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# Production of Reactive Oxygen Species and Nitric Oxide by Anticancer Agents in Rat Polymorphonuclear Leukocytes

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**Abstract :** The production of reactive oxygen species (ROS) and nitric oxide (NO) by anticancer agents in rat polymorphonuclear leukocytes (PMN) was examined. PMN treated for short term (< or = 4 h) with cyclophosphamide, cisplatin, tamoxifen and doxifluridine, respectively, exhibited an enhanced respiratory burst upon formylmethionyl-leucy1-phenylalanine (FMLP) stimulation. In the long term (> 4 h), the production of ROS was suppressed in a concentration-dependent manner. The production of superoxide anion ($O_2^-$) from the FMLP-stimulated PMN was enhanced by the treatment (for 1 hr) of cyclophosphamide, cisplatin, tamoxifen and doxifluridine, respectively. While 1 hr-treatment with cyclophosphamide, cisplatin, tamoxifen, and doxifluridine, respectively, suppressed the production of NO from the FMLP-stimulated PMN, while 8 hr-treatment enhanced the production of NO. Neomycin suppressed chemiluminescence in cisplatin-, tamoxifen- and doxifluridine-pretreated PMN, however near suppression of chemiluminescence by ethanol and genistein was observed in PMN pretreated with these agents. Staurosporine and bisindolylmaleimide suppressed chemiluminescence in cisplatin- and doxifluridine- pretreated PMN. Wortmannin has shown a slight suppression in cyclophosphamide-, cisplatin- and tamoxifen-pretreated PMN, but a strong suppression in doxifluridine-pretreated PMN. Methionine strongly suppressed in cyclophosphamide and cisplatin-pretreated PMN. In conclusion, these results indicate that long term treatment of PMN with cisplatin and doxifluridine inhibit respiratory burst through protein kinase C (PKC) translocation, phospholipase C (PLC), D (PLD) and tyrosine phosphorylation kinase (TPK) activation. Tamoxifen inhibits respiratory burst through PLC, PLD, TPK. Cyclophosphamide inhibits respiratory burst through myeloperoxidase (MPO) activity.

***Key words* :** reactive oxygen species, nitric oxide, polymorphonuclear leukocytes, anticancer agents

## Introduction

Polymorphonuclear neutrophils (PMNs) serve an important role in host defense by ingestion (phagocytosis) and destruction of infected microbial organisms by producing reactive oxygen species (ROS) (26). This process is a part of normal host defense mechanisms against infectious diseases. The generation of ROS in the respiratory burst is mediated by the multicomponent enzyme NADPH oxidase (5). The primary product of this oxidase system is one-electron-reduced oxygen, i.e., superoxide anion ($O_2^-$). Much of the $O_2^-$ formed is dismutated by superoxide dismutase (SOD) to produce oxygen and hydrogen peroxide ($H_2O_2$), which is converted by myeloperoxidase (MPO) to hypochlorous acid and chloramines inside the phagosome (14). MPO catalyzes the oxidation of Cl- by $H_2O_2$ to yield hypochlorous acid (HOCl) (41). As HOCl is a powerful oxidizing agent as well as being highly reactive, so it is regarded as obstructing the breathing of bacillus coming into the cell to act as being sterilizer. It is also well known that these ROS ($O_2$, $H_2O_2$, HOCl etc.) are reactive to kill microorganisms and subsequently prevent from being inflammatory when it occurs, but as causing a damage to DNA and other large elements (17,20,45). In this regard, ROS have bactericidal, tumorcidal and anti-inflammatory functions.

Recently, researches revealed that respiratory burst of PMN by stimulants have relations with the activation of protein kinase C (PKC), inositol phosphate and intracellular [$Ca_2^+$] (1,11,47), and that PMN when activated by chemoattractant, N-formyl-methionyl-leucyl-phenylalanine (FMLP), simultaneously released nitric oxide (NO), $O_2^-$ and $H_2O_2$ (10). Chemoattractant receptors belong to a large class of receptors which utilize the hydrolysis of polyphosphoinositides to initiate $Ca_2^+$ mobilization and cellular activation. The receptor occupancy leads to phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) yielding inositol 1,4,5-trisphosphate (IP3) and 1,2 sn-diacylglycerol (DAG) (15). These second messengers have been revealed as inducing the emission of $Ca_2^+$ as well as the activation of

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protein kinase C (PKC). The synthesis of NO from L-Arginine (L-Arg) is an ubiquitous pathway catalysed by the enzyme nitric oxide synthase (NOS) (30,39). The role of NO in the inflammatory response associated with several pathological conditions is well recognized (35). Neutrophils, which represent 50–60 % of the total circulating leukocytes, could substantially contribute to the amount of NO in the circulation. Inflammatory conditions such as glomerulonephritis (9), sepsis (36), endotoxemia (40), hepatitis (6), and pulmonary inflammation (32) are associated with the upregulation of iNOS (Inducible Nitric Oxide Synthase) and an increased level of NO in plasma and/or urine. The role of both NO and polymorphonuclear leukocytes (PMNs) in septic endotoxin shock has been demonstrated (10,38). It has been recognized by some researchers that neutrophil-derived NO might play a role during the initial stages of endotoxin shock (33,38). NO seems to have a biphasic effect (potentiating at a low concentration and inhibitory at a high concentration) on PMNs functions. Although much importance has been focused on NO, which is generated by endothelium, platelets, or other cells during interaction with neutrophils, no mention has been made of the importance of NO generated by PMNs in modulating their functions .

