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Suppression by Anticancer Agents of Reactive Oxygen Generation from Polymorphonuclear Leukocytes

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The influence of anticancer agents on signal transduction for reactive oxygen generation was examined in polymorphonuclear leukocytes (PMN). Inositol 1,4,5-trisphosphate and diacyl glycerol levels in formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated PMN were decreased by cis-diammine-dichloroplatinum (CDDP), 5-fluorouracil (5-FU), ^{137}Cs, and peplomycin (PLM, a bleomycin analog) in this order. Intracellular calcium ($[Ca^{2+}]_i$) level and protein kinase C (PKC) activity in the membrane after phorbol myristate acetate (PMA) stimulation were decreased by 5-FU and CDDP but not by ^{137}Cs and, in contrast, were increased by PLM. The level of $[Ca^{2+}]_i$ was decreased by 8 h treatment with 5-FU and CDDP. 5-FU and CDDP inhibited tyrosine phosphorylation of 83-kDa and 115-kDa proteins, however ^{137}Cs did not inhibit their phosphorylation and PLM enhanced the tyrosine phosphorylation. Short term (≤ 4 h) treatment with PLM, 5-FU and CDDP enhanced respiratory burst of PMN, whereas long term (8 h) treatment, as well as radiation, suppressed reactive oxygen generation from PMN in a dose dependent manner. Genistein suppressed chemiluminescence in 5-FU-, CDDP-, and ^{137}Cs-pretreated PMN to a greater extent than it did in PLM-pretreated PMN, however near suppression of chemiluminescence by staurosporine, 4-bromophenyl bromide and methionine was observed in PMN pretreated with these agents. In conclusion, these results indicate that long term treatment of PMN with 5-FU and CDDP inhibit respiratory burst, suppressing intracellular calcium mobilization, PKC translocation and tyrosine kinase activation, in adverse, short term treatment with PLM enhances PKC translocation and tyrosine kinase activation, but inhibits myeloperoxidase (MPO) activity, and radiation causes weak inhibition of signal transduction for respiratory burst.

Key words: polymorphonuclear leukocytes, anticancer agents, reactive oxygen generation, signal transduction

INTRODUCTION

In its present state, cancer therapy inevitably has various adverse effects, the suppression of bone marrow and leukocyte function being particularly hazardous, with leukocyte suppression occasionally resulting in life-threatening infection.[1,2] Indeed, lethal opportunistic infections frequently occur during cancer treatment.

Polymorphonuclear leukocytes (PMN) are excellent soldiers in the defense line against bacterial and fungal invasion. The bacteriocidal and fungicidal activities of PMN are closely associated with

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their reactive oxygen generation.[3–5] The generation of reactive oxygen (RO) species occurs via several pathways, associated with many enzymes and signal transducing messengers, from the cell membrane to the cytosol.[6–15] When formyl-methionyl-leucyl-phenylalanine (FMLP) binds its membrane surface receptors, guanine triphosphate (GTP)-binding protein (G-protein) moves to the receptor and stimulates phospholipase C which catalyses hydrolytic cleavage of phosphatidylinositol 4,5-diphosphate to inositol 1,4,5-trisphosphate (IP_3) and diacyl- glycerol (DG). These second messengers induce Ca^{2+} release and protein kinase C (PKC) activation, while phorbol myristate acetate (PMA) directly activates PKC and finally induces the activation of NADPH-oxidase. The importance of tyrosine phosphorylation of proteins in RO release from PMN has become clearer in recent years.[12–14]

Some anticancer drugs such as peplomycin (PLM; a derivative of bleomycin), 5-fluorouracil (5-FU), and cis-diamminedichloroplatinum (CDDP) possess immunopotentiating activity.[16–19] However, their leukocyte function-upregulating activities are exhibited only at low concentrations. However, the influence of anticancer drugs and radiation on leukocyte function has not yet been sufficiently explored.

With the aim of exploration of the influence of cancer therapy on leukocyte function, we investigated the in vitro influence of PLM, 5-FU, CDDP and ^{137}Cs on RO generation by PMN and on second messenger levels, PKC activity and protein tyrosine phosphorylation in these cells.

MATERIALS AND METHODS

Preparation of PMN

Heparinized peripheral blood was centrifuged at 400 g for 10 min. The buffy coat was collected and diluted in 3 vol of PBS and centrifuged on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) gradients according to Böyum's method.[20] The PMN pellets were resuspended in PBS, and contaminating erythrocytes were eliminated by adding PBS containing 3% (w/v) dextran for 30 min. The supernatant fraction was collected and centrifuged. Resuspending the pellet in hypotonic buffer, residual erythrocytes were removed. A PMN purity of more than 95% and cell viability of more than 98% were microscopically ascertained by Giemsa staining and trypan blue exclusion, respectively.

Chemiluminescence

Chemiluminescence of PMN was measured with a calcium analyzer (CAF-100; JASCO Ltd., Chemiluminescence mode, Tokyo, Japan). A PMN solution (5×10^5 cells/ml) containing 100 μM luminol was prewarmed for 1 min at 37°C, and 50 ng/ml PMA or 10^{-7} M FMLP was added. The chemiluminescence of the mixture was continuously measured at 37°C. Activity was expressed as the peak intensity (mV) of chemiluminescence.

Superoxide anion (O_2^-)

O_2^- was assayed spectrophotometrically by a cytochrome C (type VI, Sigma Chemical Co., St. Louis, MO) reduction method using a double-wavelength spectrophotometer (Shimadzu UV-300; Shimadzu Ltd., Kyoto, Japan) equipped with a thermostatted cuvette holder. PMN (1×10^6 cells/ml) suspended in Hanks' balanced salt solution (HBSS) and 100 μ M cytochrome C were preincubated at 37°C for 1 min and stimulated with 50 ng/ml PMA (Sigma) or 10^{-7} M FMLP (Sigma). The kinetics of cytochrome C reduction were measured by absorbance change at 540–550 nm. O_2^- concentration was calculated from the linear portion of the cytochrome C reduction curve.

Assay of IP_3, DG, and PKC

IP_3, DG, and PKC were assayed by using Amersham's commercial kits (Amersham, U.K.). The level and activity in each sample were

determined by standard curves, drawn using the standard samples in the kits.

Cytosolic Ca^{2+} level

PMN were loaded with 0.1 μ M Fura 2 AM (DOJINDO Laboratories, Kumamoto, Japan) for 30 min in medium containing 140 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 0.5 mM $CaCl_2$, 1 mM $NaHPO_4$, 5.5 mM glucose, and 20 mM HEPES, pH 7.4 (37°C), and the cells were washed twice. The cells were then resuspended in HBSS, and calcium level after stimulation with 10^{-7} M FMLP was measured with a CAF-100 Ca analyzer.

