SPANDIDOS PUBLICATIONS

Attenuation of the oxidative burst-induced DNA damage in human leukocytes by hyaluronan

H. DOROTA HALICKA[1], VADIM MITLITSKI[2], JULIE HEETER[2], ENDRE A. BALAZS[2] and ZBIGNIEW DARZYNKIEWICZ[1]

[1]Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, NY, 10595; [2]Matrix Biology Institute, 725 River Road, Suite 205, Edgewater, NJ 07020, USA

Received December 23, 2008; Accepted March 3, 2009

DOI: 10.3892/ijmm_00000182

Abstract. Oxidative burst provides the mechanism for specialized phagocytes, such as granulocytes or monocytes, to kill invading microorganisms through generation of superoxide anions. However, the oxidants generated during the burst damage DNA of the phagocytes and neighboring cells. Human blood leukocytes treated with phorbol myristate acetate (PMA) are considered to represent the experimental model of induction of oxidative burst. We recently reported that DNA damage in PMA-treated leukocytes is assessed by cytometric analysis of the induction of histone H2AX phosphorylation and Ataxia Telangiectasia Mutated (ATM) activation. In the present study we observed that hyaluronic acid (HA) of average molecular weight (MW) $5.4x10^6$ and $2x10^6$ at 0.1% (w/v) concentration significantly attenuated H2AX phosphorylation and ATM activation induced in leukocytes during oxidative burst. HA also reduced the intracellular level of PMA-induced reactive oxidants as measured by the ability of cells to oxidize 2',7'-dihydro-dichlorofluorescein-diacetate. No such effect was seen with HA of $6x10^4$ MW. The data are consistent with earlier observations that HA of high MW protects DNA from oxidative damage induced by endo- or exogenous oxidants. The anti-oxidant effect of HA seen during oxidative burst also explains its anti-inflammatory effect when used to treat arthritic joints.

Introduction

To neutralize pathogens and prevent infections from spreading, phagocytes developed a capability to rapidly generate microbicidal oxidants through a mechanism defined as respiratory- or oxidative-burst (1). This mechanism involves a transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to oxygen by NAHPH oxidase, the process that generates a superoxide anion (O_2^-) which spontaneously recombines with other molecules to produce reactive free radicals capable of killing microorganisms (1-6). Respiratory burst in phagocytes occurs also during chronic and acute inflammation and its degree in the tissue correlates with the intensity of the inflammatory reaction (7). Apart from functioning as an innate immune defense mechanism, the NADPH oxidase system was reported to also play a role in inter- and intracellular signaling in cell types other than phagocytes (1,8-10).

In vitro exposure of phagocytes to the tumor promoter phorbol myristate acetate (PMA) provides an experimental model of oxidative burst. The free radicals generated during the PMA-induced oxidative burst induce DNA damage that was observed not only in phagocytes themselves but in the adjacent cells as well (11-13). Activation of Ataxia Telangiectasia Mutated (ATM) through its phosphorylation on *Ser*1981 and phosphorylation of histone H2AX on *Ser*139 are early markers of a cell's response to DNA damage, particularly if the damage involves formation of DNA double-strand breaks (DSBs) (14,15). We recently reported that induction of oxidative burst in human peripheral blood leukocytes by PMA led to intense phosphorylation of H2AX and activation of ATM which was seen in monocytes, granulocytes as well as in lymphocytes (16). These data were consistent with earlier observations (13) that the reactive oxidants generated in phagocytes during oxidative burst were mobile and induced DNA damage in the neighboring lymphocytes.