As a combination therapy of anticancer agents has accompanied with side effects such as bone marrow depression, many studies have focused on the effect of anticancer agents in leukocyte (43). The combination of cisplatin [cis-diamminedichloridoplatinum(II)] and 5-fluorouracil (5-FU) has been widely used for combination chemotherapy in patients with squamous cell carcinoma of the head and neck (21,37). Cisplatin belongs to a group of medicines known as alkylating agents and can suppress bone marrow (23). Doxifluridine (5'-deoxy-5-fluorouridine), a prodrug of 5-FU, is an anticancer agent which is converted to 5-FU by pyrimidine nucleoside phosphorylase (PyNpase) (27). Cyclophosphamide (CPA) is a potent activator of PyNpase (19). For these reasons, combined therapy of doxifluridine and CPA appears promising for the treatment of recurrent breast cancer. Tamoxifen (TAM) has been used in the treatment of breast cancer for over a decade. Although the primary mechanism of action of TAM is believed to be through the inhibition of estrogen receptor (ER), research over the years has indicated that additional, non-ER-mediated mechanisms exist. These include the modulation of signaling proteins such as PKC, calmodulin, transforming growth factor-β (TGF β), and the proto-oncogene c-*myc* (28).

Accordingly, the present work was aimed at *in vitro* studying the effect on PMN of chemotherapy with anticancer agent and evaluating the change in the production of ROS and NO as well as the inhibition rate of phospholipase C and D, PKC, tyrosine phosphorylation kinase and phosphatidylinositol-3 kinase in signal transduction pathway. These results may allow us to understand the influence of cancer therapy on leukocyte function.

## Materials and Methods

### Materials

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), dextran, percoll (gravity 1.130), dimethyl sulfoxide, N-formyl-methionyl-leucyl-phenylalanine (FMLP), staurosporine, methionine, neomycin, wortmannin, genistein, bisindolylmaleimide (3-[1-(3-dimethylaminopropy)-indol-3-yl]-3 (indol-3-yl)maleimide) and cyclophosphamide, cisplatin, tamoxifen, doxifluridine used in the present study were purchased from Sigma Chemical Co. (USA).

### Isolation of rat PMN

Male Sprague-Dawley (SD) rats aged at 6 weeks were allowed food and water *ad libitum*. The animals were kept under controlled lighting (12 hr light-dark cycles), temperature (20-25 °C), and relative humidity (55 ± 10 %). They were acclimated to this environment for 2 weeks before the experiment. Blood samples (5 ~ 6 m*l*) were collected from anterior vena cava using the heparin-treated vacationer, mixed with 6 % dextran (Sigma, USA) at the ratio of 4 : 1 and placed in 5 % $CO_2$ incubator at 37 °C for 1 h to increase the consistency and minimize the contamination of leukocyte. The upper suspending fluids with affluent leukocytes were taken, floated with HBS (HEPES (N-2-Hydroxyethylpiperazine-N'-2'-ethanesulfonic acid) buffered solution containing 0.1 % BSA (Sigma). The leukocytes were washed with a centrifugal separation at 600 × g for 10 minutes at 20 °C. 70 % percoll (gravity 1.092) was separated by the modified percoll gravity changing method; the same procedure was done for leukocyte suspending fluids not to mix with percoll on it and centrifugally separated at 400 × g for 40 minutes at 20 °C. PMN-rich layer was collected, fixed at the level of 1 × $10^6$ cells/m*l*. The viability of the cells, tested by trypan blue exclusion assay, was never less than 95 % (24).

### Anticancer agents and experiment on proper density

Cyclophosphamide is used with the density of 500 μg/m*l*, 50 μg/m*l* and 5 μg/m*l*, cisplatin with 100 μg/m*l*, 10 μg/m*l* and 1 μg/m*l*, tamoxifen with 500 μg/m*l*, 50 μg/m*l*, 5 μg/m*l*, and doxifluridine with 500 μg/m*l*, 50 μg/m*l* and 5 μg/m*l* by diluting with HBS (0.1 % BSA) and saline.

### Measurement of Chemiluminescence response

Chemiluminescence (CL) of PMN was measured with Autolumat LB953 (Berthold Co., Germany), at 37 °C using disposable polystyrene tubes (Sarstedt, Germany) with a final 1.0 ml reaction mixture. Three separate measurements were performed simultaneously in the presence of $10^{-4}$ M luminol (4-amino-2,3-dihydro-1,4-phthalazinedione) to amplify the CL signals. The signals were then stimulated by introducing $10^{-7}$ M FMLP and measured at 37 °C pre-heated luminometer for 60 minutes after adding 50 *ml* of cyclophosphamide, cisplatin, tamoxifen and doxifluridine (13,18,31,34).

