Genotoxicity of Dioxin and its Effect on the Immune Response of Goats Vaccinated with Brucella Melitensis Vaccine

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Abstract: The few cytogenetic studies performed in both humans and animals exposed in vivo or in vitro to dioxin reported conflicting data with or without chromosome damage. Therefore the aim of this work was to fulfill the genotoxicity of dioxin and its effect on immune response of sheep vaccinated with Brucella melitensis Rev.1 vaccine . A total number of twenty female goats were divided into four groups, each group consisted of 5 animals. The first group kept as control till the end of experimental period after 3 weeks post-treated. The second group was vaccinated with the Brucella Rev 1 vaccine. The third group was given an oral dose of 4 ml of stock standard solution of dioxin for 3 successive days. The fourth group was vaccinated with the Brucella Rev 1 vaccine and then given after that the dose of dioxin for each animal for 3 successive days. Blood samples were collected for detection of micronucleus, chromosome aberrations and Brucella antibodies titer. Both cytogenetic tests gave clear indications of high levels of chromosome damage in the dioxin treated group and dioxin vaccinated group compared with the control. Serological tests revealed decreased level of antibodies titer by both Tube agglutination test (TAT) and Mercaptoethanol test (MET) in vaccinated animals plus dioxin. In conclusion, dioxin may induce chromosome damage and lower the immune response of goats vaccinated with Rev.1 vaccine. The percentage of micronuclei and chromosomal aberrations decreased after vaccination with Rev.1 vaccine plus dioxin compared with dioxin alone. [Nature and Science. 2009;7(12):15-21]. (ISSN: 1545-0740).

Key words: Dioxin, Micronucleus, Chromosome aberrations, Rev.1 vaccine.

1. Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), commonly known as "dioxin" is formed as a byproduct in the manufacture or combustion of materials made of chlorinated phenols. It is considered to be one of the most potent man-made toxicants and is the prototype for a large class of halogenated aromatic hydrocarbons. Because of the demonstrated toxicity of TCDD and its environmental persistence, dioxin is considered to be a potential hazard to human health (Hays and Aylward, 2003). The toxicity of TCDD has been well characterized: exposure causes generalized wasting syndrome, involution of the thymus, hepatic toxicity, gastric lesions, sperm toxicity, tumor promotion, teratogenicity and embryocytotoxicity (Mimura and Fujii-Kuriyama, 2003, Fisher et al., 2005 and Fouzy et al., 2007).

Cytogenetic studies of persons living within dioxin-contaminated territories occupationally exposed to dioxin resulted in contradictory data (ATSDR, 1998, Zhurkov et al., 1987, Revazova et al., 2001, Iannuzzi et al., 2004 and Baccarelli et al., 2006). The micronucleus (MN) assay and chromosome aberration have been commonly used as a predictor of genotoxicity (Moore et al., 1995). The micronucleus test is widely employed in different areas in biological monitoring. It has become a tool to evaluate the mutagenic effect of drugs before they are commercialized (Masjedi et al., 2000 and Othman and Ahmed, 2004). Moreover, micronuclei have been shown to be a sensitive measure of chromosome damaging effect of environmental pollution (Amer et al., 1997).

Brucellosis is an endemic zoonotic disease in many parts of the world, notably in Mediterranean countries and the Middle East. The Brucella vaccine is considered the only practical method for controlling and eradicating of Brucella infection in small ruminants (Stournara et al., 2007). Since first developed in the mid-1950s, the Brucella melitensis vaccine strain Rev.1 has been used worldwide and its significant value in protecting sheep and goats in endemic areas was recognized (Banai, 2002). Suppression of primary humoral immune responses is one of the most sensitive sequela associated with exposure to TCDD, a ubiquitous environmental contaminant. This suppression

is characterized by a striking reduction in plasma cell formation and immunoglobulin M (IgM) secretion, and is mediated through a direct effect by TCDD on B cells (Holsapple et al., 1986; Sulentic et al., 1998). Previous studies in mice and B cell lines that differ in AHR (aryl hydrocarbon receptor) expression demonstrated the involvement of AHR in the suppression of humoral immune responses (Vecchi et al., 1983; Kerkvliet et al., 1990; Sulentic et al., 1998; Sulentic et al., 2000).

