

## Sulphadimidine Suppresses Major Histocompatibility Complex (MHC) Expression in Broiler Chicken

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**Abstract:** The present study aimed at evaluation of the immunomodulatory effect of sulphadimidine by investigating its ability to affect constitutive expression of MHC-II molecules on B-cells, which are one determinant of antigen presentation and the vigor of immunity. Three groups of broiler chicken were used, the first two groups received 100 mg/kg and 200 mg/kg sulfadimidine, respectively, in drinking water; while the third group was left as control. Bursal samples were taken after 7 and 14 days of treatment and checked for MHC-II gene expression by RT-PCR technique. The obtained results demonstrate that oral administration of sulphadimidine inhibited the expression of MHC-II mRNA in bursal of Fabricius of broiler chicken in a dose and age dependent manner. This finding is of clinical importance as many farmers routinely add sulpha drugs to drinking water as a prophylactic measure against some infections; yet the data presented in this study doesn't recommend this managing behavior.

**Keywords:** B-cells, broiler chickens, bursa of fabricius, Major Histocompatibility Complex Class II (MHC-II), sulphadimidine

### INTRODUCTION

Major Histocompatibility Complex Class II (MHC-II) molecules are considered all-or-none gate rule for exogenous antigen presentation and consequent initiation of adaptive immunity by the virtue of their ability to present non-self peptides to the receptors of helper lymphocytes. Presentation of antigen is necessary for lymphocyte activation and differentiation (Unanue, 1992; Pieters, 1997). According to the type of antigen, the MHC molecules presented as class I or class II (Glimcher and Kara, 1992). Hence, MHC molecules play important roles in the regulation of the immune response by communicating among different cellular components of the immune system: T cells, B cells and antigen-presenting cells (Lamont, 1998).

Chicken MHC-II (called blood linked complex; BL-complex) genes are  $\alpha$  and  $\beta$  heterodimeric transmembraneous glycoproteins. The main component responsible for antigen presentation is  $\alpha$ -

molecule, while  $\beta$ -molecule is of relatively lower function. In a general sense, the bursa of Fabricius of chicken is a unique organ that provides only B-lymphocytes (B-cells), initiates the adaptive (specific) immune response and an equivalent to the bone marrow in mammals, at least with respect to B-cell development. Also the bursa of Fabricius is critical only within the first 2-3 weeks after hatching. As about 5 % of bursal cells leave the bursa per day to the periphery after that the bursa will be functionless (Lassila, 1989; Paramithiotis and Ratcliffe, 1994). MHC-II genes are one marker of B-cell receptors. Although B-cells constitutively express these surface molecules to counteract pathogens, their expression can be induced by infections or pro-inflammatory cytokines IFN- $\gamma$  and TNF- $\alpha$ .

Evidence from several laboratories in animals suggested that the level of MHC-II mRNA reflects its surface expression (Sicher *et al.*, 1995; Rohn *et al.*, 1996; Lee *et al.*, 1997; Pieters, 1997; Hobart *et al.*, 1997; Zhu *et al.*, 2002).

In spite of these facts, there was no available information regarding the effect of sulphonamides on adaptive (MHC-II) components in chickens.

## MATERIALS AND METHODS

**Experimental birds:** A day old, newly hatched male chicks (Ross strain, body weight of 40±5 g), were permitted to free access for standard nipple drinker and feed trough. Chicks were maintained on 12:12 light-dark cycle and kept in thermostatically controlled cages to match chick requirements. Starting ambient temperature was 35°C and decreased 1°C each other day.

**Measurement of MHC-II mRNA expression by RT-PCR:** Bursa of Fabricius was dissected from chicks at 7 and 14 days of treatment with sulphadimidine in drinking water (0 and 2 g/L; i.e., 3 groups). For detection of target genes (MHC-II), 14 samples from bursa of Fabricius were run together in each reverse transcription and polymerase chain reaction (RT-PCR) trial; 4 from 1 g/L-treated chicks, 2 from 7 and 2 from 14 day-treatment periods; 4 from 2 g/L-treated chicks, 2 from 7 and 2 from 14 day-treatment periods; and 4 from untreated ones (0 g/L). Two additional samples (1 from 1 g/L group and the other from 2 g/L group) that were run with no Reverse Transcriptase (RT) added (called 'RT' controls).