### Measurement of Superoxide anion ($O_2^-$)

The production of $O_2^-$ in PMS was measured using spectrophotometer (Ultraspec 3000, Pharmacia Biotech, USA) according to cytochrome c (type IV. Sigma) reduction method (12). Absorbance was measured at 550 nm after PMNs ($1 \times 10^6$ cells/m*l*) were floated on HBS (0.1 % BSA) and 100 μM cytochrome C for 1 minute at 37 °C with $10^{-7}$ M FMLP. The concentration of $O_2^-$ was calculated by the straight line graph of cytochrome c reduction curve.

### Measurement of Nitrite ($NO_2^-$)

To measure the amount of emission of NO in PMN, the concentration of nitrite was measured indirectly by the Griess reaction method (22,46). PMN suspending fluids of 100 *ml* was simply stimulated by $10^{-7}$ M FMLP. Standard nitrite (range, 1 μM~100 μM, Sigma) was used, and medium was melted with 2.5 % (v/v) concentrated $H_3PO_4$ in 1 % (w/v) sulfanilamide (Sigma Co.) at 96-well microplate, then mixed with the same amount of 0.1 % (w/v) N-(1-naphtyl)ethylenediamine dihydrochloride (Sigma, USA) to prescribe 100 *ml* of Griess reagent. The absorbance was measured at 540 nm after 10 minutes with microplate reader (ELx808, BIO-TEK Instrument, Inc., USA)

### Statistical analysis

The results were expressed as mean ± standard deviation (SD) for separate experiments, and comparisons were made by one-way ANOVA of SPSS® program. The difference between groups was determined by Duncan's test. It is considered to be statistically significant when P value was less than 0.05 or 0.01.

## Results

### Viability (%) of PMN for variable time intervals

The survival rate of the 95 % was maintained up to 8 hours without any significant change, after that it showed 87 % cell viability until 12 hours, and approx. 65 % by 48 hours (Fig 1). Therefore, most of the experiment was performed within 8 hours of PMN preparation.

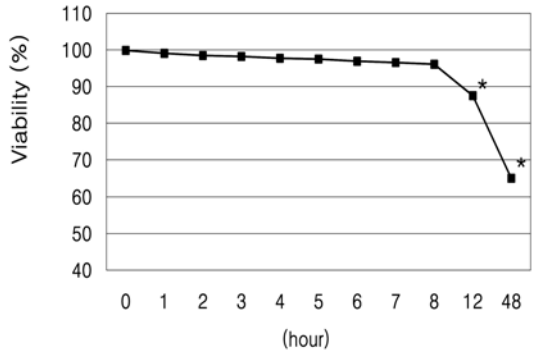


**Fig 1.** Viability of PMN according to incubation time. Data are the result of a single representative experiments, with value presented as the mean of three replicates.
*Significant differences ($p < 0.001$) in comparison with the control.

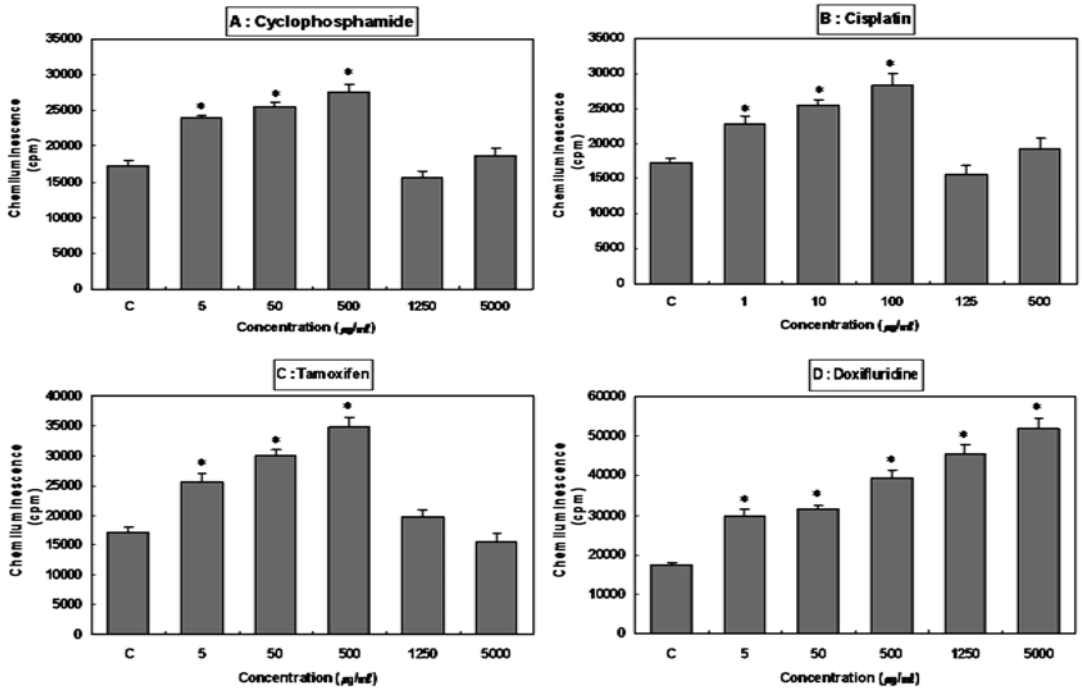


**Fig 2.** Chemiluminescence of PMN treated with different concentrations of anticancer agents. FMLP ($10^{-7}$ M) was added and Chemiluminescence was measured. Control means only FMLP-treated PMN without treatment of anticancer agent.
*Significant differences ($p < 0.05$) in comparison with the control.

