Evaluation of Inosine-Acedoben-Dimepranol as an immunomodulator in broiler chicks vaccinated with infectious bronchitis virus vaccine

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ARTICLE INFO

Keywords

IAD
Inosine Acedoben Dimepranol Infectious bronchitis Vaccine Broiler

ABSTRACT

Protecting livestock against diseases by enhancing its immunity is essential and required in poultry industry. Therefore, the aim of the present study was to evaluate the possible immunoenhancing effects of Inosine-Acedoben-Dimepranol (IAD) in broiler chicks. A total of 150 chicks were used in the present study, divided into 6 groups (25 for each) and subjected to different treatments. It has been found that IAD significantly (P≤ 0.05) increased total leukocyte count, with increased granulocyte (neutrophils, eosinophils, basophils), lymphocyte and monocyte counts compared to control chicks. IAD significantly (P≤ 0.05) increased total protein as a result of increased globulins in plasma when compared with those of control. IAD has been found to significantly (P≤ 0.05) increase immune response of IB vaccine in IAD+ IB vaccine-treated groups compared to control measured by ELISA. IAD exhibited antiviral effect indicated by increased survival percent of chicks challenged with virulent IB virus with survival 100% in the groups received IAD large dose plus vaccine. Data of the present study may indicate that supplying chicks with IAD in drinking water is a good recommendation in poultry industry based on its immune enhancing properties.

1. INTRODUCTION

Inosine Acedoben Dimepranol (IAD) is a synthetic combination of p-acetamidobenzoic acid salt of N,N-dimethylamino-2-propanol with inosine in 3:1 molar ratio. It is a purine derivative has double therapeutic effect as it possess immunostimulatory and antiviral properties. It regulates the host immune system by enhancing body resistance against various viral diseases including: subacute sclerosing panencephalitis, influenzas, human papilloma virus, herpes simplex, auto immune diseases, acute respiratory virus infection, measles (Campoli-Richards et al., 1986).

The first product was authorized in September of 1971 with license for treatment and management of cell mediated immune deficiencies associated with viral diseases. It is now marketed in many countries worldwide under different trade names as Isoprinosine®, Imunovir®, Viruxan®, Delimmun®... etc to control of viral diseases. It had become one of the first and widest immunomodulators in the world for treatment immunosuppressed patients without fear of drug toxicity (Wybrand and Appelboom, 1984).

The biochemical action of IAD is not fully known. However, the drug has been found to enhance immunity of the host as it stimulates Th1 response confirmed by increase pro inflammatory cytokines (INFγ and IL-2) in vivo and in vitro in antigen infected cell (Petrova et al., 2010). This response initiates T-lymphocyte maturation and potentiates lymphoproliferative responses (Lasek et al., 2015). In addition, it has been found that the combination of the drug and INFγ leads to inhibition of IL-10 production and other anti-inflammatory cytokines, suggesting that IAD could modulate the suppressive effect of these cytokines on innate and adaptive immunity (Sabat et al., 2010).

Introducing a drug to veterinary filed, as into poultry industry, requires establishment of efficacy and safety of such drug (Ahmed and Kasraian, 2002). The present study is a trial to assess the efficacy of IAD in protecting broiler chicks against viral respiratory infections as infectious bronchitis. The study aimed to evaluate the immunostimulant properties of IAD in one of the most utilzed farm animals in Egypt that is broiler chickens using IBV vaccine as a model. To fulfill this aim, the following objectives have been conducted: effect of IAD on total and differential leukocytes (as indicators for cellular immunity); effect of IAD on total protein, albumin, globulins, and the humoral immune response to IB vaccine by detection and titration of antibody titer.

2. MATERIAL AND METHODS

2.1. The drug:
IAD is a fine, white powder with, soluble in water and stable in sodium chloride 0.9% solution. It has obtained as the patent oral preparation Isoprinosine® produced by MUP Pharmaceutical Co., Abu Sultan, Egypt. The is formulated as liquid preparation as combination of 250 mg / 5 ml of IAD. The dosage range for human is 50-100 mg/kg/day orally, according to body weight and the severity of the

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condition. The average dose prescribed for human is 50 mg/kg/day as 3-4 divided dose was converted to its equivalent for poultry according to Paget and Barnes (1964). The converted chick dose was found to be 40 mg/kg body weight. For immunological investigation, two escalating doses have been tried; a chick weighing 100 g received a daily dose of either 40 or 80 mg/kg day after day in drinking water along the experimental course.

2.2. Chicks:
One hundred- and fifty-day-old Ross broiler chicks were used in this study. Chicks were kept in separate partitions and allowed to plenty of diets and water at temperature of 31-33 °C. After one week of acclimation, chicks reached approximate weight of 100 g and received different treatments as will be mentioned below.

