INTRODUCTION

Paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) is one of the widely used herbicides in the world. However, it has been known to be toxic to human (Christakis-Hampsas et al., 1998; Gear et al., 2001; Wesseling et al., 2001; Rahman et al., 2007) affecting lungs, liver and skin among other organs. Paraquat has been also known to induce Parkinsonism. Exposure to paraquat as a risk factor for Parkinsonism has been understood through the studies about 1-methyl-4-phenylpyridinium (MPP+), a compound with a similar structure to paraquat (Langston et al., 1984a, 1984b).

The perturbation of immunoglobulin productivity by paraquat is dependent upon its induction of the reactive oxygen species (ROS) production. Paraquat-induced ROS can cause tissue injury. Lungs are considered to be the main target organ of paraquat-induced ROS toxicity (Manktelow, 1967). Moreover, it is also known that ROS reduce IgM productivity in mouse spleen lymphocytes (Miyazaki et al., 2001). Lymphocytes bear the major responsibility for carrying out the activities of the immune system which play an important and integral role in the body’s defenses against infection and disease. An aberrant immune function can lead to diseases. However, immune function effects associated with exposure to paraquat are not yet understood.

In this study, we studied the effect paraquat on immunoglobulin productivity of mouse in vitro and in vivo. When splenocytes were cultured in vitro with various concentrations of paraquat, IgA productivity was not affected while IgG and IgM productivity decreased. On the other hand, Oral administration of paraquat for 1, 2 or 3 weeks increased IgA level but decreased IgM levels in serum of mice. Similarly IgA productivity increased while IgM productivity decreased. These results suggest that paraquat perturbs the lymphocytes immunoglobulin productivity in an immunoglobulin class-dependent manner.

Key words: Paraquat, Oral administration, Splenocytes, Immunoglobulin productivity

MATERIALS AND METHODS

Experimental animals

Four weeks old female albino mice were purchased from the Japan SLC, Inc. (Shizuoka, Japan). Mice were kept in a room at 24°C with a 12 hr light/dark cycle. Mice were allowed to acclimatize for 1 week before use in experiment. All animal experiments were carried out according to the protocols approved by Ehime University Animal Care and Use Committee, and the Law (No. 105) and Notification (No. 6) of the Japanese Government.
Lymphocytes culturing with paraquat

To determine the direct effects of different concentrations of paraquat on immunoglobulin productivity of splenocytes, mouse splenocytes were isolated from 8-week old female albino mouse according to the method described above. Then the lymphocytes were cultured in RPMI-1640 medium containing 10% fetal bovine serum with or without 10⁻⁸~10⁻² μM of paraquat for 24 hr. After culture, the medium was collected, and IgA, IgG and IgM levels were measured.

Oral administration of paraquat

To determine the effects of oral administration of paraquat to lymphocytes, Student’s t test was used to assess the significant differences between each data. In direct administration of paraquat to lymphocytes, Student’s t test was used to assess the significant differences between each data.

RESULTS AND DISCUSSION

Immunoglobulin productivity of mouse splenocytes cultured in the medium with different concentrations of paraquat is shown in Fig. 1. IgA productivity of lymphocytes was not affected by paraquat. However, IgG productivity was significantly decreased by 10⁻⁸~10⁻⁶ μM paraquat treatment. Effects of different concentrations of paraquat on IgM productivity is of the same trend as those on IgG productivity, expect that at 10⁻⁷~10⁻⁶ μM, IgM productivity was also decreased. On the other hand, no effect was observed in those treated with 10¹~10² μM paraquat. These results suggest that paraquat perturbs the productivity of immunoglobulin with dose-dependent manner. On the other hand, human lymphocytes die in the presence of 100 μM of paraquat (Rio and Velez-Pardo, 2008), suggesting that the concentration perturbing immunoglobulin productivity and cell death are not same. More studies are needed to clarify why paraquat perturbs immunoglobulin products were observed in limited concentrations.

