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Adverse effects of antimalarial drug – alpha/beta artether on growth of developing chick embryo

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Abstract
α/β artether is one of the most commonly antimalarial drug used worldwide to treat chloroquine resistant malaria and malaria falciparum. Artemisinin are the concentrates of artemisia plant found in china also called as Qinghaosu, and its subordinates are a group of drugs that have the most rapid action of all current drugs against Plasmodium falciparum, and α/β artether is one of its subordinates. The present study was performed on 153 eggs divided in to experimental groups A and B and control groups a & b. The eggs of experimental groups were exposed to α/β artether on 5th day of incubation with doses of 0.12mg and 0.24mg respectively. Whereas the control groups were treated with same amount of normal saline. On 20th day of incubation the eggs were broken and embryos were removed. The results showed no significant gross anatomical malformations but the experimental groups showed more mortality percentage, growth retardation and delayed growth.

Keywords: Artemisinins, α/β artether, Malformations, Teratogenicity.

1. Introduction

Chick embryo as a model

The only method of attaining a comprehensive understanding of embryological processes is through the study and comparison of development in various animals, and the chick is one of the most satisfactory animal on which embryological laboratory work may be based [1]. It is preferable to study embryology of chick or common fowl (Gallus gallus) because of several advantages. Eggs of chick are large in size, available through the year, can be incubated artificially and are easy to control. Chicken embryology is much like that of human in general. This character of the chicken has led to some major discoveries in embryological science [2]

Malaria

In India malaria imposes incredible socio-economic burden on humankind and with six other diseases like diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis B and pneumonia represent 85% of global infectious disease burden [3]. In the south-east Asian population of WHO, out of 1.4 billion individuals living in 11countries (area territory 8,466,600 km^2, i.e. 6% of global area), approximately 1.2 billion are presented to the danger of malaria and majority of them live in India. India reports approximately two million cases and 1000 deaths attributable to malaria yearly [4]. One of the greatest difficulties facing malaria control is the parasite resistance to old antimalarial drugs chloroquine, sulfadoxine and pyrimethamine etc.

Artemisinins

At present artemisinin group of drugs (α/β artether, Artisunate. Artemether and DHA etc.) are the drugs of choice for treating chloroquine resistant malaria and malaria falciparum. Artemisinin is the concentrates of artemisia plant found in china also called as Qinghaosu, and its subordinates are a group of drugs that have the most rapid action of all current drugs against Plasmodium falciparum. Chemically, artemisinin is a sesquiterpene lactone containing an unusual peroxide bridge which is responsible for the drug's mechanism of action.

2. Materials and method

2.1 Materials Used

Eggs of Gallus Gallus domesticus (white leg horn chicken), candle box, antimalarial drug α/β artether, tuberculin syringes, distilled water, normal saline, sprit lamp, wax, incubator with humidifier, measuring tape, magnifying glass and electronic weighing machine etc.

2.2 Methodology

Inclusion Criteria

- Proper calcified eggs with intact shell.
- Eggs having air cell at broader end.
- Egg's air cell without any blood clot.

Exclusion Criteria

- Eggs with cracked shell due to improper calcification.
- Eggs not having air cell at broader end.
- Eggs having blood clot in air cell.

The present study was designed to assess the effects of α/β Artether, an antimalarial drug on growth of developing chick embryo in 120 eggs of white leg horn chicken. The study was conducted in the department of Anatomy, Gold Field Institute of Medical Sciences and Research, Chhainsa, Ballabgarh, Faridabad, Haryana and Department of Anatomy; Santosh Medical College Ghaziabd. The eggs were obtained from Metro Feeds, Indotec Agro Products, village Dabua, Dabua Pali road, Faridabad.

The method was adopted as adopted by P.E. Natekar (2007) [5], Ritu Singroha *et al.* (2012) [6] and Sywicha Thongphanich and Jantima Roongruangchai (2013) [7] and Muktyaz Hussain (2014) [8].The eggs were candled in order to exclude defective ones and to mark outline the exact location of air cell with a pencil. For this purpose a specially made wooden box was procured. This box had a connection for a CFL and was painted black from inside. The slots for the chicken eggs made in the top. The weight of selected eggs was between 33.30 grams to 48.15 grams (mean40grams).

The selected eggs were thoroughly washed with soap water solution and put in incubator with their broad end up where chorioallantoic membrane is situated. The temperature and humidity inside the incubator were maintained at 38^0c and 80% respectively without additional CO_2 or O_2. The eggs were tilted two times a day. On the 5th day of incubation eggs were removed from incubator and candled again prior to administration of drug.