Protein tyrosine phosphorylation

PMN (2×10^6 cells/ml) suspended in HBSS were incubated with PLM, 5-FU, or CDDP at 37°C in the presence or absence of TNF-α (50 U/ml). The reaction was terminated by adding ice-cold 15% TCA solution containing 2 mM PMSF and 1 mM sodium vanadate. The precipitate was washed with ice-cold ether/ethanol (1/1), dissolved in SDS sample buffer, and subjected to SDS-PAGE. After electrophoresis (30 mA, 3 h), proteins were transferred to an Immobilon-P filter (Millipore Products Division, Bedford, MA) using the Sartorius semidry blotting apparatus. After 60-min incubation in a 5% suspension of powdered skim milk at room temperature, the filter was incubated with phosphotyrosine-specific monoclonal antibody (PY-20, ICN Biochemicals, Inc., Costa Mesa, Ca.) for 40 min. The monoconal anti-phosphotyrosine antibody was detected with peroxidase-conjugated rabbit anti-mouse IgG. Peroxidase-positive bands were detected using an ECL Western blotting detection system (Amersham). After staining with Coomassie brilliant blue, molecular weights of proteins were determined using Daiichi-Kagaku standards.

MPO activity

Intracellular MPO was assayed fluorometrically, with homovanillic acid (Sigma) as the fluorescence chromogen.[21] The fluorescence intensity was measured with a fluorimeter with excitation at 323 nm and emission at 426 nm. One unit of enzymatic activity was defined as the amount that degraded 1 μ mol peroxidase per min at 37°C.

RESULTS

PLM, 5-FU and CDDP upregulated chemiluminescence in a dose-dependent manner when PMN were treated with each drug for a short time (1 h), however with long term treatment (8 h) these agents suppressed chemiluminescence dose dependently (Figure 1). The peak intensity in control (untreated) PMN was about 140 mV 1 min after the addition of FMLP. PMN pretreated with 500 μg/ml 5-FU for 1 h released RO at a peak intensity of 180 mV, while the maximal peak intensity was decreased to about 22 mV by 8 h treatment with 500 μ g/ml of 5-FU. When PMN were treated with 10 and 100 μ g/ml of CDDP for 1 h, maximal peak intensity was 145 and 170 mV, respectively. By 8 h treatment with 10 and 100 μg/ml of CDDP, the peak intensity was decreased to 45 and 24 mV, respectively. The chemiluminescence in PMN was suppressed by ^{137}Cs in a dose dependent manner, the suppressive activity being less than that observed for the 8 h treatment with 5-FU. The maximal peak intensity was 105 and 97 mV, respectively, at 20 and 30 Gy.

The influence of duration of PMN treatment on their chemiluminescence was examined (Figure 2). For up to 2 h of treatment, all agents increased the peak intensity. After that time, the upregulated chemiluminescence was decreased and peak intensity became lower than the control level when PMN were treated with each agent for 6 h.

The generation of O_2^- was decreased by 5-FU and CDDP (Figure 3). PMN treated with 500 μg/ml of 5-FU for 1 h and stimulated with FMLP or PMA generated 80 to 90 pmol/10^4 cells/min of O_2^-, while control PMN generated about 100 to 110 pmol. When PMA was used as

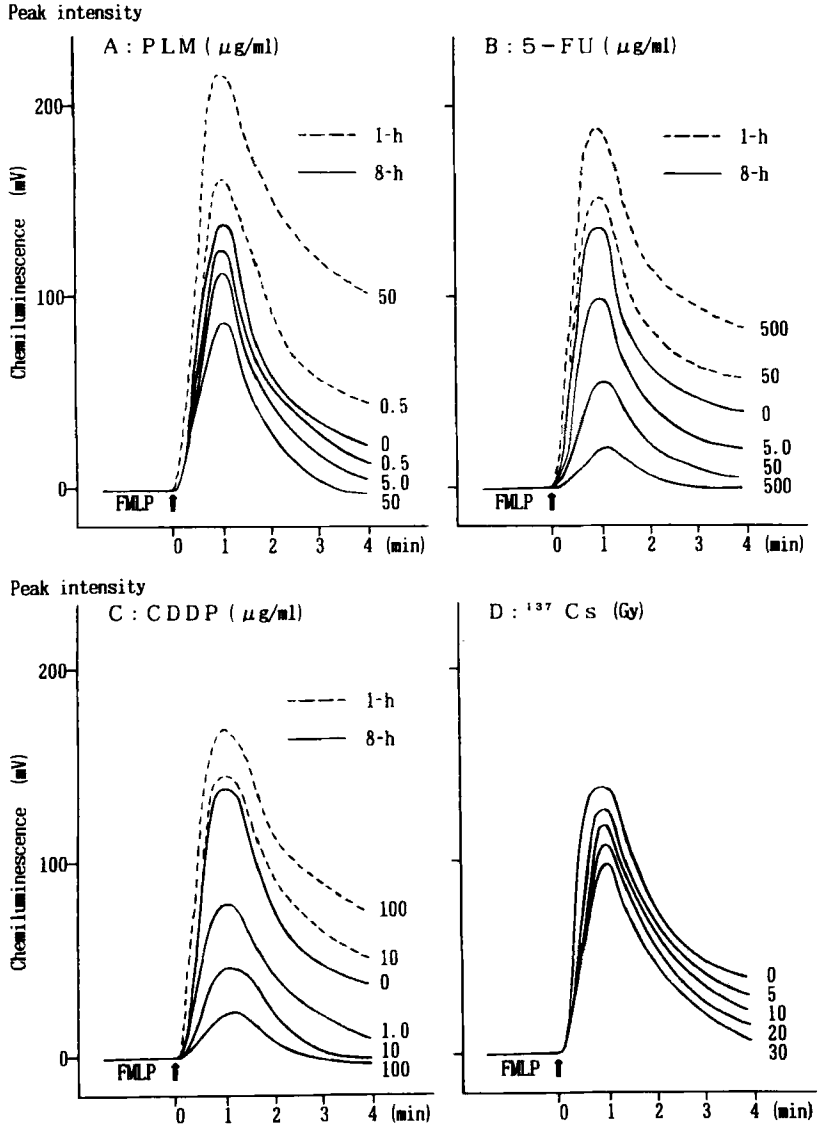

FIGURE 1 Up- and down-regulation of chemiluminescence by short (1 h) and long term (8 h) treatment of PMN with anticancer agents. PMN from 4 healthy donors were irradiated with ^{137}Cs (5–30 Gy) or treated with the indicated doses of PLM (0–50 μ g/ml), 5-FU (0–500 μ g/ml), or CDDP (0–100 μ g/ml) for 1 or 8 h. After medium change, FMLP (10^{-7} M) was added and chemiluminescence was measured. Each value represents the average peak intensity of three independent experiments.

the inducer, any decrease of O_2^- generation by CDDP was not observed. However, FMLP-induced O_2^- generation was strongly decreased, even on 1 h treatment with CDDP. PLM and ^{137}Cs scarcely inhibited O_2^- generation, and 1 h treatment of PMN with PLM slightly enhanced the release of the anion.