Enzyme-Linked Immuno Sorbent Assay (ELISA)

IgA, IgG and IgM levels in serum and culture medium were measured by ELISA. All antibodies were purchased from Zymed Laboratories (Carlsbad, CA, USA). Rabbit anti-mouse IgA, IgG and IgM antibodies were diluted 1,000 times with 50 mM sodium carbonate-bicarbonate buffer (pH 9.6). One hundred microliter of each antibody solution was added to 96 well plates and incubated at 37°C for 2 hr. The plates were blocked with 25% block-ace (Snow Bland, Hokkaido, Japan) at 37°C for 2 hr. The blocking solution was discarded before medium was dispended to the well, followed by incubation at 37°C for 1 hr. Thereafter HRP-labeled antibodies were added and incubated at 37°C for 1 hr. For staining, 100 μl of substrate solution containing 0.6 mg/ml of 2, 2’-azino-bis (etylbenzothazoline-6-sulfonic acid diammonium salt) in 0.03% H₂O₂-0.05 M citrate buffer (pH 4.0) was added to the wall. Color development was stopped by adding 100 μl/well of 1.5% oxalic acid and the absorbance was measured at 415 nm. The wells were washed three times with 0.05% Tween20-PBS between each step.

Statistical analysis

Results are expressed as means ± S.D. For oral administration experiment, Tukey’s test was used to assess the significant differences between each data. In direct administration of paraquat to lymphocytes, Student’s t test was used to assess the significant differences between each data.

RESULTS AND DISCUSSION

Immunoglobulin productivity of mouse splenocytes cultured in the medium with different concentrations of paraquat is shown in Fig. 1. IgA productivity of lymphocytes was not affected by paraquat. However, IgG productivity was significantly decreased by 10⁻⁸~10⁻⁶ μM paraquat treatment. Effects of different concentrations of paraquat on IgM productivity is of the same trend as those on IgG productivity, expect that at 10⁻⁷~10⁻⁶ μM, IgM productivity was also decreased. On the other hand, no effect was observed in those treated with 10⁻²~10² μM paraquat. These results suggest that paraquat perturbs the productivity of immunoglobulin with dose-dependent manner. On the other hand, human lymphocytes die in the presence of 100 μM of paraquat (Rio and Velez-Pardo, 2008), suggesting that the concentration perturbing immunoglobulin productivity and cell death are not same. More studies are needed to clarify why paraquat perturbs immunoglobulin products were observed in limited concentrations.

It is important to confirm whether the perturbation by paraquat against the productivity of immunoglobulins observed in vitro is also shown in vivo. Next, various concentrations of paraquat were orally administered to mice. Fig. 2 shows the body, spleen and thymus weights of mice. There were no significant differences observed in body, spleen and thymus weights between each group in every period.

IgA level in serum of mice is shown in Fig. 3. After 1 week of administration of paraquat, IgA level increased in group treated with Low and Middle doses of paraquat. After 2-week period, significant differences were not observed compared with the Control group. After 4 weeks, IgA levels were higher in Low and Middle dose groups compared with Control and High dose groups. In contrast, IgG level was not changed by the administration
of paraquat after 1 or 2 weeks of administration. After 4 weeks, IgG level was lower in High dose group compared with Control group, however, significant difference was not observed (Fig. 4). On the other hand, IgM decreased after 2 or 4 weeks of paraquat administration. Especially after 2 weeks, IgM level was significantly decreased in High dose group (Fig. 5). It is very interesting that conspicuous difference was observed after 2 weeks, and more data are required to discuss about this result.

Fig. 6 shows the effect of paraquat on the IgA productivity of splenocytes. In all period of paraquat administration, IgA productivity was higher compared with Control group. This tendency was remarkable on 1 and 4 weeks administration. On the other hand, there were no significant differences in IgG productivity between each group (Fig. 7). While IgM productivity in treatment groups tend to be lower than those of the Control group (Fig. 8).

Some chemicals, for example, water soluble fraction of heavy oil (Nishimoto et al., 2009) has been known to exhibit toxicity to immune function. About herbicides, there have been some reports. Agent Orange, an herbicide used in Vietnam War was also found to be toxic to immune function (Kim et al., 2003). Pentachlorophenol, one kind of herbicide induces humoral immunodeficiencies, which is associated with the increased blood levels of Pentachlorophenol (Daniel et al., 2001). 3,4-dichloropropionaniline is also a herbicide, and suppresses several inflammatory parameters, including TNF-alpha production to suppresses normal macrophage function (Ustyugova et al., 2007). A mixture containing the herbicides 3,4-dichloropropionanilide and 2,4-dichlorophenoxyacetic acid induce the Loss of pre-B and IgM(+) B cells in the bone marrow (de la Rosa et al., 2003). About the effect of paraquat on productivity of IgA IgG and IgM, there has not been enough information. There is some risk that such kinds of chemicals are taken through polluted foods, thus it is important to know the health effect of those chemicals including paraquat, which is one of the widely used herbicides in the world. In this study, we showed for the first time that paraquat perturbs the productivity of IgA IgG and IgM, in vivo and in vitro. This result suggests that it is required to study about the risk of herbicides against the productivity of immunoglobulin to keep the safety of plants.