2.3. Experimental design:
A parallel study design has been applied, where acclimatized chicks were divided into 6 groups, 25 for each, and were treated differently as follows:
Group 1: chicks were kept on normal conditions of water and feed, and received no drugs, kept as control group.
Group 2: chicks received a routine Infectious Bronchitis (IB) disease virus vaccine (Servac IB vaccine H-120) from VSVRI, Abbaseia, Cairo, Egypt, in drinking water; kept as vaccine group only.
Group 3: chicks received small dose of IAD (40 mg/kg day after day/ 4 weeks) in drinking water; kept as drug group only.
Group 4: chicks received large dose of IAD (80 mg/kg day after day/ 4 weeks) in drinking water, kept as drug group only.
Group 5: chicks received small dose of IAD (40 mg/kg) with IB vaccine in drinking water, kept as small dose drugged vaccinated group.
Group 6: chicks received large dose of IAD (80 mg/kg) with IB vaccine in drinking water, kept as large dose drugged vaccinated group.

2.4. Sampling:
Two types of blood samples were collected. Blood for total and differential leukocytic counts on the 7th, 14th, 21st and 28th days after a week from accommodation along the course of the experiment. Blood for plasma was collected on the same time points for determination of antibody titre of IB virus and measuring of total protein and albumin. Samples were collected from medial metatarsal vein by means of 3 ml syringe with 20G needle. Clear plasmas were separated by centrifugation at 900 xG for 5 minutes and then harvested in Eppendorf tubes using automatic pipettes and kept frozen (-20°C) till analysis. The chicks (13/group) were subjected to challenge test (Figure 1).

2.5. Analysis:
2.5.1. Haematological analysis was performed by manual method using improved Neubauer haemocytometer according to Feldman et al. (2000). Stained blood films by Giemsa stain were examined and differential leucocytic counts were performed using cross sectional method. Total and differential leukocytic counts were taken as indicator for cellular immunity.

2.5.2. Protein analyses (total and albumin) were performed after the method described by Henry (1964) using kits from spectra GmbH company (Kleinfeld, Germany.). Globulins and A/G ratio were calculated mathematically.

2.5.3. Antibody titration has been determined spectrophotometrically in plasma samples of different groups using diagnostic ELISA kits and microtiter plate reader. Kits were supplied by ID Screen® (IDvet, 310 rue Louis Pasteur – Grabels- FRANCE) following the instructions of the manufacturer (ID.vet, 2022).

2.6. Challenge test:
Chick groups were challenged post treatment by virulent IBV containing 10^4 EID50/ml bird, by Eye drop method. Virulent IBV strain as classical (M41) was kindly supplied from the central laboratory for evaluation of veterinary biologics (CLEVB) that was used for challenge of groups of chicken. The challenged birds were observed for 7 days post inoculation, dead birds through this time were recorded and examined for post-mortem lesions (National Research Council U.S., 1971). The protection % in every group has been calculated using the following formula:
Protection % = (Number of survived birds / Total number of challenged birds) x 100

2.7. Statistical analysis:
Data were expressed as mean ± S.E of 3 observations (immunological parameters) which are calculated using GraphPad® software. Protection in challenge test was calculated as % of 13 birds. The obtained data were statistically analysed using ANOVA followed by Tukey’s post-hoc to express the differences (P ≤ 0.05) among groups.

3. RESULTS
3.1. Total leukocytic count:
There was a significant (P ≤ 0.05) increase in total leukocytic count in samples collected from chicks administrated IAD small dose (40 mg/kg day after day, for 4 weeks) and large dose (80 mg/kg, po, day after day, for 4 weeks) and IB vaccine (10^6 EID50/ml, every 10 days) on the days 7th, 14th, 21st and 28th, compared to those of negative control chicks. Co-administration of IAD at both doses and IB vaccine resulted in significant (P ≤ 0.05) increase in total leukocytic count compared to the control group along the experimental course, and to the vaccinated group only on the days 21st and 28th.

Table 1: Effects of IAD on total leukocytic counts (×10^9/L).

<table>
<thead>
<tr>
<th>Control</th>
<th>Vaccinated</th>
<th>IAD-SD</th>
<th>LAD-SD</th>
<th>Vaccine + IAD-SD</th>
<th>Vaccine + LAD-SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td>20.06 ± 0.88</td>
<td>26.83 ± 1.46</td>
<td>23.8 ± 0.56</td>
<td>25.2 ± 1.56</td>
<td>27.9 ± 1.86</td>
</tr>
<tr>
<td>Day 14</td>
<td>20.4 ± 0.81</td>
<td>27.2 ± 1.56</td>
<td>24.8 ± 1.56</td>
<td>26.2 ± 1.56</td>
<td>28.9 ± 1.76</td>
</tr>
<tr>
<td>Day 21</td>
<td>20.3 ± 0.66</td>
<td>29.2 ± 1.56</td>
<td>27.8 ± 1.56</td>
<td>28.6 ± 1.56</td>
<td>31.06 ± 1.76</td>
</tr>
<tr>
<td>Day 28</td>
<td>22.03 ± 1.76</td>
<td>42.5 ± 1.2</td>
<td>41.3 ± 0.96</td>
<td>51.9 ± 1.36</td>
<td>53.1 ± 1.36</td>
</tr>
</tbody>
</table>