Staurosporine (a kinase inhibitor, 50 nM), 4-bromo-phenyl bromide (4-BPB, an inhibitor of phospholipase C, 50 nM), and methionine (an inhibitor of MPO, 500 nM) inhibited FMLP-induced chemiluminescence to 61.1%, 69.0% and 70.9%, respectively, of the peak intensity in untreated PMN (Figure 4). The suppression of

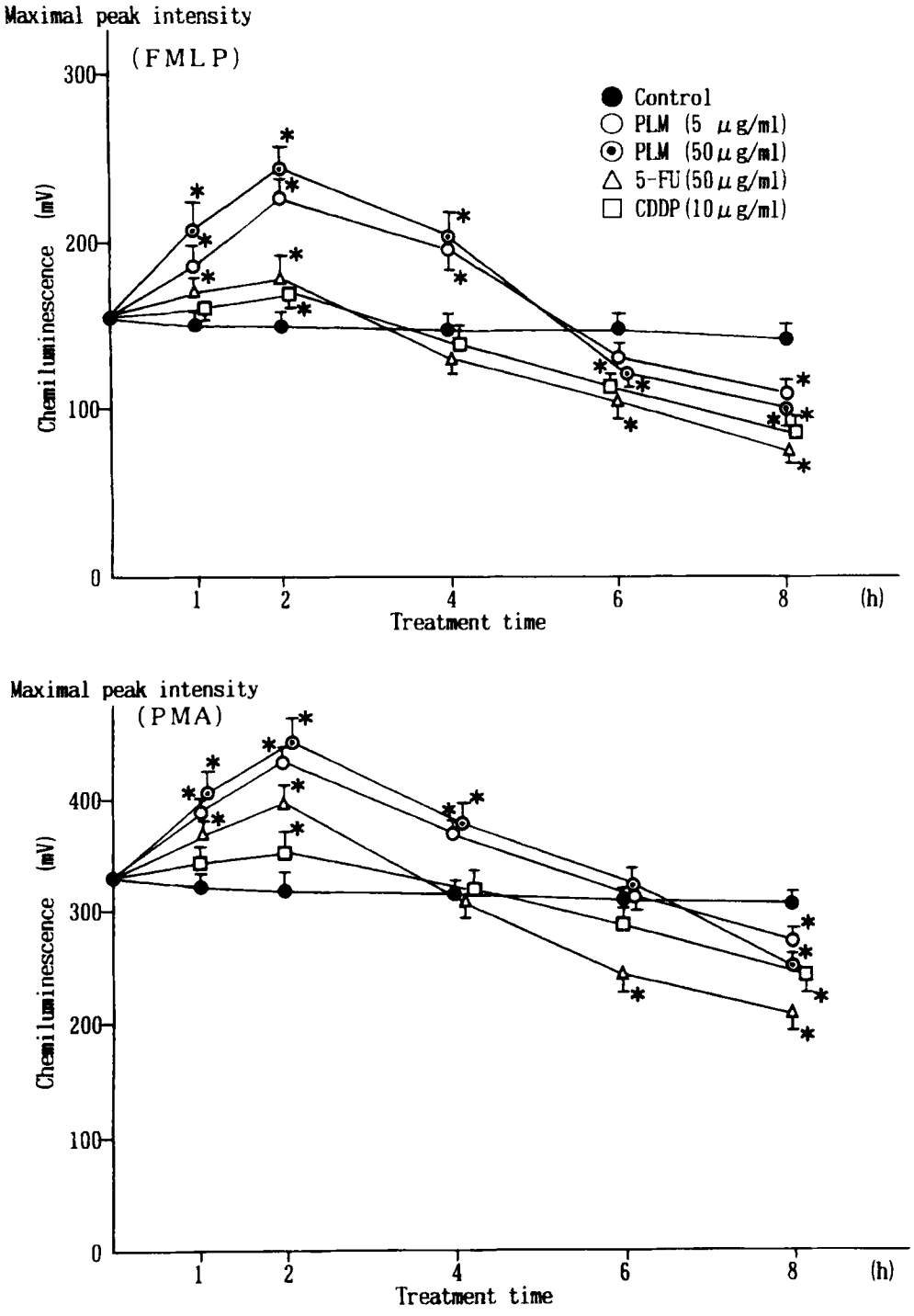

FIGURE 2 Influence of anticancer agents on chemiluminescence in PMN. PMN from 4 healthy donors were treated with each anticancer drug for the indicated times. After being washed, the PMN were stimulated with 10^{-7} M FMLP or 50 ng/ml PMA. Each bar indicates standard deviation (SD) of triplicate experiments. *: $P<0.01$ (vs control, by t-test).