Within the tissue, cells reside in the environment of intercellular matrix that has a modulating effect on reactive oxidants. The glycosaminoglycans, such as chondroitin-4-sulfate and hyaluronan (hyaluronic acid, HA), inhibit lipid peroxidation caused by oxidative stress and attenuate inflammatory reactions mediated by reactive oxidants (17-20). The mechanism of the anti-inflammatory effect of HA seen after its administration into the inflamed arthritic joints (21) is likely to be also mediated through neutralization of the oxidants generated during inflammation-driven oxidative burst in leukocytes and macrophages. We recently reported that HA protected DNA in A549 and WI-38 cells from oxidative damage induced by exogenous oxidants, such as H_2O_2, as well as from constitutive

Correspondence to: Dr Zbigniew Darzynkiewicz, Brander Cancer Research Institute, New York Medical College, Valhalla, NY 10595, USA
E-mail: darzynk@nymc.edu

Key words: DNA damage, DNA double-strand breaks, free radicals, ROIs, anti-oxidants, oxidative stress, hyaluronic acid, granulocytes, monocytes

DNA damage caused by metabolically generated endogenous reactive oxygen species (ROS) (22).

The aim of the preset study was to explore whether HA modulates the extent of DNA damage of leukocytes induced by the oxidative burst. Similar to prior studies, the extent of DNA damage was assessed by the cytometric analysis of H2AX phosphorylation and ATM activation (16,22-25), the early events of the DNA damage response (14,15,26). The data show significant attenuation of both H2AX phosphorylation and ATM activation by HA, consistent with the earlier evidence of the protective effect of this constituent of intercellular matrix on oxidative DNA damage in other cell systems (17-22).

Materials and methods

Cell isolation and treatment. Human peripheral blood was collected from healthy volunteers by venipuncture into heparinized syringes which were then maintained upright for 1 h at room temperature to allow erythrocytes to sediment at 1 x g. The plasma containing white blood cells (WBCs) was collected. Then cells were rinsed with RPMI-1640 medium (Gibco-BRL Life Technologies, Inc., Grand Island, NY) . Cells (10^6) were then suspended in 1 ml of RPMI-1640 supplemented with 10% fetal calf serum in 17x120 mm nonpyrogenic Falcon polystyrene conical tubes (Becton-Dickinson Labware, Franklin Lakes, NJ). HA (0.5% w/v in PBS; Matrix Biology Institute, Edgewater, NJ) was added to some suspensions to obtain the final 0.1% w/v HA concentration. The respective control cultures were supplemented with the equivalent volumes of PBS. HA of three different MWs, $6x10^4$, $2x10^6$ and $5.4x10^6$ were used throughout the experiments, as listed under Results section and in Figure legends. Phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich, St Louis, MO) was added at a concentration of 1 μM to the suspensions and cells were incubated at 37˚C in an atmosphere of 5% CO_2 in air for 1 h. HA was added 1 h prior to addition of PMA and was present throughout the incubation with PMA.

Immunocytochemical detection of γH2AX, activated ATM and caspase activation. After 1 h of PMA stimulation, cells were washed in phosphate-buffered saline (PBS) (centrifugation at 300 x g, 5 min) and then fixed in suspension in 1% ice-cold methanol-free formaldehyde (Polysciences, Inc., Warrington, PA), dissolved in PBS for 15 min followed by suspension in 80% ice-cold ethanol in which they were stored overnight at -20˚C. Cells were washed twice in PBS (300 g, 5 min) and incubated first in 0.1% Triton X-100 (Sigma-Aldrich) for 15 min at room temperature, then in 1% solution of bovine serum albumin (BSA) for 30 min at room temperature to suppress nonspecific antibody (Ab) binding. Cells were then incubated with 100 μl of 1% BSA in PBS containing 1:100 diluted anti-phospho-histone H2AX (*Ser*-139) anti-mouse Ab or 1:100 diluted anti-phospho-ATM (*Ser*-1981) anti-mouse Ab (Millipore/Upstate, Temecula, CA) or 1:100 cleaved caspase-3 (*Asp*-175) anti-rabbit Ab (Cell Signaling Technology, Inc., Danvers, MA), and incubated for 2 h at room temperature. Cells were rinsed with PBS and incubated with 100 μl of 1% BSA in PBS containing 1:100 Alexa Fluor 488 $F(ab')_2$ fragment of goat anti-mouse IgG (H+L) or 1:100 Alexa Fluor 488 $F(ab')_2$ fragment of goat anti-rabbit IgG (H+L) (Invitrogen, Carlsbad, CA) for 40 min at room temperature in the dark. After washing with PBS, cells were counter-stained with 10 μg/ml propidium iodide (PI; Invitrogen/Molecular Probes, Eugene, OR) in the presence of 100 μg/ml of RNase A (Sigma-Aldrich) for 20 min at room temperature in the dark.

