

Inhibition of γ -Irradiation Induced Adhesion Molecules and NO Production by Alginate in Human Endothelial Cells

Eun-Wha Son¹, Chul-Koo Cho², Dong-Kwon Rhee¹, and Suhkneung Pyo¹

¹Division of Immunopharmacology, College of Pharmacy, Sungkyunkwan University, Suwon, Kyunggi-do 440-746 Korea and ²Department of Radiation Oncology, Korea Cancer Center Hospital, Seoul 139-706, Korea

(Received July 1, 2001)

Inflammation is a frequent radiation-induced reaction following therapeutic irradiation. Treatment of human umbilical endothelial cells (HUVEC) with γ -irradiation (γ IR) induces the expression of adhesion proteins such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. Since the upregulation of these proteins on endothelial cell surface has been known to be associated with inflammation, interfering with the expression of adhesion molecules is an important therapeutic target. In the present study, we demonstrate that high mannuronic acid-containing alginate (HMA) inhibits γ IR induced expression of ICAM-1, VCAM-1, and E-selectin on HUVEC in a dose dependent manner. HMA also inhibited γ IR induced production of Nitric oxide (NO). These data suggest that HMA has therapeutic potential for the treatment of various inflammatory disorder associated with an increase of endothelial leukocyte adhesion molecules.

Key words: Alginate, γ -Irradiation, Endothelial cells, ICAM-1, VCAM-1, E-selectin, NO

INTRODUCTION

Alginate, extract of seaweeds, is linear polymer of polysaccharides with gel-forming properties composed of β -(1-4)-D-mannosyluronic acid (M), α -(1-4)-L-glucosyluronic acid (G), and alternating (MG) blocks (Davidson *et al.*, 1976). Alginate has been used for immobilization of Langerhans islets for treatment of experimental diabetes mellitus in rats (Tze *et al.*, 1982; Soon-Shiong *et al.*, 1994). Microencapsulation of hormone-producing cells into calcium alginate gel was useful for the treatment of diabetes mellitus and parathyroid disease (Darquy *et al.*, 1987; Fan *et al.*, 1990). In addition, it has been suggested that sodium alginate has a reparative effect on healing of radiation-induced stomatitis (Hasegawa *et al.*, 1989). Recently, alginate suspension containing poraprezine has been shown to have anti-inflammatory activity as tested in severe gingivostomatitis (Katayama *et al.*, 1998).

Ionizing radiation damage is partially characterized by the generation and the maintenance of an inflammatory

reaction (Hruza and Pentland, 1993). An important event in this inflammatory response is the localization of leukocytes at the sites of inflammatory lesions through a multistep process. The endothelial cell adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), play an important role in leukocyte adhesion and transendothelial migration at sites of inflammation. Endothelial expression of these molecule has been known to be elevated in both a temporal and spatial association with inflammatory cell infiltrates (Carlos *et al.*, 1994). When activated by inflammatory cytokines, endothelial cells exhibit an upregulation of specific adhesion molecules on their surface, the ligands for which are borne on circulation leukocyte (Bevilacqua *et al.* 1985; Springer *et al.*, 1990). A logical target for new drug development would be the design of compounds that would interfere with adhesion molecule interactions. It has been suggested that various small molecules including glucocorticoids, aspirin and pentoxifylline inhibit the upregulation of adhesion protein expression and have a protective effect on inflammatory diseases (Brostjan *et al.*, 1997; Weber *et al.*, 1995; Neuner *et al.*, 1997).

Nitric oxide (NO) is synthesised by many cell types in various tissues and it acts as a vascular relaxing agent, a

Correspondence to: Suhkneung Pyo, Sungkyunkwan University, College of Pharmacy, Suwon, Kyunggi-do, 440-746, Korea
E-mail: snpyo@skku.ac.kr

neurotransmitter and an inhibitor of platelet aggregation (Moncada *et al.*, 1991). In addition, NO is generated during immune and inflammatory responses (Moncada *et al.*, 1991). NO may induce toxic reactions against other tissue of the host and since it is generated at high levels in certain types of inflammation, it has been implicated as pro-inflammatory agent. Equally, it may act as an anti-inflammatory or immunosuppressive agent via its inhibitory or apoptotic effects on cells. However, there has been little reported that has assessed the roles of NO in γ -irradiation-induced inflammation.