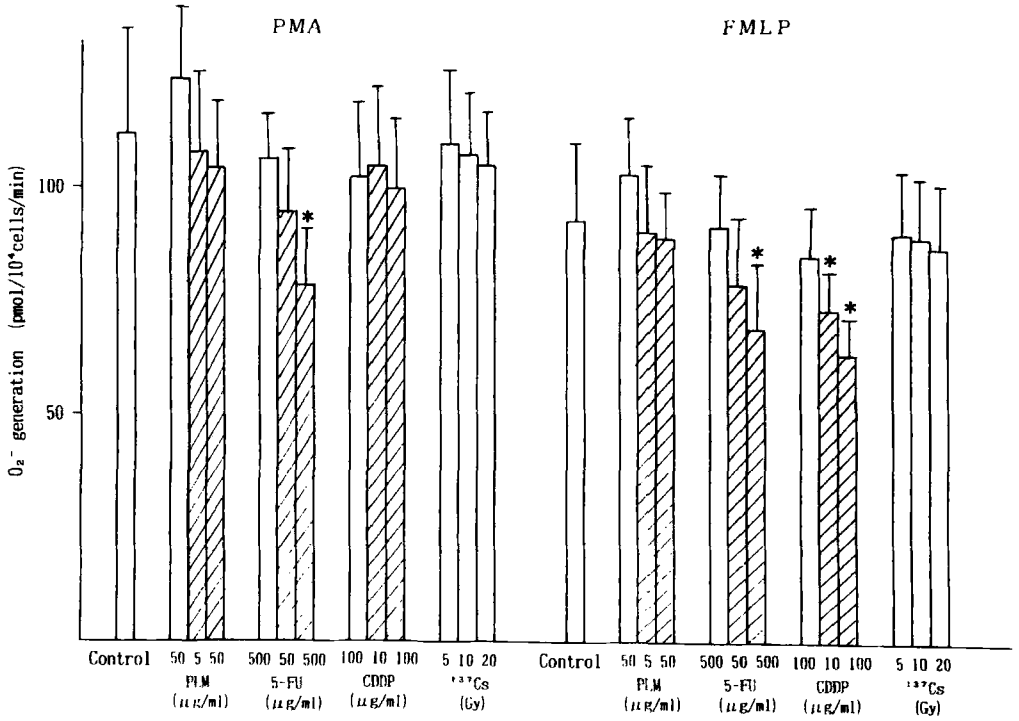

FIGURE 3 Influence of PLM, 5-FU, CDDP and ^{137}Cs on O_2^- generation from PMN. PMN from 4 healthy donors were irradiated with ^{137}Cs or treated with the indicated doses of PLM, 5-FU or CDDP for 1 h (□) or 8 h (▨). After washing and the addition of 50 ng/ml PMA or 10^{-7} M FMLP, O_2^- generated was measured. Each column and bar indicate the average pmol/10^4 cells/minute ± SD of three independent experiments. *: P<0.05 (vs control, by t-test).

chemiluminescence by these inhibitors in PMN pretreated with each anticancer agent was similar to that seen in control PMN. However, chemiluminescence in PLM (5 μ g/ml)-pretreated PMN was suppressed by staurosporin to a greater extent than that in PMN pretreated with any other agents. PMA- and FMLP-induced chemiluminescence were equally inhibited by the above inhibitors. Genistein (an inhibitor of tyrosine phosphorylation, 5 μ M) suppressed FMLP-induced chemiluminescence to near half the control level in both untreated and 5-FU-, CDDP-, and ^{137}Cs-pretreated PMN. However, the suppression of chemiluminescence by genistein was slight in PLM-pretreated PMN, the peak intensity level being 83.0% of the control. Different suppression of PMA-induced chemiluminescence by genistein was also observed in PMN pretreated with PLM and with other drugs. The PMA-induced chemiluminescence in untreated PMN and PMN pretreated with 5-FU, CDDP or ^{137}Cs was suppressed by genistein to a level less than 20% of the control (without genistein), while in PMN pretreated with PLM, the chemiluminescence was suppressed to only 73.7% of the control level. The influence of high dose (50 μ g/ml) PLM in the chemiluminescence was similar with that of low dose (5 μ g/ml) PLM.

Intracellular MPO activity was dose-dependently suppressed by CDDP and PLM. However, the activity of the enzyme was scarcely suppressed by ^{137}Cs and 5-FU (Figure 5). In control PMN cultured for 1 h without any agent, MPO activity was about 0.76 U/10^7 cells, and the MPO activity was decreased to 0.68, 0.66 and 0.61 U/10^7 cells on 1 h treatment with 50 μ g/ml of PLM, and with 10 μ g/ml and 100 μ g/ml of CDDP, respectively. After that time, linear decreases of MPO

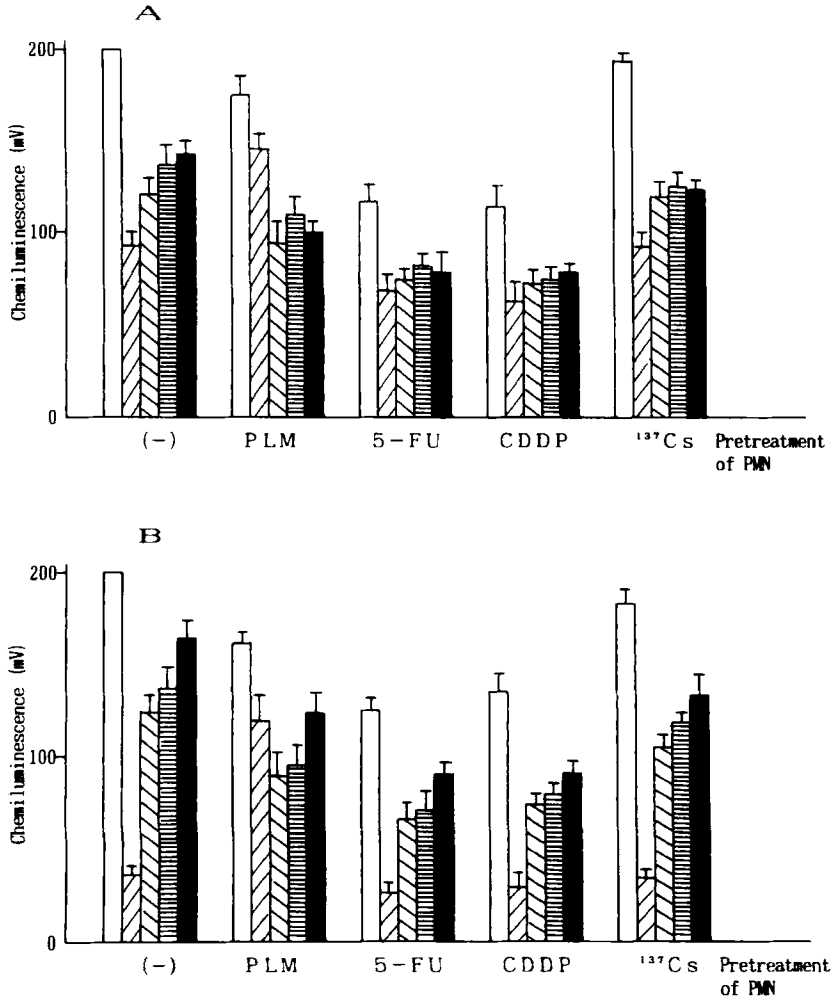

FIGURE 4 Characterization of reactive oxygens released from PMN pretreated with PLM, 5-FU, CDDP or ^{137}Cs. PMN from 4 healthy donors were irradiated with ^{137}Cs (20 Gy) or treated with PLM (5 μ g/ml), 5-FU (50 μ g/ml) or CDDP (10 μ g/ml) for 8 h. The PMN were then cultured for 5 min in the presence of each inhibitor indicated, and FMLP (A)- and PMA (B)- induced chemiluminescence was measured. Each column and bar represent the average peak intensity ± SD of three independent experiments. □: medium, ▨: genistein (5 μ M), ▧: staurosporine (50 nM), ▤: 4-BPB (50 nM),: ■ methionine (500 nM).

activity were observed with steep slopes in PLM and CDDP treatment.

