Original Article - Evaluative Study

Effect of Kenacort on Pregnant Wistar Albino Rats

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Abstract

Kenacort is a well-known synthetic glucocorticoid used in today's medical practice for various therapeutic uses. The drug has been known to induce cleft palate in rodents in specific doses. This study uses triamcinolone acetonide (TAC) as a teratogen for inducing cleft palate in rat embryos. The study discusses the molecular level action of TAC on the palatal mucosa in the rat palate. This study substantiates that the TAC given during the first trimester between 29th and 38th day of gestation can induce many congenital anomalies. The study shows an animal model to depict the drug toxicity on the pregnant Wistar albino rats.

Keywords: Cleft palate, teratogenicity, toxicity, triamcinolone acetonide

Introduction

Triamcinolone acetonide (TAC) is the acetonide salt form of triamcinolone, a synthetic glucocorticosteroid with immunosuppressive and anti-inflammatory activity.[1] TAC is the drug of choice for diseases such as rheumatoid arthritis, rheumatic fever, gout, autoimmune, bronchial asthma, severe infections, and intestinal infections and malignancies. TAC given during pregnancies can induce various congenital abnormalities such as cleft palate, hare lip, cardiac septal defects, and neurological and behavioral disturbances, especially during the second trimester of pregnancy.[2]

TAC binds to specific cytosolic glucocorticoid receptors and subsequently interacts with glucocorticoid receptor response element on DNA and alters gene expression. This results in an induction of the synthesis of certain anti-inflammatory proteins while inhibiting the synthesis of certain inflammatory mediators. Consequently, an overall reduction in chronic inflammation and autoimmune reactions is accomplished.[1] TAC is used in therapeutic doses for immunosuppressant and anti-inflammatory activity.

Experiment

Twenty Wistar albino rats were used all of them were female rats. The study was carried out after getting permission from the Institutional Ethical Committee and Animal Ethical Committee (IEC/Research board, Saveetha University). The rats were weighing 150–160 g on an average. The rats were 22-week-old rats. They were divided into two groups. Group I received TAC at a dosage of 0.5 mg/kg bodyweight and Group II received 0.05 mg/kg bodyweight. A rat of 150 g received approximately 0.075 mg [Figure 1].

The female rat was allowed to mate with male rat during its esterase cycle. The day of the vaginal plug was calculated as day 0. The pregnant rats belonging to Group I were given TAC at a dosage of 0.5 mg/kg bodyweight on the 10th–14th day of gestation. Pregnant rats belonging to Group II were given the same drug at a dosage of 0.05 mg/kg bodyweight on 10th–14th days of gestation but on the alternate days.

- Average body weight of Indian strain male wister albino rat is 185g and female rat is 150g

Drug dosage calculation

0.5 mg/kg bodyweight is 0.5/1000 = 0.005 mg of drug can be given for rat weighing 150g 0.005* 150 = 0.075mg.

0.5mg/kg bodyweight triamcinolone acetonide (TAC) given intraperitoneally to the pregnant rats during the 10-14th day of gestation for inducing nonsyndromic cleft palate animal model. The rats were weighing around 150–165 g on an

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average. Control group animals did not receive any drug and had normal gestation period. Group I animals had various side effects. There was significant weight loss, subcutaneous bleeding, severe gastritis, ulcerations in the gastrointestinal tract, cervical bleeding, dural venous sinus engorgement, and spontaneous abortion of the fetus. Group II animals received TAC at a dosage of 0.05 mg/kg bodyweight. There were reduced side effects when compared to Group I animals [Tables 1-2 and Figures 2-5].

The rat pups born to the mothers received 0.05 mg/kg bodyweight. The pups were abnormal in appearance. There was zygomatic protrusion and maxillary hypoplasia. Their birth weight was less when compared with the control group animals [Figure 6].