Since alginate has anti-inflammatory activity and expression of adhesion molecules and NO play an important role in inflammation, we determined whether alginate modulates the expression of adhesion proteins and NO release in irradiated-human umbilical vein endothelial cells (HUVEC). The results of the present study suggest that alginate inhibits the upregulation of γ IR-induced adhesion protein expression. We also observed that alginate blocked the production of NO induced by γ IR.

MATERIALS AND METHODS

Reagents

HMA (A0682) (polyuronic acid of hydrophilic colloid having anhydro-D-mannuronic acid residue (1 \rightarrow 4) from *Macrocystis pyrifera* (Kelp) was purchased from Sigma Chemical Co. (St Louis, USA). Anti-ICAM-1 (BBA3), anti-VCAM-1 (BBA6) and anti-E-selectin (BBA1) antibodies were purchased from R & D Systems, USA. Anti-mouse IgG-HRP and p-nitrophenyl phosphate were purchased from Sigma Chemical Co. Fetal bovine serum was purchased from Gibco, USA.

Cells and cell culture

HUVEC were purchased from Clonetics (San Diego, CA) and were grown in EGM-2 medium (Clonetics) in gelatin coated tissue culture flasks. For subculturing, the cells were detached using 0.125% trypsin containing 0.01 M EDTA. Cells used in this study were from the first to third passage.

γ -Irradiation on cells

Cells were irradiated by a previous method of Gaugler *et al.* (1997). Just before irradiation, the medium of confluent cells was changed and replaced with new medium. The cells were then uniformly irradiated at room temperature with various doses of a ^{137}Cs γ -source (dose rate of 5.94 Gy/min) (IBL 437 C type H, CIS biointernational, France). The culture medium was renewed after irradiation. For each dose, control cells were simultaneously exposed to sham irradiation.

Cytotoxicity evaluation

Alginate and γ IR at concentrations presented herein were shown to be non-toxic by the following procedure. Viability of HUVEC treated with alginate and/or γ IR was determined using MTT assay. Irradiated HUVEC were cultured in gelatin coated 96-well microplate (Costar Products, Cambridge, MA) until confluent. Cells were treated with or without alginate in quadruplicate for indicated time and subsequently 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added for 4 h. Also, a visual inspection of the cells was performed by trypan blue exclusion staining under inverted microscope.

ELISA for measurement of adhesion molecules

The cell surface expression of adhesion molecules on endothelial monolayers was quantified using ELISA by modification of the methods described previously (Gupta and Ghosh, 1999). After irradiation, HUVEC were seeded at a concentration of 2×10^4 cells/well in 96-well, flat bottom, gelatin-coated plates (Costar Products, Cambridge, MA). The cells were incubated without or with various doses of alginate for the time indicated in the text to measure ICAM-1, VCAM-1 and E-selectin expression. Following incubation, the cells were washed with phosphate buffer saline pH 7.4 (PBS) and fixed with 10% glutaraldehyde for 30 min at 4°C. Bovine serum albumin (1.0% in PBS) was added to the cells to reduce non-specific binding. Cells were incubated with anti-ICAM-1, anti-VCAM-1 and anti-E-selectin monoclonal antibody or isotype matched control antibody (0.25 μ g/ml, diluted in blocking buffer) overnight at 4°C, washed with PBS followed by incubation with alkaline phosphatase-conjugated goat anti-mouse secondary antibody (1 μ g/ml, diluted in PBS). The cells were then washed with PBS and exposed to the peroxidase substrate (p-nitrophenyl phosphate 1 mg/ml in 0.1 M glycine buffer, pH 10.4 containing 1 mM MgCl_2 and 1 mM ZnCl_2). Absorbance was determined at 405 nm by a Molecular device microplate reader (Menlo Park, CA). The absorbance values of isotype matched control antibody were taken as blank and subtracted from the experimental values.

Nitrite determination

Irradiated HUVEC were treated with various doses of alginate for times indicated in the text and the accumulation of nitrite in culture supernatant was measured using the assay system described by Ding *et al.* (1998). Briefly, 100 μ l supernatant was removed from each well into an empty 96-well plate. After the addition of 100 μ l Griess reagent to each well, absorbance at 540 nm was measured using an a Molecular device microplate reader. Nitrite concentration was calculated from a NaNO_2

standard curve. The levels of absorbance are indicative of NO production. Griess reagent was prepared by mixing one part 0.1% naphthylethylenediamine dihydrochloride in distilled water plus one part 1% sulfanilamide in 5% concentrated H_3PO_4 .

