The role of calcium in human lymphocyte DNA repair ability

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DNA repair ability is reduced in a variety of pathologic conditions. In addition, in some of these diseases a disturbance in cellular Ca homeostasis occurs or cytosolic (Ca^{2+}) responses to various stimuli are impaired. The leading environmental cause for genomal DNA damage is ultraviolet (UV) irradiation. The aims of the present study were (1) to evaluate a possible dependence of UV-induced DNA repair ability on cytosolic Ca^{2+} in human lymphocytes and (2) to assess the direct effect of UV irradiation on Ca^{2+} homeostasis in these cells. UV-induced DNA repair ability in lymphocytes was maximal at 1 mmol/L $CaCl_2$ in the medium. Suppression of DNA repair ability occurred after elevation or reduction of cellular (Ca^{2+}) when various methods were used, including changes in Ca^{2+} concentration in the medium, cellular Ca^{2+} depletion by ethyleneglycol-*bis*-(βaminoethylether)-N,N,N′,N′-tetraacetic acid, excessive Ca^{2+} concentration induced by ionophore, and shortening of Ca^{2+} presence time during repair synthesis. UV irradiation caused an immediate and significant rise in cytosolic (Ca^{2+}) that was the result of both enhanced Ca^{2+} uptake and inhibition of plasma membrane Ca-adenosine triphosphatase activity. The tyrosine kinase inhibitor genistein inhibited both UV-induced DNA repair and UV-induced cytosolic (Ca^{2+}) elevation. These results emphasize the importance of a precise cellular Ca^{2+} level regulation for the optimal DNA repair process. UV irradiation, by inducing cellular Ca^{2+} rise, may activate DNA repair as soon as DNA is damaged. (J Lab Clin Med 1997;130:33-41)

Abbreviations: ANOVA = analysis of variance; ATPase = adenosine triphosphatase; BSA = bovine serum albumin; (Ca^{2+}) = Ca^{2+} concentration; EGTA = ethyleneglycol-*bis*-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid; HEPES = N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; PBS = phosphate-buffered saline solution; Pi = inorganic phosphorus; RPMI = Rosewell Park Memorial Institute 1640 tissue culture medium; TCA = trichloroacetic acid; UV = ultraviolet

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The repair of DNA is a process that enables the organism to overcome spontaneous mistakes in the DNA sequence as well as breaks in DNA caused by various environmental agents. UV irradiation constitutes one of the leading threats to the integrity of the genomal DNA. Suppressed DNA repairability accompanies a variety of diseases. These include Down's syndrome, Fanconi's anemia, xeroderma pigmentosum, ataxia telangiectasia,[1-3] end-stage renal disease,[4,5] different kinds of malignancies,[6-8] and more.[9]

Some of these diseases are malignant and the others predispose the subjects to a high risk for the

Table I. The effect of external Ca^{2+} concentration on UV-induced DNA repair ability (cpm/10^6 cells) of normal lymphocytes

External (Ca^{2+})	0 μmol/L	0.1 μmol/L	1 μmol/L	5 μmol/L	10 μmol/L	50 μmol/L	100 μmol/L
Without ionophore	105.9 ± 9.4	182.8 ± 8.3	196.3 ± 5.1	—	245.4 ± 11.1	—	289.8 ± 11.6
With ionophore	66.6 ± 6.2	133.9 ± 4.0	146.6 ± 3.0	189.3 ± 3.9	202.2 ± 6.6	233.6 ± 9.0	159.5 ± 8.6

Each point is the mean ± SEM of 5 to 9 experiments. Significant differences ($p < 0.02$) as measured by the repeated measures methods existed between the points 0, 1 μmol/L, 10 μmol/L, 100 μmol/L, 1 mmol/L, and 5 mmol/L without ionophore, and 0, 0.1 μmol/L, 5 μmol/L, 50 μmol/L, 100 μmol/L, and 1 mmol/L with ionophore.

development of cancer.[5,10,11] An association between the propensity for the development of malignancy and the depressed ability to overcome DNA damage has been suggested.

One of the methods to activate the genomal DNA repair system in lymphocytes is to irradiate them with sublethal doses of UV.[5-8] It has been shown that such sublethal UV irradiation may also affect cytosolic Ca^{2+} levels.[12-15]

UV has been shown to cause Ca^{2+} signaling by inducing tyrosine phosphorylation within seconds after irradiation.[14] Disturbed Ca^{2+} metabolism and a defective response to Ca^{2+} stimulators have been reported to exist in some of the patients with DNA repair deficiency.[16-20] The general aim of the study was to examine the association of DNA repairability with cytosolic Ca^{2+} in human lymphocytes. The specific objectives were (1) to investigate, by using various approaches, the effect of changes in cytosolic Ca^{2+} on DNA repairability, and (2) to evaluate the effect of UV irradiation, which triggers DNA repair synthesis, on several components of the Ca^{2+} homeostasis system. To evaluate the association between UV-induced DNA repairability and UV-induced cellular [Ca^{2+}] elevation, the inhibition of tyrosine kinase activity after UV irradiation was studied.