Name of Drug-Artether

Availability -150 mg / 2ml

Name of the manufacturer - Injection E-Mal (Themis Medicare)

Recommended dose -150 mg by intramuscular route for 3 consecutive days (Adults)

-3 mg / kg of body weight by intramuscular route for 3 Consecutive days (Children)

Dose Titration

-1 Kg body weight (1000gm) requires 3mg of α/β artether

-40 gm body weight requires - 3X 40 / 1000 =0.12 mg

- Solution was made by- 2ml Artether + 48 ml Distilled water = 50 ml

-150 mg α/β artether is present in = 50 ml solution

- 12 mg α/β artether is present in = 50 x .12 / 150 = .04 ml

Method for drug administration

Eggs were divided into two groups experimental group A and B having 50 eggs each and two control group a and b having 10 eggs each. Eggs from experimental group A were exposed to α/β artether with dose of 0.12 mg and experimental group B to 0.24 mg in a volume of .04ml, and .08ml. whereas eggs from control groups were treated with same volume of normal saline respectively on the 5th day of incubation.

The solution was taken in a tuberculin syringe. The broad end of eggs was wiped with sterile gauze pad moistened with 70% isopropyl alcohol solution. A hole was drilled in eggshells in the centre of surface over the air cell with a lancet, It was taken care not to damage the shell. The needle of the syringe was inserted horizontally into the air cell and the solution was injected. The needle of the syringe was sterilized after wiping with sterile isopropyl alcohol 70% swab between each injection. The hole of the shell was sealed with candle melted wax immediately after injection.

After the injection of drug, sealed eggs were kept again into incubator at 38 ^{0}C and 80% humidity and tilted two times a day. On the 20th day of incubation eggs were removed from the incubator for sacrifice. The egg shells were broken with a scalpel and the embryos were removed. The number of live and dead embryos was noted. Crown rump lengths (length from base of the beak up to the tip of the coccyx through median plane on dorsal aspect[5]were measured with a thread and a centimeter scale, weights were measured by electronic weighing machine and following gross anatomical malformations were observed with necked eyes and magnifying glass.

- Macrocephaly (Enlargement of head)
- Microcephaly (Small size of head)
- Exophthalmia (Bulging eye)
- Micropthalmia (Small eye)
- Narrow Neck
- Twisted Neck
- Long Beak
- Short Beak
- Subcutaneous Hemorrhage (hematoma)
- Scanty Feathers
- Retraction of yolk sec

3. Observations

The weight and CRL of the chick embryos were measured and gross anatomical malformations were observed. The percentage of lethality was compared in all groups and shown in Table 2.

The decrease in weight and CRL is given in Table no. 3.

4. Tables and Figures

Table1: Showing exclusion criteria

S. No	Exclusion Criteria	Total no. of eggs taken (153)
1	Eggs with cracked shell	1
2	Eggs not having air cell at broader end	3
3	Eggs having blood clot in air cell	1
4	Unfertilized eggs	28
5	Total No. of eggs included in the study	120

Table 2: Showing lethal effects induced by α/β artether in developing chick embryo

Name of the groups	No. of fertile eggs used	No. of dead embryos	No. of live embryos	Percentile lethality
Control a	10	00	10	0%
Experimental A	50	02	48	4%
Control b	10	01	09	10%
Experimental B	50	07	43	14%

Table 3: Showing abnormal weight and crown rump length due to effects of α/β artether

S. No.	Groups	Weight of embryo (gm.)	CR Length of embryo (cm)
1	a	20.77 ± 1.24	7.05 ±0.59
2	A	19.18 ± 3.94	5.57 ± 1.00
3	b	18.64 ± 2.33	6.41 ± 1.24
4	B	23.43 ± 3.88	5.83 ± 1.64

Fig 1: Weighing of eggs.

Fig 2: Marking out line the exact location of aircell.

