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Aldicarb Suppresses Macrophage but not Natural Killer (NK) Cell-Mediated Cytotoxicity of Tumor Cells

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Aldicarb is a highly effective carbamate pesticide used extensively all over the world. Aldicarb has high acute mammalian toxicity mediating its effect as an acetylcholinesterase inhibitor. Aldicarb has been shown to contaminate drinking water in several states of the United States of America, often exceeding 100 parts per billion (ppb) (Milex and Delfino 1985; Harkin et al 1984). Due to this reason, aldicarb recently became the first pesticide to be regulated by the Environmental Protection Agency (EPA) to protect the drinking water (Sun 1988).

Although aldicarb is highly toxic with an oral LD50 value of 0.9 - 1mg/kg body weight for rats, it has been reported to have no long-term adverse health effects. (U.S. EPA 1984; U.C. Ag. Prod. Co. 1983). However, very little is known about the effect of aldicarb on the immune system. Recent reports suggested that aldicarb administered via drinking water suppressed the B cell responses (Olson et al 1987). In contrast, Thomas et al (1987) found that aldicarb had no significant effect on T and B cell responses to mitogens and on T cells responding in the mixed-lymphocyte culture.

In the present study, we investigated the immunotoxic effects of aldicarb on the capacity of macrophages and natural killer (NK) cells to mediate cytotoxicity against some tumor cells. Since macrophages and NK cells constitute two important natural effector cells involved in defense against cancer, and since the effect of aldicarb on these cell populations has not been investigated before, we initiated these studies. The data presented in this paper suggests that aldicarb suppresses selectively the macrophage-mediated cytotoxicity of tumor cells without altering the cytotoxic functions of NK cells.

MATERIALS AND METHODS

Six to eight week old female C3H mice were obtained from National Cancer Institute, Bethesda, Md. Mice were maintained in a sterile environment (Animal Storage Isolators, Nu Aire Inc., Plymouth, MN).

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Aldicarb was obtained from Chem Service, PA. The stock solution was prepared in phosphate buffered saline (PBS), pH 7.2. The purity and concentration was analyzed by HPLC technique using a Beckman 344 system with a 3μ ultra phase column (4.6 x 7cm). The compound was eluted from the column using 45% Methanol: water solvent with a flow rate of 0.5 ml/min. The compound was detected at 210 nm with a retention time 5.5 ± 0.09 min. With these conditions, the purity of aldicarb was found to be 99.4%. The stock solution was stored a 4°C and used within 3 months during which period the compound was found to be in stable condition. Groups of 5-10 mice were injected intraperitoneally (ip) with 0.1 ml of PBS containing 0.1, 1, 10, 100 or 1000 ppb of aldicarb. The control mice were injected with PBS alone.

Peritoneal exudate cells (PEC) were obtained from the peritoneal cavity of control or aldicarb-treated mice by washing the peritoneal cavity repeatedly with cold PBS, as described elsewhere (Nagarkatti et al 1988). To study the antibody-dependent macrophage-mediated cytotoxicity (ADCC), LSA tumor cells were used as targets. Since LSA tumor cells were thy-1^+, anti-thy-1 antibodies were used at a final dilution of 1:10 for the ADCC assay (Nagarkatti et al 1988).

Single cell suspensions of spleen were prepared using a laboratory blender (Stomacher, Tekmar Co., Cincinnati, OH) in medium RPMI-1640 supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, NY), 10mM HEPES, 1mM glutamine and 40 μg/ml gentamycin sulfate (Seth et al 1988). The red cells were lysed using 0.83% ammonium chloride and the cells were resuspended in approximately 5ml of complete medium after two washings (Seth et al 1988).

LSA, a spontaneous lymphoma syngeneic to C57BL/6 mice and resistant to NK cell-mediated cytotoxicity and YAC-1, a NK-sensitive target were maintained *in vitro* in complete medium as described elsewhere (Nagarkatti et al 1988).