Discussion

The normal fetal oral cavity develops by opening of cranial pore rupture of the oropharyngeal membrane, which occurs on the 29th day of gestational age and 11th Carnegie stage. Crown and rump length is around 2.5–4.5 mm.[3] Formation of the upper lip occurs in the 38th day of gestational age and 16th Carnegie stage. The crown and rump length may be 8–11 mm.[3]

Hillard *et al.* in 2005 had stated that secondary palate develops in embryonic day 11.5 in rats.[4] Palatogenesis is completed when the palatine shelves make contact and adhere with each other in the midline forming the epithelial union. In the midline the fusion occurs and osseointegration takes place. Definitive palate fuses with the primitive palate and nasal septum in the anterior and midline regions, and this separates the oropharynx from the nasopharynx. This complex mechanism of palatal fusion involves multiple steps and failure during any one step can cause defective palatal fusion leading to cleft palate. Derivates of first and second rhombomere can be involved in the defective palatal fusion.[5] In rat, the frequency of its occurrence is 1 in 7000 live births which is very rare. The affected pup usually cannot survive. To prepare a nonsyndromic cleft palate animal model, Wistar albino rats were selected and drug teratogenicity was used to induce cleft palate.

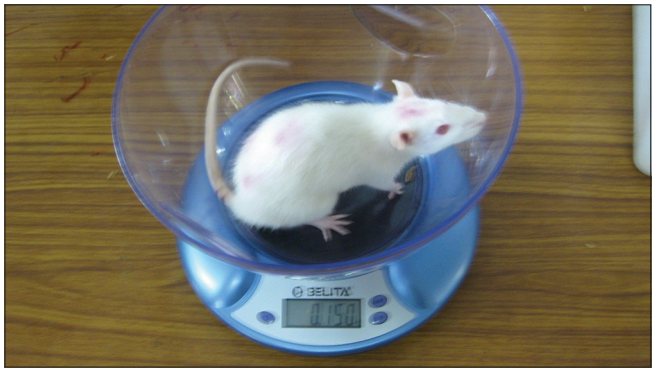

Figure 1: Wistar albino rat

Figure 3: Intraperitoneal injections being given in a Wistar albino rat

Table 1: Experimental groups in the study

Group name	Drug dosage	Number of animals	Type of injection
Group I	0.5 mg/kg	10 female rats	Intraperitoneally
Group II	0.05 mg/kg	10 female rats	Intraperitoneally
Control group	No injections given	2 female rats	No injections given

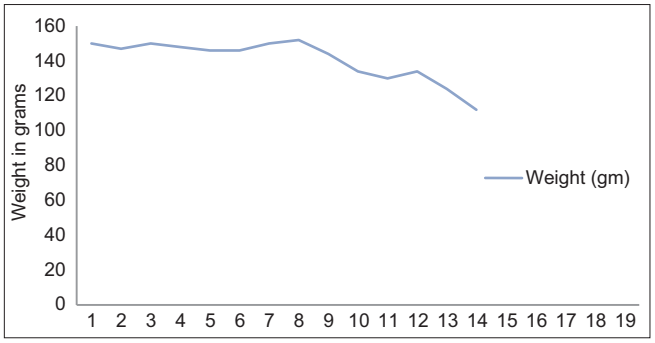

Figure 2: Significant weight loss in Group I animals which received 0.5 mg/kg bodyweight X-axis: Weight in grams; Y-axis: Gestational age

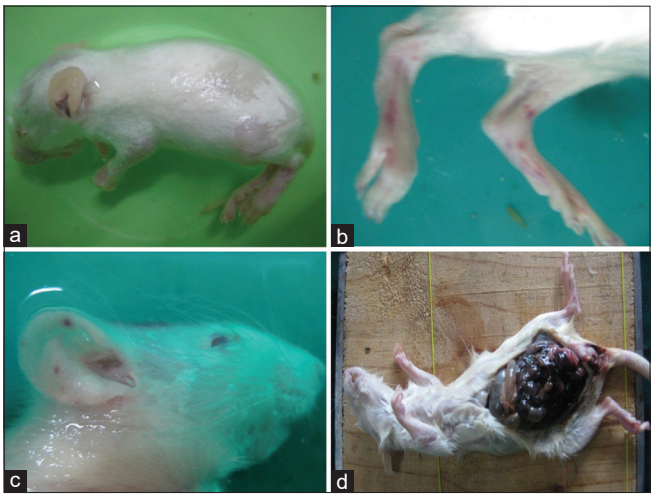

Figure 4: (a) Significant hair loss in the Group I animal. (b) Bleeding in the lower limb and in the pinnae, (c) subcutaneous bleeding spots in the pinnae, (d) the abdominal viscera appearing black in the postmortem