Statistical analysis

Each data were given as means \pm S.E.M. Statistical difference between groups was determined by one-way analysis of variance (ANOVA) and significant values were represented by an asterisk (* p <0.05, ** p <0.01).

RESULTS

Endothelial cell viability and growth after γ R

Although the effect of radiation on endothelial cells has been extensively reported (Rubin *et al.* 1989; Eissner *et al.* 1995; Gaugler *et al.* 1997), the radiosensitivity of HUVEC was assessed in our culture conditions. Viability of the adherent endothelial cells was >95% at the different times tested after exposure. However, the number of irradiated cells was decreased with time after exposure, while the number of non-irradiated cells was stable. 3 days after a 8-Gy irradiation, the number of irradiated cells represented 80% of the number of control cells. This percentage was decreased to 10% by 5 days after irradiation (Fig. 1). In addition, when the cells were treated with various doses of γ R, the expression of ICAM-1 was maximally induced at 8-Gy irradiation (data not shown). Therefore, in subsequent studies 8-Gy irradiation was used.

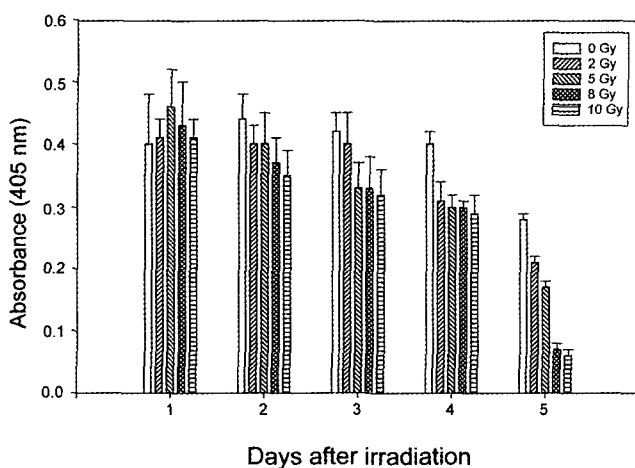


Fig. 1. Effect of γ -irradiation on cell viability. Cells were irradiated with various doses of γ -ray for various times. Each experiment was performed in quadruplicate. The results are mean \pm S.E.M from three independent experiments. * p < 0.05, ** p <0.01; significantly different from control (sham-irradiated cells).

Alginate inhibits γ R-induced ICAM-1 expression on endothelial cells in a dose dependent manner

To examine the effect of alginate, HUVEC were incubated without or with various concentrations of alginate for 3 days after γ R. The time of incubation and concentration of alginate used in these experiments had no effect on the viability as determined by trypan blue staining and morphology of the endothelial cells (data not shown). These concentrations are in complete agreement with the optimum concentrations of alginate needed in previous studies (Otterlei *et al.*, 1993; Son *et al.*, 1999). As detected by ELISA, ICAM-1 was expressed at low levels on unstimulated endothelial cells and was significantly induced by γ R (Fig. 2). Alginate did not have effect on the basal level of ICAM-1 expression, whereas it led to a reduction in the γ R-induced ICAM-1 expression in a dose dependent manner (Fig. 2).

Alginate also inhibits γ R-induced VCAM-1 and E-selectin expression

In addition to ICAM-1, γ R also induced VCAM-1 and E-selectin expression in endothelial cells. To examine the effect of alginate on γ R-induced expression of E-selectin and VCAM-1, HUVEC were incubated with various concentrations of alginate for 6 h for the expression of E-selectin and for 3 days for VCAM-1. As measured by ELISA, the unstimulated cells expressed detectable amounts of E-selectin and VCAM-1 (Fig. 3). Upon induction with γ R, a significant increase in the expression of E-selectin was observed. Treatment with alginate inhibited