Reactive oxygen species (ROS) detection. Untreated cells as well as cells treated with HA alone or with PMA in the absence or presence of HA for 1 h were incubated for the final 30 min with 10 μM 2',7'-dihydro-dichlorofluorescein-diacetate (H2DCFDA) (Invitrogen/Molecular Probes) at 37˚C. Cellular green fluorescence was then measured by flow cytometry. Following oxidation by ROS and peroxides the non-fluorescent substrate H2DCFDA is converted within cells to the highly fluorescent derivative DCF (27).

Determination of MW and polydispersity of HA samples. The tissue culture medium to which HA of MW $2x10^6$ was added and in which cells were incubated in the absence or presence of PMA was separated from cells by centrifugation (300 x g, 5 min) and the MW and the polydispersity of the HA molecules in the medium were determined by agarose gel electrophoresis on 0.5% agarose gel as recently described by us (22). Monodisperse internal standards (Hyalose, LLC, Oklahoma City, OH) were applied for each run.

Fluorescence measurements. Cellular green (Alexa Fluor 488 or DCF) and red (PI) fluorescence was measured using a FACScan flow cytometer (Becton-Dickinson, San Jose, CA) using the standard optics of the FACScan. CellQuest software (Becton-Dickinson) was used to analyze the data. Granulocytes and mononuclear cells were distinguished by their characteristic properties to scatter laser light in the forward and right angle (side) direction (27). Cells (10^4) were analyzed per each sample. Each sample was run in duplicate or triplicate and experiments was repeated at least three times. The SD was estimated based on Poisson distribution of cell populations. The inter-sample variations were not statistically significant. The representative raw data in form of bivariate distributions (scatterplots) are shown in Figs. 1-4.

Results

The induction of histone H2AX phosphorylation in leukocytes by PMA, in the absence and presence of HA of MW $5.4x10^6$, is illustrated in Fig. 1. The data show the distinct increase in expression of γH2AX in cells treated with PMA. This increase, however, was markedly attenuated when cells were treated with PMA in the presence of HA. HA alone had no apparent effect on the level of γH2AX expression. A very similar pattern of response was also seen when cells were treated with PMA in the presence of HA of MW $2x10^6$ (Fig. 2). Here again the PMA induced a distinct increase in extent of H2AX phosphorylation which was essentially prevented by HA. However, HA of low MW ($6x10^4$) had little effect on the PMA-induced H2AX phosphorylation as it reduced the expression of γH2AX by only 10% compared to PMA alone (data not shown).

We have also measured the effect of PMA in the absence and presence of HA on activation of ATM as measured by

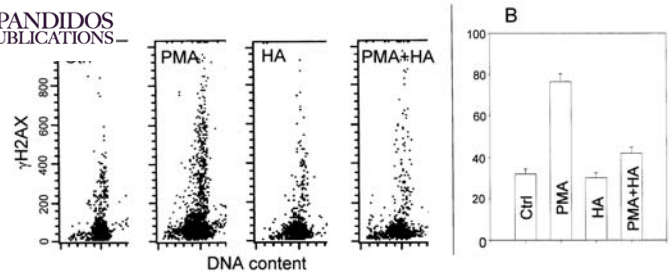

Figure 1. Effect of PMA in the absence and presence of HA (MW 2x10^6) on induction of γH2AX in human leukocytes. Human peripheral white blood cells suspended in culture medium were either untreated (Ctrl), treated with PMA alone (PMA), with HA alone (HA) or with PMA in the presence of HA (PMA+HA). (A) Bivariate distributions (scatterplots) illustrating the expression of γH2AX vs DNA content of the measured cells. (B) presents the mean values of γH2AX IF (+SD) of the measured cells.

