Health, 2015, 7, 830-837
Published Online July 2015 in SciRes. http://www.scirp.org/journal/health
http://dx.doi.org/10.4236/health.2015.77098

Effect of Aldicarb Exposure on Cellular Immunity and Antioxidant Capacity in Kunming Mice

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Received 1 June 2015; accepted 11 July 2015; published 14 July 2015

Abstract

Immune function and antioxidant defense play an important role in protecting animals against pathogens and in controlling oxidative stress, respectively. Aldicarb is of great concern for human health due to its toxic nature, its extensive usage and consequent pollution. The hypothesis that aldicarb exposure would suppress immune function and antioxidant capacity in Kunming mice was to be tested in the present study. Twenty-three adult male mice were randomly divided into the control (n = 11) and the aldicarb treated (n = 12) groups. Food and water were provided *ad libtum* for both groups, while the aldicarb treated mice drank aldicarb solution (0.097 mg/L) for 22 days. Cellular immunity assessed by phytohaemagglutinin (PHA) response did not differ between the control and the aldicarb treated groups. Similarly, white blood cells were not influenced by aldicarb treatment. Moreover, aldicarb exposure had not significant effect on body mass, all organ masses detected. However, aldicarb treatment suppressed total antioxidant capacity in liver but not in kidneys. In summary, aldicarb treatment did not affect immune function, but suppressed liver antioxidant capacity in Kunming mice.

Keywords

Aldicarb, Antioxidant Capacity, Kunming Mice, Phytohaemagglutinin Response

1. Introduction

Pesticides are hazardous pollutants which are abundant in soil, water, atmosphere and agricultural products. Be-

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How to cite this paper: Tian, Y.F., Liu, Y.M., Zhou, Y.Y., Liu, Y.P., Lü, G.L., Zheng, X.X. and Xu, D.L. (2015) Effect of Aldicarb Exposure on Cellular Immunity and Antioxidant Capacity in Kunming Mice. *Health*, **7**, 830-837.
http://dx.doi.org/10.4236/health.2015.77098

cause of their toxic nature, their extensive usage and consequent pollution, great environmental concerns have developed [1]. Aldicarb, an *N*-methyl carbamate pesticide, has many hazardous effects on humans and animals [2] [3]. In the past decades, control actions to ban or severely restrict aldicarb use have been achieved by Europe and other countries due to its toxic effects [4]. However, aldicarb is still used in agriculture in some developing countries including China [5] [6]. Thus, aldicarb is of great concern to human health because of its highly toxic effects.

The immune system defends human and animals against environmental pathogens, which plays an important role in maintaining health [7]. Some researchers have investigated the impact of aldicarb on immune function in human and animals. For example, Fiore *et al.* (1986) assessed the effects of chronic ingestion of low-level aldicarb-contaminated groundwater on the immune function of humans and found that abnormalities in T-cell subsets were related with the consumption of aldicarb-contaminated groundwater in women [8]. A follow-up study showed that changes in T-lymphocyte distribution were associated with ingestion of aldicarb-contaminated drinking water [9]. Moreover, Dean *et al.* (1990) found that aldicarb could inhibit the stimulatory activity of macrophages without affecting the T-cell responses in the syngeneic mixed lymphocyte reaction [10]. However, some other investigators got different results. For instance, aldicarb exposure had no significant effect on the ability of splenic natural killer cells, the percentages and absolute numbers of total T-cells, T-suppressor, T-helper, and B-cells in female B6C3F1 mice [11]. Many immunological parameters such as humoral, cellular and nonspecific immunity in mice were also not affected by chronic low level of aldicarb exposure [12]. Therefore, further researches are needed to clarify these discrepancies.

Phytohaemagglutinin (PHA) response has been used to assess mammalian cellular immunity, which belongs to adaptive immune system [14] [15]. Thymus is responsible for primary T cell development [16], and a larger spleen represents stronger immunity [17]. Total white blood cells (or leukocytes, WBC) are also used to evaluate the overall health [7].