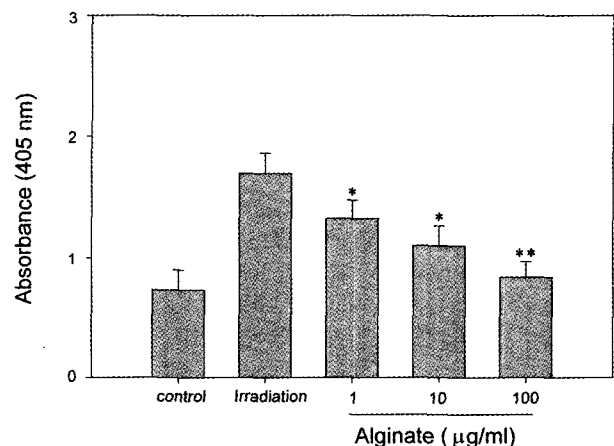


Fig. 2. Dose dependent inhibition of γ -irradiation induced ICAM-1 expression by alginate. Three independent experiments were performed in which confluent HUVEC were sham- or 8 Gy-irradiated without or with indicated concentrations of alginate and the expression of ICAM-1 measured by ELISA. The results are mean \pm S.E.M from three independent experiments. * p <0.05, ** p <0.01; significantly different from irradiation-treated control.

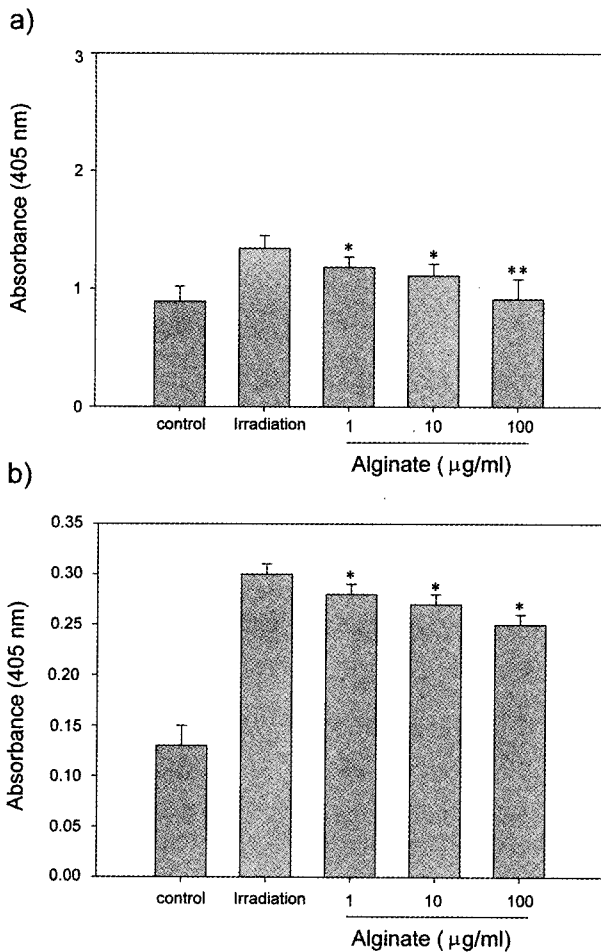


Fig. 3. Effect of alginate on γ -irradiation induced VCAM-1 (a) and E-selectin (b) expression by HUVEC. Three independent experiments were performed in which confluent HUVEC were sham- or 8 Gy-irradiated without or with indicated concentrations of alginate and the expression of VCAM-1 or E-selectin measured by ELISA. The results are mean \pm S.E.M from three independent experiments. * $p < 0.05$, ** $p < 0.01$; significantly different from irradiation-treated control.

slightly but significantly the expression of VCAM-1 induced by γ IR in a dose dependent manner (Fig. 3A). Similarly, γ IR-induced E-selectin expression was also inhibited by alginate (Fig. 3B). Taken together, these results suggest that alginate is effective in blocking the induced level of expression of ICAM-1, VCAM-1 and E-selectin.

Alginate inhibits γ IR-induced NO production

Since NO has been known to be an important modulator of the inflammatory response to various stimuli, we determined the effect of alginate on NO production in endothelial cells. As shown in Fig. 4, treatment of HUVEC with IR results in an increase of NO release that was inhibited by alginate in a dose dependent manner.

