IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com

Original Article

Effect of Acyclovir on the Viability of Chicken Embryo During the Developmental Stage

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ARTICLE INFO

Article history:
Received: 08 Feb, 2015
Revised: 15 Mar, 2015
Accepted: 25 Apr, 2015
ePublished: 30 May, 2015

Key words:
Acyclovir
Chicken
Embryo
Pathology

ABSTRACT

Objective: In recent years, antiviral drugs have been used widely in the glob to prevent and treat viral diseases. They injected into eggs to eliminate pathogens and prevent of egg transmission of disease, but the adverse effects of drugs have always been a major concern. There is little information available about the safety of antiviral drugs in the embryonated eggs of birds. The objective of this study was to evaluation of the effect of acyclovir on the viability of chicken embryo during the first trimester of the incubation period. **Methods:** Fertile chicken eggs were divided into two equal treatment groups. The phosphate buffered saline injected group and acyclovir-injected group whose individuals were injected with acyclovir solution at a dosage of 80 mg per Kg egg-weight. Embryos monitored daily during incubation until day 10 after which; they were examined for viability. **Results:** Results showed that all of the embryos were dead in the acyclovir-injected group. Based on findings, it is concluded that acyclovir at above-mentioned concentration has a severe lethal effect on the chicken embryo. So, acyclovir egg-injection cannot be used to eliminate pathogens and prevention of egg transmission of the disease.

1.INTRODUCTION

Raising chickens for their eggs and meat is becoming a popular and lucrative business in many countries including Iran. Viral diseases are the most common and destructive disease of the chicken (Swayne et al. 2013). So, different types of antiviral drugs were used to prevent and treatment of viral diseases in this industry (Swayne, Glisson et al. 2013; Tavakkoli et al. 2104).

Acyclovir belongs to the guanosine analogue antiviral medication and is on the World Health Organization's List of Essential Medicines. It is primarily used for the treatment of herpes simplex virus infections, chickenpox and shingles (Tavakkoli et al. 2014a). Other uses including prevention of cytomegalovirus infections following transplant, infections due to Epstein-Barr virus, genital herpes simplex, cold sores, shingles, acute chickenpox in immunocompromised patients, herpes simplex encephalitis, acute mucocutaneous HSV infections in immunocompromised patients and herpes of the eye and herpes simplex blepharitis (Richardson 1997; Sweetman et al. 2009; Tavakkoli et al. 2014c; Tavakkoli, Derakhshanfar et al. 2104). Acyclovir trials show that this agent has no role in preventing HIV transmission, but it can help slow HIV disease progression in people not taking anti-retroviral therapy (Richardson 1997; Conway et al. 2013; Wightwick et al. 2013). In veterinary medicine, acyclovir is used to prevent disease and cure animals and birds (Ahrens et al.

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2013; Swayne, Glisson et al. 2013). In hatcheries, the hygienic process in association with injecting drugs into the egg, result in eliminating infection and preventing egg transmission of pathogens. Alternatively, eggs may be dipped in the drug solutions for controlling the disease transmission before hatching.

Adverse effects of drugs have always been a major concern. There is little research in the literature describing the effect of acyclovir on the viability of bird embryos, and further studies still need to be undertaken to determine this aspect. In this regard, in the present study, we evaluated the effect of acyclovir on the viability of the chicken embryo during the first trimester of the incubation period. We believe that results in this study will contribute to our better understanding of the safety of antiviral drugs for in ovo administration in the bird's egg.

2. MATERIALS AND METHODS

2.1. Drug and eggs

Acyclovir powder was diluted in phosphate buffered saline solution. A volume of 0.5 mL of phosphate buffered saline solution with 80 mg acyclovir was inoculated per Kg egg-weight. Fertile chicken eggs (Ross 308) with the average egg-weight of 50 ± 1g and with the same age were purchased from a local breeder farm. In this farm, birds were kept and grown up under the standard condition of breeding.

2.2. Experimental protocol

Eggs were incubated at 37.5ºC and 55% relative humidity. The eggs were randomly assigned to two equal treatment groups, 10 eggs each, as follows. Group 1: phosphate buffered saline injected group, embryonated eggs injected with sterile phosphate buffered saline of 0.5 ml/egg into the yolk sac. On day 4 of incubation, the eggs of group 2 treated with acyclovir solution at a dosage of 80 mg acyclovir per Kg egg-weight. Embryos received treatment by direct injection into the yolk sac according to the standard techniques (Hamburger 1942). Embryos were re-incubated post-treatment and allowed to develop. The viability of the embryos was checked throughout the incubation period by candling. At the end of the experiment, on day 10, the eggs were opened at the wider end and monitored for the viability of the embryos. The treatment protocols and procedures in this study were conducted according to local ethical guidelines, and were approved by the Animal Ethics Committee of the Research Council of Shahid Bahonar University, Iran.

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 20. The Chi-Squar test was used to determine the significant differences in embryo-viability between experimental groups. A P-value of <0.05 was considered as statistically significant.