Oxidative stress commonly defined as the imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the organism is deleterious to the structure and function of the cell and tissue, which is widely believed to be involved in many diseases [18] [19]. Therefore, antioxidant capacity prioritizing self-maintenance in animals plays a key role in maintaining their health [20].

In the present study, the hypothesis that aldicarb would have great influence on immune function and total antioxidant capacity in Kunming mice was tested. We expected that cellular immunity, thymus and spleen mass, white blood cells and total antioxidant capacity in liver and kidneys would be suppressed in aldicarb treated mice compared with the controls. The purpose of this study was to evaluated whether aldicarb exerted influences on immunity and antioxidant capacity in mice. We adopted the integrative research method including the morphological and biochemical method. We found that different parameters responded differently to adicarb exposure. These finding had implications for human health.

2. Materials and Methods

2.1. Animals and Experimental Design

All animal procedures were carried out according to the Institutional Animal Care and Use Committee of Qufu Normal University. Male Kunming mice (age: 2 months) used in this study were obtained from the Animal Breeding Center in Lukang Pharmaceutical Group Co., Ltd. of Shangdong province. The experiment was carried out from March 8 to April 4 in 2014. Mice were housed individually in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding. The raising conditions are semi-natural and the photoperiod was natural light. Animals had free access to water and food (Standard rat pellets chow, provided by Animal Breeding Center in Lukang Pharmaceutical Group Co., Ltd. of Shangdong province). After body mass stabilized, 23 mice were randomly divided into the control group (n = 11) and the aldicarb (manufactured by Shandong Huayang Technology Co., Ltd) treated group (n = 12) in which each mouse drank aldicarb solution (0.097 mg/L). The reason we chose this drank aldicarb solution concentration was that the content of aldicarb in one batch of gingers in Guangdong Jiangnan Fruit and Vegetable Wholesale Market was 0.097 mg/kg (http://baike.baidu.com/view/5560243.htm). The residue content of aldicarb in these gingers has exceeded that of the maximum residue content of aldicarb in vegetables (0.03 mg/kg) according to the “National food safety standard-Maximum residue limits for pesticides in food” (GB2763-2012) in China. The period of the experiment was 22 days. Day 0 and day n represented initial day and n days of treatment, respectively.

2.2. Organ Index

Organs were measured as described previously [21]. In brief, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, testes, epididymis, seminal vesicals and the digestive organs with contents (*i.e.*, stomach, small intestine, caecum and colon) were dissected and weighed (±1 mg). The stomach, small intestine, caecum and colon were rinsed with saline to eliminate all the gut contents, before being weighed.

2.3. White Blood Cells Assays

At the end of the experiment, after collecting trunk blood, 20 μL whole blood was diluted immediately in 0.38 ml solution containing 1.5% glacial acetic acid, 1% crystal violet (Sigma) and the leukocytes were counted in an improved Neubauer chamber using microscope. The total number of WBC was determined by counting all leucocytes in the four corner large-squares of the Neubauer chamber, and multiplying the raw data by 5×10^7 to obtain the final values (10^9 cells/L) [22].

2.4. Antioxidant Capacity Assays

Liver and kidneys were homogenized using ice-cold 0.9% NaCl solution. The homogenates were centrifuged at 3000 rpm for 20 min and the supernatant was taken for the later assay. Total antioxidant capacity (T-AOC) and protein content in liver and kidney was measured using kits (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions. One unit of T-AOC was defined as the extent to which optical density is increased by 0.01 per milligram protein per minute.

2.5. Cellular Immunity Assays

PHA response indicative of cellular immunity was evaluated as described previously [15] [21]. Specifically, mice in the control and aldicarb treated groups on day 19 were caught, then we measured their footpad thickness of the left hind foot with a micrometer (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547 - 301, Japan) to ± 0.01 mm. Immediately thereafter, mice in both groups were injected subcutaneously 0.1 mg of PHA (PHA-P, Sigma L-8754) dissolved in 0.03 ml of sterile saline (pH7.4) in the middle of the footpad. After 6 h, 12 h, 24 h, 48 h and 72 h injection, we measured footpad thickness. The PHA response (*i.e.*, cellular immunity) was calculated as the difference between pre- and post-injection measurements divided by initial footpad thickness (PHA response = (post PHA – pre PHA)/pre PHA). Six measures of footpad thickness were taken to obtain the value of each mouse [21].