The IP_3 level in PMN was increased within 15 sec after stimulation with FMLP, the level being about 13 pmol/6 × 10^6 cells in untreated PMN (Figure 6). On 1 h and 8 h treatment with PLM (5 μg/ml), 5-FU (50 μ g/ml), and CDDP (10 μg/ml), as well as on irradiation with ^{137}Cs (10 Gy), the IP_3 level was decreased, however, this decrease was not significant statistically. Of these agents, CDDP induced the greatest suppression, and 5-FU, ^{137}Cs and PLM followed in this order.

The decrease of DG was most prominent in PMN treated with CDDP followed by PMN treated with 5-FU (Figure 7). Fifteen sec after FMLP stimulation, DG in untreated PMN was increased to about 80 pmol/6 × 10^6 cells, and decreased to near the original level at 30 sec. From 30 to 60 sec after stimulation with FMLP, DG level increased rapidly, and was then maintained at a

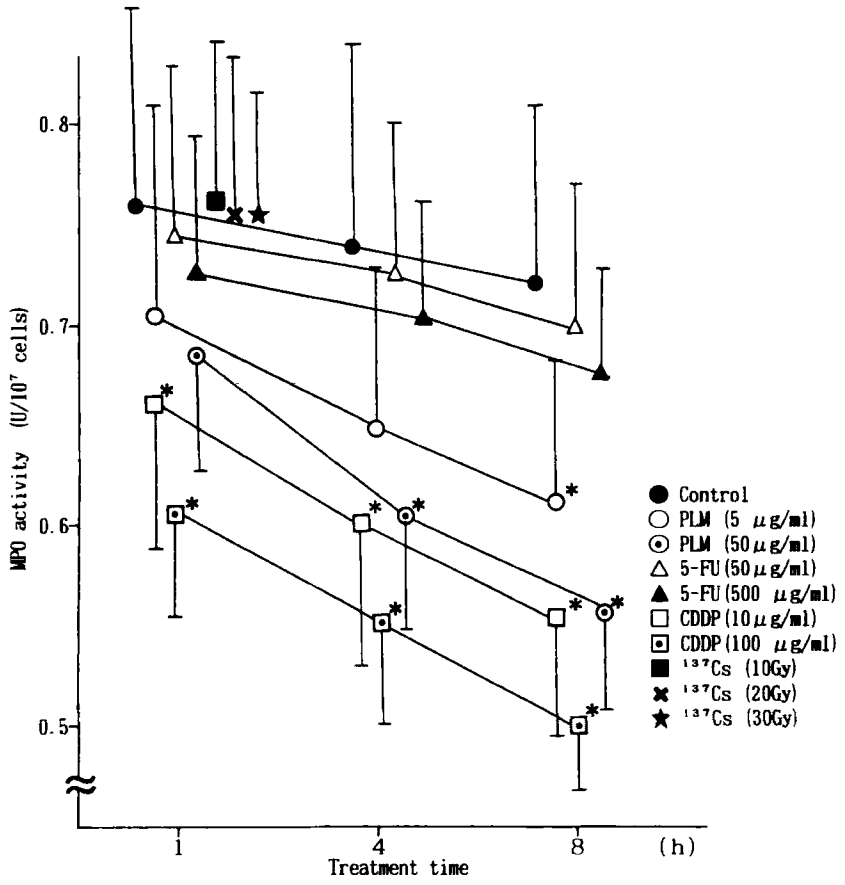

FIGURE 5 Intracellular myeloperoxidase activity in PMN treated with PLM, 5-FU, CDDP and ^{137}Cs. PMN from 3 healthy donors were treated with the indicated doses of ^{137}Cs, PLM, 5-FU or CDDP for 1, 4 or 8 h, and myeloperoxidase activity was assayed by the method described in Materials and Methods. Each bar indicates SD of triplicate experiments. *: $P<0.05$ (U-test).

high level. The kinetics of DG in PMN treated with ^{137}Cs and PLM (5 μ g/ml) for 1 h were similar to those in untreated PMN, although the levels were slightly lower than the control level. In PMN treated with CDDP (10 μ g/ml), 5-FU (50 μ g/ml) and with long term PLM, DG levels were lower than the control level.

When PMN were not stimulated with FMLP, intracellular ionized carcium ($[Ca^{2+}]_i$) level did not change 1 h after cell treatment with PLM, however a slight increase and decrease of $[Ca^{2+}]_i$ in PMN pretreated with 50 μ g/ml PLM and 100 μ g/ml CDDP, respectively, for 1 h was detected by the addition of FMLP (Figure 8). Eight hour treatment with 5-FU and CDDP caused statistically significant decreases of $[Ca^{2+}]_i$ even without stimulation with FMLP, however PLM caused only minimal decreases of $[Ca^{2+}]_i$ in both the presence and absence of FMLP.

In control PMN, PKC activity in the membrane fraction was increased by PMA from 122 pmol/10^7 cells to 256 pmol/10^7 cells 1 min after the addition of PMA (Figure 9). Correspondingly, the activity in the cytosol fraction decreased (data not shown). The PKC shift was enhanced by the pre-treatment of PMN with PLM for 1 h, in which cells PKC activity 1 min after PMA stimulation was higher (294 pmol/10^7 cells) than the activity in control