The bursa of Fabricius was cooled in ice-cold distilled water and homogenized. Total cellular RNA was extracted from tissue homogenates by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) (Chomczynski and Sacchi, 1987). The RNA concentration was determined on the basis of absorbance at 260 nm and its purity was evaluated by the ratio of absorbance at 260/280 nm (>1.8). After digestion of genomic DNA by treatment with deoxyribonuclease I (Invitrogen), first-strand cDNA was synthesized from 2 µg of total RNA by using Superscript II RNase, reverse transcriptase and oligo (dT) primer (Invitrogen). To validate successful deoxyribonuclease I treatment, the reverse transcriptase was omitted in control reactions. The absence of PCR-amplified DNA fragments in these samples indicated the isolation of RNA free from genomic DNA contamination.

For performing PCR, 1 µL of template cDNA was added to a PCR cocktail consisting of 10 mM Tris-HCl, 50 mM KCl, 1.25 mM MgCl<sub>2</sub>, 10 mM deoxy-NTPs, 200 nM primers and 0.05 U Taq DNA polymerase (Takara Bio, Otsu, Japan) for a final volume of 10 µL.

Primers set for housekeeping β-actin gene (GenBank accession number; NM\_031144) were as follows: sense, 5'-gtg ttc cac cag gag atg ttg-3' and anti-sense, 5'-ctc ctg ccc atc gag ttc gtc-3' (predicted size = 434 bp); BL-alpha (GenBank accession number; AY357253), sense 5'-ggg ccg tcc tga agccgc ac-3' and antisense, 5'-ttc cgg ctg cca cat cct ct-3' (predicted size = 410 bp). BL-beta (GenBank accession number; AY510090), sense; 5'-gcg ggg gcc gtg ctg gc-3' and antisense; 5'-acg tag cac gcc aga cgg tc-3' (predicted size = 560 bp). PCR was performed with a thermal cycler (GeneAmp. PCR system 2700, Applied Biosystems, Foster City, CA, USA). A first incubation at 94°C for 3 min was performed for initial denaturation. This was followed by 25 cycles of the following sequential steps: 30 s at 94°C (denaturation), 1 min at 55°C or 51°C for β-actin or BL-alpha and at 57°C for BL-beta. respectively (annealing) and 1 min at 72°C (extension); the final incubation was at 72°C for 7 min (final extension). The reaction products were electrophoresed on 1% agarose gels and stained with ethidium bromide (0.2 µg/mL). The gels were exposed to UV with a UV transilluminator (UVP Laboratory Products, Upland, CA, USA) and the images were picked up using a camera and analyzed only qualitatively.

## RESULTS

To match doses of the drug routinely used by farmers, 2 doses (1 and 2 g/L) were selected in drinking water along their concept that they protect their chicks against possible infections. Because the genes encoding MHC-II molecules are regulated in tight fashion at mRNA level to meet with the local requirements for the adaptive immune response, measurement of MHC-II by RT-PCR was adopted. As shown in Fig. 1, administration of sulphadimidine for first 7 days of age resulted in suppression of MHC-II molecules indicated by absence of the bands representing β-component of the molecule with non-significant effect on β-component. The effect was evident at both doses of sulfadimidine used (1 and 2 g/L).

Unexpectedly, the suppressive effect of the sulpha drug used on MHC-II molecules after 14 days of treatment in drinking water, was relatively less than that recorded after 7 days of treatment, however the suppressive effect was still evident. Again β-component was not affected while the whole effect was only on the main β-component (Fig. 2). The effect was unrelated the dose of sulphadimidine used as the effect was similar in 1 and 2 g/L groups.