A modification of the ^{51}Cr-release assay as previously described (Nagarkatti & Kaplan 1988; Nagarkatti et al 1988) was used to study the macrophage and NK cell-mediated cytotoxicity. Briefly, varying numbers of effector cells in 0.1 ml were seeded in triplicate, into the wells of 96-well round-bottomed micro-titre plates (Flow Laboratories, Inc., McLean, VA). Tumor targets were labeled with ^{51}Cr by incubating 1 x 10^7 tumor cells in 0.5 ml medium with 20 μCi of sodium chromate (specific activity, 200 to 500 ci/g; New England Nuclear, Boston, MA) at 37°C for I hr. The tumor cells were next washed 4 times and 0.1ml of the ^{51}Cr-labeled tumor targets were added to each well. The plates were incubated for 4 hr and 37°C. After incubation, the supernatants were harvested with the Titertech collecting system (Flow Laboratories, Rockville, MD) and the radioactivity was measured with a gamma counter (Gamma Trac 1191, TM Analytic, Elk Grove, Illinois). Percentage of specific cytotoxicity was calculated using the formula: % specific cytotoxicity = (experimental release - control release)/(total release - control release) x 100. Control release was measured by incubating ^{51}Cr-labeled targets alone. The control release was usually less than 20% of the maximum release. Maximum release was determined by incubating labeled tumor cells with sodium dodecyl sulfate.

In all experiments, PEC or spleen cells from groups of 5-10 aldicarb treated or control mice were pooled. The cytotoxicity assay was performed in triplicate and the mean percent cytotoxicity and standard errors (S. E.) were calculated. The various experimental groups were compared with the control group of mice using student's t-test and any differences with p values less than 0.05 were considered to be statistically significant. All experiments were repeated at least three times with consistent results.

RESULTS AND DISCUSSION

Since aldicarb levels found in the drinking water have been reported to be generally less than 100 ppb, initial studies were conducted to test the effect of aldicarb on macrophage and NK cell-mediated cytotoxicity at doses of less than 10 ppb. Initial studies were restricted to short term toxicity of aldicarb in which groups of 5-10 mice were injected ip with 0.01, 0.1, 1 or 10 ppb of aldicarb everyday for 7 days. Control mice received PBS for seven days. Twenty-four hours later mice were sacrificed and the macrophage-mediated antibody-dependent cytotoxicity was studied. The data shown in Fig. 1 suggested that at all effector:target (E:T) ratios tested, macrophages from mice treated with 0.01 to 10 ppb, all demonstrated a decreased cytotoxicity against LSA tumor cells when compared to the cytotoxicity observed with control group of mice ($p < 0.05$; all aldicarb treated groups versus control). The percent decrease in cytotoxicity in aldicarb treated groups ranged from 64-100% when compared to the control responses.

We next investigated the effect of aldicarb on NK cell-mediated cytoxicity. Mice were injected with different doses of aldicarb or PBS (control) for seven days as described above and 24 hr later, NK cell- mediated cytotoxicity was studied using NK-sensitive YAC-1 targets. The data shown in Fig. 2 suggested that aldicarb failed to alter the NK cell- mediated cytotoxicity at all doses tested. In contrast to the macrophage mediated cytotoxicity which was severely suppressed in aldicarb treated mice at 0.01 to 10 ppb (Fig 1), the NK-cell-mediated cytotoxicity was not altered at these doses, and furthermore, increasing the aldicarb doses to 100 or 1000 ppb, still failed to bring about any change in the NK activity.

When acute toxicity testing was performed using a single injection of aldicarb at 0.1 to 1000 ppb and the macrophage-mediated cytotoxicity analyzed 24 hr later, it was observed that all doses of aldicarb from 0.1 to 1000 ppb inhibited the macrophage mediated killing significantly at all E:T ratios ($p < 0.05$; aldicarb treated groups versus respective controls). However, at lower E:T ratios of 25:1, the percent decrease in cytotoxicity in aldicarb treated mice appeared to be more than the decrease observed at higher E:T ratios of 100:1. Also, at lower E:T ratio, the percent decrease was similar in all aldicarb treated groups (approximately 33%) when compared to the decrease seen at higher E:T ratio of 100:1, where, % suppression ranged from 40% (0.1ppb) to 11% (10ppb). It should be noted that at 100:1 E:T ratio, although the decrease in cytotoxicity was very little in magnitude (for example at 10 ppb of aldicarb, the % decrease was only 11%), it was statistically significant ($p = 0.019$). In summary, following single dose treatment with aldicarb, the % suppression in macrophage-mediated cytotoxicity was to a lesser extent (Fig. 3) than when multiple doses of aldicarb were administered (Fig. 1).