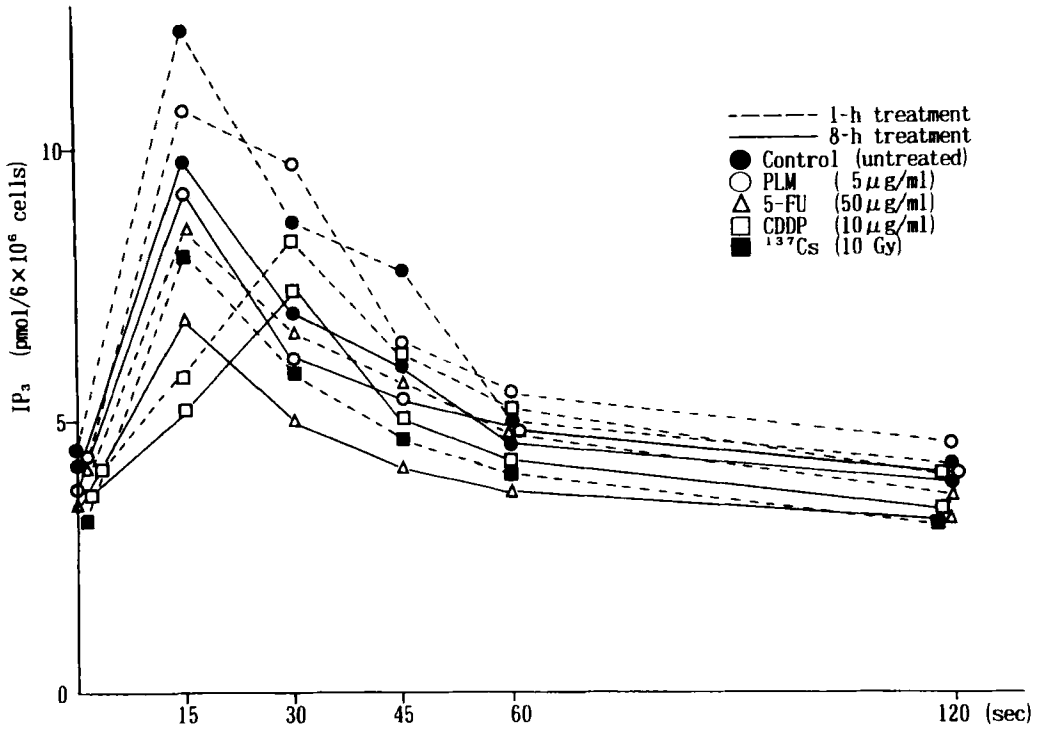

FIGURE 6 Influence of PLM, 5-FU, CDDP and ^{137}Cs on IP_3 level in PMN. PMN (6×10^6 cells) from 3 healthy donors were irradiated with ^{137}Cs or treated with PLM, 5-FU or CDDP for 1 h (- - -) or 8 h (——). Being washed, the PMN were resuspended in HBSS containing 1 mM $CaCl_2$ and stimulated with 10^{-7} M FMLP at 37°C, and IP_3 was measured by the method described in Materials and Methods. In the control, PMN were preincubated for 1 or 8 h without any agents. Each point represents the mean pmol of 4 independent experiments.

PMN. In contrast, 5-FU and CDDP decreased PKC activity in the membrane (192 pmol/10^7 cells and 208 pmol/10^7 cells, respectively, 1 min after PMA stimulation) although not significantly, when PMN were treated with each drug for 8 h. Low dose (10 Gy) irradiation did not suppress PKC activity, while high dose (30 Gy) irradiation had a slight, but not significant suppressive effect, the peak PKC activity being 220 pmol/10^7 cells.

The tyrosine phosphorylation of a 115-kDa protein was upregulated by PLM, but downregulated by 5-FU (Figure 10A). However, 5-FU enhanced the tyrosine phosphorylation of 60-kDa, 55-kDa and 43-kDa proteins. CDDP and ^{137}Cs suppressed the tyrosine phosphorylation of these proteins, however ^{137}Cs did not suppress the phosphorylation of the 115-kDa protein. The tyrosine phosphorylation of these proteins, particularly the 115-kDa protein in PLM-pretreated PMN, was suppressed to a greater extent by genistein than the phosphorylation in untreated PMN (Figure 10B).

DISCUSSION

Both PMN and macrophages play an important role in the host defense against microbial invasion. They possess various functions essential for killing of microorganisms. The bacteriocidal and fungicidal activity of these phagocytic cells is closely correlated with their production of RO in particular.[3–5, 22–24]

Cancer therapy-induced immunosuppression is very serious and a few studies dealing with RO generation by PMN have been performed in cancer patients.[25,26] We have previously reported that peripheral and salivary PMN from oral cancer

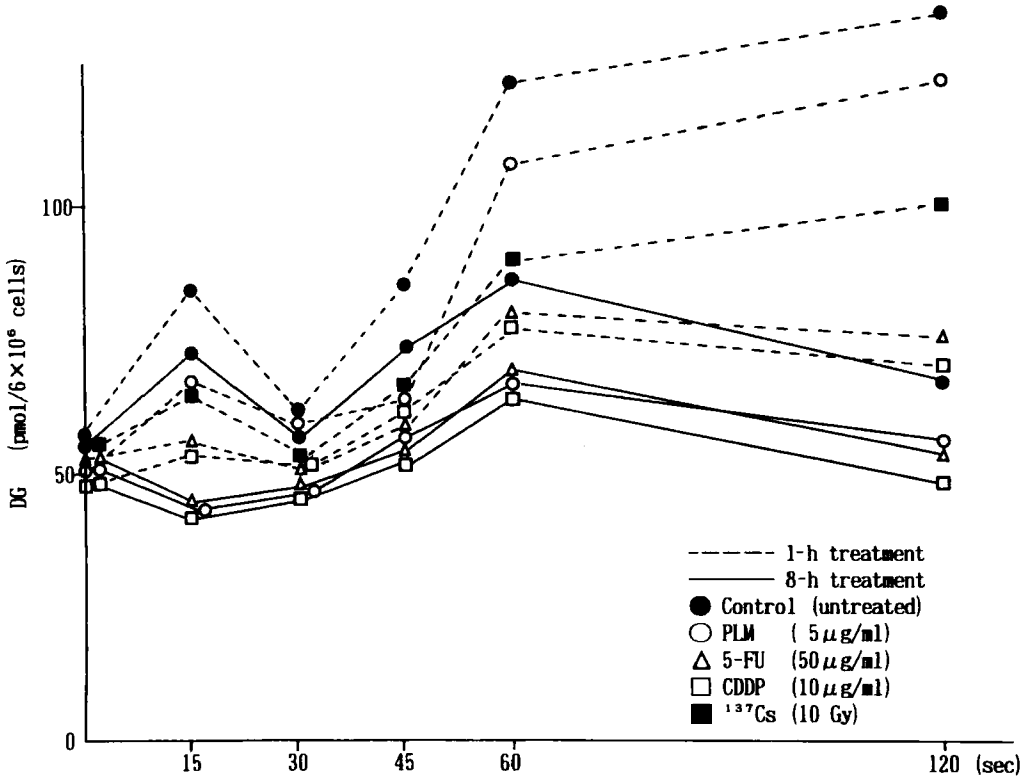

FIGURE 7 Influence of PLM, 5-FU, CDDP and ^{137}Cs on DG level in PMN. Six × 10^6 PMN were treated in the same manner as described in Figure 6. Being washed, the PMN were resuspended in HBSS containing 1 mM $CaCl_2$ and stimulated with 10^{-7} M FMLP at 37°C, and DG level was measured by the method described in Materials and Methods. Each point represents the mean pmol of 4 independent experiments.

patients generated lower levels of RO than PMN from healthy individuals, and that the RO generation by PMN was further suppressed by cancer treatment being correlated with the candidacidal activity of these cells.[25] However, the critical mechanism involved in this suppression of RO generation has not yet been explored.

