Effects of 2-Carboxyethylgermanium Sesquioxide (Ge-132) as an Immunological Modifier of Post-Surgical Immunosuppression in Dogs

Youko NAKADA, Toshifumi KOSAKA, Masato KUWABARA, Shigeo TANAKA\textsuperscript{1}, Kei SATO\textsuperscript{1}, and Fusaoki KOIDE

Departments of Veterinary Radiology and \textsuperscript{1}Surgery, College of Agriculture and Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252, Japan

(Received 9 November 1992/Accepted 14 June 1993)

\textbf{ABSTRACT.} The effect of 2-carboxyethylgermanium sesquioxide (Ge-132) as an interferon-\(\gamma\) (IFN-\(\gamma\)) inducer on post-surgical immunosuppression was evaluated from the immunological response augmented in canine neutrophils, macrophages and peripheral blood lymphocytes (PBL) using the chemiluminescence test. Experimental gastrotomy was performed on dogs in two groups; one group was subjected to sham-surgery without any medication, and the other was treated by Ge-132 administration at 24 hr before operation. Although the phagocytic activities of neutrophils, macrophages and PBL in the sham-operated group were depressed transiently after surgery, those in the Ge-132-administered group (Ge-132 group) were enhanced for a long time after surgery. It appeared that the generated free radical, which blocked the phagocytosis of macrophages and PBL, was activated by IFN-\(\gamma\). These results suggest that Ge-132 pre-treatment may be efficacious and useful in preventing the multifaceted clinical symptoms induced by post-operative immunosuppression in dogs.—\textit{KEY WORDS:} chemiluminescence, Ge-132, macrophage, neutrophil, PBL.


Based on the measurement of mitogen-induced lymphocyte blastogenesis, immunosuppression by anesthesia and surgery in various animal species has been reported in both human iatrology and veterinary medicine [3, 4, 8, 15–19, 21, 23]. Immunosuppression, which usually occurs immediately after surgery, persists from a few days to as long as 2 weeks [18, 23]. Lymphocyte blastogenesis can be based on the response for multiplication of lymphocytes against mitogen, and this technique eliminates the immunological activation of neutrophils and monocytes and lymphocytes [10]. Immunological functions are rendered active by macrophages, lymphocytes, neutrophils and mononuclear cells [6, 7]. Therefore, it is considered that neutrophils, macrophages and natural killer cells (NK cells) play important roles in the biophylactic mechanism of surgical stress. The effects of surgery and anesthesia on the activation of neutrophils, macrophages and NK cells have been little reported. Many clinically undesirable symptoms are induced by immunosuppression after surgery, and the introduction of pre-operative immunotherapy may be a possible innovation. Interferon-\(\gamma\) (IFN-\(\gamma\)) is one of the most effective lymphokines in the cellular immunological system [1, 12, 20, 22, 24]. IFN-\(\gamma\) is an activator of macrophages [13], NK cells [22] and cytotoxic T cells [20] \textit{in vivo}. An IFN-inducing agent, 2-carboxyethylgermanium sesquioxide (Ge-132) [2, 12], was used as an immunological activator in this study.

This report describes the immunological effects of Ge-132 on surgically operated dogs in which the immunological function was suppressed.

\textbf{MATERIALS AND METHODS}

\textit{Animals:} Twenty-seven normal mongrel dogs of both sexes weighing 9–11 kg (raised in our laboratory) were used. Experimental gastrotomy was performed on dogs in two groups. Dogs in a control group were sham-operated without any medication, and dogs in a Ge-132 group were treated by the administration of 50 mg/kg of Ge-132 (kindly donated by Asai Geranium Inst. Co., Ltd., Japan) in p.o. at 24 hr before operation (Ge-132 group). Each group was composed of 18 (\(\updownarrow\) 10; \(\updownarrow\) 8) or 9 (\(\updownarrow\) 5; \(\updownarrow\) 4) animals.

\textit{Target cell:} The CL-1 cell line derived from canine thymus type lymphosarcoma [6] was maintained at 37°C in a humidified atmosphere with 5% \(\text{CO}_2\) in RPMI 1640 (Nissui Co., Ltd., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Co., Ltd., New York, U.S.A.) and 0.3 g/l of glutamine.