The impairment of the functional outcome of B cell differentiation, (*i.e.,* IgM secretion) by TCDD was previously shown to occur at TCDD concentrations that only modestly suppressed B cell proliferation, giving rise to the notion that TCDD impairs terminal B cell differentiation (Holsapple et al., 1986; Luster et al., 1988). However, little is known about the mechanism by which TCDD-mediated suppression of B cell differentiation occurs, and what other aspects of B cell differentiation, besides the IgM response, are impacted by TCDD treatment. In previous studies TCDD treatment of LPS-activated CH12.LX cells was shown to markedly reduce the mRNA levels of IgH, Igκ and IgJ as well as protein levels of XBP-1 **(**Yoo et al., 2004).

Although 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to influence immune responses, the effects of low-dose TCDD on the development of autoimmunity are unclear **(**Ishimaru et al., 2009). Therefore the aim of this work was to fulfill the genotoxicity of dioxin and its effect on immune response of sheep vaccinated with Brucella melitensis Rev.1 vaccine.

2. Materials and methods

2.1. Animals

Twenty mature female baladi goats (over 3 years old and about 30 kg live body weight) were used. Animals were kept under the routine mangemental system and fed on commercial concentrate mixture with rice straw and barseem *ad libitum*.

2.2. Dioxin Standard

The stock standard solution contained Pg WHO- TEQ (PCDD/PCDFS) of 17 congeners labelled with C13 and 17 native congeners at equal preparation. Total is 57.7826 Pg WHO. It was obtained from Freiburg, Germany (Rainer, 2002).

2.3. Brucella melitensis Rev.1 vaccine

Live attenuated Brucella melitensis strain. The recommended dose was 1x 10^9 colony forming units (cfu). It was obtained from Meral, Lyon, France.

2.4. Experimental Design

Goats were divided into four groups, each group consisted of 5 animals.

- The first group was kept as a control group till the end of experimental period lasted for 3 weeks post-treatment.
- The second group was vaccinated with the Brucella Rev 1 vaccine (2 ml for each animal).
- The third group was given an oral dose of 4 ml of stock standard solution of dioxin diluted with 5 ml distilled water , The amounts of stock standard solution of dioxin given to the goats were 6.9 µg which represent 0.23 µg /body weight and equal (1/3 of LD50) for guinea-pig (0.6 µg/kg body weight), Kociba et al. (1978) for 3 successive days .
- The fourth group was vaccinated with the Brucella Rev 1 vaccine and then given after that the dose of dioxin for each animal (4 ml of stock standard solution of dioxin diluted with 5 ml distilled water) for 3 successive days.

2..5. The in vitro micronuclei (MN) test

Blood samples were collected in vials containing heparin as anticoagulant. The in vitro micronuclei test with goat peripheral blood lymphocytes was carried out according to Fenech and Morley (1985). Whole blood cultures from the four groups were set up by adding 0.4 ml whole blood to 5 ml culture medium consisting of RPMI 1640 supplemented with 15 % fetal bovine serum, 2mM l-glutamine, antibiotics (100 units/ml penicillin and 100 µg streptomycin/ml) and 1.0 % phytohemagglutinin. Cytochalasin B was add to the cultures at 44 h post initiation at final concentration 5 µg/ml. 24 h later the cells were centrifugated, resuspended in hypotonic saline (75 mM KCL), centrifuged again and fixed twice in fixative (acetic acid and methanol 1:3) for 20 min. the cell suspension was dropped on wet slides and the air dried preparations were stained with 4 % Giemsa in Sorensen's buffer, pH 7.4. Scoring was done at 100 X magnification. 1000 binucleated cells/ experiment were counted for the presence of micronuclei. The data were statistically analyzed using Fisher exact test. Replicative index (RI), a measure of cell division kinetics was calculated by scoring 500 cell/sample , by counting the percent of cells containing 1,2,3 or more nuclei / individual.

RI= [(1x % mononuclear cells)+ (2x % bi) + (3 x% tri) + (4x % tetra)]/n.

2.6. Chromosomal aberrations

Blood samples were collected via sterile syringes from the four groups of goats. Lymphocyte cultures were prepared according

to Halnan (1977). Blood cells were cultured for 72 h at 38°C in 5 ml TCM-199, 1ml fetal calf serum and 0.1 ml phytohaemagglutinin (PHA). After incubation, cells were treated with colchicines (0.05%) for 2 h, then with a hypotonic (0.075M KCL) for 30 min. After fixation in acetic acid: ethanol (1: 3) solution, the cells suspension were dropped on wet slides then flammed to dry. The slides were stained with Giemsa stain and covered with DPX mounting media for chromosomal analysis. Chromosomal abnormalities were recorded in at least 100 metaphase spreads for each animal.