As shown in Fig. 3, the effect of sulphadimidine is specific to the drug as the control β-actin bands were intact in both treated and control groups.

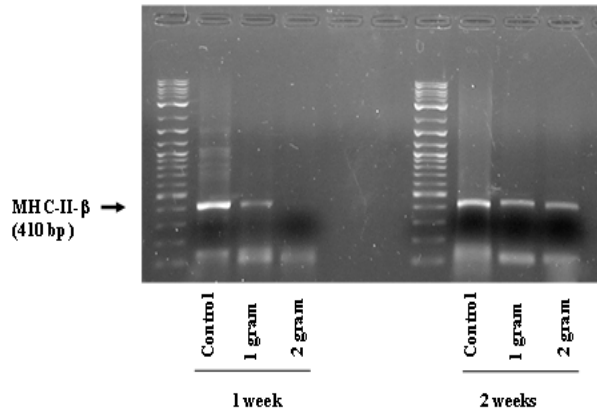


Fig. 1: Effect of administration of sulphadimidine in drinking water (1 and 2 g/L) to chicks for 1 week and 2 weeks on expression of MHC-II ( $\beta$ -component) by bursal cells

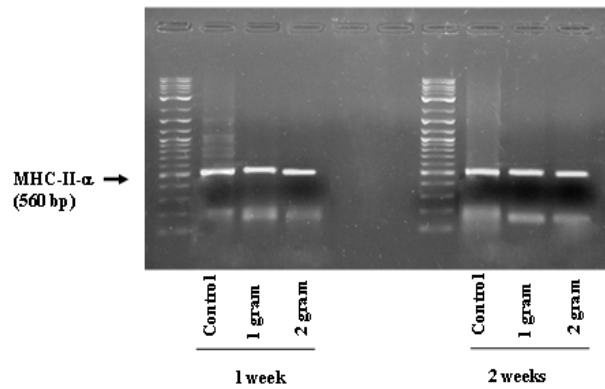


Fig. 2: Effect of administration of sulphadimidine in drinking water (1 and 2 g/L) to chicks for 1 week and 2 weeks on expression of MHC-II ( $\alpha$ -component) by bursal cells

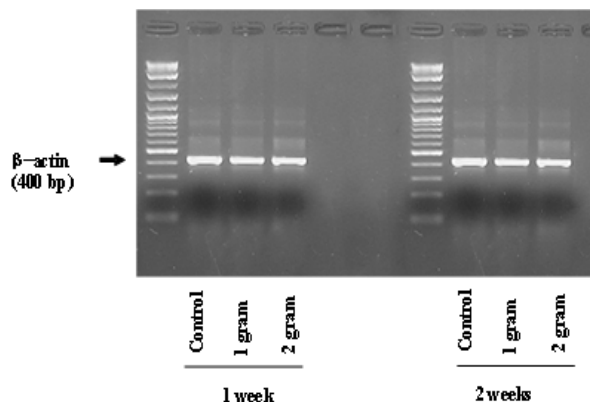


Fig. 3: The non-effect of administration of sulphadimidine in drinking water (1g/L and 2g/L) to chicks for 1 week and 2 weeks on expression  $\beta$ -actin by bursal cells; indicating specific action of the drug used

## DISCUSSION

Long term ago, some farming problems as coccidiosis are being controlled by routine supplying sulpha drugs, such as sulphadimidine, into drinking water to chicks. Although this prophylactic measure was successful sometimes and unsuccessful other times, our finding in this study strongly recommend avoiding sulpha drug administration to chicks especially in newly hatching chicks. This recommendation comes from data of this manuscript which clearly showed the immunosuppressive effect of sulphadimidine by inhibiting MHC class II mRNA expression. As a result, this drug could suppress the antigen presentation function of bursal immune cells. It is well established that any antigen is not processed as it is in immune pathways in the body; any antigen must be firstly presented by antigen presenting cells such as macrophages, lymphocytes and fibroblasts to molecules recognizable by lymphocytes that are MHC-II molecules (Unanue, 1992; Glimcher and Kara, 1992; Riley *et al.*, 1995; Pieters, 1997; Zhu *et al.*, 2002). This crucial function of the MHC in the immune response makes these molecules a promising candidate for genetic selection to improve avian immunity.

