



## A short preliminary experimental study on teratogenic effect of methenamine in embryonic model

Hadi Tavakkoli<sup>1\*</sup>, Amin Derakhshanfar<sup>2</sup>, Samaneh Noori Gooshki<sup>3</sup>

<sup>1\*</sup> Department of Avian Medicine, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

<sup>2</sup> Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

<sup>3</sup> School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

### ABSTRACT

Methenamine salts have been traditionally used in the veterinary and human medicine, but its use is sometimes associated with some side effects. Toxicopathological effects of methenamine salt have always been a major concern. There is scanty information available about the lesions of methenamine compound in embryo. The objective of this study was to determine the teratogenic effects of methenamine in the chicken embryo. Fertile chicken eggs were divided into four equal treatment groups, 10 eggs per each, as follow: group 1: no injected group. Group 2: needle-injected group; the needle was insert into yolk sac without any injection. Group 3: phosphate buffered saline-injected group; whose individuals were injected with phosphate buffered saline solution. Group 4, whose individuals were likewise injected with methenamine at dosage of 30 mg/egg at day 4 of incubation. The teratogenic effect of methenamine was revealed as cyst formation instead of abdominal and thoracic body structures. Not recognizable organ was existed in abdominal/thoracic cyst. The normal brain tissue was replaced by large cavity with rim of nervous tissue. The feather formation was also defected. Based on these findings, it is concluded that methenamine at above-mentioned concentration are teratogenic for the chicken embryo. Current study also advises caution in the extended use of methenamine compounds. Further studies are needed to clarify the teratogenic effects of this drug on the development of human fetus.

**Key words:** Chicken, Egg, Embryo, Teratogenic effect, Methenamine

### INTRODUCTION

Methenamine is an antibacterial drug which has been used for many years. At an acid pH, it decomposes to ammonia and formaldehyde. The formaldehyde has bactericidal property. Methenamine application had been reduced for several years, due to side effects, but its use has now been re-approved because of the occurrence of antibiotic resistance of bacteria to commonly used antibiotics (Sweetman et al. 2009; Lee et al. 2012; Ahrens and Martin 2013). In human, methenamine is used in the treatment of urinary

tract infections, prophylaxis and bacteriuria, because bacteria are susceptible to formaldehyde. (Sweetman et al. 2009; Chung et al. 2010; Williams et al. 2012; Al-Badr and Al-Shaikh 2013; Hickling and Nitti 2013). In avian medicine, methenamine often dispense in the treatment of nephritis, pyelitis, pyelonephritis, gout, ascitis, edema and hepatitis. It has also diuretic and anti-bleeding properties for treatment of sulpha-drug intoxication, infectious bursal disease and coccidiosis (Ahrens and Martin 2013). Despite the use of methenamine in veterinary (Litster et al. 2011; Ahrens and Martin 2013; Smee et al. 2013) and human medicine (Chung et al. 2010; Masunaga et al. 2010; Williams et al. 2012; Christofides et al. 2013; Hickling and Nitti 2013), it's use is sometimes associated with adverse effects such as skin rashes, pruritus, cerebral vasoconstriction syndrome, hemorrhagic cystitis, gastrointestinal disturbances, nausea, vomiting, and diarrhea (Sweetman et al. 2009; Davies et al. 2013). Following administration, methenamine crosses from the placental barrier in humans and small amounts may be distributed into breast milk. In this regard, it is necessary to identify the benefits and risks of using methenamine during pregnancy, especially, for fetus. Although increasing consumption and production of methenamine salts are predicted in medical and veterinary medicine, there is little information about the toxic effects of these compounds on the embryo. Thus, the current study aimed to determine the teratogenic lesions of methenamine salt in the chicken embryo. Furthermore, since the embryogenesis in chick is similar to human beings, the study was performed in chicken embryo as a model to investigate teratogenic effect of methenamine in human fetus. We believe that this experiment will give important information about the toxic effects of methenamine compounds as a basic embryo-pathological study.

## MATERIALS AND METHODS

### Hatching eggs

A total of 40 fertile chicken eggs (Ross 308) with the average egg-weight of  $50 \pm 0.2$  g and same age were purchased from a local breeder farm whose birds were maintained under standard conditions.

### Experiment protocol

Eggs were incubated at 37.7°C and 60% relative humidity. The eggs were randomly assigned to four equal treatment groups, 10 eggs per each, as follow: group 1: no injected group; embryonated eggs do not receive any treatment at all. Group 2: needle-injected groups, the needle (22-gauge) were inserted into yolk sac without any injection. Group 3: phosphate buffered saline-injected groups, embryonated eggs were injected with sterile phosphate buffered saline solution of 0.5 ml/egg into yolk sac. Group 4: eggs were treated with sterile methenamine (Shchekinoazot Co., Tula.) at dosage of 30 mg/egg at day 4 of incubation. 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 embryos were necropsied at day 18 of incubation. All treatments and procedures in this study were conducted according to local ethic guidelines, and were approved by the Animal Ethics Committee of the Research Council of Shahid Bahonar University, Iran.