2.7. Serological examination for Brucella antibodies titer

Blood samples from vaccinated group and vaccinated plus dioxin group were collected and centrifuged at 3000 rpm /15 min. The obtained sera were kept at -20 ºC till used for detection of Brucella antibodies titer. Tube agglutination test (TAT) and Mercaptoethanol (MET) were made according to Alton et al. (1998) and Brucella antigens were supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.

2.8. Statistical analysis

Data were subjected to statistical analysis according to Snedecor and Cochran (1982).

3. Results

The clinical symptoms of goat drenched dioxin (alone or with Brucella vaccine) were ranging from general depression, different degrees of inappetaness, poor body condition, pale mucous membranes, staggering gaits and respiratory manifestations.

A significant ($p<0.01$) increase in percentage of micronuclei in binucleated lymphocyte was observed in dioxin group than control (Table 1). In group of goats vaccinated and given dioxin, there was a significant ($p<0.05$) decrease in percentage of micronuclei than dioxin group.

Chromosomal aberrations in goat lymphocytes for all groups are presented in Table 2. The frequencies of chromosomal abnormalities increased significantly ($p<0.01$) in dioxin treated goat than control. The percentage reached 8.0 ± 0.51 in treated animals compared with 2.66 ± 0.58 for the control. The percentage of chromosomal aberration significantly ($p<0.05$) decreased in dioxin plus vaccinated group than dioxin group.

Serological examination of vaccinated goats with or without dioxin treatment using serological tests revealed decreased level of antibodies titer by both TAT and MET in vaccinated animals plus dioxin (Table 3). Results showed that the titer of antibodies by TAT significantly decreased in goats vaccinated and drenched dioxin (28±10.95) than in vaccinated (72±16). Meanwhile the titer of antibodies by MET showed non significant decreased in animals vaccinated and drenched dioxin (24±8.94) than in vaccinated (36±8.94).

Table (1): Percentage of micronuclei (MN) in binucleated goat blood lymphocytes vaccinated with Rev.1 vaccine and treated with dioxin.

Treatment	Number of Exp	No of binucleated cells	No of MN in binucleated cells	% of MN in binucleated cells ± S.E
Control	6	6000	32	0.53 ± 0.43
Vaccine	18	18000	109	0.60 ± 0.55
Dioxin	6	6000	194	3.23 ± 0.45**
Dioxin + Vaccine	10	10000	188	1.88 ± 0.31●

** Highly significant P< 0.01 comparing to control. ● Significant P< 0.05 comparing to dioxin.

Table (2): The types and mean percentage of chromosome aberrations in cultured goat blood lymphocytes vaccinated with Rev.1 vaccine and treated with dioxin.

Treatment	Number of Exp	Number of metaphases	Number of abnormal metaphases	Chromosome aberrations (Mean % ± S.E) without gaps	Number of abnormal metaphases			
					Gaps	Fragment and / or break	Deletion	polyploidy
Control	6	300	14	2.66 ± 0.58	6	4	4	-
Vaccine	18	900	46	3.55 ± 0.54	14	26	6	-
Dioxin	6	300	32	8.0 ± 0.51**	8	14	2	8
Dioxin + Vaccine	10	500	39	5.80 ± 0.42●	10	22	3	4

** Highly significant P< 0.01 comparing to control (T-test). ● Significant P< 0.05 comparing to dioxin (T-test).

Table (3): The titer of antibodies in vaccinated and/or vaccinated plus dioxin groups by using the serological tests.

	Tube agglutination test (TAT)		Mercaptoethanol test (MET)	
	Vaccinated	Vaccinated and drenched dioxin	Vaccinated	Vaccinated and drenched dioxin
The titer of antibodies	72* ± 16	28 ± 10.95	36 ± 8.94	24 ± 8.94

***** Significant P< 0.05 comparing to vaccinated and drenched dioxin by the same test.

4. Discussion

In the present study, dioxin exposed goats show mild signs of adverse healthy conditioned. Fouzy et al. (2007) reported similar findings in goats. Such clinical signs could be due to appetite suppressive effect of TCDD which related to its feedback mechanism originating in the periphery and not to a direct effect on appetite-regulating areas of the brain (Stahl and Rozman, 1990).