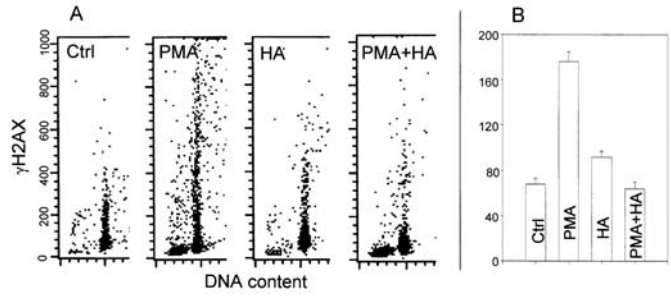

Figure 2. Effect of PMA in the absence and presence of HA (MW 2x10^6) on induction of γH2AX in human leukocytes. The cells were untreated (Ctrl), treated with PMA alone (PMA), or with PMA in the presence of HA (PMA+HA). (A) Scatterplots presenting the expression of γH2AX vs DNA content of the measured cells. (B) Presents the mean values of γH2AX IF (+SD) of the measured cells.

phosphorylation on Ser-1981. The data show that, similarly, as in the case of H2AX phosphorylation, the treatment with PMA led to an increase in expression of ATM-1981^P and the extent of the increase was reduced by HA (MW 2x10^6) (Fig. 3). The effects, however, were somewhat less pronounced compared to the induction of γH2AX.

To assess the intracellular level of reactive oxidants in leukocytes treated with PMA and HA we measured the cells' capability to oxidize the fluorescent probe H2DCFDA (Fig. 4). Treatment of cells with PMA alone led to an overall increase in intensity of DCF fluorescence with a distinct bimodal response as evident by the presence of two peaks. The peak fluorescence intensity of the subpopulation of cells that responded maximally (46% cells) was two orders of magnitude greater compared to the subpopulation represented by the lower intensity peak. However, even this lower-intensity peak was distinctly shifted towards higher CDF fluorescence values compared to the untreated cells. Incubation of cells with HA alone had a relatively minor effect on their CDF fluorescence although a shift towards higher values was apparent and there was an increase in proportion of cells with higher CDF values (7%). The induction of oxidative burst by PMA in the presence of HA (5.4x10^6 MW) lowered the proportion of cells with high CDF fluorescence from 46 to 35%. Similar results were obtained using 2x10^6 MW HA, but no distinct effects were seen with 6x10^4 MW HA (data not shown).

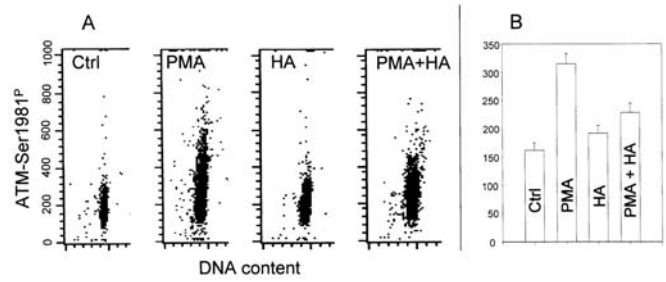

Figure 3. Effect of PMA in the absence and presence of HA (MW 5.4x10^6) on phosphorylation of ATM human leukocytes. The cells were untreated (Ctrl), treated with PMA alone (PMA), or with PMA in the presence of HA (PMA+HA). (A) Scatterplots presenting the expression of ATM-S1981^P vs DNA content of the measured cells. (B) Presents the mean values of ATM-S1981^P IF (+SD) of the measured cells.