Table 2: Drug dosages used in the study

	Group I (TAC dosage 0.5 mg/kg bodyweight)	Group II (TAC dosage 0.05 g/kg bodyweight)
Number of animals	10	10
Drug dosage	0.5 mg/kg bodyweight	0.05 mg/kg bodyweight
Mode of injection	Intraperitoneally	Intraperitoneally
Average loss of weight	60-70 g loss on an average	40 g loss of weight
Subcutaneous bleeding	Subcutaneous bleeding seen on the pinnae and the lower limb	No subcutaneous bleeding seen
Severe gastritis/ulcerations in GIT	Gaseous bubbles seen in the inflamed intestines	Not seen
Cervical bleeding	Spontaneous cervical bleeding was evident	Cervical bleeding not seen
Dural venous sinus engorgement	Dural venous sinus engorgement seen	No venous engorgement evident
Infant pups	Did not deliver the infant pups	Delivered infant pups, but they had maxillary hypoplasia, protruded zygomatic arches, growth rate was retarded
Cleft palate and lip	Seen	Not seen

TAC=Triamcinolone acetonide; GIT=Gastrointestinal tract

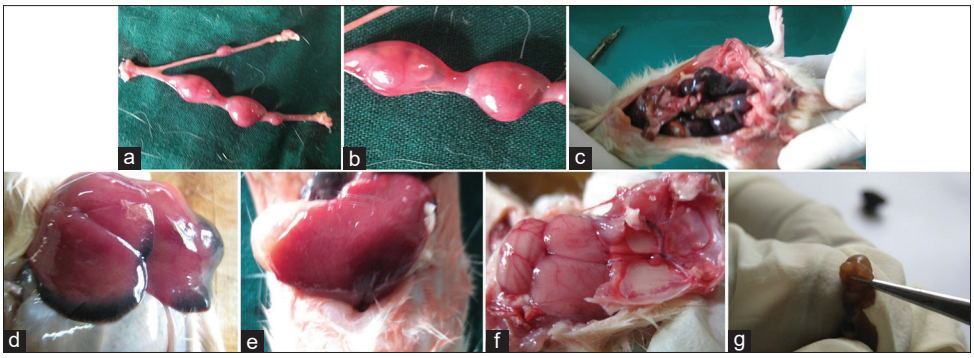

Figure 5: (a) Two 16-day-old fetus in the bicornate uterus, (b) 16-day-old fetus seen in the bicornuate uterus, (c) inflamed intestines within the animals, (d) hepatomegaly with nodulations, (e) liver showing nodulations, (f) intracranial hemorrhage and engorgement of dural venous sinus, (g) fetus showing cleft palate

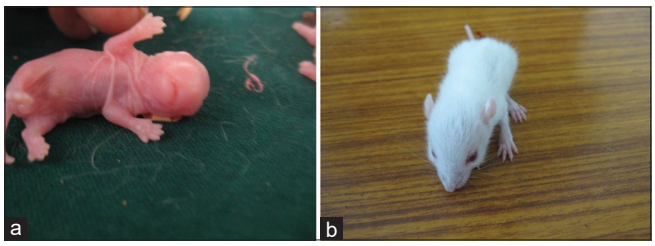

Figure 6: (a) Pups delivered for the Group II animals, (b) delivered infant with abnormal face, note the zygomatic arch protrusion, altered maxillary and mandibular arch hypoplasia