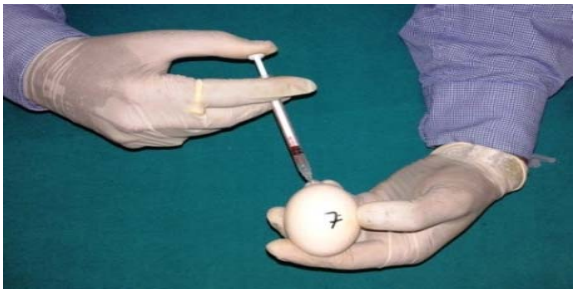

Fig 3: Drug administration

Fig 4: Normal chick embryo

Fig 5: Dead and growth retarded embryo

5. Discussion

Infection with plasmodium Falciparum and chloroquine resistant plasmodium Vivax is dangerous so effective and safe treatment is required. At present α/β artether is drug of choice for the treatment of Malaria Falciparum and chloroquine resistant malaria Vivax because no other drug is available for the same. Due to non availability of any other drug and more over due to insufficient health infrastructure in hugely populated India α/β Artether and other drugs of its group are being using very frequently resultant α/β artether resistance malaria cases are reporting. Under the circumstances people are helpless to take α/β artether in higher dose or for a long period for the treatment of malaria falciparum.

The role of α/β artether is controversial in many previous studies. In a study α/β artether and other drugs of its group were found embryo fetal and angiogenesis inhibitor in rats [10]. By using artemisinin combination therapy during 1st trimester in 685 pregnant Senegal found 2.6% stillbirth, 2,8% miscarriages and in 1.6% Senegal major congenital malformations were identified [11]. In previous animal experiments there are clear evidence of death of embryos and some evidence of gross anatomical abnormalities [9] by using α/β artether and drugs of its group.

There are so many research fellows [12, 13] who worked on teratogenic effects of α/β artether and other drugs of its group with respect to pregnancy and concluded toxic in early pregnancy and safe in 2nd and 3rd trimester. As far as teratogenic effects of higher dose of same drug is concern the data is very much insufficient almost negligible. In present study the percentage of lethality (mortality) in experimental groups A (4%) and B (14 %) is significant as compare to control groups a (0%) and b (10%).

As compare with control group decrease in weight of the eggs and CR length of embryos were observed in experimental groups which indirectly implies the growth of the embryos. No significant gross anatomical malformations mentioned above were observed neither control nor in experimental groups.

6. Conclusion

In present study increased mortality percentage and decrease in weight and crown rump length due to double dose of the drug indicate to do more work with a large sample size to asses the adverse effects of α/β artether.

7. References

1. The chick embryo past, present and future as a Yuji model system in developmental Biology, Elsevier Ireland limited, Mechanism of development 121 (2004) 1011-1013
2. R.L. Kotpal/ Modern Text Book of Zoology ; 3rd Edition 2013; page no.651-662
3. Estimation of True Malaria Burden in India, A profile of National Institute of Malaria Research Page no.91-99
4. National Vector Born Disease Control Programme. Directorate General of Health Services, Ministry of Health & Family Welfare. 2014
5. P.E. Natekar/ Methotrexate Induced Gross Malformations in Chick Embryos J. Hum. Ecol., 21(3): 223-226 (2007)
6. Ritu Singroha, S.K. Srivastava1, Pankaj Chhikara/ Effect of Gentamicin on kidneys in developing chicks, Eur J Anat, 16 (2): 119-126 (2012).
7. Suwicha Thongphanich and Jantima Roongruangchai, The effects of Alphacypermethrin Pesticide on the development of in Ovo Gallus domesticus, The 29thNational Graduate Research Conference, Mae Fah Luang University, 24-25 October 2013
8. Muktyaz Hussein, Vishram Singh, Prerna Gupta, Birendra Yadav, Singh AK. Developmetal and Biochemical Effects of Imidacloprid On Developing Chick Embryos; JASI,22(2):Dec 2014;12
9. Li, Q, Si, Y, Smith, Zeng Q, Weina P.J/ Embryotoxicity of artesunate in animal species related to drug tissue distribution and toxicokinetic profiles,. Birth Defects Res. Reprod. Toxicol, 2008;83: 435-445
10. Bhabagrahi Rath. Reproductive profile of artemisinins in albino rats. Indian J Pharmacol. 2010; 42(3):192-193
11. Dellicour S, Brasseur P, Thorn P. Gaye O, Olliaro P, Badiane M. Problastic record Linkage for monitoring the safety of artemisinin –based combination therapy in first trimester Of pregnancy in Senegal; Drug. Saf. 2013; 36(7):505-513.
12. Qigui Li, Peter J, Weina. Severe embryotoxicity of Artemisinin Derivatives in Experimental Animals, but Possibly Safe in Pregnant Women. 2010; 15:40-57
13. Phillips- Howard PA, Wood D. The safty of antimalarial drugs in pregnancy. Drug Saf. 1996; 14(3):131-145.