the transmigration of circulating cells through the vessel wall into the subendothelial space [6]. Recently, it has been shown that the AGEs react with specific receptors called receptors for advanced glycosylated end products (RAGEs). These receptors are expressed on the surface of a number of cells of different lineage, e.g. monocytes and endothelial cells [7].

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The aim of this study was to follow up the effect of AGEs on the activity of cell adhesion molecules on lymphoid cells. It was shown that the presence of AGEs in the tissue culture medium stimulates homotypic adhesion of monocyte-macrophage cells (line U937).

Materials and methods

Cell lines. A monocyte-macrophage-like cell line (U937) was kindly donated by INSERM U 435, Paris, France; the T-cell lymphoma cell line (Jurkat) was supplied by the National Bank for Microbial Strains and Tissue Cultures, Bulgaria; B-cell lymphoblastoid cell line W1-729-HF2 was provided by the Department of Obstetrics and Gynecology, Hyogo Medical College, Hyogo, Japan. All cell lines were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine, sodium pyruvate and antibiotics, purchased from Sigma (St. Louis, MO, USA).

Non-enzymatic glycosylation of human serum albumin (HSA) was carried out as described by others [8, 9]. Briefly, HSA (0.2 g, Behringwerke, Marburg, Germany) was dissolved in 5 ml 0.2 M phosphate buffered saline (PBS, pH 7.4) containing 1.1 M D-glucose and 6 mM sodium azide and incubated for 45 days at 37 °C. The reaction mixture was dialysed extensively against PBS at 4 °C. Glycosylated HSA (glcHSA) was aliquoted and stored at −20 °C.

HSA incubated with no glucose under the same conditions was used as a control. To register the formation of AGEs, fluorescence spectroscopy [9] and polyacrylamide gel electrophoresis (PAGE) [9, 10] in 10% gel were applied.

Homotypic adhesion assay. The quantitative adhesion assay was performed in 96-well microtitre plates in general as already described [11]. The cells resuspended in culture medium at concentration 2×10^6 cells/ml were counted in a haemocytometer. Samples of 50 µl glcHSA (2 mg/ml) or HSA (2 mg/ml) were mixed with 50 µl RPMI 1640 medium and 100 µl cell suspension in Titertek (Labsystems, Helsinki, Finland) plates to a final concentration of the ingredients 500 µg/ml for the glcHSA or HSA and 10^6 cells per 200 µl medium per well. Wells containing phorbol myriastate acetate (PMA, 20 ng/ml, Sigma, St. Louis, MO, USA) served as adhesion positive controls, while wells with tissue culture medium were used as negative controls for the spontaneous adhesion of lymphoid cells.

Samples of 100 µl were taken after gentle resuspension at 15, 30, 45 and 60 min intervals and counted in a haemocytometer. Cell aggregation was evaluated by the aggregation index:

$$AI = [1 - (\text{number of free cells/number of input cells})] \times 100.$$

In a parallel experiment specific monoclonal antibodies (anti-CD18 and anti-CD54, Immunotech, Marseille, France) in concentration 10 µg/ml were added to the above mixture, and the experiment was performed under the same conditions. The AI values were analysed by the Newman-Keuls test of the ANOVA program.

Results

Following incubation for 45 days at 37 °C the HSA/glucose mixture was extensively dialysed against PBS, pH 7.4.

The in vitro glycosylation of HSA was confirmed by PAGE showing that the electrophoretic mobility of the glcHSA was slower than that of the non-glycosylated HSA. The glcHSA band was located about 0.5 cm higher in the gel as compared to the position of the native HSA (fig. 1). This electrophoretic mobility shift is explained by glycosylation of the HSA leading to an increase in the molecular weight of the glcHSA.

The extent of in vitro glycosylation of the HSA was also assayed by fluorescence measurements. When HSA or glcHSA with an equal protein content were at 370 nm and their emission spectra were read in the interval 400–480 nm, significant differences in their fluorescence intensities were recorded. Whereas the fluorescence intensity of the HSA solution was equal to about 0.2 arbitrary units, the intensity of the glcHSA solution was as high as 1.6 fluorescence units. The eightfold difference in the fluorescence of the glcHSA solution was taken as evidence for the formation of AGEs.