2.6. Statistical Analysis

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively. The ratio values such as PHA response were subjected to arcsine transformation. The differences of body mass between the control and aldicarb treated groups were analyzed by independent-samples t-test. Group differences in wet organ mass with body mass as the covariate were analyzed by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests. Group differences in other parameters (PHA response, WBC, T-AOC) were analyzed by independent-samples t-test. Results were expressed as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Body Mass

On day 0, body mass between the control and aldicarb treated groups was not different ($t = -0.185$, $df = 21$, $P = 0.855$). There was no difference of body mass between these two groups from day 1 ($t = 0.189$, $df = 21$, $P = 0.852$) to day 22 ($t = -0.016$, $df = 21$, $P = 0.987$) (**Figure 1**).

3.2. Organs

Aldicarb treatment had no significant effect on the masses of heart, lungs, liver, kidneys, stomach, small intestine, caecum, colon, testes, epididymis, seminal vesicals and immune organs including thymus and spleen (**Table 1**).

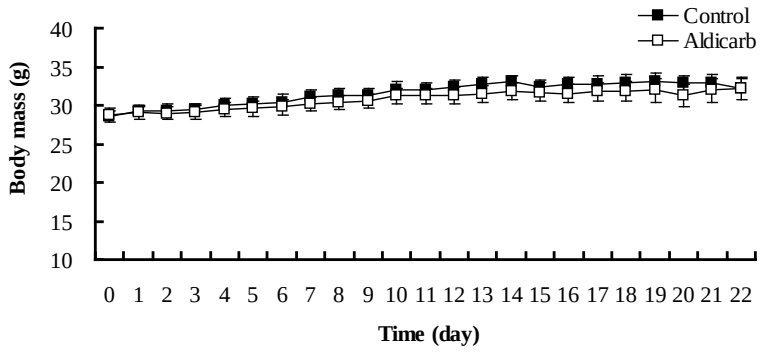

Figure 1. Changes of body mass in mice during aldicarb treatment. Values are means ± SE. Body mass on day 0 between the control and aldicarb treated groups did not differ significantly.

Table 1. Effect of aldicrab on wet organ mass in Kunming mice.

Parameters	Control	Aldicarb	Statistical summary	
Sample size	**11**	**12**	**$F_{1,20}$**	**P**
Heart (g)	0.161 ± 0.007	0.174 ± 0.007	1.685	0.209
Lungs (g)	0.282 ± 0.030	0.315 ± 0.029	0.617	0.441
Thymus (g)	0.057 ± 0.005	0.043 ± 0.005	3.985	0.060
Liver (g)	1.664 ± 0.059	1.650 ± 0.056	0.031	0.863
Spleen (g)	0.106 ± 0.014	0.128 ± 0.013	1.261	0.275
Kidneys (g)	0.471 ± 0.022	0.484 ± 0.021	0.166	0.688
Stomach with contents (g)	0.559 ± 0.074	0.555 ± 0.071	0.002	0.969
Stomach (g)	0.232 ± 0.015	0.215 ± 0.014	0.754	0.396
Small intestine with contents (g)	2.242 ± 0.122	2.201 ± 0.117	0.058	0.812
Small intestine (g)	1.483 ± 0.110	1.315 ± 0.106	1.220	0.282
Small intestine length (cm)	65.500 ± 1.785	64.333 ± 1.709	0.223	0.642
Caecum with contents (g)	0.568 ± 0.030	0.613 ± 0.029	1.149	0.297
Caecum (g)	0.200 ± 0.018	0.162 ± 0.018	2.224	0.151
Caecum length (cm)	3.455 ± 0.135	3.141 ± 0.130	2.799	0.110
Colon with contents (g)	0.549 ± 0.050	0.613 ± 0.048	0.858	0.365
Colon (g)	0.285 ± 0.015	0.317 ± 0.014	2.429	0.135
Colon length (cm)	9.455 ± 0.420	9.799 ± 0.402	0.350	0.561
Total digestive tract (g)	1.969 ± 0.124	1.794 ± 0.119	1.038	0.320
Total digestive tract length (cm)	78.410 ± 1.910	77.274 ± 1.828	0.185	0.672
Testes (g)	0.219 ± 0.012	0.197 ± 0.011	1.866	0.187
Epididymis（g)	0.051 ± 0.010	0.034 ± 0.009	1.580	0.223
Seminal vesical (g)	0.161 ± 0.016	0.161 ± 0.015	0.001	0.997