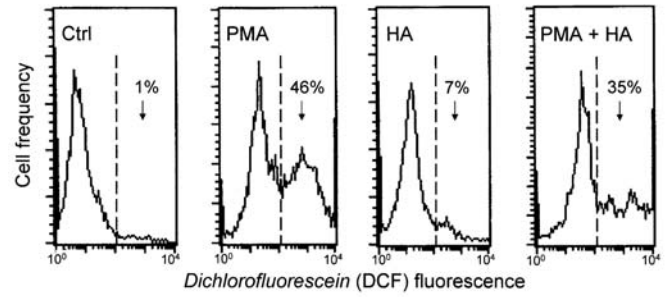

Figure 4. Effect of PMA in the absence and presence of HA (MW 5.4 x10^6) on the capability of leukocytes to oxidize 2',7'-dihydro-dichlorofluorescein-diacetate (H2DCFDA). The leukocytes were suspended in culture medium (Ctrl) or treated with PMA alone (PMA), HA alone (HA) and PMA in the presence of HA (PMA+HA), and during the final 30 min of incubation 10 μM H2DCFDA was included into the media. Cellular green fluorescence was then measured by flow cytometry. On these bi-modal frequency distributions the percent of cells with high intensity of CDF fluorescence (above the threshold marked by the dashed line) is shown. Note exponential scale of the CDF fluorescence intensity.

We also measured the effect of cell incubation in the absence and presence of PMA on possible degradation of HA that was present in cell suspensions by measuring its MW and polydisperity. Towards this end HA MW 2x10^6 with the media, in which the cells were suspended, was incubated without or with PMA. After removal of cells by centrifugation these solutions were subjected to gel electrophoresis and MW of HA from these samples was compared. Identical distribution curves with similar matching MW (1.4x10^6) as well as comparable polydisperity, were seen in both PMA treated and untreated samples (data not shown).

Discussion

Our present data confirm an earlier observation that the induction of oxidative burst in leukocytes by PMA leads to distinct DNA damage response as evidenced by phosphorylation of histone H2AX on Ser-139 and activation of ATM through its phosphorylation on Ser-1981 (16). The induction of ROS in cells undergoing the oxidative burst was detected using the permeant H2DCFDA probe which becomes fluorescent upon oxidation (27). Based on the intensity of CDF

fluorescence two distinct cell subpopulations become apparent (Fig. 4). By the analysis of the light scatter signal in forward versus right angle ('side') direction, similar as before (16), we were able to identify the cells with very high CDF fluorescence intensity as granulocytes and monocytes and the cells with much lower fluorescence intensity, represented by a sharp peak on the frequency histograms of PMA-treated cells as predominantly lymphocytes.

Interestingly, a rather minor but distinct increase in intensity of CDF fluorescence was observed in cells incubated with H2DCDFA in the presence of HA alone as compared with the cells probed in the absence of HA (Fig. 4). It is unlikely that the apparent increase in CDF fluorescence was due to the HA-mediated rise in the level of ROS. Probably this was the consequence of steric volume exclusion effect exerted by HA (28). Namely, the extensive binding of H_2O molecules by HA as a result of HA hydration left fewer free H_2O molecules and thereby increased effective concentration of H2DCDFA in free H_2O that in turn led to a greater uptake of this probe by the cells.

The present data show that the induction of oxidative burst in cells suspended in HA of high MW attenuated the DNA damage response, both in terms of H2AX phosphorylation and activation of ATM. Also reduced was the proportion of cells with high CDF fluorescence intensity upon treatment with PMA in the presence of HA. These data thus suggest that HA had a neutralizing effect on the induction of ROS and thereby protected DNA, at least in some cells, from the oxidative damage. This observation is consistent with our recent findings that HA of high MW ($5.4x10^6$) attenuated oxidative DNA damage in WI-38 fibroblasts and pulmonary carcinoma A549 cells induced by exogenously applied H_2O_2 as well as by the endogenous, metabolically generated oxidants (22). It should be noted that DNA damage by the endogenous ROS generated in mitochondria during aerobic metabolism triggers distinct DNA damage response manifesting as constitutive ATM activation and H2AX phosphorylation the extent of which is modulated by ROS scavengers and caloric restriction mimetics (23-26).