We found here that PLM, 5-FU, and CDDP upregulated RO generation by PMN in a dose-dependent manner when treatment was short term (≤ 4 h). Generally, PMN generate RO in the presence of stimulants such as antigens, up-regulatory cytokines and chemicals,[27-37] and they are stimulated to release O_2^- even by hypotonic shock.[38] The electric charge in the membrane may be changed by the binding of molecules of the agents examined in the present study and signals essential for RO generation thus appear to be transduced. However, there is an influx of these agents into the cells and the cell metabolism is eventually inhibited, leading to the suppression of RO generation.

PLM exhibited the strongest upregulation of RO generation on 1 h treatment of PMN, and its RO generation suppressive activity on 8 h treatment was the weakest of the agents examined. However both IP_3 and DG levels in FMLP-stimulated PMN were slightly decreased by PLM, PKC activity in the membrane fraction was not decreased by PLM, and $[Ca^{2+}]_i$ level in PLM-treated PMN was rather higher than the level in untreated PMN. In addition, PLM upregulated protein tyrosine phosphorylation. These results indicate that short term treatment with PLM stimulates certain signals essential for RO generation, however such treatment slightly suppresses signal transduction from

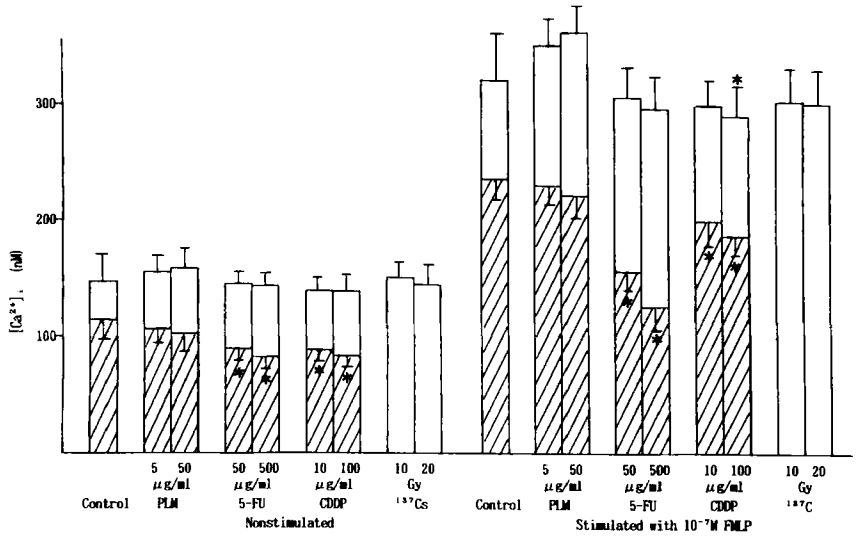

FIGURE 8 Influence of PLM, 5-FU, CDDP and ^{137}Cs on $[Ca^{2+}]_i$ level in PMN. PMN were treated in the same manner as described in Figure 6. The assay was triplicated, and each bar shows mean ± standard deviation of the triplicate experiments on PMN from 3 healthy donors. □: 1 h treatment, ▨: 8 h treatments. *: $P<0.05$ (U-test).

phosphorylated inositols. On the other hand, ^{137}Cs seems to suppress all signal transduction for RO generation, since both the second messengers, PKC activity and $[Ca^{2+}]_i$ were decreased by 10 Gy irradiation. Compared to ^{137}Cs, both 5-FU and CDDP more suppressed IP_3 and DG levels, and also $[Ca^{2+}]_i$ level and PKC activity which were associated with largely suppressed chemiluminescence. Staurosporine and 4-BPB inhibited FMLP-induced chemiluminescence to nearly 50%. Together with the decrease of second messenger levels produced by 5-FU and CDDP, suppression of the RO generation by these drugs seems to depend on the inhibition of signal transduction via phospholipase C.

In both FMLP- and PMA-induced chemiluminescence, respiratory burst was decreased by genistein to about 50% and 20%, respectively, of the control (without genistein) level in both untreated PMN and PMN pretreated with each agent, except for PLM-pretreated PMN, in which FMLP- and PMA-induced chemiluminescence was decreased by genistein to about 85% and 80%, respectively, of the control level. These values appear to be contradictive to the result in the western blots where protein tyrosine phosphorylation was enhanced by PLM but suppressed by other agents. However, the influence of genistein can be reasonably understood when 5-FU, CDDP and ^{137}Cs suppress not only tyrosine phosphorylation but other pathways and PLM upregulated the signals, in addition to tyrosine phosphorylation, for the respiratory burst. If so, the suppressive effect of genistein in chemiluminescence becomes apparently slight in PLM-pretreated PMN and severe in other agent-pretreated PMN. This hypothesis is supported by the results presented in this study and others reported.

Tyrosine phosphorylation in the 115 kDa protein was enhanced by TNF-α and, furthermore, was upregulated by the addition of both TNF-α and PLM, but was decreased by 5-FU and CDDP. It has recently been reported that protein tyrosine phosphorylation is essential for the activation of many kinds of cells, and that tyrosine