Data of the effect of IAD on total leukocytic count (TEL) in 6 groups of broilers every 7 days of different treatment. Values are presented as means ± SE of 3 chicks/group. Different superscript letters in each row indicate significance (P ≤ 0.05, ANOVA followed by Tukey’s post-hoc test). IAD-SD (Inosine-Acedoben-Dimepranol, 40 mg/kg orally); LAD-SD (Inosine-Acedoben-Dimepranol, 80 mg/kg, orally).

3.2. Differential leukocytic count:
There was a significant (P ≤ 0.05) increase in lymphocytes, monocytes and granulocytes in samples collected from...
chicken administrated IAD small dose, IAD large doses and IB vaccine on the days 7th, 14th, 21st and 28th after a week of accommodation along the course of the experiment compared to those of negative control chickens. Co-administration of IAD at both doses and IB vaccine resulted in significant (P ≤ 0.05) increase in different types of leukocytes compared to the control group along the experimental course, and to the vaccinated group only on the days 21st and 28th (Lymphocytes) and the days 14th, 21st and 28th (Other cells) (Table 2).

Data of the current study revealed a significant (P ≤ 0.05) increase in antibody titer in different types of leukocytes compared to the control group along the experimental course, and to the vaccinated group only on the days 21st and 28th (Lymphocytes) and the days 14th, 21st and 28th (Other cells) (Table 2).

### Table 2: Effects of IAD on leukocyte counts (%)

<table>
<thead>
<tr>
<th>Day</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>7 0.2 a</td>
<td>0.1 a</td>
<td>0.01 b</td>
</tr>
<tr>
<td>21</td>
<td>0.1 a</td>
<td>0.2 a</td>
<td>0.1 b</td>
</tr>
<tr>
<td>28</td>
<td>0.2 a</td>
<td>0.3 a</td>
<td>0.3 a</td>
</tr>
</tbody>
</table>

### Table 3: Effects of IAD on total protein, albumin and globulins (g/l)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>IAD SD</th>
<th>IAD LD</th>
<th>Vaccine + IAD SD</th>
<th>Vaccine + IAD LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2.7 ± 0.2</td>
<td>2.96 ± 0.2</td>
<td>3.36 ± 0.2</td>
<td>3.56 ± 0.2</td>
<td>3.81 ± 0.2</td>
</tr>
<tr>
<td>14</td>
<td>0.1 a</td>
<td>0.2 a</td>
<td>0.1 a</td>
<td>0.1 a</td>
<td>0.1 a</td>
</tr>
<tr>
<td>21</td>
<td>0.1 a</td>
<td>0.2 a</td>
<td>0.1 a</td>
<td>0.1 a</td>
<td>0.1 a</td>
</tr>
<tr>
<td>28</td>
<td>0.2 a</td>
<td>0.3 a</td>
<td>0.2 a</td>
<td>0.2 a</td>
<td>0.3 a</td>
</tr>
</tbody>
</table>

Data expressed the effect of IAD on total protein, albumin and globulin levels in 6 groups of broilers every 7 days of different treatments. Values are presented as means ± SE of 3 chicks/group. Different superscript letters in each row indicate significance (P ≤ 0.05, ANOVA followed by Tukey’s post-hoc test). IAD-SD (Inosine-Acedoben-Dimepranol, 40 mg/kg orally); IAD-LD (Inosine-Acedoben-Dimepranol, 80 mg/kg orally).

### 3.4. IB Antibody titre

Data revealed a significant (P ≤ 0.05) increase in antibody titre in samples collected from chicken administrated small and large doses and IB vaccine, on the days 7th, 14th, 21st and 28th along the course of the experiment compared to those of negative control chickens. Co-administration of IAD and IB vaccine significantly (P ≤ 0.05) increased antibody titre compared to the control group along the experimental course, and to the vaccines group only on the days 7th, 14th, 21st and 28th (Table 4).