The results suggest that DNA repairability in lymphocytes in vitro was optimal at the physiologic extracellular [Ca^{2+}]. It decreased when cellular [Ca^{2+}] was either substantially reduced or increased. UV irradiation caused elevation of lymphocyte cytosolic [Ca^{2+}] by increasing Ca^{2+} uptake and decreasing plasma membrane Ca-ATPase activity. Both UV-induced DNA repair and UV-induced cytosolic [Ca^{2+}] elevation were inhibited by a tyrosine phosphorylation inhibitor. Thus an impaired DNA repairability may result in part from a disturbed Ca^{2+} homeostasis.

METHODS

Reagents and instruments. NaCl, KCl, $MgCl_2$, $CaCl_2$, NaH_2PO_4, Na_2HPO_4, glucose, HEPES, BSA, the Ca^{2+} chelator EGTA, hydroxyurea, Triton X100, TCA, Tris, ATP-Na_2, Ca ionophore A23187, anhydrous dimethyl sulfoxide, and genistein were obtained from Sigma Chemical Co., St. Louis, Mo. PBS without Ca^{2+} and Mg^{2+}, RPMI, fetal calf serum, glutamin solution, antibiotic solution (containing penicillin, streptomycin, and amphotericin B), Ham's F-10 Nutrient Mixture, and serum-free medium supplement Biogro-1 were obtained from Biologic Industries, Beth Haemek, Israel. Fura2-AM, in vials of 50 μg, was supplied by Molecular Probes Inc., Eugene, Ore. Lymphoprep was obtained from Nycomed Pharma AS, Oslo, Norway. Scintillation liquid was obtained from Optifluor, Packard Co., Groningen, The Netherlands. Me^3H-thymidine was supplied by Rotem Industries, Negev, Israel. Whatman CF/C 25 mm glass microfiber filters were obtained from Tamar, Jerusalem, Israel. The liquid scintillation counter was from LKB Wallac, Turko, Finland, supplemented with a Facit 4420 video terminal. A Shimadzu RF-5000 spectrofluorometer was used for cytosolic [Ca^{2+}] determination. UV irradiation at a wavelength of 254 nm was produced by a Philips TUV 15W G1538 lamp, and the UV intensity was measured by a UVX digital radiometer from UVP Inc., San Gabriel, Calif. For cell homogenization, a motor-operated glass-polytef homogenizer was used.

Peripheral blood lymphocytes. For studying the dependence of DNA repairability on external [Ca^{2+}], buffy coats from apparently healthy blood donors from the local blood bank were used. For the other experiments, 20 to 40 ml of heparinized blood was collected by venopuncture at 8 AM from the local medical staff or from other apparently healthy volunteers. Informed consent was obtained from all participants. The fresh blood was diluted with PBS, and the lymphocytes were separated on a Lymphoprep gradient[21] and washed three times with PBS. In case of erythrocyte contamination, a short hemolysis in hypotonic saline solution was performed. Viability was assessed by using Trypan blue dye.

DNA repairability measurements. Cells were diluted with PBS to 4×10^6/ml, and DNA repairability was measured as previously described.[5] In brief, quadruplicates of 2×10^6 cells were UV irradiated at a wavelength of 254 nm and dosage of 25 joules/m^2 to damage the DNA and to initiate the repair process. The small amount of PBS did not reduce the intensity of the irradiation. Nonirradiated cells served as blanks. The whole procedure, except for the incubation, was performed in the cold and in the dark. A 2 ml sample of complete medium (15% fetal calf serum,

500 μmol/L	1 mmol/L	2.6 mmol/L	5 mmol/L
322.9 ± 4.5	347.8 ± 27.6	326.4 ± 26.4	138.6 ± 18.4
—	77.5 ± 4.0	—	—

1% of each glutamine, and antibiotic solutions in RPMI containing 2.6 mmol/L [Ca^{2+}]) was added and supplemented with 10 mmol/L hydroxyurea to avoid scheduled DNA replication. After 30 minutes of incubation at 37° C in a 5% CO_2 humidified atmosphere, 25 μl of tritiated thymidine solution (specific activity 49 Ci/mmol) to a final concentration of 10 μCi/ml was added. After incubation for another 2 hours, during which the DNA repair process occurred, the cells were cooled, collected, washed, hydrolyzed for 30 minutes in 0.5N NaOH at 37° C, neutralized with 0.5N HCl, precipitated with ice-cold TCA (20% solution), collected on glass microfiber filters, finally washed with 10% cold TCA and with cold 95% ethanol, and counted for tritium in scintillation liquid. The DNA repairability was expressed as mean cpm of tritiated thymidine incorporation per 1×10^6 irradiated cells, after the cpm of the nonirradiated blanks was subtracted.

To find out the degree of dependence of DNA repair on external [Ca^{2+}], this procedure was repeated with EGTA-regulated external [Ca^{2+}] of 0, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, 5×10^{-2}, 0.1, 0.5, 1, 2.6, and 5 mmol/L $CaCl_2$, with 5 to 9 replications at each point. The medium used in Ca^{2+}-regulated experiments was Ham's F-10 Nutrient Mixture containing 2% serum-free medium supplement Biogro-1 and 1% of each glutamine and antibiotic solution. The same concentration curves were also performed in the presence of 2 μmol/L Ca-ionophore A23187. In other experiments the incubation of the UV-irradiated lymphocytes started without external Ca^{2+}. A 1 mmol/L $CaCl_2$ final concentration, which gave optimum results in the former concentration curves, was added at 0, 30, 60, 90, 120, or 150 minutes of the overall 150-minute incubation time needed to allow the repair process to occur and to be measured. DNA repairability was also measured in the presence of either EGTA alone or Ca-ionophore alone, or in a combination of both solutions.