Monocytes and granulocytes express the hyaluronate receptor CD44 on their plasma membrane (29,30). The presence of CD44 mediates internalization of this biopolymer via endocytosis (31). It is likely therefore that the observed effects of HA, namely the attenuation of DNA damage response and reduction of ROS, are the consequences of HA internalization by these phagocytes and the intracellular neutralization of the reactive oxidants by this biopolymer. However, because the ROS, generated during the oxidative burst is mobile and has the ability to damage DNA of neighboring cells (13,16) it is also possible that the observed effect of HA was due to ROS neutralization outside the cells in the media in which the cells were suspended. We were unable however, to detect any differences in degradation of HA in tissue culture media samples in which the cells were suspended and treated with PMA versus the media from the untreated cells. Namely, MW as well as polydisperity of HA, all were the same in media that contained untreated and PMA treated cells. No degradation of extracellular HA during the induction of oxidative burst, was thus detectable. However, the reduction of MW of HA in the samples incubated with tissue culture media plus the cells was evident as the MW changed from $2x10^6$ to $1.4x10^6$ during 2 h incubation with the cells. The polydispersity changed with it. This most likely was caused by the change in the composition of the media due to the activity of cells.

Two mechanisms may explain the protective effect of HA against DNA damage during oxidative burst. One mechanism involves the capability of the polyanionic HA molecule to chelate Fe^{++} and Cu^{++} (32,33). These ions are essential in Fenton's reaction during which the superoxide and hydrogen peroxide, which themselves are not highly reactive with DNA, are converted into the strongly reactive with DNA, hydroxyl radical (OH^*) (34,35). The direct scavenging by HA of reactive oxidant molecules, particularly the reactive products of Fenton's reaction, such as OH^*, is the second mechanism by which HA attenuated DNA damage during oxidative burst. The ROS scavenging activity by HA would deplete the pool of oxidants that otherwise will damage DNA. The ROS scavenging mechanism results in a breakdown of HA molecules that are detected by a decrease of its MW (36). It was proposed by us (37) that one of HA's biological functions is to give protection against cellular damage caused by radicals generated by oxidative reductive systems or ionizing radiation. Consistent with this proposal is the observation that exposure of synovial fibroblasts to low concentrations of H_2O_2 enhances the rate of HA synthesis (38). Apparently HA degradation by H_2O_2 triggers compensatory production of high MW HA thereby enhancing the antioxidant defense.

HA was shown to promote wound healing (39,40) and attenuating inflammatory processes (18-20,41-43). Also, the interaction of some ligands, including antibodies with the hyaluronate cell surface receptor CD44 on phagocytes, was shown to modulate their inflammatory response (33,44,45). Since ROS generated during oxidative burst of phagocytes/macrophages play a key role in inflammation (16,46), it is likely that the palliative effect of HA in inflammation is mediated to a large extent by its capability to protect cells from reactive oxidants.

Acknowledgements

Supported by NCI RO1 CA 28704 and Matrix Biology Institute. We thank Millipore Corp. for providing samples of antibody reagents.

References

1. Quinn MT and Gauss KA: Structure and regulation of the neutrophil respiratory burst: comparison with nonphagocytic oxidases. J Leukocyte Biol 76: 760-781, 2004.
2. Cross AR and Jones OT: Enzymic mechanisms of superoxide production. Biochim Biophys Acta 1057: 281-298, 1991.
3. Janeway CA Jr and Medzhitov R: Innate immune recognition. Annu Rev Immunol 20: 197-216, 2002.
4. Ricevuti G: Host tissue damage by phagocytes. Ann NY Acad Sci 832: 426-448, 1997.
5. Roos D, Van Bruggen R and Meischl C: Oxidative killing of microbes by neutrophils. Microbes Infect 5: 1307-1315, 2003.
6. Babior BM: NADPH oxidase. Curr Opin Immunol 16: 42-47, 2004.
7. Maor I, Rainis T, Lanir A and Lavey A: Oxidative stress, inflammation and neutrophil superoxide release in patients with Crohn's disease: distinction between active and non-active state. Dig Dis Sci 53: 2208-2214, 2008.
8. Bokoch GM and Knaus UG: NADPH oxidases: not just for leukocytes anymore! Trends Biochem Sci 28: 502-508, 2003.