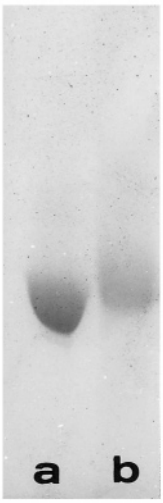

Figure 1. Polyacrylamide gel electrophoresis (10%) under non-reducing conditions. Lanes: (*a*) native human serum albumin (HSA); (*b*) Non-enzymatically glycosylated HSA (glcHSA).

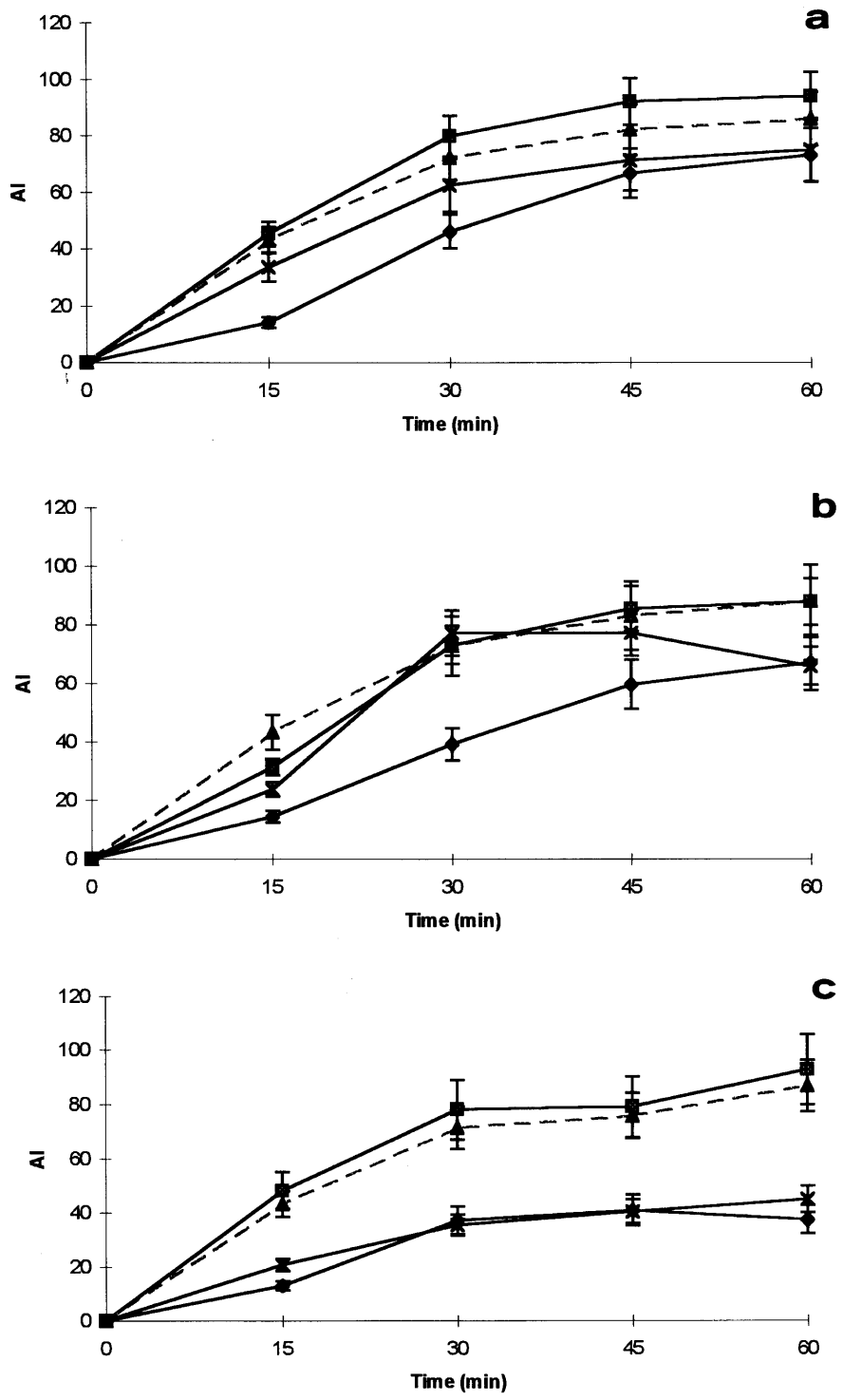

Figure 2. Aggregation index (AI) values of WL-729-HF2 (*a*), Jurkat (*b*) and U937 (*c*) cells incubated for different time intervals in the presence of: phorbol myriastate acetate (PMA, –■–■–■–); glcHSA (–▲–▲–▲–); HSA (– × – × – × –) and control (untreated) cells (–◆–◆–◆–). Error bars represent SEM.

The effect of advanced glycosylated end products on cell adhesion was studied by the homotypic adhesion assay. When W1-729-HF2 cells were incubated with glcHSA (500 μg/ml) for different time intervals, the aggregation index (AI) increased significantly in comparison with the control cells. After 15 min of incuba-

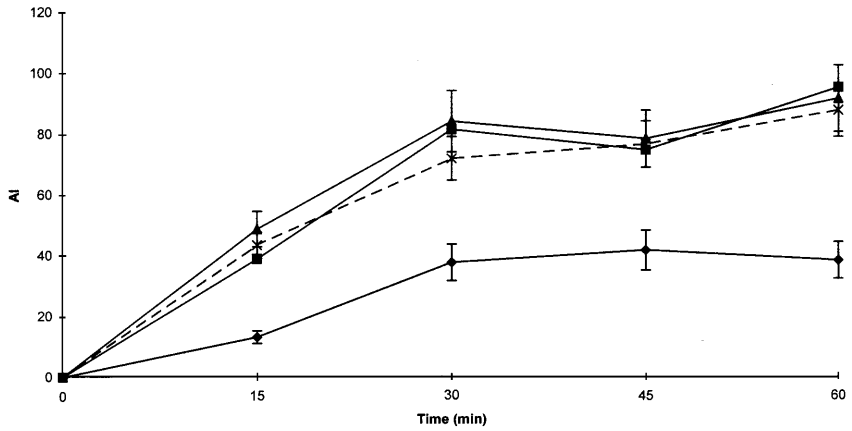

Figure 3. Effect of glcHSA on the homotypic adhesion of U937 cells. The U937 cells were incubated in the presence of different concentrations of glcHSA: 10 μg/ml (–■–■–■–); 100 μg/ml (–▲–▲–▲–); 500 μg/ml (– × – × – × –) and control cells (–◆–◆–◆–). Error bars as in figure 2.