**Time kinetic changes of Chemiluminescence in PMN treated with various concentrations of anticancer agents**

Cyclophosphamide, tamoxifen and doxifluridine, respectively was treated to PMNs with the concentration of 5000 μg/m*l*, 1250 μg/m*l*, 500 μg/m*l*, 50 μg/m*l* and 5 μg/m*l* and cisplatin with 500 μg/m*l*, 125 μg/m*l*, 100 μg/m*l*, 10 μg/m*l* and 1 μg/m*l*, and CL of these PMNs was measured (Fig 2). Cyclophosphamide and tamoxifen showed a significant increase in chemiluminescence at the concentrations of 500 μg/m*l*, 50 μg/m*l* and 5 μg/m*l*, and cisplatin did at 100 μg/m*l*, 10 μg/m*l*, 1 μg/m*l*. Unlike other anticancer agents showing a biphasic response, doxifluridine showed a continuous increase of chemiluminescence throughout the tested concentrations ($P < 0.05$) at the ranges of 5 ~ 5000 μg/m*l*.

**Change of respiratory burst of PMN treated with anticancer agents**

After treating rat PMN with the anticancer agents of cyclophosphamide 500 μg/m*l*, cisplatin 100 μg/m*l*, tamoxifen 500 μg/m*l* and doxifluridine 500 μg/m*l*, luminol-amplified CL was measured at FMLP-stimulated PMN. In the case of FMLP, cyclophosphamide treatment enhanced PMN chemiluminescence with a significance ($P<0.05$) as 27,500 ± 200 cpm rather than 22,200 ± 300 cpm of control (PMN + FMLP). Cisplatin treatment showed 28,300 ± 200 cpm of peak intensity which is slightly higher than cyclophosphamide one. On the other hand, doxifluridine and tamoxifen showed a strong increase of CL with 39,500 ± 300 and 34,700 ± 200 cpm, respectively (Fig 3).

**Change of respiratory burst according to treatment time and concentration of the anticancer agents**

CL in PMN treated with 500 μg/m*l* of cyclophosphamide, tamoxifen and doxifluridine for 1 hour was much higher than that treated with 50 μg/m*l* or 5 μg/m*l*. The higher was the

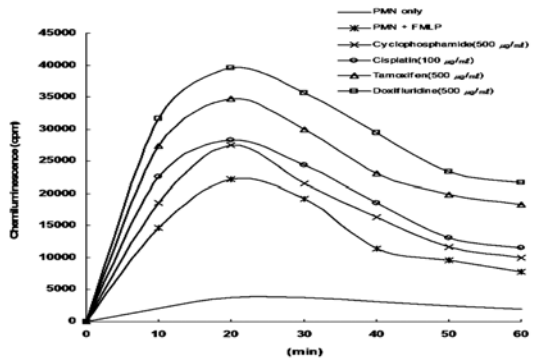


**Fig 3.** Time kinetics of chemiluminescence response of rat PMN in the absence or presence of anticancer agents. The cells were incubated with luminal, and then chemiluminescence was measured as an expression of respiratory burst. Data are the result of a single representative experiments, with value presented as the mean of three replicates.