Values are means ± SE. Values for a specific parameter that share different superscripts are significantly different at $P < 0.05$, determined by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests with body mass as the covariate.

Lengh of small intestine, caecum, colon was also not influenced by aldicarb treatment (**Table 1**).

3.3. White Blood Cells

Aldicarb exposure had no significant influence on white blood cells ($t = 0.222$, $df = 21$, $P = 0.827$) (**Figure 2**).

3.4. Total Antioxidant Capacity

Aldicarb exposure suppressed total antioxidant capacity (T-AOC) in liver ($t = 6.451$, $df = 21$, $P < 0.001$) (**Figure 3(a)**) but not in kidneys ($t = 0.293$, $df = 21$, $P = 0.772$) (**Figure 3(b)**).

3.5. Cellular Immune Response

PHA response in the control and the aldicarb treated group did not differ after 6 h ($t = 1.133$, $df = 21$, $P = 0.270$), 12 h ($t = -0.549$, $df = 21$, $P = 0.589$), 24 h ($t = -0.398$, $df = 21$, $P = 0.695$), 48 h ($t = 0.651$, $df = 21$, $P = 0.522$), 72 h ($t = 1.804$, $df = 21$, $P = 0.086$) of PHA injection (**Figure 4**).

4. Discussion

Contrary to our expectation, cellular immunity, thymus and spleen mass and white blood cells were all not affected by aldicarb treatment in Kunming mice. However, aldicarb exposure suppressed total antioxidant capacity in liver but not in kidneys in mice.

4.1. Immunity and Aldicarb

Our findings that cellular immunity, thymus and spleen mass and white blood cells were not response to aldicarb exposure were consistent with other researches in which many immunological parameters including humoral,

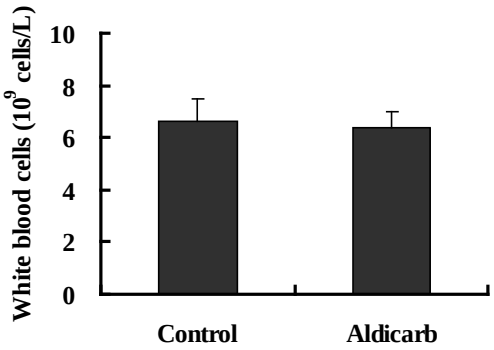

Figure 2. Effect of aldicarb treatment on white blood cells in mice. Values are means ± SE. WBC did not differ between the control and the aldicarb treated groups.

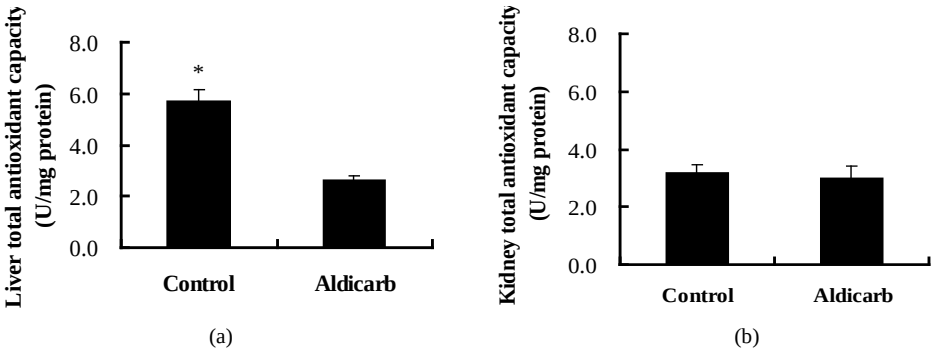

Figure 3. Effect of aldicarb treatment on total antioxidant capacity in liver (a) and kidneys (b) in mice. Values are means ± SE. An asterisk (*) indicates statistical differences at $P < 0.05$.