tion of W1-729-HF2 in the presence of glcHSA, the AI reached 43 ± 4.6 ($n = 10$), while the AI for the control cells was 14.1 ± 8.1 ($n = 10$) and the difference was statistically significant ($p < 0.00126$). The effect of glcHSA on cell aggregation was found to be comparable to that of 2 ng/ml PMA ($AI = 45.375 \pm 5.6$, $n = 9$). When HSA was added to the culture medium, the AI was 33.667 ± 6.2 ($n = 9$), i.e. a significant difference was found in comparison with the control cells ($p < 0.002073$) but not with the cells incubated in the presence of glcHSA ($p = 0.115746$) or PMA ($p = 0.144190$). The differences in the extent of homotypic adhesion of glcHSA- or PMA-treated W1-729-HF2 cells and the negative control cells were observed at later periods of incubation as well, while HSA did not cause a clear-cut effect (fig. 2a). A similar effect of glcHSA on homotypic cell adhesion was observed also with the Jurkat cell line (fig. 2b). It should be mentioned that statistically significant differences in AI between HSA-treated and control cells were found after 30 and 45 min of incubation, whereas at the early stages (15 min) the AI values did not differ significantly. The comparison of the AI values obtained with HSA- and glcHSA-treated cells indicated a similar effect of both preparations on homotypic cell adhesion.