\textit{Effector cells:} Peripheral blood lymphocytes (PBL) and macrophages were isolated from heparinized blood by Lymphoprep (NYCOMED PHARAMA AS., Oslo, Norway) centrifugation at 350–400 \(\times\) g for 40 min. Isolated PBL were washed with Dulbecco's phosphate-balanced salt solution prior to centrifugation at 100–150 \(\times\) g for 5 min. These PBL were resuspended in 2 ml of PRMI 1640 and incubated for 1 hr at 37°C in a 35-mm plastic petri dish (Falcon Co., \#3003, New Jersey, U.S.A.). Adherent and nonadherent cells were collected as macrophages and PBL, respectively.

\textit{Anesthesia:} All the dogs were premedicated with 0.04 mg/kg of atropine sulfate (Tanabe Pharm. Co., Ltd., Osaka; s.c.) and 0.5 mg/kg of chlorpromazine chloride (Comtomin\textsuperscript{b}; Yositomi Pharm. Co., Ltd., Osaka; i.m.), prior to anesthetic induction with 12.5 mg/kg of thiopental sodium (Ravona 5G; Tanabe Pharm. Co., Ltd., Osaka; i.v.). Anesthesia was maintained with 1.5% halothane (Fluothane\textsuperscript{c}; Takeda Pharm. Co., Ltd., Tokyo) and 300 ml/oxygen/respiration for 90 min, followed by gastrotomy. After the operation, 0.1 ml/kg of penicillin (Mycillin\textsuperscript{d};

NII-Electronic Library Service
Zenyaku Co., Ltd., Fukushima) was injected i.m. into the operated dogs for 3 consecutive days.

Preparation of heparinized blood: Blood was collected every day at 12:00 noon for 14 days via a heparinized catheter inserted into the cephalic vein.

Chemiluminescence (CL) assay: Whole blood (0.5 mL), 6x10^6 macrophages per mL, and 4x10^6 PBL per mL were incubated in plastic vials (12x47 mm) at 37°C for 10 min for adaptation to the dark. Then 50 μL of luminol solution was added to each sample and the background CL was measured for 10 min. Luminol solution was prepared in the following way. One hundred mg of luminol and 50 μL of triethylamine (Wako Pure Chemicals, Osaka) were added to 50 mL of RPMI 1640 and then allowed to dissolve. After complete dissolution with sufficient agitation, the solution was passed through a 0.45 μm membrane filter (Japan Millipore Co., Tokyo) and stored in the dark at -20°C until use. KAC-2 solution (Nihon Antibody Laboratory, Gunma; 200 μL) was added as the whole blood or macrophages stimulant, whereas CL-1 solution (200 μL, 1x10^6 cells/mL) was added as PBL stimulant prior to CL determination which was carried out at 60 and 90 min after luminol addition, respectively. Transitional CL activity was calculated by means of the following equation and compared thereafter:

\[
CL = \frac{\text{total count after stimulation}}{\text{total count before stimulation}} \times 100
\]

where the count was the total CL from the time of injecting the stimulant to the time when the whole blood and macrophages attained the maximum counts. The total count for PBL was the total CL from 20 to 80 min after injection of the stimulant.

RESULTS

Changes in white blood cell count: Figure 1 shows the variations in white blood cell count in the blood from 27 clinically healthy adult dogs. The number of white blood cells showed a significant but reversible transient decrease immediately after the operation (p<0.001). In the Ge-132 group, the number of white blood cells which decreased immediately after the operation reversed to a 1.4-fold increase on day 1 post-operation. The number of white blood cells was subsequently maintained at a reasonably high level for 14 days. Dynamic changes in CL were induced by leukocytes after operation (Fig. 3). In both groups, the relative numbers of lymphocytes and monocytes were maintained at a stationary level. However, the number of neutrophils slightly increased in the Ge-132 group at 24 hr post-operation. From the results of two groups expressed in ratios of white blood cells (Fig. 2), the post-operative increase in the white blood cell count in the Ge-132 group was mainly attributed to the increase in the relative number of neutrophils.