SPANDIDOS PUBLICATIONS

...th JD: NOX enzymes and the biology of reactive oxygen. ...v Immunol 4: 181-189, 2004.
10. Gwinn MR and Vallyathan V: Respiratory burst: role in signal transduction in alveolar macrophages. J Toxicol Environ Health B Crit Rev 9: 27-39, 2006.
11. Birnboim HC: DNA strand breakage in human leukocytes exposed to tumor promoter, phorbol myristate acetate. Science 215: 1247-1249, 1982.
12. Birnboim HC and Sandhu JK: Levels of DNA strand breaks and superoxide in phorbol ester-treated human granulocytes. J Cell Biochem 66: 219-228, 1999.
13. Shacter E, Beecham EJ, Covey JM, Kohn KW and Potter M: Activated neutrophils induce prolonged DNA damage in neighboring cells. Carcinogenesis 9: 2297-2304, 1988.
14. Rogakou EP, Pilch DR, Orr AH, Ivanova VS and Bonner WM: DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273: 5858-5868, 1998.
15. Burma S, Chen BP, Murphy M, Kurimasa A and Chen DJ: ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J Biol Chem 276: 42462-42467, 2001.
16. Tanaka T, Halicka HD, Traganos F and Darzynkiewicz Z: Phosphorylation of histone H2AX on Ser139 and activation of ATM during oxidative burst in phorbol ester-treated human leukocytes. Cell Cycle 5: 2671-2675, 2006.
17. Arai H, Kashiwagi S, Nagasaka Y, Uchida K, Hoshii Y and Nakamura K: Oxidative modification of apolipoprotein E in human very-low density lipoprotein and its inhibition by glycosaminoglycans. Arch Biochem Biophys 367: 1-8, 1999.
18. Campo GM, Avenoso A, Campo S, Ferlazzo AM, Altavilla A and Calatroni A: Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. Arthritis Res Ther 5: R122-R131, 2003.
19. Campo GM, Avenoso A, Campo S, Ferlazzo AM, Altavilla D, Micali C and Calatroni A: Aromatic trap analysis of free radicals production in experimental collagen-induced arthritis in the rat: protective effect of glycosaminoglycans treatment. Free Radic Res 37: 257-268, 2003.
20. Albertini R, Passi A, Abuja PM and De Luca G: The effect of glycosaminoglycans and proteoglycans on lipid peroxidation. Int J Mol Med 6: 126-136, 2000.
21. Balazs EA: Viscosupplementation for treatment of osteoarthritis: from initial discovery to current status and results. Surg Technol Int 12: 278-289, 2004.
22. Zhao H, Tanaka T, Mitlitski V, Heeter J, Balazs EA and Darzynkiewicz Z: Protective effect of hyaluronate on oxidative DNA damage in WI-38 and A549 cells. Int J Oncol 32: 1159-1169, 2008.
23. Zhao H, Tanaka T, Halicka HD, Traganos F, Zarebski M, Dobrucki J and Darzynkiewicz Z: Cytometric assessment of DNA damage by exogenous and endogenous oxidants reports the aging-related processes. Cytometry A 71: A905-A914, 2007.
24. Tanaka T, Halicka HD, Huang X, Traganos F and Darzynkiewicz Z: Constitutive histone H2AX phosphorylation and ATM activation, the reporters of DNA damage by endogenous oxidants. Cell Cycle 5: 1940-1945, 2006.
25. Zhao H, Traganos F, Albino AP and Darzynkiewicz Z: Oxidative stress induces cell cycle-dependent Mre11 recruitment, ATM and Chk2 activation and histone H2AX phosphorylation. Cell Cycle 7: 1490-1495, 2008.
26. Tanaka T, Kajstura M, Halicka HD, Traganos F and Darzynkiewicz Z: Constitutive histone H2AX phosphorylation and ATM activation are strongly amplified during mitogenic stimulation of lymphocytes. Cell Prolif 40: 1-13, 2007.