The stimulating effect of glcHSA on cell adhesion was pronounced with the U937 cell line. The portion of aggregated cells in cultures treated with PMA or glcHSA was similar at all times, and the profile of the kinetic curves was the same (fig. 2c). It should be noted that the presence of HSA in the culture medium did not change the AI values for the U937 cells. The AI of HSA-treated cells reached 20.714 ± 4.723 ($n = 8$) after 15 min of incubation and rose to 45.143 ± 7.317 ($n = 8$) after 60 min, whereas with the untreated control cells the AI values were 13.00 ± 8.41 ($n = 10$) and 37.556 ± 3.415 ($n = 10$), respectively. Well-pronounced differences in AI were found between glcHSA-treated cells and the cells treated with HSA or negative controls. The effect of glcHSA on the U937 cell adhesion was confirmed in experiments where low concentrations of glcHSA were used. It was found that concentrations as low as 10 μg/ml glcHSA had an enhancing effect on the aggregation of the cells used (fig. 3).

It has been shown that homotypic cell adhesion is mediated by specific molecules and is mainly due to the interaction of the integrins LFA-1 or Mac-1 with the cell adhesion molecule ICAM-1 [11]. The observation that cell treatment with glcHSA enhances homotypic adhesion of U937 cells gives us reason to assume that it might be due to activation of these cell adhesion molecules. To test this hypothesis, specific monoclonal antibodies anti-CD18 (anti-β_2 integrin) and anti-CD54 (anti-ICAM-1) were added to the culture medium at a concentration of 10 μg/ml. Our results showed that the anti-CD18 antibody markedly blocked the homotypic adhesion of cells cultured in the presence of PMA. The AI value thus obtained was 23.12, while the AI value corresponding to the control cells was 78.05. A similar inhibitory effect of the antibody was recorded when U937 cells were stimulated with glcHSA. In this case the AI value was 20.23 for the anti-CD18-treated cells and 71.14 for the antibody-untreated cells. Those values represent the average mean of six independent experiments. The inhibitory effect of the second monoclonal antibody

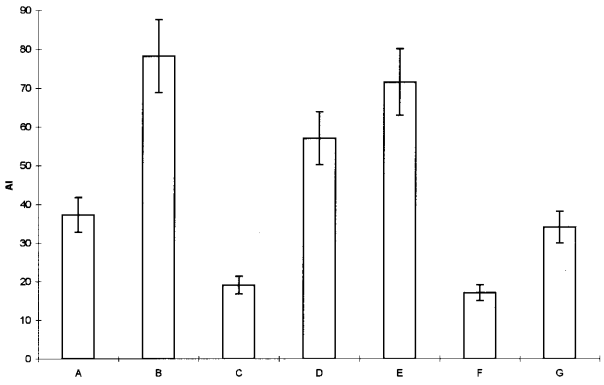

Figure 4. Effect of specific monoclonal antibodies anti-CD18 and anti-CD54 on the homotypic adhesion of U937 cells. The U937 cells were incubated for 30 min in non-supplemented medium (*A*) or medium supplemented with PMA (*B*); PMA and anti-CD18 (*C*); PMA and anti-CD54 (*D*); glcHSA (*E*); glcHSA and anti-CD18 (*F*); glcHSA and anti-CD54 (*G*). Error bars as in figure 2.

anti-CD54 was studied under similar experimental conditions. As seen in fig. 4, its effect was less pronounced.

Discussion

It has been shown recently that AGEs can induce activation of cell adhesion molecules expressed on the surface of in vitro cultured endothelial cells [8]. On the other hand, the effect of AGEs on the activity of integrins on lymphoid cells might be considered as a prerequisite for their interplay with endothelial cells, the extracellular matrix and the interactions between the lymphoid cells in the immune response.

In our experiments HSA was glycosylated in vitro, and the extent of glycosylation was monitored by fluorescence.

The AGEs thus obtained had an enhancing effect on homotypic adhesion of lymphoid cells, and this effect was better pronounced with cells from the monocyte-macrophage cell line U937 in comparison with the T-cell lymphoma cell line (Jurkat) or B-cell lymphoblastoid cell line (W1-729-HF2). These results could be explained by the ability of monocyte-macrophage cells to react with the modified self proteins by specific receptors for AGEs (RAGE) which are expressed on the cell surface.