To examine the effect of tyrosine kinase inhibition on DNA repair, the tyrosine kinase inhibitor genistein was used. A 200 μmol/L sample of genistein (from a stock solution of 1.35 mg/ml dimethyl sulfoxide) was added at different time points of the DNA repair incubation period. In other experiments a dose-response curve was established by adding 0, 25, 50, or 100 μmol/L genistein to the cell suspension immediately after the UV irradiation.

Cytosolic (Ca^{2+}) measurements. The methods for cytosolic [Ca^{2+}] determination in Fura-2-loaded lymphocytes have been extensively discussed in the last few years, and our procedure was based on the experiments of Gaciong,[18] Genot,[22] and Woogen.[23] Washed lymphocytes were suspended in 2 ml of buffer-Ca-BSA, which was composed of 127 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, 1.2 mmol/L NaH_2PO_4, 10 mmol/L glucose, 10 mmol/L HEPES, 1 mmol/L $CaCl_2$ and 0.1% BSA, pH 7.4. A 50 μg sample of Fura-2-AM was freshly dissolved in 25 μl anhydrous dimethyl sulfoxide. The Fura-2-AM solution was added to the cell suspensions to a final concentration of 4 μmol/L. The tubes were protected from daylight and incubated in a shaking water bath for 25 minutes at 37° C. The cells were then diluted with buffer-Ca-BSA at 37° C, and incubation continued for another 25 minutes to turn the incorporated Fura-2-AM to Fura-2. The dyed cells were washed twice with 20 ml cold buffer-Ca-BSA, once more with buffer-Ca (without BSA), suspended in 300 μl buffer-Ca, and soon after stored in a light-protected ice bucket until cytosolic [Ca^{2+}] was measured.

The spectrofluorometric measurements were performed within 2 hours of the Fura-2 loading. Fura-2-loaded cells (4×10^6) were diluted to 2 ml in a stirred quartz cuvette adjusted with a thermostat to 37° C, and spectrofluorometric measurements started at once. The cell suspension was excited alternately at 340 nm and 380 nm, and the emission was at 505 nm, with a slit of 10 nm and a response time of 0.02 seconds. The ratio of the fluorescence was monitored during 1 to 2 minutes for the baseline. Cells were then lysed with 10 μl Triton X100 solution (0.1% final concentration) to determine the ratio at maximum [Ca^{2+}] and were then Ca-chelated with 20 μl EGTA solution (pH 8)(7.5 mmol/L in the cuvette) to determine the ratio with zero [Ca^{2+}]. Cytosolic [Ca^{2+}] was calculated according to the equation of Grynkiewicz et al.[24] with a dissociation constant of 224. In preliminary experiments the optimum conditions for Fura-2 loading and monitoring were established as follows: if the loaded cells were kept on ice in darkness and measured within 2 hours, and if the temperature of the cuvette was strictly kept at 37° C or just below, no additional washing of the cells and no corrections for leakage or autofluorescence were needed.

The effect of UV irradiation on cytosolic (Ca^{2+}). The immediate effect of UV irradiation on cytosolic [Ca^{2+}] in lymphocytes was determined as follows: 4×10^6 Fura-2-loaded cells in 100 μl adequate buffer were placed in a quartz cuvette and UV irradiated, whereafter they were immediately diluted to 2 ml, and cytosolic [Ca^{2+}] was measured at once. Nonirradiated cells were treated likewise and served as blanks. Preliminary experiments proved that the UV irradiation penetrated the quartz cuvette of the spectrofluorometer without any loss of intensity. It was further revealed that UV irradiation of Fura-2-loaded cells at 254 nm with the intensity used in the DNA repair experiments did not interfere with the cytosolic [Ca^{2+}] determination (in agreement with Sakai et al.[25]).

The conditions for cytosolic [Ca^{2+}] measurements in UV-irradiated lymphocytes with successive DNA repair process were established after preliminary experiments in which cytosolic [Ca^{2+}] and DNA repairability were measured simultaneously. In these preliminary experiments,