27. Rothe and Klouche M: Phagocyte functions. Methods Cell Biol 75: 679-708, 2004.
28. Louie D and Serwer P: Quantitation of the effect of excluded volume on double-stranded DNA. J Mol Biol 242: 547-558, 1994.
29. Levesque MC and Haynes BF: In vitro culture oh human peripheral blood monocytes induces hyaluronan binding and upregulates monocyte variant CD44 isoform expression. J Immunol 156: 1557-1565, 1996.
30. Hutas G, Bajnok E, Gal I, Finnegan A, Glant TT and Mikecz K: CD44-specific antibody treatment and CD44 deficiency exert distinct effects on leukocyte recruitment in experimental arthritis. Blood 112: 4999-5006, 2008.
31. Thankamony SP and Knudson W: Acetylation of CD44 and its association with lipid rafts are required for receptor and hyaluronan endocytosis. J Biol Chem 281: 34601-34609, 2006.
32. Balogh GT, Illes J, Szekely Z, Forrai E and Gere A: Effect of different metal ions on the oxidative damage and antioxidant capacity of hyaluronic acid. Arch Biochem Biophys 410: 76-82, 2003.
33. Nagy L, Yamashita S, Yamaguchi S, Sipos P, Wakita H and Nomura M: The local structures of Cu(II) and Zn(II) complexes of hyaluronate. J Inorg Biochem 72: 49-55, 1998.
34. Koppenol WH: The Haber-Weiss cycle - 70 years later. Redox Rep 7: 55-57, 2002.
35. Valko M, Morris H and Cronin MT: Metals, toxicity and oxidative stress. Curr Med Chem 12: 1161-1208, 2005.
36. Greenwald RA and Moy WW: Effect of oxygen-derived free radicals on hyaluronic acid. Arthritis Rheum 23: 455-463, 1980.
37. Balazs E, Davies J, Phillips G and Young M: Transient intermediates in the radiolysis of hyaluronic acid. Radiat Res 31: 243-255, 1967.
38. Hutadilok N, Smith MM and Ghosh P: Effect of hydrogen peroxide on the metabolism of human rheumatoid and osteoarthritic synovial fibroblasts in vitro. Ann Rheum Dis 50: 219-226, 1991.
39. Chen WY and Abatangelo G: Functions of hyaluronan in wound repair. Wound Repair Regen 7: 79-89, 1999.
40. Haider AS, Grabarek J, Eng B, Pedraza P, Ferreri NR, Balazs EA and Darzynkiewicz Z: In vitro wound healing analyzed by laser scanning cytometry. Accelerated healing of epithelial cell monolayers in the presence of hyaluronate. Cytometry A 53: A1-A8, 2003.
41. Balazs EA and Darzynkiewicz Z: The effect of hyaluronic acid on fibroblasts, mononuclear phagocytes and lymphocytes. In: Biology of the Fibroblast, Papers of the Symposium held in Turku, Finland, 1972; Kulonen E and Pikkarainen J (eds). Academic Press, London, pp237-252, 1973.
42. Suzuki Y and Yamaguchi T: Effects of hyaluronic acid on macrophage phagocytosis and active oxygen release. Agents Action 38: 332-337, 1993.
43. Fukuda K, Takayama M, Ueno M, Oh M, Asada S, Kumano F and Tanaka S: Hyaluronic acid inhibits interleukin-1-induced superoxide anion in bovine chondrocytes. Inflam Res 46: 114-117, 1997.
44. Teder P, Vandivier RW, Jiang D, Liang J, Cohn L, Pure E, Henson PM and Noble PW: Resolution of lung inflammation by CD44. Science 296: 155-158, 2002.;
45. Mytar B, Siedlar M, Woloszyn M, Colizzi V and Zembala M: Cross-talk between human monocytes and cancer cells during reactive oxygen intermediates generation: the essential role of hyaluronan. Int J Cancer 94: 727-732, 2001.
46. Quinn MT and Gauss KA: Structure and regulation of the neutrophil respiratory burst: comparison with nonphagocytic oxidases. J Leukocyte Biol 76: 760-781, 2004.