### Table 4: Effects of IAD on antibody titre

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>IAD SD</th>
<th>IAD LD</th>
<th>Vaccine + IAD SD</th>
<th>Vaccine + IAD LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2.9 ± 0.3</td>
<td>4.11 ± 0.3</td>
<td>3.57 ± 0.3</td>
<td>3.76 ± 0.3</td>
<td>4.85 ± 0.3</td>
</tr>
<tr>
<td>14</td>
<td>0.16 ± 0.1</td>
<td>0.18 ± 0.1</td>
<td>0.26 ± 0.1</td>
<td>0.16 ± 0.1</td>
<td>0.26 ± 0.1</td>
</tr>
<tr>
<td>21</td>
<td>0.2 ± 0.2</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>28</td>
<td>0.2 ± 0.2</td>
<td>0.4 ± 0.4</td>
<td>0.4 ± 0.4</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
</tr>
</tbody>
</table>

Data expressed the effect of IAD on antibody titre in 6 groups of broilers every 7 days of different treatments. Values are presented as means ± SE of 3 chicks/group. Different superscript letters in each row indicate significance (P ≤ 0.05, ANOVA followed by Tukey’s post-hoc test). IAD-SD (Inosine-Acedoben-Dimepranol, 40 mg/kg orally); IAD-LD (Inosine-Acedoben-Dimepranol, 80 mg/kg orally).

### 3.5. Challenge test

Only one chick out of 13 (7.6%) has been survived in the control group. On the other hand, all chicks have been survived upon treatment with IAD large dose plus vaccine (100%). Other groups showed intermediate survival rates, being 76.9% (Vaccine only), 46.2% (IAD-SD), 53.8% (IAD-LD), 92.6% (IAD-SD plus vaccine).
Poultry industry is one of the most important fields in veterinary practice. Protecting this livestock against diseases by enhancing its immunity becomes essential and always required. Farming poultry without immune umbrella may render them subjective to variety of diseases especially respiratory ones, causing consequent economic losses and health hazards. Farmers, therefore, always follow strict vaccination programs and keep trying to enhance immunological status of their flocks by all possible means (Schwabenbauer and Rushton, 2007).

Ginsberg and Glasky (1977) reported that there is balance between invading viruses and natural defence mechanisms of the host. The cell-mediated immunity is considered the important one. Thus, the therapeutic agent that is intended to inhibit viral replication must act with these defence tools to maximize their effectiveness.

More than a trial has been conducted in order to discover immunostimulants in chickens such as polysaccharides isolated from Rhizopus (Yu et al., 2016), Astragalus polysaccharides (Shan et al., 2019), Lymphocyte-derived Transfer factors (Mohymen and Mechanical, 2019), and herbal immune boosters (Andriani et al., 2022).

The present study was designed to evaluate the immunopotentiation potential of IAD in the most important species in poultry industry that is broiler chickens, either vaccinated or unvaccinated against IB virus.

Data revealed that day after day administration of IAD increased total and differential leukocytic counts of broiler chicks on the days 7th, 14th, 21st and 28th after one week of accommodation. Such finding has been found to further increase upon combination with IB vaccine. These finding may be supported by De Simone et al. (1982) & De Simone (1985) who stated that IAD enhances cell mediated immunity, by stimulating the differentiation of T lymphocytes into T cytotoxic cells and T helper cells and increasing cytokine production. This assists the body to mount an effective defence. Further support could be given by Campoli-Richards et al. (1986) who reported that IAD potentiates neutrophil, monocyte and macrophage chemotaxis and phagocytosis. Our finding may be parallel to those reported by AbdelMaksoud et al. (2019), who found that inosine pranobex has immunostimulant action in terms of increased total and differential leukocytic counts in broilers either vaccinated or not with Newcastle disease virus.

Data of the present study showed that there was a significant increase in plasma total protein, albumin and globulin of chicks received IAD and vaccine+IAD on the day 7th, 14th, 21st and 28th of experiment if compared with the control and vaccine only groups indicating the immune-enhancing effect of IAD especially when combined with IB vaccine. The finding may be attributed to that IAD increases the humoral immune response by stimulating B-lymphocyte differentiation of into plasma cells and enhancing antibody production, and increases the production of IL-1, IL-2 and IF-γ (Milano et al., 1991) & (Petrova et al., 2010). Our findings may be consistent with those of Stenzel et al. (2011) in pigeons vaccinated with paramyxovirus where there were significant increases in antibody titers in inosine pranobex-treated groups; the authors added that such effect was dose-dependent.

Survival of chicks upon infection with IB virus differed according to previous incubation treatment. Almost all chicks have been died in the control group, and all chicks have been survived upon treatment with IAD large dose plus vaccine. Protection could be explained based on increased specific immunity (by vaccine) and nonspecific immunity (by IAD).

Data of the present study may indicate that IAD enhances both nonspecific and vaccine-induced specific immune responses in broiler chicken, in terms of increased both cellular and humoral immunities. The study recommends IAD as immuno-enhancer in poultry industry especially when given synergistically with concurrently administered vaccines.