Aldicarb, a carbamate pesticide, has been used extensively throughout the world. It has one of the highest acute mammalian toxicites when compared

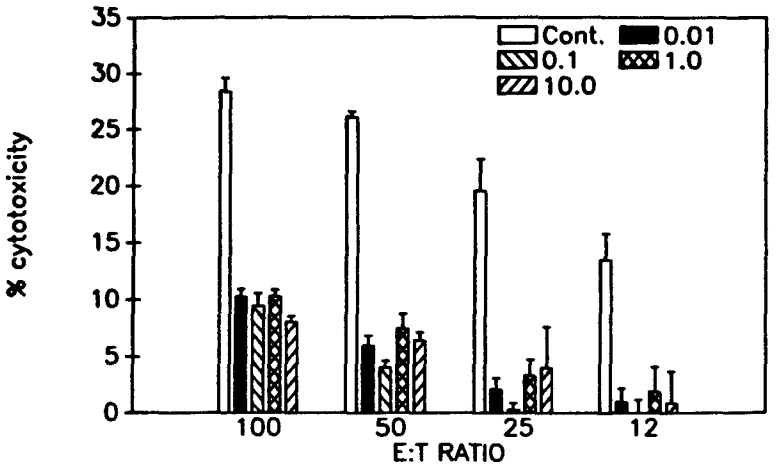

Figure 1. Antibody-dependent macrophage-mediated cytotoxicity in mice treated with aldicarb. Groups of 10 C3H mice were injected either with PBS (control) or with 0.01, 0.1, 1, or 10 ppb of aldicarb ip daily for 7 days. Twenty four hours later, PEC (effector cells) were collected, mixed with antibody-coated ^{51}Cr-labeled LSA tumor targets at different effector cell:target cell (E:T) ratios. The percent cytotoxicity was calculated by measuring the amount of ^{51}Cr-released as described in methods. The vertical bars represent mean percent cytotoxicity ± S.E.

to other pesticides and it leaches easily into ground water, thereby contaminating the drinking water. Aldicarb has been responsible for the largest recorded North American outbreak of foodborne pesticide illness (MMWR 1986). Although, recently, some studies have reported the toxic effects of aldicarb (MMWR 1986; DePass et al 1985; Gonzales and Matos 1987; Pant et al 1987), there is insufficient data assessing the effect of aldicarb on the immune system. Such studies are highly essential because any alterations in the immune system can lead to increased susceptibility to infections, development of autoimmune disorders and increased susceptibility to cancer (Nagarkatti and Nagarkatti 1987).

In the present study we investigated the effect of aldicarb on macrophage-and NK-mediated killing of tumor cells and found that aldicarb could suppress the macrophage-mediated cytotoxicity but not the NK mediated cytotoxicity. The effect on macrophages was more prominent during short-term toxicity testing, when, mice received 7 daily injections of 0.01 to 10 ppb of aldicarb (Fig 1). When tested for acute toxicity, with single injection of aldicarb at 0.1 to 1000 ppb, at lower E:T ratios, the macrophage responses were significantly decreased. However, using higher E:T ratios, although 0.1 ppb of aldicarb could still induce significant inhibition, doses above 1ppb, caused minimal inhibition of the responses (Fig 2). The differences observed at lower and higher E:T ratios can be explained by the fact that when large number of effector cells were used, there were optimal numbers of effectors in aldicarb treated groups to cause cytotoxicity comparable to that seen in controls. It is interesting to note that at higher E:T ratios, the suppression was more prominent with the lowest dose of aldicarb tested when compared to the higher doses. Recently, a similar inverse dose - dependent suppression in the splenic plaque forming cell responses were reported in aldicarb treated mice.