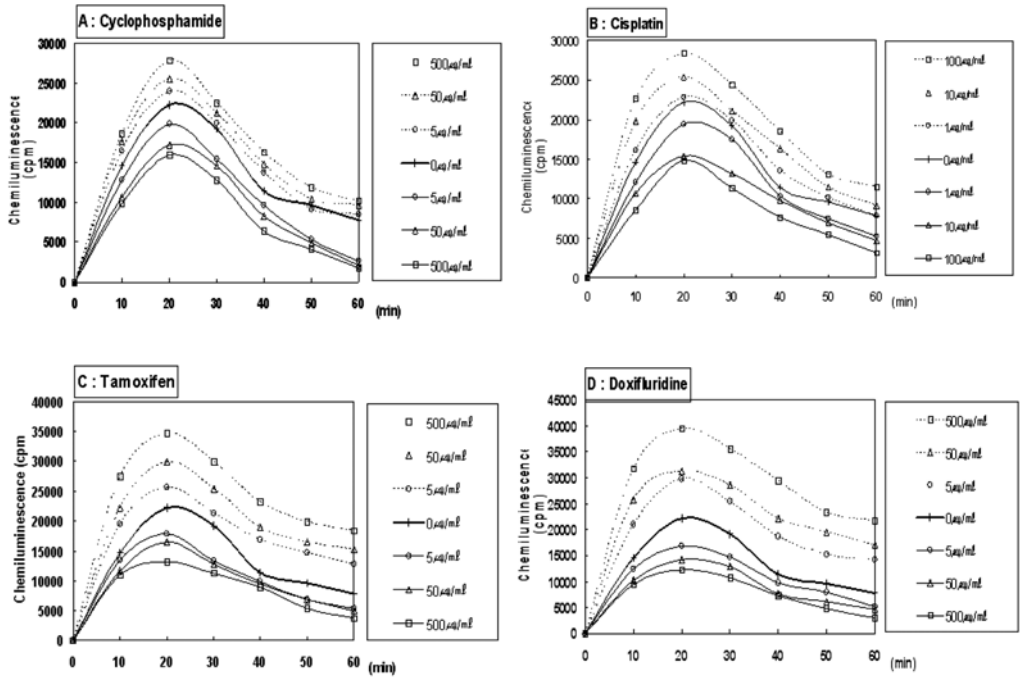


**Fig 4.** Up- and down-regulation of Chemiluminescence by 1 hr (…) and 8 hrs (—) treatment of PMN with anticancer agents. PMN from 4 normal SD rats were treated with the indicated concentrations of cyclophosphamide (0-500 μg/m*l*), cisplatin (0-100 μg/m*l*), tamoxifen (0-500 μg/m*l*) or doxifluridine (0-500 μg/m*l*) for 1hr (…) or 8hrs (—). After medium change, FMLP ($10^{-7}$ M) was added and chemiluminescence was measured. Data are the result of a single representative experiments, with value presented as the mean of three replicates.

concentration of anticancer agents, the lower was the respiratory burst in PMN treated for 8 hours (Fig 4A, C and D). Cisplatin showed a similar pattern with 100, 10 and 1 μg/m*l* concentrations (Fig 4B).

**Change of Chemiluminescence in PMN treated with anticancer agents by time**

Each anticancer agent was tested for checking the effect of CL according to the duration treated in PMN (Fig 5). The peak intensity of all agents was increased until 4 hours when each agent was treated, gradually decreased, and from 6 hours, showed lower value than control (only FMLP-treated PMN).

**Change in the production of Superoxide ($O_2^-$) in PMN treated with anticancer agents**

The production of Superoxide ($O_2^-$) was suppressed when each anticancer agent was treated with PMN (Fig 6). Control (PMN + FMLP) emitted $O_2^-$ of 6.3 ± 0.47 nmol/$10^6$cells/min. The emission of $O_2^-$ was increased to 7.5 ± 0.6 nmol/$10^6$cells/min by treating 500 μg/m*l* of cyclophosphamide for 1 hour, but decreased to 4.5 ± 0.9 nmol/$10^6$cells/min for 8 hours. The tamoxifen and doxifluridine showed similar pattern with cyclophosphamide. In the case of cisplatin, the production of $O_2^-$ was increased by treating with 100 μg/m*l* for 1 hour, but decreased for 8 hours.

**Change in the production of Nitric Oxide in PMN treated with anticancer agents**

As a result of microplate assay, control (PMN + FMLP) had 6.5 ± 0.3 μM for 1 hour; however, the production of $NO_2^-$ when stimulating with $10^{-7}$ M FMLP after treating cyclophosphamide, tamoxifen and doxifluridine with the concentration of 500 μg/m*l* to PMN was decreased by 5.4 ± 0.5, 3.1 ± 0.5, 3.7 ± 0.3 μM for 1 hour, but increased for 8 hours. Also cisplatin was more decreased by 4.5 ± 0.7 μM for 1 hour at the concentration of 100 μg/m*l* (than PMN + FMLP), but increased for 8 hours (Fig 7).

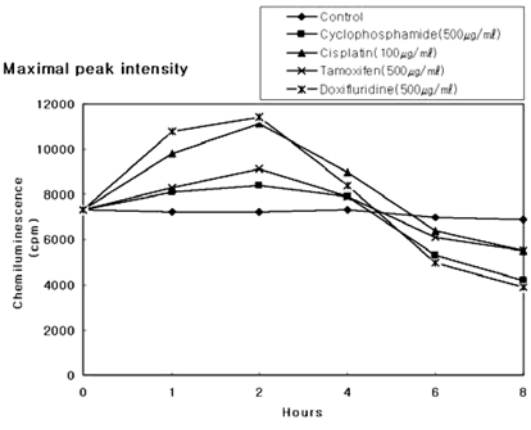


**Fig 5.** Effect of anticancer agents on Chemiuminescence in PMN treated for 1 hr to 8 hrs. PMN from 4 normal rats were treated with each anticancer agent for the indicated times. After being washed, the PMN were stimulated with $10^{-7}$ M FMLP. Control means only FMLP-treated PMN without treatment of anticancer agent. Data are the result of a single representative experiments, with value presented as the mean of three replicates.