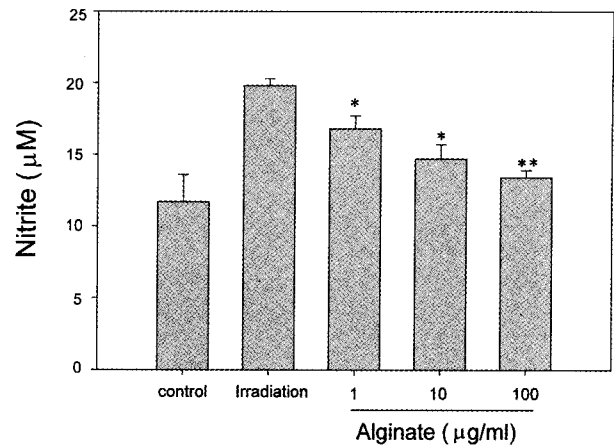


Fig. 4. Effect of alginate on γ -irradiation induced NO production in HUVEC. Three independent experiments were performed in which confluent HUVEC were sham- or 8 Gy-irradiated without or with indicated concentrations of alginate. The conditioned medium was collected, and the nitrite concentration was determined by means of the Griess reagent. The results are mean \pm S.E.M from three independent experiments. * $p < 0.05$, ** $p < 0.01$; significantly different from irradiation-treated control.

DISCUSSION

Sodium alginate is an agent with a promotive effect on healing of inflammation of the gastric and esophageal musoca. Hasegawa *et al.* (1989) revealed that orally administered alginate in irradiated mice has a pharmacologically reparative effect on radiation induced stomatitis. Although alginate is found to have anti-inflammatory properties, very little is known in regard to its effect on the induction of cell adhesion molecules by γ IR. In the present study, alginate blocked γ IR-induced expression of leukocyte adhesion molecules, ICAM-1, VCAM-1 and E-selectin. Thus, alginate possesses anti-inflammatory effect on the expression of adhesion protein induced by radiation. This compound also inhibited the NO production in γ -irradiated HUVEC.

Radiation has been shown to induce the expression of a number of genes that participate in the inflammatory response. These include TNF- α and IL-1 which have been known to induce the expression of adhesion molecules such as E-selection when added to endothelial cells in culture (Sherman *et al.*, 1991; Brach *et al.*, 1993; Bevilacqua *et al.*, 1989). When the effect of γ IR on cytokine production was examined in HUVEC, it did not induce the production of TNF- α in irradiated cells (data not shown). Recently, Hallahan *et al.* (1995) demonstrated that E-selectin gene induction by ionizing radiation is independent of cytokine induction. In accordance with their report, our data confirmed that adhesion protein expression did not require cytokine synthesis.

Nitric oxide is a biologically active gas that is synthe-

sized by a variety of cells, including vascular endothelium, from the guanido group of L-arginine. This reactive nitrogen molecule has been invoked as a mediator of vascular phenomena such as arteriolar dilation, platelet aggregation, and platelet-leukocyte adhesion (Moncada, 1992). It has been suggested that NO is able to inhibit LPS-induced ICAM-1 expression (Spiecker *et al.*, 1998). In addition, Kawachi *et al.* (1999) demonstrated that iNOS^{-/-} mice injected with TNF- α showed an enhancement of VCAM-1 expression in 50% of all tissues compared to wild-type controls. Based on these findings NO could inhibit the expression of adhesion molecules. Our data showed that γ IR induces the production of NO and alginate inhibits NO release. Recently, it has been shown that UVB radiation acts as a potent stimulator of NO in human endothelial cells and NO is involved in skin erythema and inflammation (Deliconstantinos *et al.*, 1996). Thus, a role could be proposed for NO either in inhibiting or promoting inflammation. At present the precise mechanisms accounting for these modulation are unknown. It may be possible, however, to override inhibiting by enhancing inflammation. Moreover, the involvement of NO in the modulation and regulation of adhesion molecules expression in inflammation may be dependent on the source of NO, the cells involved and type of stimulus used to induce inflammation.