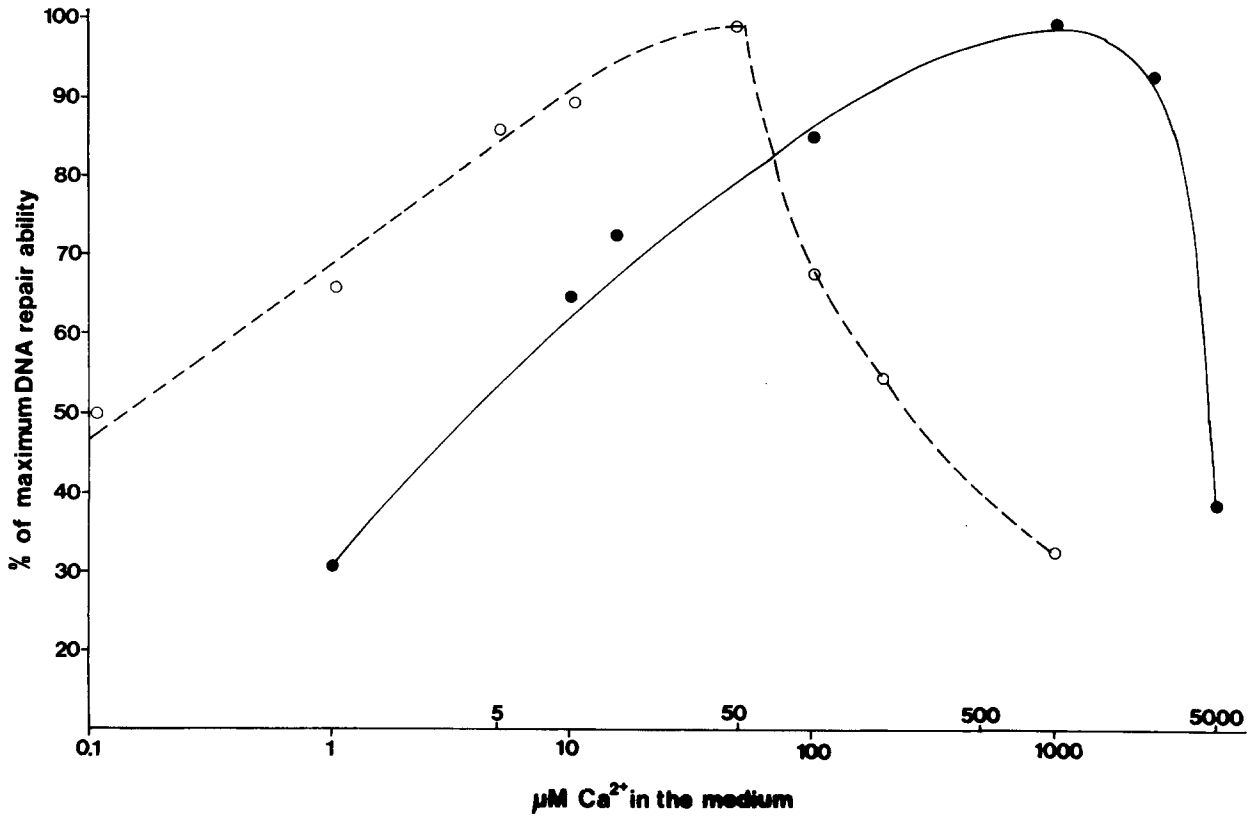

Fig. 1. Results of one representative experiment demonstrating the effect of external $[Ca^{2+}]$ on DNA repairability in lymphocytes. ●, Without ionophore; ○, with 2 μmol/L ionophore A23187.

4×10^6 cells in 100 μl Fura-2 loading buffer were UV irradiated and then diluted to 2 ml. Tritiated thymidine and Fura-2-AM were added, and the incubation was performed in a shaking water bath at 37° C, followed by cytosolic $[Ca^{2+}]$ and DNA repairability measurements, as previously described. Nonirradiated cells, which otherwise were treated alike, served as blanks. Although the DNA repairability measured by this procedure was reduced by 10% to 15% in comparison with the results with standard conditions, it still proved that DNA repairability took place in UV-irradiated cells during the Fura-2 loading incubation. The measurements of cytosolic $[Ca^{2+}]$ in UV-irradiated lymphocytes with successive DNA repair were then performed without tritiated thymidine.

In some experiments these cytosolic $[Ca^{2+}]$ measurements were performed in the presence of 100 μmol/L genistein, which was added immediately after UV irradiation.

Ca^{2+} uptake measurement. Ca^{2+} uptake was measured in Ca-depleted Fura-2-loaded lymphocytes. These were prepared by Fura-2-AM loading, diluting, and washing of the cells with buffer-EGTA-BSA, which contained 0.1 mmol/L EGTA instead of the 1 mmol/L $CaCl_2$ used in cytosolic $[Ca^{2+}]$ measurements. The dyed cells were washed once in buffer-EGTA (without BSA), diluted in 300 μl buffer-EGTA, and shortly thereafter kept on ice in the dark. A 100 μl sample of the Ca-depleted cells was then diluted to 2 ml and subjected to cytosolic $[Ca^{2+}]$ measurement. Monitoring of basal $[Ca^{2+}]$ lasted for 60 seconds, whereafter 10 μl $CaCl_2$, 1 mmol/L final concentration, was added to the cuvette. The time interval up to maximal Ca^{2+} uptake was carefully recorded. Ca^{2+} uptake is the elevation rate of cellular Ca^{2+}. This was calculated by dividing the increase in cytosolic $[Ca^{2+}]$ by the number of seconds the slope of the increase lasted. Ca^{2+} uptake was thus expressed as nmol/L/second. Triton X100 and EGTA were added as before. The immediate effect of UV irradiation on Ca^{2+} uptake was evaluated by cytosolic $[Ca^{2+}]$ monitoring of UV-irradiated Ca-depleted cells. Ca^{2+} uptake could not be measured in UV-irradiated DNA-repaired cells because DNA repair did not occur in Ca-depleted cells.