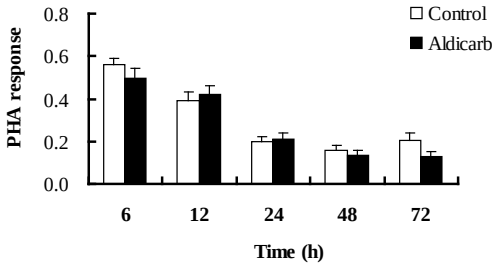

Figure 4. Effect of aldicarb treatment on PHA response in mice. Values are means ± SE.

cellular and nonspecific immunity in mice [11]-[13]. The reason might be due to the mice used in our and other studies were adult animals. Generally, juvenile animals are often more sensitive to the toxic effects of aldicarb [23]. The absence of significant effects on any of these parameters implies that aldicarb at low exposure concentrations did not impair immune function in rodents. However, our results disagreed with other findings in which abnormalities in T-cell subsets were associated with the consumption of aldicarb-contaminated groundwater in women [8] [9]. Moreover, the stimulatory activity of macrophages was inhibited by aldicarb exposure [10]. The discrepancies in different researches might be due to the differences in subjects investigated, the treatment mode and the immune parameters measured.

4.2. Antioxidant Capacity and Aldicarb

In the present study, aldicarb exposure decreased total antioxidant capacity in liver in Kunming mice. This result agreed with other research, in which aldicarb exposure induced a significant decrease in antioxidant capacity such as the glutathione reductase, the glutathione peroxidase and the glutathione S-transferase activities in Chinese Hamster Ovary (CHO-K1) cells [24]. However, total antioxidant capacity in kidney was not affected by aldicarb treatment, implying that effects of aldicarb exposure on antioxidant capacity were tissues specific. In addition, aldicarb increased malondialdehyde (MDA) production indicative of lipid peroxidation in CHO-K1 cells [24]. Yarsan *et al.* (1999) also found that high doses of aldicarb stimulated lipid peroxidation in a mammalian test species after subacute, subchronic and chronic expositions. Lipid peroxidation in liver and kidneys were not detected in our study. Thus further researches were required to clarify whether oxidative stress such as lipid peroxidation occurred in liver and kidneys and other organs after aldicarb exposure.

4.3. Body Composition and Aldicarb

Body mass and all organ masses detected including heart, lungs, liver, kidneys, stomach, small intestine, caecum, colon, testes, epididymis, seminal vesicals, thymus and spleen indicated that aldicarb exposure had no significant effect on body composition. Aldicarb treatment had no significant influence on lengh of small intestine, caecum, colon and contents with stomath, small intestine, caecum, colon, implying that the digestive capacity in mice was not affected by aldicarb exposure.

5. Conclusion

In summary, aldicarb exposure had no significant effect on immunological parameters including cellular immune response, thymus, spleen and white blood cells, kidney total antioxidant capacity, body mass and many organ masses detected in Kunming mice. However, aldicarb exposure suppressed total antioxidant capacity in liver in mice. Taken together, aldicarb exposure exerts different impact on different biological processes.

Acknowledgements

We are grateful for the detailed comments and suggestions provided by each of the reviewers. The present study was supported by grants from the National Natural Science Foundation of China (No. 31370427), Natural Science Foundation of Shandong province (ZR2013CM019) and Undergraduate Scientific Research and Training Program of Qufu Normal University (201410446004; 2013A050).

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