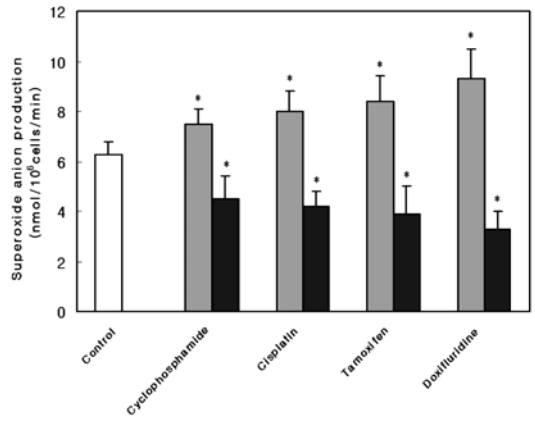


**Fig 6.** Effect of cyclophosphamide, cisplatin, tamoxifen and doxifluridine on $O_2^-$ production from PMN. PMN from 4 healthy SD rats were treated with the indicated concentrations of anticancer agents for 1hr (■) or 8hrs (■). After washing and the addition of $10^{-7}$ M FMLP, $O_2^-$ production was measured. Control (□) means only FMLP-treated PMN without treatment of anticancer agents. Each bar indicates standard deviation (SD) of triplicate experiments; initial reaction velocity is expressed as $\triangle_{excitation}$ at 550 nm/min; error bar denotes SD.
* Significant differences ($p < 0.01$) in comparison with the control.

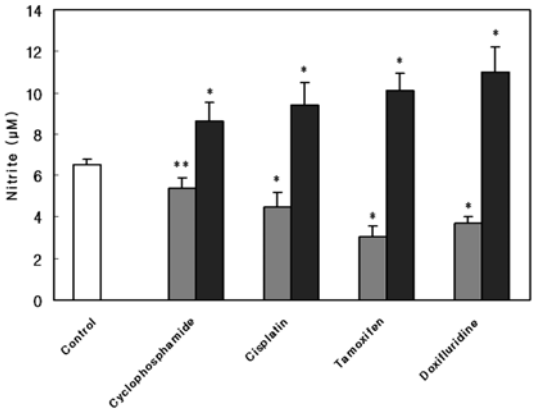


**Fig 7.** Effect of cyclophosphamide, cisplatin, tamoxifen and doxifluridine on nitric oxide production from PMN. PMN from 4 normal rats were treated with the indicated concentrations of anticancer agents for 1hr (■) or 8hrs (■). After washing and the addition of $10^{-7}$ M FMLP, NO generation was measured. Control (□) means only FMLP-treated PMN without treatment of anticancer agents. Each column and bar indicates the average μM ± SD of three replicate experiments.
*, ** Significant differences ($p < 0.01$ and $p < 0.05$, respectively) in comparison with the control.

**Effect on Phospholipase C, Phospholipase D, Protein Kinase C, Tyrosine Phosphorylation Kinase and Phosphatidylinsitol-3 Kinase treated with anticancer agents**

The inhibitors suppressing the secondary signal transduction enzyme lying in the cell membrane of PMN, before being stimulated by FMLP, were treated with PMN for 5 minutes. Neomycin (inhibitor of phospholipase C, 1 mM) (29) suppressed chemiluminescence in cisplatin-, tamoxifen- and doxifluridine-pretreated PMN to a greater extent than it did cyclophosphamide-pretreated PMN (Fig 8A), however near suppression of chemiluminescence by ethanol (1 %) (Inhibitor of respiratory burst mediated by phospholipase C) (8) and genistein (inhibitor of tyrosine phosphorylation kinase, 100 μM) (3) was observed in PMN pretreated with these agents (Fig 8C and D). Staurosporine and bisindolylmaleimide (7) (inhibitor of protein kinase C, phospholipid/calcium-dependent, 50 nM and 500 nM) suppressed chemiluminescence in cisplatin- and doxifluridine- pretreated PMN (Fig 8B). Wortmannin [(inhibitor of FMLP-stimulated respiratory burst due to inhibition of phosphatidylinositol 3-kinase), (inhibitor of PLD activity in human neutrophils, 25 nM)] (4) has shown a slight suppression in cyclophosphamide-, cisplatin- and tamoxifen-pretreated PMN, but a strong suppression in doxifluridine-pretreated PMN (Fig 8E). Methionine (inhibitor of myeloperoxidase, 500 μM) strongly suppressed in cyclophosphamide and cisplatin-pretreated PMN (Fig 8F).

## Discussion

There exist innumerable numbers of microorganism in the environment surrounding the living body and some of them may intrude into the body to cause critical influence. Also within the body survive the superannuated cells and organism to obstruct metabolism or for part of cells during segmentation to be transformed into those of cancers to threat the lives. These phenomena lead to generate inflammatory activity or immune activity for which polymorphonuclear leukocytes and macrophage are represented as acting protective activity against microorganism and cancer cell intruded