When direct exposure of mouse splenocytes to paraquat showed no effect on IgA productivity, but IgG and IgM productivity decreased in the presence of 10^-4~10^-8 μM of paraquat. On the other hand, paraquat was orally administrated to mice, IgA productivity increased and IgM productivity decreased. It has been known that paraquat is detected in spleen of rats fed paraquat containing diets (Minakata et al., 1993). And it has been known that ROS reduces IgM productivity of mouse splenocytes (Miyazaki et al., 2001). Hence it might be possible that ROS may mediate paraquat-induced the perturbation of immune function. About serum paraquat concentration, there has been known about a study in mouse. 60 min after the 200 mg/kg body weight of paraquat administration, serum paraquat level was 9.096 ng/ml, and paraquat concentration decreased to about 3,000 ng/ml (Tsuchiya et al., 1989), suggesting that paraquat exist in serum after oral administration and is able to affect to lymphocytes in blood. More studies are still needed to clarify the mecha-
Fig. 2. Effect of oral administration of paraquat on body, thymus and spleen weight in mice. Mice were administered 0 (Control), 0.12 (Low), 1.2 (Middle) and 4.8 (High) mg/kg body weight of paraquat for 1, 2 or 4 weeks, and body, thymus and spleen weights were measured.

Fig. 3. Effect of oral administration of paraquat on serum IgA concentration. Mice were administered 0 (Control), 0.12 (Low), 1.2 (Middle) and 4.8 (High) mg/kg body weight of paraquat for 1, 2 or 4 weeks, and blood samples were collected. Then serum was separated and used for the measurement of serum IgA level.

Fig. 4. Effect of oral administration of paraquat on serum IgG concentration. Mice were administered 0 (Control), 0.12 (Low), 1.2 (Middle) and 4.8 (High) mg/kg body weight of paraquat for 1, 2 or 4 weeks, and blood samples were collected. Then serum was separated and used for the measurement of serum IgG level.
Paraquat perturbs immunoglobulin productivity

![Graph showing serum IgM concentration](image)

**Fig. 5.** Effect of oral administration of paraquat on serum IgM concentration.
Mice were administered 0 (Control), 0.12 (Low), 1.2 (Middle) and 4.8 (High) mg/kg body weight of paraquat for 1, 2 or 4 weeks, and blood samples were collected. Then serum was separated and used for the measurement of serum IgM level. Data not sharing common letter are significantly different at $p < 0.05$.

![Graph showing splenocytes IgA productivity](image)

**Fig. 6.** Effect of oral administration of paraquat on splenocytes IgA productivity.
Mice were administered 0 (Control), 0.12 (Low), 1.2 (Middle) and 4.8 (High) mg/kg body weight of paraquat for 1, 2 or 4 weeks, and splenocytes were collected and cultured. Then the medium was harvested and used for the measurement of IgA productivity.

![Graph showing splenocytes IgG productivity](image)

**Fig. 7.** Effect of oral administration of paraquat on splenocytes IgG productivity
Mice were administered 0 (Control), 0.12 (Low), 1.2 (Middle) and 4.8 (High) mg/kg body weight of paraquat for 1, 2 or 4 weeks, and splenocytes were collected and cultured. Then the medium was harvested and used for the measurement of IgG productivity.
nism of paraquat effects on the immunoglobulin productivity of lymphocytes.

Oral administration of paraquat increased IgA productivity and reduced IgM productivity in mice. IgA inhibits the absorption of antigens and pathogens in small intestine to prevent allergic reaction (Shorter and Tomasi, 1982) thus many studies have been carried out to establish anti-allergic functional foods, such as food oil, (Lim et al., 1996; Yamasaki et al., 1999, 2003) and Vitamin E (Gu et al., 1999; Kaku et al., 1999). These food elements are known to be safe in usual intake. However, the increasing of IgA productivity by paraquat should be treated carefully because of the perturb effect of paraquat. The decrease in IgM productivity by the administration of paraquat shows the risk of exposure to paraquat, implying a compromised immune system.

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