Changes in whole blood cells CL: Changes in KAC-2-induced CL with time in whole blood cells are illustrated in Fig. 3. The phagocytic activity of whole blood cells was reduced immediately after operation, and rapidly recovered thereafter. The response curves for the operated dogs with or without Ge-132 manifested the same equipment activity, although the neutrophil count in peripheral blood in the Ge-132 group was enhanced on day 1 post-operation (Fig. 2). In both groups, the whole blood cell activity initially increased but gradually diminished with post-operative time and recovered to the pre-operative level on day 10 post-operation. The CL activity per a white blood cell was expressed by dividing the CL activity in whole blood cells by the number of total white blood cells (Fig. 4). The CL index for whole blood cells had increased by 24 hr post-operation. In the Ge-132 group, however, this index was completely reduced compared to that of the operation group. Therefore, the
EFFECTS OF GE-132 ON SURGICAL STRESS

Fig. 3. Changes in phagocytotoxic activities in whole blood cells after operation with or without Ge-132 administration. A transient reversible phenomenon in neutrophils is indicated. Refer to Fig. 1 for explanation of each point.

Fig. 4. Changes in chemiluminescence (CL) activity per a white blood cell after operation with or without Ge-132 administration. Refer to Fig. 1 for explanation of each point and symbols.

Fig. 5. Changes in macrophage CL activities after operation with or without Ge-132 administration. (C) and (A) have been described in Fig. 1. Vertical bars represent standard errors.

Fig. 6. Changes in peripheral blood lymphocyte (PBL) activities caused by the CL technique after operation with or without Ge-132 administration. The patterns of immunological augmentation of PBL displayed by both groups resembled those indicated by macrophages. Refer to Fig. 1 for explanation of each point and symbols.

Discussion

Effects of surgical stress on the immunocompetence of patients have been focused on recently [3, 4, 8, 15-19, 21, 23]. Mitogen-induced lymphocyte blastogenesis is an in vitro procedure extensively used for studying the function status of lymphocytes [3, 4, 8, 15-19, 21, 23]. However, the phagocytic activities of macrophages and neutrophils, as well as the cytotoxic activities of T and NK cells, have not been demonstrated as yet. Helfand et al., Kosaka et al., and Kuwabara et al. [5, 9, 11] have described the luminol-aided CL technique as a simple and sensitive method to measure free radical content produced by phagocytic or cytotoxic cells. This method also allows measurement of the immunological activation of neutrophils, macrophages and NK cells. There were similar patterns in the CL of both whole blood cells and
neutrophils (data not shown). As the majority of total white blood cells were found in blood, the phagocytic activity of whole blood cells was closely associated with that of neutrophils. We observed changes in the free radical production in neutrophils, macrophages and NK cells after surgery. Nakasushi et al. and Uematsu et al. have demonstrated that lymphocyte blastogeneic activity was decreased immediately after the operation, and recovered within 48 hr post-operation, then decrease again on day 4 post-operation [18, 23]. Employing the CL technique, the present study showed that the immunofunctions of macrophages and PBL were considerably suppressed from immediately after the operation to 48 hr post-operation, although an increase was recorded on day 3 post-operation. Our CL technique indicated that the immunosuppressive condition appeared earlier than the period of lymphocyte blastogenesis [23] in the surgically operated dogs. In the Ge-132 group, the activities of neutrophils, macrophages and PBL remained higher than in the surgery-treated group without Ge-132. By Ge-132 treatment, immunosuppression of macrophage and PBL activities after operation was distinctly prevented. In fact, the decrease in immunosuppression which was observed very clearly was sharply expressed from 24 hr post-operation. Ge-132 which has been reported as an IFN-γ inducer [2, 12], can serve as an immunomodulatory activator as well. IFN-γ, which is produced mainly by helper T-cells and NK cells, affects various white blood cells, such as macrophages, NK cells, and cytotoxic T-cells [13, 20, 22]. Moreover, IFN-γ induces some cytokines, such as IL-1, IL-2, MAF and GM-CSF [1, 24]. The period of maximum IFN-γ induction is attained 24 hr post-treatment with Ge-132, and Ge-132 is excreted from the body system within 72 hr post-treatment [14]. Meanwhile, its effect on enhancing the macrophage and NK cell activities lasted until 14 days after operation. It is therefore expected that macrophage and NK cell are activated by IFN-γ formed positive feedbacks, inhibiting the post-operative immunosuppression of macrophage and PBL activities following Ge-132 treatment. Our results showed that Ge-132 not only avoided the initial 24 hr of post-operative suppression of immunological function in macrophages and PBL, but also enhanced the post-operative immunofunction for 14 days after the operation. Hence, pre-operative treatment with Ge-132 (an immunopotentiator) may be able to prevent many undesired clinical symptoms, such as post-operative infection and other immunological disorders.

ACKNOWLEDGEMENT. We thank Miss Hisako Ezaki who helped in this work.

REFERENCES