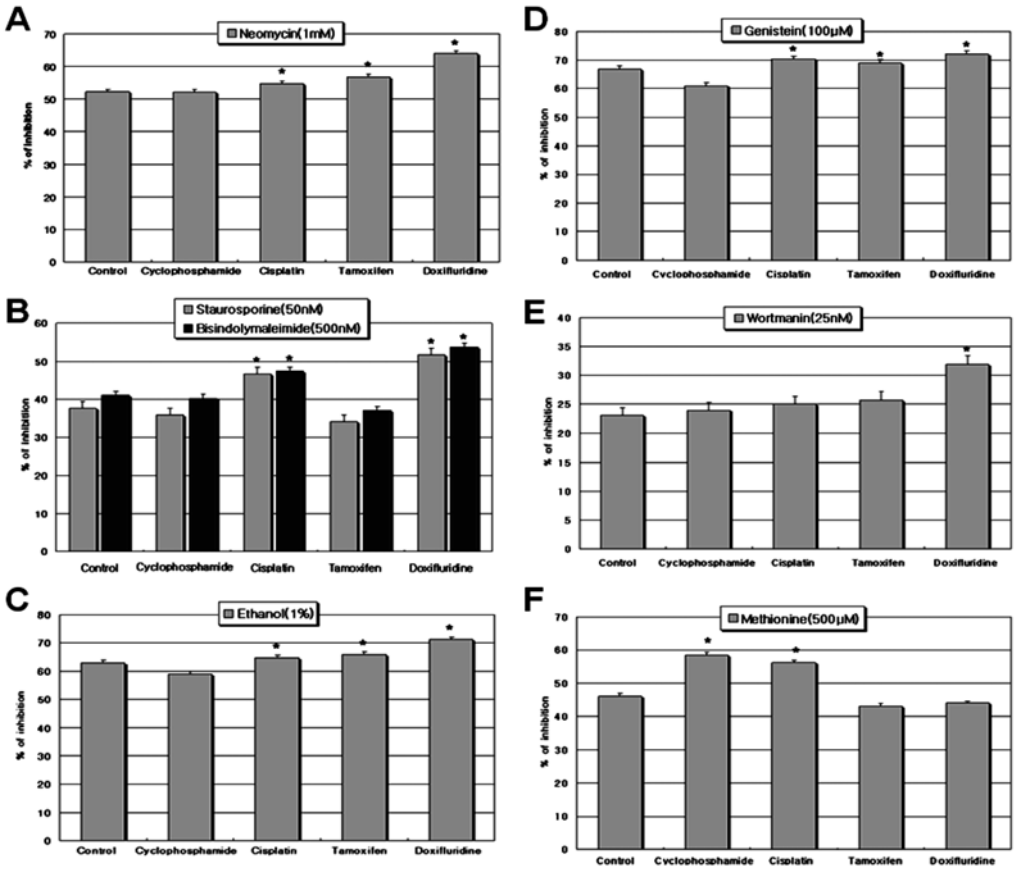


**Fig 8.** Inhibitory effect of characterization of reactive oxygens species released from PMN pretreated with anticancer agents. PMN from 4 normal rats were treated with cyclophosphamide (500 μg/m*l*), cisplatin (100 μg/m*l*), tamoxifen (500 μg/m*l*) or doxifluridine (500 μg/m*l*) for 8 hrs. The PMN were then cultured for 5 min in the presence of each inhibitor indicated, and $10^{-7}$ M FMLP-stimulated Chemiluminescence was measured. Control means only FMLP-treated PMN without treatment of anticancer agents. Each column and bar represents the average peak intensity ± SD of three replicate experiments.
*, ** Significant differences ($p < 0.01$ or $p < 0.05$, respectively) in comparison with the control.

into the body at the frontline.

PMN and Reactive Oxygen Species (ROS) of macrophage in case damage of cell and organism and degeneration of various diseases have been known to us as providing important anticancer activity in the formation of the new cell organism (17,20,45). Immune suppression caused by anticancer treatment is so critical that several studies on the emission of ROS at PMN have been carried out for some of the cancer patients (25,42). In the previous studies showed ROS at abdominal and salivary PMM of the patients with the cancer of the mouth emitted lower value than those of healthy person and ROS emission at PMN had shown the suppression when treating cancer of which these cells were related with candicidal activity (42). However, any definite mechanism concerning the suppression of ROS emission has not been revealed yet. This study surveyed the increase of ROS emission at PMN when treating cyclophosphamide, cisplatin, tamoxifen and doxifluridine for 1 hour. When there exist the stimulators like cancer agents or cytokine or chemical substances, in general, PMN emitted ROS and the emission of $O_2^-$ might been stimulated even in hypotonic shock (16). The electric charge of membrane of polymorphonuclear leukocytes could be changed by the molecules' combination of anticancer agents used in this study and thus the emission of ROS could be regarded as having the possibility of changing in signally excellent. It is assumed that these agents are permeated into the cells to suppress the emission of ROS, accordingly resulting in the suppression of cell metabolism can be suppressed (43).