Ca-ATPase activity measurements. Ca-ATPase was measured in plasma membrane proteins that had been prepared from lymphocytes, either immediately after their UV irradiation or after their UV irradiation and subsequent DNA repair process, or from nonirradiated lymphocytes. Washed lymphocytes were suspended in complete medium at a concentration of 4×10^6/ml. A 0.5 ml sample of the cell suspension was applied on each 10 cm diameter plate (as many plates as possible until all suspension was used) and kept cold with avoidance of day-

light. One quarter of the plates were UV irradiated at 25 joules/m^2, 8 ml of complete medium was added, and these plates were incubated for 150 minutes as described earlier, to allow DNA repair to occur. Nonirradiated plates served as blanks. Another quarter of the plates were UV irradiated, and processing continued at once, while nonirradiated cells were treated alike. The ice-cold cells were transferred to 15 ml tubes and washed twice with cold PBS. The plasma membrane protein was prepared from ice-cold homogenates[26] in hypotonic Tris and ethylenediaminetetraacetic acid buffer (0.1 mmol/L each, pH 7.4), followed by differential centrifugation in the cold. A 20 μl sample of the fraction was used for protein determination according to the method of Lowry et al.,[27] and the rest served as the plasma membrane protein in Ca-ATPase activity measurements, which were performed without delay. The enzyme activity was evaluated by its ability to hydrolyze ATP while producing Pi. The reaction mixture contained 20 mmol/L Tris, 30 mmol/L KCl, 4 mmol/L $CaCl_2$, 0.5 mmol/L EGTA, and 15 to 30 μg freshly prepared plasma membrane. The final volume was 0.5 ml and the pH was 8.0, which had been found to be the optimal pH after several preliminary experiments of changes in the Ca-ATPase activity as a function of pH. The reaction, which was run in triplicate, was started by the addition of 5 mmol/L pH-adjusted ATP-Na_2 into the ice-cold tubes; they were then kept in a shaking water bath at 37° C for 30 minutes. The reaction was stopped by ice cooling and the addition of 0.1 ml of 25% TCA. The amount of Pi in the supernatants was evaluated by the Fiske-Subbarow method,[28] and the Ca-ATPase activity was expressed as nanomoles of Pi per milligram of protein per 30 minutes, after the appropriate blanks were subtracted.

Statistical analysis. The statistical tests were chosen in accordance with the size of the samples, the distribution of the results, and their interdependence. Parametric tests—Student's *t* test, the paired *t* test, the *t* test of repeated measures, and one-way ANOVA—and parallel nonparametric tests were used, each when appropriate. In ANOVA, the Levene test for homogeneity and the Scheffe procedure for post hoc comparison were applied. The SPSS statistical package was used. A two-tailed $p < 0.05$ was considered significant.

RESULTS

Association of UV-induced DNA repairability with extracellular (Ca^{2+}). UV-induced DNA repairability of normal lymphocytes showed a strong dependence on [Ca^{2+}] in the incubation medium during the 150 minutes in which the repair of UV-damaged DNA was measured (Table I). The maximal DNA repairability was 347.8 ± 27.6 cpm/10^6 cells in a medium containing 1 mmol/L external Ca^{2+} concentration, while in a medium containing 2.6 mmol/L, the repair was slightly and insignificantly less (326.4 ± 26.4 cpm/10^6 cells) than the maximum, but it was strongly inhibited to 138.6 ± 18.4 cpm/10^6 cells when the external [Ca^{2+}] was further elevated to 5 mmol/L. When the [Ca^{2+}] in the medium was gradually reduced, the DNA repairability also significantly declined until it reached a minimum of 105.9 ± 9.4 cpm/10^6 cells in a medium with no external [Ca^{2+}] (in the presence of 0.1 mmol/L EGTA). In the presence of 2 mol/L Ca-ionophore A23187, the whole concentration curve was shifted to the left (Table I, Fig. 1), and the maximal DNA repairability was achieved at 50 μmol/L external [Ca^{2+}], while at 1 mmol/L there existed only a minor ability.

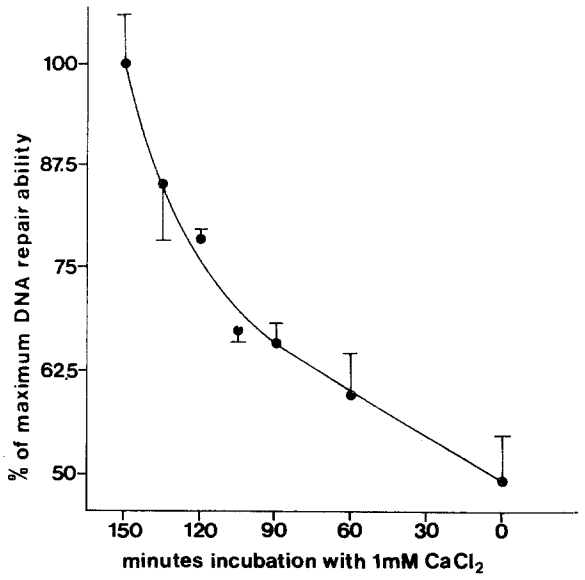

Fig. 2. The dependence of DNA repairability in lymphocytes on the time of the addition of 1 mmol/L $CaCl_2$ into the incubation medium. The $CaCl_2$ was added to the medium at different time points along the incubation period (maximal DNA repairability was referred to as 100% when $CaCl_2$ was added at the start). All the points are significantly ($p < 0.01$) different from each other with the exception of 90 vs 105 minutes.