Since the homotypic adhesion is mediated by the interaction between the integrins (LFA-1, Mac-1) and ICAM-1, one can assume that the enhanced homotypic adhesion is due to the activation of cell adhesion molecules. AGEs would most probably react with specific RAGEs which have been found on the surface of a number of cell types [7, 12], thus leading to the activation of the β_2-integrins and their binding to the ICAM-1 molecule. This assumption is supported by the fact that interaction of the T-cell receptor with the peptide-MHC complex upregulate the activity of β_2-integrins [13]. As was demonstrated in our studies, the blocking effect of the monoclonal antibody anti-CD18 (anti-β_2 chain) on homotypic adhesion of AGE-stimulated U937 cells was considerably stronger than the effect of the anti-CD54 (anti-ICAM-1) antibody. This finding supports our assumption that the enhancing effect of AGEs is most probably due to activation of the β_2-integrins. Such mechanism of activation of cell adhesion molecules might be involved in the pathogenesis of vascular damage in diabetes and ageing [5]. Furthermore, it has been reported that advanced glycosylation end products induce expression of vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells [8]. The results presented above show that AGEs have a stimulating effect on the integrins expressed on lymphoid cells which would strongly stimulate the interactions between endothelial cells and circulating blood mononuclear leukocytes. This is the reason to speculate that the activation of cell adhesion molecules by AGEs seems to be a phenomenon affecting many cell types and might be one of the important factors involved in the pathogenesis of vascular disease in diabetes, atherosclerosis and ageing.

Acknowledgments. This study was supported by Grant no. L-513 of the Bulgarian National Research Fund.

1 Factor S., Segal B. and van Hoeven K. (1992) Diabetes and coronary vascular disease. Coron. Art. Dis. **3:** 4–10
2 Day J. F., Ingebresten C. G., Ingebresten W. R., Baynes J. W. and Thorpe S. R. (1980) Non-enzymatic glycosylation of serum proteins and hemoglobin: response to changes in blood glucose levels in diabetic rats. Diabetes **29:** 524–527
3 Yue D. K., Morris K., McLennan S. and Turtle R. J. (1980) Glycation of plasma proteins and its relation to glycated haemoglobin in diabetes. Diabetes **29:** 296–300
4 Dolhofer R. and Wieland O. H. (1980) Increased glycation of serum albumin in diabetes mellitus. Diabetes **29:** 417–422
5 Vlassara H., Bucala R. and Striker L. (1994) Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and ageing. Lab. Invest. **70:** 138–151
6 Cybulski M. I. and Gimbrone M. A. Jr. (1991) Endothelial expression of mononuclear leukocyte adhesion molecule during atherogenesis. Science **251:** 788–791
7 Schmidt A. M., Hori O., Brett J., Yan S. D., Wauteir J. L. and Stern D. (1994) Cellular receptors for advanced glycation endproducts. Arterioscler. Thromb. Vasc. Biol. **14:** 1521–1528
8 Schmidt A. M., Hori O., Chen J. X., Li J. F., Crandell J., Zhang J. et al. (1995) Advanced glycation end products interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. J. Clin. Invest. **96:** 1395–1403
9 Westwood M. E. and Thornalley P. J. (1995) Molecular characteristics of methylglyoxal-modified bovine and human serum albumins. Comparison with glucose-derived advanced glycation endproduct-modified serum albumins. J. Protein Chem. **14:** 359–372

10 Cohen M. P. and Hud E. (1989) Production and characterization of monoclonal antibodies against human glycoalbumin. J. Immunol. Methods **117:** 121–129
11 Rothlein R. and Springer T. A. (1986) The requirement for lymphocyte function associated antigen 1 in homotypic leukocyte adhesion stimulated by phorbol ester. J. Exp. Med **163:** 1132–1149
12 Schmidt A. M., Yan S. D., Brett J., Mora R., Nowygrod R. and Stern D. (1993) Regulation of mononuclear phagocyte migration by cell surface binding proteins for AGEs. J. Clin. Invest. **92:** 8 2155–2168
13 Dustin M. L. and Springer T. A. (1991) Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. Annu. Rev. Immunol. **9:** 2–66