In contrast with ROS, the production of NO was decreased when treated with anticancer agents for 1 hour but increased for 8 hours. This can be thought as the interaction between ROS and NO relating to signal transduction pathway. FMLP-induced CL at PMN is thought of depending on phosphatidylinositol 3-kinaseas and as the reaction such that the activation of $Ca_2^+$-dependent phospholipase D and/or phospholipase C and protein kinase C occurs prior to that of NADPH oxidization enzyme (44). The suppression against ROS emission by cisplatin, tamoxifen and doxifluridine has been coming from that of signal transduction pathway across phospholipase C. Neomycin (29) has shown much more effect on the suppression of FMLP-stimulated at PMN with anticancer agents treated than that without treatment. Ethanol (8), inhibitor of phospholipase D, was suppressed FMLP-stimulated CL at PMN with cisplatin, tamoxifen and doxifluridine treated. Other effect in the membrane might be one of the causes in this process; however, respiratory burst stimulated by PMN was not entirely suppressed in the existence of ethanol (data not shown). It's assumed that ethanol itself does not give any effect on suppression. Staurosporine (PKC inhibitor) has been adopted in several studies for the clarification of the relation with PKC in receptor-mediated activation of NADPH oxidization enzyme, but failed to reach any corresponding result. So in this study more peculiar PKC inhibitor, bisindolylmaleimide (49) was used and by experiment compared with staurosporine and bisindolylmaleimide at the same time. It is presumed that Bisindolylmaleimide suppresses FMLP-stimulated CL at cisplatin and doxifluridine to give an important influence on the activation process of NADPH oxidase of PKC not by a stimulator. Genistein (3), Tyrosine phosphorylation kinase inhibitor was suppressed at PMN with cisplatin, tamoxifen and doxifluridine treated. This can be presumed as promoting protein tyrosine phosphorylation. Genistein as tyrosine phosphorylation inhibitor can be regarded as being suppressed by tyrosine phosphorylation by means of cisplatin, tamoxifen and doxifluridine as well as other pathway. Protein tyrosine phosphorylation is necessary for the activation of various cells and 115 kDa protein tyrosine phosphorylation at PMN, the study had proved these (2). Wortmannin has been known as activating phosphatidylinositol-3 kinase and suppressing respiratory burst which is intermediated by FMLP[52]. Wortmannin has shown a slight suppression on PMN with cyclophosphamide, cisplatin and tamoxifen treated and a powerful for doxifluridine. Myeloperoxidase (MPO) has played an important role in emitting and scavenging $O_2^-$ and $H_2O_2$ and the activation of these enzymes has been calculated by bactericidal activity at PMN and macrophage. The inhibitor of MPO, methionine has been suppressed when treating at PMN cyclophosphamide and cisplatin for 8 hours and suppressing the ROS emission can be judged as having a relation with the inactivation of MPO.

Anticancer agents in the suppression of signal transduction enzyme have strongly suppressed PKC activity and protein tyrosine phosphorylation kinase. With this reason it can be thought of as anticancer agents act as the dangerous element in the cases of viral infection. This study aims at defining the nature of ROS emission in relation to signal transduction when respiratory burst of PMN comes out by anticancer agents and the following results can be helpful for reducing the exposure to infection during cancer treatment. However, in order clinically to apply these especially for the development of immunomodulator regulating the signal transduction pathway, further continuous and systematic research should be required, judged by this study.

In conclusion, the emission of ROS and the change in the production of NO at the time of respiratory burst of PMN by anticancer agents can be regarded as being interrelated reaction with the signal transduction pathway and in order to make clinical application to include the development of immunomodulator regulating the signal transduction pathway based on the differentiation of inactivated enzyme, further in-depth study should be followed.

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# 항암제에 의한 흰쥐 다형핵백혈구의 활성산소종(reactive oxygen species) 및 산화질소(nitric oxide)의 생성

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**요 약** : 항암제에 의해 흰쥐에서 다형핵 백혈구(polymorphonuclear leukocytes, PMN) 의 활성산소종(reactive oxygen species, ROS)과 산화질소(nitric oxide, NO)의 생성변화에 대해 연구하였다. 최근 자극유도인자에 의한 PMN의 호흡방출은 protein kinase C (PKC)의 활성, inositol phosphate transduction pathway의 활성, 그리고 intracellular [$Ca^{2+}$]와 관계가 있다고 밝혀졌으며, 본 연구에서 사용된 항암제(cyclophosphamide, cisplatin, tamoxifen, doxifluridine)중 일부는 화학치료제로써 비특이적으로 면역을 억제하는데 사용되고 있다. 암 치료 시 백혈구의 방어기능에 미치는 영향을 연구하기 위한 목적으로 *in vitro*에서 각 항암제를 처리한 PMN을 배양하여 ROS와 NO의 생성변화와 이차적 신호전달계인 phospholipase C(PLC), D(PLD), PKC, tyrosine phosphorylation kinase (TPK)와 phosphatidylinositol-3 kinase의 억제율을 측정하였다. PMN에 각각cyclophosphamide, cisplatin, tamoxifen, doxifluridine을 short term(≤4hrs) 처리 시, formylmethionyl-leucy1-phenylalanine (FMLP) 자극에 의해 호흡방출의 증가가 나타났다. 반면, long term (8hrs) 처리 시, ROS의 생성은concentration-dependent 방법으로 감소되었다.

**주요어** : 활성산소종, 산화질소, 다형핵 백혈구, 항암제.