In other experiments the DNA repair incubation started without external Ca^{2+}, and a $CaCl_2$ solution, to a final 1 mmol/L external [Ca^{2+}], was added at different time points along the 150-minute incubation period. The results (Fig. 2) show that the shorter the time $CaCl_2$ was present in the incubation medium, the lower the DNA repairability of the cells. The maximum of 100% ± 5.8% at 150 minutes of incubation with $CaCl_2$ declined significantly ($p < 0.01$) to 85.3% ± 6.8% at 135 minutes of incubation, was further reduced to 78.1% ± 1.2% at 120 minutes, 67.5% ± 1.2% at 105 minutes, 65% ± 2.2% at 90 minutes, 59.4% ± 5% at 60 minutes, and down to a minimum of 48.4% ± 5.1% when no $CaCl_2$ was added. The minimum ability of 48.4% is higher than the minimum of 30.4% (105.9 cpm) reported in Table I, presumably because of residual amounts of

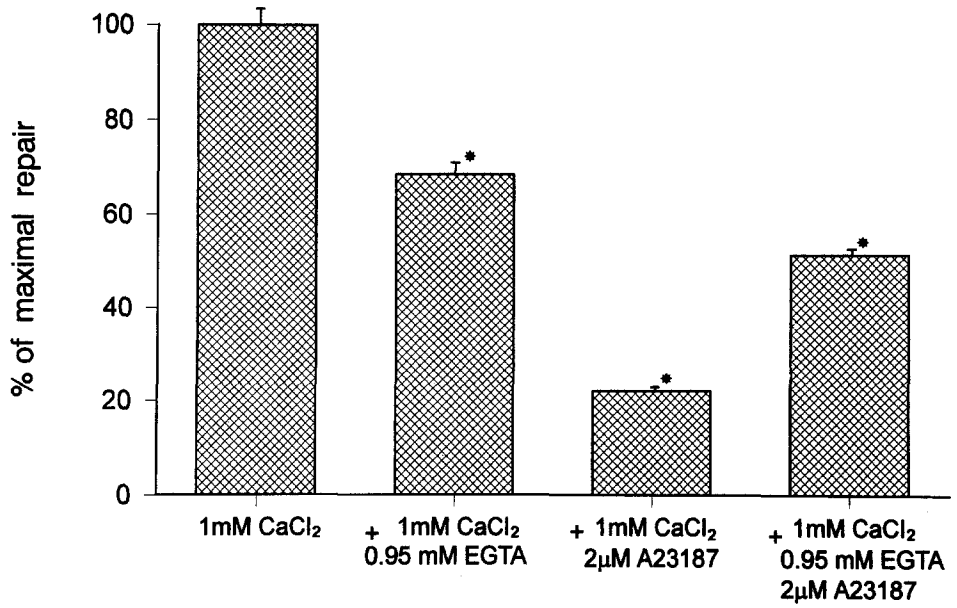

Fig. 3. DNA repairability in lymphocytes in incubation medium containing 1 mmol/L Ca^{2+}. The effect of 0.95 mmol/L EGTA, 2 μmol/L Ca-ionophore A23187, and a combination of the two (% of maximal DNA repairability, $*p < 0.01$).

Table II. Effects of UV irradiation, immediately after irradiation and after DNA repair, on three components of Ca homeostasis in lymphocytes

	Without UV	Immediately after UV	After UV and incubation
Cytosolic [Ca^{2+}] (nmol/L) (n = 22)	65.8 ± 3.0	76.5 ± 7.2*	98.0 ± 9.1*†
Ca^{2+} uptake (nmol/L/sec) (n = 15)	1.93 ± 0.8	2.27 ± 0.11*	—
Ca-ATPase activity (nmol Pi/mg protein/0.5 h) (n = 10)	199.6 ± 21.6	111.5 ± 15.0‡	166.3 ± 19.6†§

*$p < 0.02$ versus Without UV.
†$p < 0.02$ versus Immediately after UV.
‡$p < 0.005$ versus Without UV.
§$p < 0.05$ versus Without UV.

Ca^{2+}, because no EGTA was used in these experiments.

As mentioned, when UV-irradiated normal lymphocytes were incubated at 37° C in the presence of 1 mmol/L $CaCl_2$ for 150 minutes, maximal DNA repairability was demonstrated. When 0.95 mmol/L EGTA was added to the incubation in addition to the 1 mmol/L $CaCl_2$, the DNA repairability was significantly reduced, from 100% ± 2.7% to 68.7% ± 1.6% (Fig. 3), because the addition of EGTA caused partial Ca depletion of the cells. On the other hand, when Ca-ionophore was added to these cells instead of EGTA, the DNA repairability was reduced from 100% ± 2.7% to 22.2% ± 0.4% as a result of enhanced Ca^{2+} influx that resulted in a very high cytosolic [Ca^{2+}]. The addition of EGTA to the Ca-ionophore-containing 1 mmol/L $CaCl_2$ solution modulated the inhibition caused by the excess of cytosolic Ca^{2+} created by the Ca-ionophore, resulting in a DNA repairability of 51.6% ± 1.5% of the maximal value ($p < 0.01$ by nonparametric ANOVA).