### Pathological examination

Eggs were opened at the wider end. After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormality on the external body surface. The membranes and yolk sac were also inspected. Then, the tissues of embryos were dissected out and examined for gross lesions.

**Statistical analysis**

Statistical analysis was performed using SPSS version 20. The Fisher's exact test was used to determine the significant differences in lesion occurrence between experimental groups. A p-value of <0.05 was considered as statistically significant.

**RESULTS**

The embryos were normal in groups 1, 2 and 3 (figure 1). The teratogenic effect of methenamine was revealed as cyst formation instead of abdominal and thoracic body structures (figure 2). The cyst was made from the very thin epithelial tissue with prominent vessels and was filled by excessive fluid. Not recognizable organ was existed in abdominal/thoracic cyst. The head, neck and limbs were formed in embryo. The normal brain tissue was replaced by large cavity with rim of nervous tissue (figure 3). The formation of feather was also defective.



**Fig. 1** The chick embryo is injected with sterile physiologic saline solution of 0.5 ml/egg into yolk sac. The embryo is normal with no lesions.



**Fig. 2** The chick embryo is injected with methenamine at dosage of 30 mg/egg into yolk sac. Note to the cyst formation instead of abdominal and thoracic body structures.



**Fig. 3** The chick embryo is injected with methenamine at dosage of 30 mg/egg into yolk sac. The normal brain tissue is replaced by large cavity with rim of nervous tissue.

## DISCUSSION

Methenamine salts are therapeutic agents and have bactericidal effect. Most gram-positive and gram-negative pathogens are susceptible (Sweetman et al. 2009; Ahrens and Martin 2013; Al-Badr and Al-Shaikh 2013; Hickling and Nitti 2013). In many countries such as, United States, Austria, Polish, Canada, Denmark Germany, Spain, France, Turkey, Africa and China methenamine compounds has been used successfully for several decades (Sweetman et al. 2009; Basger et al. 2012; Lee et al. 2012; Hickling and Nitti 2013). The Food and Drug Administration (FDA) has approved oral methenamine only for the treatment of urinary tract infections because of limited urotropine-related clinical research. Furthermore, methenamine is in the FDA pregnancy category C. This means that it is not known whether it will be harmful to an unborn baby. In some countries, this drug may only be approved for veterinary use (Lee et al. 2012; Williams et al. 2012; Al-Badr and Al-Shaikh 2013). Nevertheless, considering the adverse effects of methenamine on animal and human health still needs to be justified. Determining the side effects of drugs on the development of chick embryo is a useful method for studying the biological properties of drugs. There is scanty information available about toxicopathological effects of methenamine in embryo. Current study is focused upon the teratogenic lesions induced by administration of methenamine in chicken embryo. This research can further help us to investigate the toxic potential of methenamine salts in human fetus. In our study, gross abnormality on the external body surface consists of impaired feather growth. Cyst formation was also seen in the embryos. In the brain, normal tissue was replaced by large cavity with rim of nervous tissue. Our study established that methenamine at dosage of 30 mg/egg resulted in embryo-teratogenic effect. No such lesions have been reported in avian embryo by previous workers. Methenamine pharmacokinetic has not been studied in avian species. Tissue distribution of methenamine in avian embryo is also unknown and there was not any comparable report. Based on our results, we conclude that methenamine can distribute into the brain and various tissues of chicken embryo. The mechanism behind urotropine-induced teratogenic lesions in embryo is not exactly known. These alterations may be due to cytotoxic properties and adverse effect of methenamine in the various organs and cells. Further investigations are needed to elucidate the underlying mechanisms. In human model, it was showed that methenamine distributes well into the various tissues (Sweetman et al. 2009). Adverse effects of methenamine salts, such as dysuria, bladder irritation, painful and frequent micturition, albuminuria, and gross hematuria in human have previously reported (Sweetman et al. 2009; Nosseir et al. 2012; Chen and Lee 2013; Davies et al. 2013). It was also showed that methenamine crosses

the placenta (Sweetman et al. 2009), so, further studies still need to be undertaken to determine the pharmacokinetic and teratogenic effects of methenamine on the development of human fetus.

## Conclusions

Results of the present study describe different alterations induced by the administration of methenamine in chicken embryos. Current study also advises caution in the extended use of methenamine compounds. Further studies are needed to clarify the toxic effects of this drug on the development of human fetus.

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Galley Proof