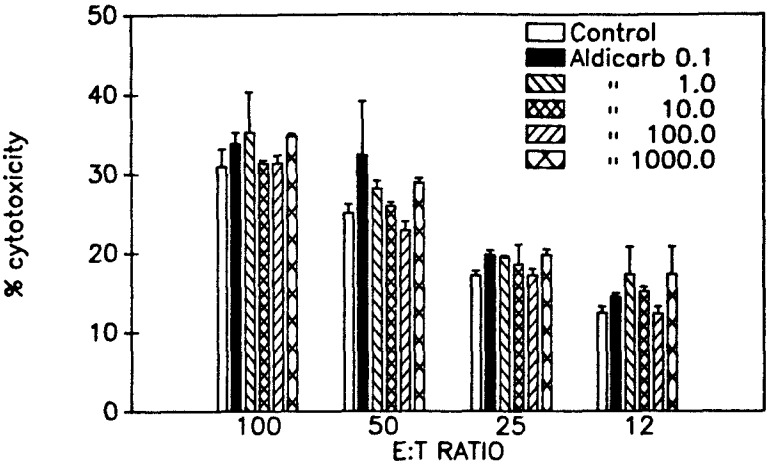

Figure 2. NK cell-mediated cytotoxicity in aldicarb treated mice. Groups of 5 C3H mice were injected either with PBS (control) or with 0.1, 1, 10, 100 or 1000 ppb of aldicarb ip daily for 7 days. Twenty-four hours later, spleens were harvested, pooled and tested for cytotoxicity against ^{51}Cr-labeled YAC-1 tumor cell targets. Varying numbers of spleen cells (effector cells) were mixed with a constant number of tumor cells (target cells) to achieve effector:target (E:T) cell ratios from 100 to 25. The percent cytotoxicity was calculated as described in Fig 1. The vertical bars represent mean percent cytotoxicity ± S.E.

Although the reason for this phenomenon observed were not clear (Olson et al 1987). Recently, there have been two reports on the immunomodulating activities of aldicarb. In the first study, it was demonstrated that aldicarb suppressed the B cell responses to sheep red blood cells. In contrast, Thomas et al (1987) reported that aldicarb at doses of 0.1 to 1000 ppb had no effect on B cell responses to sheep erythrocytes. In addition, these authors found no change in the capacity of B and T lymphocytes to respond to mitogens, and in the gross and histopathologic examination of thymus, spleen, and lymph nodes. The reason for the discrepancy in the above two studies is not clear. One possible reason could be that in the former study outbred white mice were used, while, in the latter study, B6C3F1 mice were employed. This may not be uncommon since, we have observed mouse-strain differences, in the susceptibility to immunotoxic effects of dioxin (Nagarkatti et al 1984).

In the present study we observed a significant effect on macrophages especially in mice receiving 7 daily doses of 0.1 to 10 ppb of aldicarb. The mechanism by which aldicarb suppresses the macrophage-mediated cytotoxicity is not clear and needs further investigation. The extracellular mechanism by which macrophages cause lysis of tumor targets has been suggested to be due to the production of thymidine, toxic oxygen metabolites, arginase, proteases, complement components, tumor necrosis factor, etc. (Nagarkatti et al 1988). The relative importance of any of these factors appears to be a function of the method of macrophage activation and the cytotoxic system used. Further studies are essential to delineate whether aldicarb affects the production and release of extracellular factors produced by the macrophages which cause tumor cell-lysis.

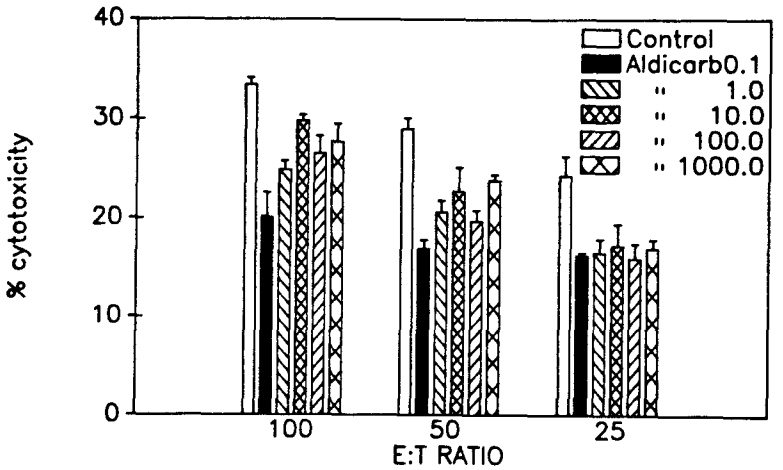

Figure 3. Antibody-dependent macrophage-mediated cytotoxicity in mice treated with a single dose of aldicarb. Groups of 10 C3H mice were injected with either PBS (control) or with a single dose of 0.1, 1, 10, 100 or 1000 ppb of aldicarb. Twenty four later, the cytotoxicity assay was performed as described in fig 1. The vertical bars represent mean percent cytotoxicity ± S.E.