The effect of genistein on DNA repairability. DNA repairability was strongly inhibited when 200 μmol/L genistein was added at different time points during the procedure. With only vehicle added, DNA repair was referred to as 100%. When genistein was added at the time the cell suspension was prepared, the DNA repairability significantly decreased to 14.6% ± 3.2%. When genistein was added immediately after UV irradiation, DNA repairability was reduced to 20.9% ± 3.8%, and when it was added together with the tritiated thymidine, the repairability was 11.7% ± 0.63%. The differences between these low levels were not significant. Dimethyl sulfoxide alone has no effect on DNA repairability

when applied in the same amounts as used in the genistein solutions.

A dose-response curve (Fig. 4) showed that at 25 μmol/L genistein, which was added immediately after UV irradiation, the DNA repair decreased to 93.4% ± 7.9% of the maximum ability; at 50 μmol/L genistein, DNA repair decreased to 49.6% ± 2%, and at 100 μmol/L, it exhibited only 15.5% ± 0.4% of the repairability without genistein.

Effect of UV irradiation on Ca homeostasis in lymphocytes. The previous results showed that UV-induced DNA repairability was Ca dependent. In the following section the effect of UV irradiation (254 nm) on Ca homeostasis in lymphocytes was investigated twice. First, it was investigated immediately after irradiation, which produced DNA damage, and second, it was investigated after irradiation followed by incubation at 37° C, during which DNA repair synthesis was taking place.

Effect of UV irradiation on cytosolic [Ca^{2+}]. The cytosolic [Ca^{2+}] in lymphocytes in regular 1 mmol/L $CaCl_2$ buffer without UV, immediately after UV irradiation, and after UV irradiation with successive UV-induced DNA repair process is exhibited in Table II. UV irradiation caused an immediate small but significant elevation in cytosolic [Ca^{2+}], from 65.8 ± 3.0 nmol/L to 76.5 ± 7.2 nmol/L, and it continued to rise during the DNA repair incubation, to 98.0 ± 9.1 nmol/L. In the presence of 100 μmol/L genistein, this elevation in cytosolic [Ca^{2+}] was abolished and was similar (105% ± 4%) to the [Ca^{2+}] level in nonirradiated cells.

Effect of UV irradiation on Ca^{2+} uptake. The cells for Ca^{2+} uptake evaluation had been Ca depleted by EGTA, which reduced their cytosolic [Ca^{2+}] level to 3.6 ± 1.1 nmol/L. The Ca^{2+} uptake significantly increased, from 1.93 ± 0.08 nmol/L/sec in nonirradiated cells to 2.27 ± 0.11 nmol/L/sec immediately after irradiation (Table II). Ca^{2+} uptake after UV irradiation followed by DNA repair incubation could not be measured, because Ca-depleted cells, in which no DNA repair synthesis takes place, were used for the Ca^{2+} uptake method.

Effect of UV irradiation on plasma membrane Ca-ATPase activity. As shown in Table II, immediately after UV irradiation, Ca-ATPase activity was inhibited significantly, from 199.6 ± 21.6 nmol Pi per milligram of protein per 0.5 hour down to 111.5 ± 15.0 nmol Pi per milligram of protein per 0.5 hour. The Ca-ATPase activity measured after the cells had been allowed to repair their UV damaged DNA improved significantly as compared with nonrepaired cells (elevation from 111.5 ± 15.0 nmol Pi per milligram of protein per 0.5 hour to 166.3 ± 19.6 nmol Pi per milligram of protein per 0.5 hour). Yet even after the improvement during incubation, Ca-ATPase activity did not reach the level of the nonirradiated lymphocytes, this activity remained significantly reduced.

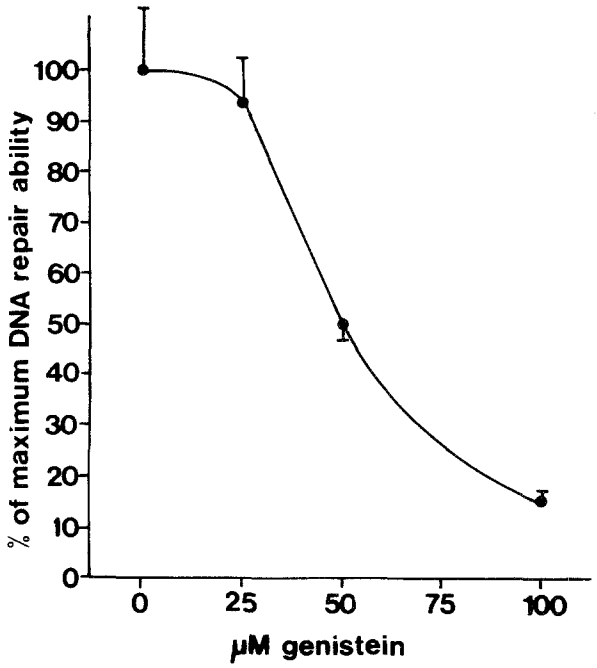

Fig. 4. Inhibition of DNA repairability by various concentrations of genistein in the incubation medium (maximal repair was referred to as 100% when vehicle only was added).