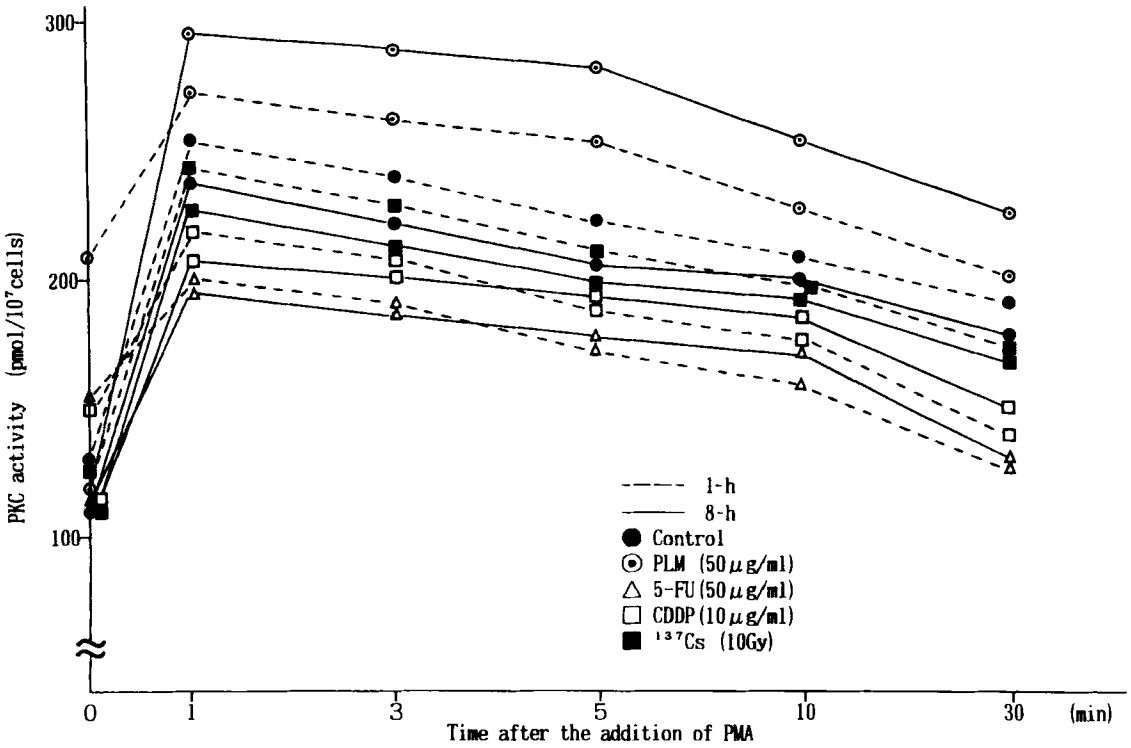

FIGURE 9 PKC activity in PMN treated with PLM, 5-FU, CDDP and ^{137}Cs. PMN were treated in the same manner as described in Figure 6. Being washed, cell membrane was separated and PKC activity in the membrane fraction was measured 0, 1, 3, 5, 10 and 30 min after the addition of PMA (50 ng/ml).

phosphorylation of the 115-kDa protein is a critical pathway for O_2^- generation in PMN.[12] In the signal transduction via tyrosine phosphorylation, there are two pathways, one that is $p21^{ras}$-dependent and one that is -independent.[39,40] In the latter, the JAK-STAT kinase system has been shown to be the critical pathway for cytokine signals.[41–45] In PMN, the activation of JAK family proteins is suspected, although the induction of JAK2 and JAK3 has not been demonstrated (data not shown). The association of JAK-STAT kinases in the signal transduction of respiratory burst in PMN must be investigated to gain further understanding of RO generation.

MPO plays an important role in the generation and scavenging of O_2^- and H_2O_2, and the activity of this enzyme is correlated with the bacteriocidal activity of macrophages and PMN. The examination using an inhibitor of MPO, methionine, revealed that suppression of the RO generation by long term PLM treatment depended on suppression of MPO and that the suppression of chemiluminescence by CDDP was due, to some extent, to the inactivation of MPO. As well as exerting this suppression, CDDP strongly suppressed PKC activity and protein tyrosine phosphorylation. It can, therefore, be concluded that CDDP is dangerous in terms of allowing microbial infection.

The present study has elucidated the nature of the impairment of PMN by anticancer agents, and the results obtained should be helpful in the prevention of opportunistic infections during cancer therapy. However, further in depth investigations are needed for the benefits of these findings to be applied clinically.

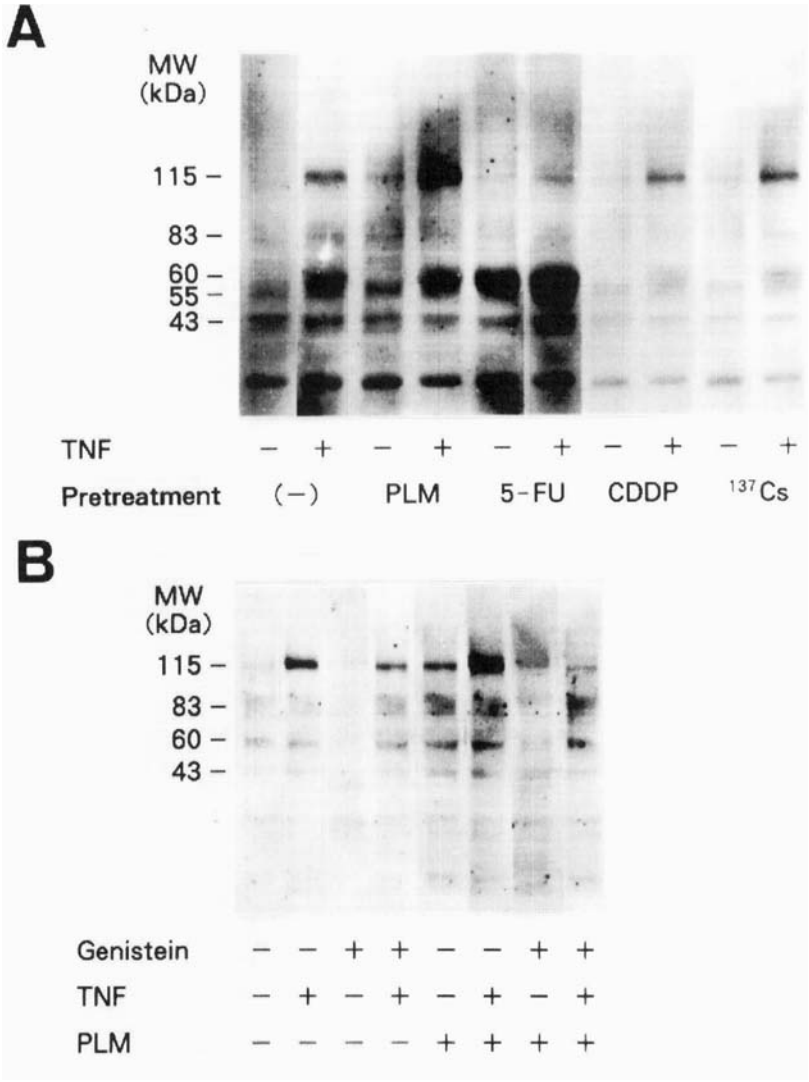

FIGURE 10 Protein tyrosine phosphorylation in PMN treated with PLM, 5-FU, CDDP or ^{137}Cs. (A). PMN (10^7 cells) were irradiated with ^{137}Cs (20 Gy) or treated with PLM (50 μ g/ml), 5-FU (50 μ g/ml) or CDDP (10 μ g/ml) for 8 h, and cultured for 30 min in the presence or absence of 50 U/ml TNF-α. (B). Some samples of PLM-pretreated cells were treated with 5 μ M genistein for 1 h. Total proteins were then extracted from the cells, and Western blotting was performed, using anti-phosphotyrosine antibody.

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