During severe injury, infection, or ischemia and reperfusion damage, spillover of chemoactivators in the systemic circulation results in cellular activation, leading to the release of injurious agents which damage host tissues. These inflammatory mediators can alter the functional integrity of the vascular system which may be due to the upregulation of expression of cell adhesion molecules. Thus various strategies, such as monoclonal antibodies against adhesion molecules, soluble receptors, soluble counterreceptors, peptides derived from adhesion molecules to prevent receptor-ligand interactions, and antisense oligonucleotides have been employed to inhibit the cell adhesion molecules (Weiser *et al.*, 1997). Flavonoids, glucocorticoid, benzothioephene-carboxamide and vitamin A have been shown to inhibit cytokine- or irradiation-induced cell adhesion molecule expression (Gerritsen *et al.*, 1995; Brostjan *et al.*, 1997; Cobb *et al.*, 1996; Redlich *et al.*, 1998). Here we demonstrated that alginate effectively blocked the expression of leukocyte adhesion molecules. These studies suggest that alginate may serve as a potential therapeutic tool toward radiation-induced inflammation. Further studies are needed to clarify how this modulation occurs and to what extent it occurs *in vivo*.

ACKNOWLEDGEMENTS

This work was supported by a National Nuclear R & D Program from the Ministry of Science and Technology of Korea.

REFERENCES

- Bevilacqua, M. P., Poher, J. S., Wheeler, M. E., Cotran, R. S., and Gimbrone, M. A. Jr. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes and related cell lines. *J. Clin. Invest.*, 76, 2003-2009 (1985).
- Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A. Jr, and Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science*, 243, 1160-1165 (1989).
- Brach, M. A., Gruss, H. J., Kaisho, T., Asano, Y., Hirano, T., and Herrmann, F. Ionizing radiation induce expression of interleukin 6 by human fibroblasts involving activation of nuclear factor-kappa B. *J. Biol. Chem.*, 268, 8466-8472 (1993).
- Brostjan, C., Anrather, J., Csizmadia, V., Natrajan, G., and Winkler, H. Glucocorticoids inhibit E-selectin expression by targeting NF-kB and not ATF/c-jun. *J. Immunol.*, 158, 3836-3844 (1997).
- Carlos, T. and Harlan, J. M. Leukocyte-endothelial interactions. *Blood*, 84, 1765-1792 (1994).
- Cobb, R. R., Felts, K. A., McKenzie, T. C., Parry, G., and Mackman, N. A benzothioephene-carboxamide is a potent inhibitor of IL-1 induced VCAM-1 gene expression in human endothelial cells. *FEBS Lett.*, 382, 323-326 (1996).
- Darquy, S. and Sun, A. M., Microencapsulation of parathyroid cells as a bioartificial parathyroid in vitro studies. *ASAIO J.*, 33, 356-358 (1987).
- Davidson, I. W., Sutherland, Z. W., and Lawson, C. J. Purification and Properties of an alginate lyase from a marine bacterium. *Biochem. J.*, 159, 707-713 (1976).
- Ding, A. H., Nathan, C. F., and Stuehr, D. J. Release of reactive nitrogen intermediates and reactive oxygen intermediated from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J. Immunol.*, 141, 2407-2412 (1988).
- Eissner, G., Kohlhuber, F., Grell, M., Ueffing, M., Scheurich, P., Hieke, A., Milthoff, G., Bornkamm, G. W. and Holler, E. Critical involvement of transmembrane tumor necrosis factor- in endothelial programmed cell death mediated by ionizing radiation and bacterial endo-toxin. *Blood*, 86, 4184-4193 (1995).
- Fan, M. Y., Lum, Z. P., Fu, X. W., Levesque, L., Tai, I. T., and Sun, A. M. Reversal of diabetes in BB rats in transplantation of encapsulated pancreatic islets. *Diabetes*, 39, 519-522 (1990).
- Gaugler, M. H., Squiban, C., Van der Meeren, A., Bertho, J. M., Vandamme, M., and Mouthon, M. A. Late and persistent up-regulation of intercellular adhesion molecule-1 (ICAM-1) expression by ionizing radiation in