2. RESULTS

Candling of the hatching chicken eggs during the incubation period and examination of the eggs or embryos is a useful tool for hatchery managers. In the current study, the developing blood vessels were seen in the ebryonated eggs at the early stage of incubation (figure 1). In the next phase of incubation, the more advanced circulatory system was not developed. Results of our experiments showed that the early developing blood vessels were seen in 80% and 70% of chicken eggs received phosphate buffered saline and treated with acyclovir, respectively. At the end of the experiment, on day 10, the eggs were opened at the wider end and monitored for the viability of the embryos. As can be seen in the figure 2, there was centrally located dead embryo in the chicken egg treated with acyclovir into the yolk sac. The circulatory system and the blood vessels of the extra-embryonic membranes were not developed, while in the chicken egg treated with phosphate buffered saline, the embryo and blood vessels were normal.

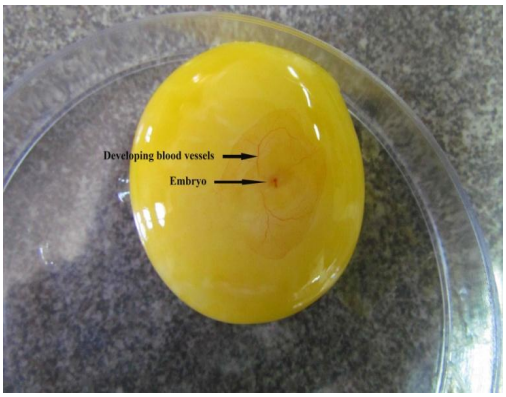

Fig. 1. The developmental stage of a normal chicken embryo. The embryo and developing blood vessels are seen (arrows).

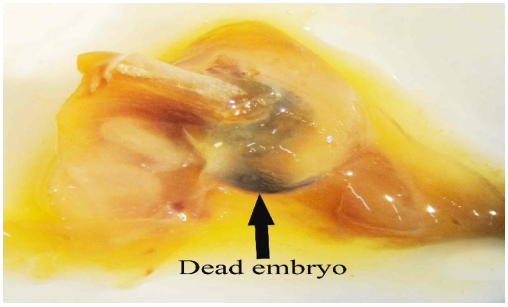

Fig. 2. The chicken egg treated with acyclovir into the yolk sac. A centrally located dead embryo is seen.

4. DISCUSSION

Despite reports of an expanding chicken industry across the globe, pathogenic agents can decrease the hatchability rate during embryonic development.

Embryonic death causes serious economic losses to the poultry industry (Swayne, Glisson et al. 2013).

For many years, researchers have been using different antipathogenic compounds to restrict pathogens and enhance the performance of different poultry species, including young chicken (Colomer-Lluch et al. 2011; Sapkota et al. 2011; Obeng et al. 2012; Banerjee et al. 2013), chicken (McDougald et al. 2012; Crespo et al. 2013; Rigobelo et al. 2013), turkey (Altunsoy et al. 2011; Erdem et al. 2012; Buscaglia 2013), broiler (MacDonald et al. 2011; Agunos et al. 2012; Lee et al. 2012; Tavakkoli et al. 2014b; Tavakkoli, Derakhshanfar et al. 2014c), layers (Hasan et al. 2011; Lee et al. 2013; Nemati 2013) and poultry breeder (Kabir 2010; Priyantha et al. 2012; Jones et al. 2013; Tavakkoli, Derakhshanfar et al. 2104).

Antiviral drugs have an increased role as therapeutic agents against avian viruses. They have virucidal effect and a wide antiviral spectrum. Most viruses are susceptible (Sweetman, Pharm et al. 2009; Ahrensand Martin 2013). Acyclovir belongs to the guanosine analogue antiviral group. It has been used successfully for several decades in many countries such as Canada, Spain, France, Austria, Polish, Denmark, Germany, Turkey, Africa, United States and China. In recent years, its use has increased rapidly in the Iranian pet bird industry, but there is little information available about the effects of injecting the acyclovir solution into the bird's egg. In the present study, we have evaluated the effect of acyclovir on the viability of the chicken embryo during the first trimester of the incubation period.

Up to now, drug-egg-treatment has been examined and described in different situations (Ghazikhanian et al. 1980; Sheeks et al. 1992; Kleven 2008; Singroha et al. 2012; Singroha et al. 2013; Tavakkoli et al. 2013; Tavakkoli, Derakhshanfar et al. 2014b). The results of these studies show that injecting drugs into hatching eggs can eliminate pathogens and prevent vertical transmission of disease. Some injection sites that are present in fertile eggs at day 4 of incubation are the air cell and yolk sac. Our results obviously showed severe lethal effect was seen in the chicken eggs treated with acyclovir solution by the yolk sac rout. Therefore, these findings suggest that acyclovir is not safe for injection into the ovo by the yolk sac route. Nevertheless, further efforts are needed to evaluate in ovo administration of various antiviral drugs for prevention and eliminate pathogenic microorganisms. In conclusion, based on findings, it is concluded that acyclovir cannot be used for the success of the prevention and treatment scheme in chicken embryo.

Acknowledgment

Authors wish to thank Dr. A. Hamidi for improving the use of English in the manuscript.

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