Failure to present antigens to immune cells, results in weakness or even absence of immune defense mechanisms adopted upon antigen invading with serious outbreaks and economic losses. In addition, the immunological response to a given vaccine will be also drastically affected. In fact there is an association between the chicken MHC and antibody production against a variety of antigens (Dunnington *et al.*, 1992; Lakshmanan *et al.*, 1997; Lamont 1998; Karaca *et al.*, 1999; Weigend *et al.*, 2001), since long-term selection for antibody response to SRBC was accompanied by a correlated change in MHC allelic frequencies in the chicken (Dunnington *et al.*, 1984; Pinar *et al.*, 1993; Yonash *et al.*, 1999). Furthermore, genetic selection of optimal MHC genotypes was used for improvement of humoral immunity (Zhou and Lamont, 2003). Interestingly, chicken MHC is roughly 20 fold smaller than the human MHC, therefore, this crucial property (smaller size and simplicity) makes it possible to study the striking MHC-determined pathogen specific disease resistance at the molecular level (Briles *et al.*, 1983; Kaufman and Lamont, 1996). Among markers of B-cells is MHC-II which is expressed on their membranes to counteract pathogens and also can be induced by infection, pro-inflammatory cytokines IFN- $\gamma$  and TNF- $\alpha$ . MHC molecules could be measured at genetic level in

terms of their mRNA expression (Sicher, *et al.*, 1995; Rohn *et al.*, 1996; Hobart *et al.*, 1997). Unlike viral infection which down-regulates the surface expression of MHC class I or destroys B-cells in bursa of Fabricius cells in chicken early life (Cohen, 1998; Hunt *et al.*, 2001), sulphadimidine inhibits the constitutive expression of MHC-II in chicken.

The classical chains alpha and beta of chicken MHC-II are not necessarily to be linked genetically and the beta chains are highly variable between individuals (Kumanovics *et al.*, 2003). In mammalian species,  $\beta$ -component is the main component while  $\alpha$ -component comes in the second rank. The reverse is true in chicken where the main component responsible for antigen presentation is  $\beta$ -molecule, while  $\alpha$ -molecule is of relatively lower function. Therefore, the more conserved L-alpha chain was the target of (Kumanovics *et al.*, 2003) to investigate the regulation procedures of these genes in chicken. The fact that BL-alpha chains are more conserved has been reproduced in present study (Fig. 2). On the other hand, BL-beta was affected by our treatment constantly in 7 and 14 day-old chicken as shown in Fig. 1.

The bursa of Fabricius of chicken provides only B-lymphocytes that initiate the adaptive (specific) immune response upon invading antigen or vaccination. It strongly determines the resistance and susceptibility to infectious diseases (Kaufman *et al.*, 1999). Because role of the bursa of is critical only within the first 2-3 weeks after hatching; after that that it becomes functionless (Lassila, 1989; Paramithiotis and Ratcliffe, 1994), therefore, we focused our research on the first 2 weeks after hatching. In the present study the constitutive expression of MHC-II (BL- $\beta$ ) mRNA by bursal cells was severely inhibited upon administration of sulphadimidine for 7 and 14 days at 1 and 2 g/L drinking water. However, the suppressive effect of sulphadimidine on MHC-II mRNA expression was not only dose dependent (more evident at high dose) but also age dependent (1 week-old chicks were affected more than 2-weeks old chicks).

In conclusion, in spite of that the effectiveness of sulphonamides against some microbes is out of debate and the use of these drugs could be inevitable. However, our finding strongly recommends avoiding sulphadimidine administration to chicks especially in the first week after hatching as this may lead, on the other hand, to drastic immune effect in the form of decreased antigen presentation due to inhibition of MHC expression; the matter that renders chicks more liable to microbes to which sulpha is not a protector.

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