In the present study we observed that aldicarb affected selectively, the macrophage-mediated cytotoxicity but not the NK cell-mediated cytotoxicity. Several lines of evidence suggest that NK and cytotoxic T cells may share basically a similar recognition, binding and lytic mechanisms (Henkart 1987), which may be different from the one used by activated macrophages. Thus isolated cytoplasmic granules from NK or cytotoxic T cells have similar cytolytic properties (Henkart 1987) and differ from the extracellular mechanism by which macrophages mediate lysis of tumor cells, such as, through reactive oxygen intermediates. In a previous study, we observed that treatment of tumor cells with an anti-cancer drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) affected only the macrophage-mediated cytotoxicity but not that of NK cells. (Nagarkatti et al 1988).

In summary, the present study suggests that aldicarb when administered in low doses, similar to those found in contaminated ground water, suppresses the macrophage-mediated killing of tumor cells without affecting the NK cell-mediated lysis. Since, activated cytotoxic macrophages play an important role in defense against neoplasia and infections, our studies suggest the possible health risks involved following consumption of water, vegetables or fruits contaminated with aldicarb.

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REFERENCES

De Pass L R,Weaver E V, Mirro E J (1985) Aldicarb sulfoxide/Aldicarb sulfone mixture in drinking water of rats: effects on growth and acetylchloinesterase activity. J. Toxicol. Environ. Health. 16: 163-172.

Gonzalez C,Matos E (1987) Chromosomal aberrations in cultured human lymphocytes treated with aldicarb, a carbamate pesticide. Mutation Res. 191: 99-103.

Harkin J M, Jones F A,Fathulla R, Dzantor E K, O'Neill F J (1984) Pesticides in ground water beneath the central sands plains of Wisconsin. Wisconsin Water Resources Center, Technical Report. WIS WRC 83-01: 3, 24, 26, 42-43.

Henkart P A (1985) Mechanism of lymphocyte-mediated cytotoxicity. Ann. Rev. Immunol. 3: 31-58.

Milex C J, Delfino J J (1985) Fate of aldicarb, aldicarb sulfoxide and aldicarb sulfone in Floridan ground water. J. Agricult. Food Chem., 33: 455-460.

Morbidity and Mortality Weekly Report (1986) Aldicarb food poisoning from contaminated melons - California. 35: 175-176.

Nagarkatti P S, Sweeny G D, Gauldie J, and Clark D A (1984) Sensitivity to suppression of cytotoxic T cell generation by 2, 3, 7, 8 - tetrachlorodibenzo-p-dioxin (TCDD) is dependent on the Ah genotype of the murine host. Toxicol. Appl. Pharmacol. 72: 169-176.

Nagarkatti P S, Nagarkatti M (1987) Immunotoxicology: Modulation of the Immune system by xenobiotics. Def. Sci. J. 37 (1987) 235-244.

Nagarkatti M, Kaplan A M (1988) The role of suppressor T cells in BCNU-mediated rejection of a syngeneic tumor. J. Immunol. 135: 1510-1517.

Nagarkatti M, Nagarkatti P S, Kaplan A M (1988) Differential effects of BCNU on T cell, macrophage, NK and lymphokine activated killer cell activity in mice bearing a syngeneic tumor. Cancer Immunol. Immunotherapy. 27: 38-46.

Olson L J, Erickson B J, Hinsdill R D, Wyman J A, Porter W A, Binning L K, Bidgood R C, Nordheim E V (1987) Aldicarb immunomodulation in mice: An inverse dose - response to parts per billion levels in drinking water. Arch. Envrion. Contamin. Toxicol. 16: 433-439.

Pant J, Tewavir M, Gill T S (1987) Effect of aldicarb on the blood and tissues of a freshwater fish. Bull. Environ. Contamin. Toxicol. 38: 36-41.

Seth A, Pyle R H, Nagarkatti M, Nagarkatti P S (1988) Expression of the J11d marker on peripheral lymphocytes of MRL -1pr/lpr mice. J. Immunol. 141: 1120-1125.

Sun M (1988) EPA proposes state action on aldicarb. Science, 241: 22.

Thomas P T, Ratajczak H V, Eisenberg W C, Furedi-Machacek M, Ketels K V, Barbera P W (1987) Evaluation of host resistance and immunity in mice exposed to the carbamate pesticide aldicarb. Fundamental Appl. Toxicol. 9: 82-89.

Union Carbide Agricultural Products Company, Inc (1983) Research Triangle Park, NC. Temik Aldicarb Pesticide: A Scientific Assessment 44-57.

U.S. Environmental Protection Agency (1984) Pesticide Fact Sheet. No. 19: 1-12.

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