The results of this study showed a significant increase in percentage of micronuclei in binucleated lymphocyte in dioxin and dioxin with vaccine groups. Micronuclei represent whole chromosomes or chromosome fragments that have been lost from the cell nucleus during mitosis or meiosis (Kirsch-Volders et al., 1997 and Junk et al., 2002). Heddle et al. (1991) suggest that micronuclei may form by one of four basic mechanisms:1) mitotic or meiotic loss of an acentric fragment; 2) a variety of mechanical consequences of chromosomal breakage and exchange; 3) mitotic or meiotic loss of whole chromosomes; 4) as a result of apoptosis. In this respect, Patterson et al. (2003) found that induction of apoptosis was accompanied by dioxin exposure.

Our data demonstrate that, there is a significant increased in structural and numerical chromosomal aberrations in dioxin treated group and group vaccinated with dioxin. Similarly, Perucatti et al. (2006) cytologically examined two herd of sheep with high levels of dioxins in the milk (50.65 and 39.51 pg/g of fat, respectively). Increases of both chromosome abnormalities (gap, chromosome and chromatid breaks) (17 and 8 times higher in the two exposed herds, respectively). Also, Iannuzzi (2004) recorded a significant percentages of chromosomal aberrations in the same two herds exposed to lower levels of dioxins (5.27 pg/g). Bertazzi et al. (2001) reported cytogenetic abnormalities in human and found to be linked to TCDD exposure. Ingel et al. (2001) found high level of correlation between emotional stress and individual dioxins blood contents (up $P \leq 0.001$) as well as between emotional stress and individual chromosome aberration level (up $P \leq 0.05$). In contrast, Revazova et al. (2001) found no personal correlation related to dioxins exposure in human by chromosome aberrations and micronuclei.

The result of micronucleus assay coincide also with chromosome aberrations in inducing

DNA damage. TCDD-induced oxidative stress and DNA damage may, in part, contribute to TCDD-induced carcinogenesis (Lin et al., 2007). The group of vaccinated animal with dioxin has decreased rate of chromosomal abnormalities than group of dioxin alone. The primary explanation for these effect could be the increasing general immune response of the animals due to vaccine lead to decrease the chromosomal damage. Gupta et al. (2007) cited that increasing immune response of goats vaccinated with brucella melitensis vaccine. But TCDD induce oxidative stress (Jin et al., 2008) which contribute to DNA damage (Lin et al., 2007).

In this study, we used Brucella vaccine, which is live attenuated bacterial vaccine, as a model of bacterial infection and estimation of the immune response due to TCDD exposure. Our results demonstrate that the Dioxin markedly suppresses the humoral immune response in the form of decreased titer of antibodies in the serum of goat experimentally exposed to Dioxins. Previous studies have demonstrated that the suppression of humoral immune responses is one of the most sensitive sequela associated with TCDD exposure. They demonstrated that B cells are directly targeted by TCDD (Holsapple et al., 1986; Sulentic et al., 1998) and that the AHR is required for suppression of the IgM response (Vecchi et al., 1983; Kerkvliet et al., 1990; Sulentic et al., 1998). However, the molecular mechanism responsible for the suppression of humoral immune responses by TCDD remains undeciphered. Collectively, these studies demonstrate that the suppression of the IgM response by TCDD is due to the impairment of B cell differentiation by dysregulation of Pax5 resulting in high-level expression of the Pax5a isoform, a potent repressor of XBP-1, IgH, Igκ and the IgJ chain (Yoo et al., 2004). Pax5 is known to induce genes responsible for the mature B cell phenotype, while suppressing genes involved in their terminal differentiation into plasma cells. Consequently, suppression of Pax5 promotes the terminal B cell differentiation program (Nera et al., 2006).

With respect to the two tests used in our study, Several serological tests have been used for detecting specific serum antibodies of brucellosis. The tube agglutination test (TAT), Rose Bengal test, Mercaptoethanol (MET), complement fixation test, indirect Coombs test, enzyme immunoassay (ELISA) and, more recently, an immunocapture-agglutination test (Díaz and Moriyon, 1989, Orduña et al., 2000 and Rubio et al., 2001). However, the interpretation of differences in results among these tests is due to every test depend on specific type of immunity.

In conclusion, dioxin may induce chromosome damage and lower the immune response of goats vaccinated with Rev.1 vaccine. The adverse effect of dioxin on chromosomes decreased in vaccinated animals.

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