- human endothelial cells *in vitro*. *Int. J. Radiat. Biol.*, 72, 201-209 (1997).
- Gerritsen, M., Carley, W., Ranges, G. E., Shen, C., Phan, S. A., Ligon, G., and Perry, C. Flavonoid inhibit cytokine-induced endothelial cell adhesion protein gene expression. *Am. J. Pathol.*, 147, 278-292 (1995).
- Gupta, B. and Ghosh, B. *Curcuma longa* inhibits TNF- α induced expression of adhesion molecules on human umbilical vein endothelial cells. *Int. J. Immunopharmac.*, 21, 745-757 (1999).
- Hallahan, D., Clark, E. T., Kuchibhotla, J., Gewertz, B. L., and Collins, T. E-selectin gene induction by ionizing radiation is independent of cytokine induction. *Biochem. Biophys. Res. Commun.*, 217, 784-795 (1995).
- Hasegawa, T., Takahashi, T., Inada, Y., Yamada, C., and Tanaka, Y. Reparative effects of sodium alginate (Alloid G) on radiation stomatitis. *Nippon Igaku Hoshasen Gakkai Zasshi*, 49(8), 1047-1051 (1989).
- Hruza, L. L. and Pentland, A. P. Mechanisms of UV-induced inflammation. *J. Invest. Dermatol.*, 100, 355-415 (1993).
- Katayama, S., Ohshita, J., Sugaya, K., Hirano, M., Momose, Y., and Yamamura, S. New medicinal treatment for severe gingivostomatitis. *Int. J. Mol. Med.*, 2(6), 675-679 (1998).
- Kawachi, S., Cockrell, A., Laroux, F. S., Gray, L., Granger, D.N., Van Der Heyde, H.C., and Grisham, M.B. Role of inducible nitric oxide synthase in the regulation of VCAM-1 expression in gut inflammation. *Am. J. Physiol.*, 277, G572-G576 (1999).
- Moncada, S. The L-arginine:nitric oxide pathway. *Acta Physiol. Scand.*, 145, 201-227 (1992).
- Neuner, P., Klosner, G., Pourmojib, M., Knobler, R., and Schwarz, T. Pentoxifylline *in vivo* and *in vitro* down-regulates the expression of the intercellular adhesion molecule-1 in monocytes. *Immunology*, 90, 435-439 (1997).
- Otterlei, M., Sundan, A., Skjak-Braek, G., Ryan, L., Smidsrod, O., and Espevik, T. Similar mechanism of action of defined polysaccharides and lipopolysaccharides: characterization of binding and tumor necrosis factor alpha induction. *Infect. Immun.*, 61, 1917-1925 (1993).
- Redlich, C. A., Rockwell, S., Chung, J. C., Sikora, A. G., Kelley, M., and Mayne, S. T. Vitamin A inhibits radiation-induced pneumonitis in rats. *J. Nutr.*, 128, 1661-1664 (1998).
- Rubin, P., Johnston C. J., Williams, J. P., McDonald, S. and Finkelstein, J. N. A perpetual cascade of cytokines post-irradiation leads to pulmonary fibrosis. *Int. J. Radiat. Biol.*, 33, 99-109 (1995).
- Sherman, M. L., Datta, R., Hallahan, D. E., Weichesbaum, R. R., and Kufe, D. W. Regulation of tumor necrosis factor gene expression by ionizing radiation in human myeloid leukemia cells and peripheral blood monocytes. *J. Clin. Invest.*, 81, 1506-1510 (1991).
- Son, E. H., Yoon, Y. S., and Pyo, S. Immunomodulating activity of alginate. *J. Appl. Pharmacol.*, 7, 377-384 (1999).
- Soon-Shiong, P., Henitz, R. E., Merideth, N., Yao, Q. X., Zheng, T. Murphy, M., Moloney, M. K., Schmehl, M., and Harris, M. Insulin independence in a type I diabetic patient after encapsulated islet transplantation. *Lancet*, 343, 950-951 (1994).
- Spiecker, M., Darius, H., kaboth, K., Hubner, F., and Liao, J. K. Differential regulation of endothelial cell adhesion molecule expression by nitric oxide donors and antioxidants. *J. Leuk. Biol.*, 63, 732-739 (1998).
- Springer, T. A. Adhesion receptors of the immune system. *Nature*, 346, 425-434 (1990).
- Tze, W. J. and Tai, J. Biocompatibility and immunological studies of microencapsulation with cross-linked alginate capsules. *Transplantation*, 33, 563-564 (1982).
- Weber, C. Erl, W., Pietsch, A., and Weber, P. C. Aspirin inhibits nuclear factor-kB mobilization and monocyte adhesion in stimulated human endothelial cells. *Circulation*, 91, 1914-1917 (1995).
- Weiser, M. R., Gibbs, S., and Hechtman, H. B. Strategies to inhibit cellular adhesion molecules: In Paul, L.C., and Issekutz, T. (Eds.) Adhesion molecules in Health and Disease. Marcel Dekker Inc., New York, pp. 55-86, (1997).