DISCUSSION

Several methods used in the present study suggest that UV-induced DNA repairability in human lymphocytes is in large part Ca dependent. DNA repair was at its peak in a medium containing 1 mmol/L [Ca^{2+}] (Table I), which approximates the free [Ca^{2+}] in the serum. At this external Ca^{2+} level, the cytosolic [Ca^{2+}] was 65.8 ± 3.0 nmol/L. The DNA repairability was reduced to 30% of its maximum in cells that were Ca depleted by extracellular EGTA and whose cytosolic [Ca^{2+}] dropped to 3.4 ± 1.1 nmol/L. The DNA repairability was also significantly reduced as a result of cytosolic Ca^{2+} excess brought about by either Ca-ionophore or by elevation of the cytosolic [Ca^{2+}] in the extracellular medium (Table I). The DNA repair synthesis was never completely abolished in the Ca-depleted cells in a medium without Ca^{2+}, presumably because of prolonged emptying of Ca^{2+} stores into the cytosol[29] or because of the existence of an alternative pathway. The DNA repair was activated by UV irradiation.

UV irradiation has a dual effect on living cells. It affects both the nucleus and surface molecules. Thus UV causes DNA damage and simultaneously causes Ca^{2+} signaling by inducing tyrosine kinase activity.[14,30,31] Because UV-induced DNA repairability was found to be Ca dependent, the direct and the prolonged effects of UV irradiation on cellular Ca^{2+} was further investigated. Thus, cellular [Ca^{2+}] was measured immediately after UV irradiation and after irradiation with successive incubation, which allowed the DNA repair synthesis to occur. UV irradiation was found to elevate cytosolic [Ca^{2+}] at these two time points. The UV irradiation led to the elevated cellular [Ca^{2+}], probably by stimulating Ca^{2+} uptake, with a concomitant inhibition of the Ca pump activity (Table II). Ca^{2+} uptake and Ca-ATPase together regulate most of the Ca^{2+} fluxes through the plasma membrane in lymphocytes,[32] while the existence of a Ca^{2+}/Na^{+} exchanger is claimed[33] but is still doubtful.[29] These two enzymatic systems may have been manipulated by reactive oxygen species produced by UV irradiation.[31]

After the incubation during which DNA repair occurred, the Ca-ATPase activity increased but was still significantly reduced as compared with that in nonirradated cells. At this point in time Ca^{2+} uptake could not be measured but was presumably stimulated both by the direct effect of UV on the plasma membrane and by the Ca^{2+} extrusion from internal stores into the cytosol.[34] This Ca-ATPase reduction, together with a possible induction of Ca^{2+} uptake, may be the reason for the elevated cytosolic [Ca^{2+}] found after the incubation ended. Because the incubation period of the UV-irradiated cells was artificially established to provide standard conditions for DNA repairability measurements, it is not certain that the DNA repair system returned to its unstimulated state after the end of this period. This standard incubation period might explain why the cytosolic [Ca^{2+}] was still elevated and the Ca-ATPase activity was still reduced and both had not yet returned to their baseline levels.

The immediate effect of the UV irradiation on cytosolic [Ca^{2+}] was small but significant. It seems that Ca^{2+} is the signal transducer of UV stimulation, and this small Ca^{2+} transient initiates stimulation of the DNA repair system at once, to avoid replication of genomal errors. The cellular [Ca^{2+}] elevation after UV irradiation is caused by induction of tyrosine kinase activation.[14,30,31] The association between the UV-induced cytosolic [Ca^{2+}] elevation and the UV-induced DNA repair synthesis, which was Ca^{2+}-dependent, was demonstrated by the inhibition of DNA repair synthesis with the tyrosine kinase inhibitor genistein. Because genistein inhibits UV-induced [Ca^{2+}] elevation by inhibiting UV-induced tyrosine phosphorylation, and because genistein also inhibits DNA repair synthesis, it is suggested that the Ca^{2+} signaling produced by UV irradiation is essential for DNA repair. Further support for this association between UV-induced Ca^{2+} signaling and DNA repair is suggested by the following observations in lymphocytes. UV irradiation induced both a much higher [Ca^{2+}] elevation[14] and a much higher DNA repair synthesis[5] as compared with gamma irradiation. The results also reveal that higher Ca^{2+} levels inhibit DNA repair, and thus the small elevation of [Ca^{2+}] observed after UV irradiation seems suitable for its purpose. The effect of UV irradiation is less pronounced than the effect of other mitogens such as phytohemagglutinin,[35] which more than doubles the cellular Ca^{2+} level. The UV-induced DNA repair synthesis is intended to repair excision and not to cause cell replication, whereas the effect of PHA and other mitogens on lymphocytes is not immediate but is long lasting, with massive replication of cells. In these cases the high levels of Ca^{2+} exert their effect through a different mechanism.

In conclusion, optimal operation of the UV-induced DNA repair system necessitates a precisely regulated cellular [Ca^{2+}]. This delicate regulation is dependent in large part on Ca^{2+} uptake and Ca-ATPase activity. UV irradiation causes genomal DNA damage but simultaneously activates Ca^{2+} uptake and decreases Ca-ATPase activity to an extent that leads to a cellular [Ca^{2+}] that signals the induction of the DNA repair system. Thus the UV irradiation provides a means to repair the damage that it causes.

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