Phylogenetically, in birds and in some reptiles, cleft palate is an ancestral trait.[4] The reason for selecting rat as an animal model for nonsyndromic cleft palate has been supported by Sehupbach.[6] He stated that human and rodents share greater similarity in palatogenesis. Poswillo *et al*. in 1968[7] also supported the striking resemblance between naturally occurring cleft palate in human and those produced by rat in teratogenic experiments. The teratogens affecting the human fetus at the 11th–16th Carnegie stage develop cleft lip and palate. Drugs such as glucocorticoids given by oral or parental route during the first trimester can affect the maxillofacial development of the fetus. Tripathy Textbook of Pharmacology[2] states that glucocorticoids given during second trimester can produce congenital defects.

The rodent animal model used for the development of cleft palate clearly indicates that glucocorticoids given during 10th–16th day of gestation in Wistar albino rat produced cleft palate. The animal model clearly depicts that TAC administered through the tail vein of the pregnant maternal rat can produce improper fusion of the palate. Glucocorticoids also had significant systemic side effects on the maternal and the rat fetus. The rat pup born to the pregnant rats in group II had significant growth retardation, delay in milestones, altered maxillofacial bone growth and defective facial skeletal development.

Group I animals which received TAC dosage at a concentration of 0.5 mg/kg bodyweight had many side effects. The animals that received high dosage of TAC 0.5 mg/kg bodyweight has suffered drug toxicity suffered with severe weight loss. Loss of appetite, subcutaneous bleeding in the limb and pinnae.[2] He also further reported cleft palate, cleft lip, cardiovascular, and musculoskeletal deformity in TAC-induced cleft palate when given during the second trimester of life. Hilliard *et al*. in 2005 and Gu *et al*. in 2008[8] have stated that the critical period in the palotogenesis is embryonic day 11.5. The palatine shelves elevate during the embryonic period 14.5–15.5 days in rats. During this interval, a series of steps take place that involve the fusion of palate. TAC interrupts these steps to cause a cleft defect in rats. A series of molecular steps occur in the

differentiation of the palate one or more of which is blocked by TAC, thus causing cleft palate. Hence the second group of animals was given 0.05 mg/kg bodyweight. The second group of animals had no toxic symptoms.

The animal had reduced bodyweight and decrease in appetite. Behavioral changes were also noted. The rat pups delivered by the Group II animals had distinct cleft palate and maxillofacial bony deformities. The TAC acts on the medial edge epithelial cells (MEE) and prevent apoptosis of the MEE, thereby preventing the fusion of the palatal shelves.[9] Epithelial–mesenchymal interaction is prevented thereby interrupting palate formation. There is evident cross-talk between the Wnt and transforming growth factor (TGF)β3 signaling during the epithelium and mesenchymal interaction. TGF β3-mediated phosphorylation of SMAD 2 inhibits the fusion of the palatal shelves. The TAC forms a complex with the receptor present in the cell membrane. This receptor complex helps the TAC to enter the cell and nucleus. It translocates the nucleus, which then binds to chromatin acceptor sites and alters transcription factors such as MSx 1. The study utilizes the drug to induce cleft palate in Wistar albino rats and reconstruct it with bone marrow mesenchymal stem cells.

Role of Triamcinolone Acetonide on the Immune System

Classical cytotoxic immunosuppressant acts by inhibiting DNA synthesis. Others may act through activation of T-cells or by inhibiting the activation of helper cells. While immunosuppression has been brought about in the past primarily to prevent rejection of transplanted organs, new applications involving effects of interleukins and other cytokines will emerge in future.

Results

TAC is a potent synthetic glucocorticoid that it is widely used in today's medical practice for various inflammatory reactions. The drug is to be avoided in use especially in first trimester of pregnancy (29th–38th day of pregnancy). The TAC has the potency to inhibit the palatal MEE interrupting the palatal fusion molecular signaling, thereby causing cleft or improper fusion of the palatine shelves. Further clinical trials are needed to be performed to avoid using the teratogen during the gestational period.

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Conflicts of interest

There are no conflicts of interest.

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