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TERATOGENIC EFFECTS OF MODAFINIL ON EARLY EMBRYONIC DEVELOPMENT OF GALLUS GALLUSDOMESTICUS

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ABSTRACT

Modafinil is a wakefulness promoting drug prescribed for narcolepsy and sleep apnea. Our experiment was aimed to study the effects of Modafinil on early developmental stages of chick embryos (*Gallus gallus domesticus*). For this, early developmental stages of chick embryos were treated with 10 mM and 100 mM of Modafinil. Modafinil induces mild to severe teratogenesis in a dose-dependent manner. This study can be extrapolated for the evaluation of the effects of Modafinil on gestating and lactating women, so as to prevent teratogenesis in the developing foetus. More studies on Nootropics like Modafinil and its derivatives need to be carried out to study their effects on developmentally regulated gene expression.

KEYWORDS: Modafinil, Teratogenesis, Chick Embryos, NTDs, Nootropics, Hamburger-Hamilton(HH) staging system.

INTRODUCTION

Modafinil (2-[(diphenylmethyl) sulfinyl] acetamide)[1] is a novel psychostimulant waking drug used in treatment of narcolepsy. Along with waking effects it also generates mood-brightening and memory-enha- ncing effects. Modafinil is a racemic compound consisting of R-enantiomer and S-enantiomer.[2]

The exact mechanism of action or the site of action of Modafinil is not yet known, however it is found that it targets sodium dependent dopamine transporter and alpha 1B adrenergic receptors.[3,4] Research conducted on rats resulted in elevated levels of dopamine in the

prefrontal cortex, norepinephrine in hypothalamus andhistamine via hypocretin cells.[5]

The effects of Modafinil on the body helps to overcome fatigue, improve working and episodic memory in both depressed and healthy patients, enhance alertness and restore cognitive performance and also improve overall mood.[4]

Modafinil is administered to patients suffering from narcolepsy, sleep-work disorder, obstructivesleep apnea, and other mental illnesses.[6] Behavioral studies on mice showed that, Modafinil induces hyperactivity and hyperreactivity without stereotypical behavior.

Another study conducted on mice showed that Modafinil did not produce any significant behavioral or peripheral change at lower doses (2-8mg/kg, i.p.) while at higher doses, hyperactivity and hyperreactivity was observed. Similar observations were found in monkeys.[7] Modafinil treated cats showed extremely less FOS immunoreactivity in the brain. No FOS labeling as a result of Modafinil administration in the basal forebrain, thalamus, posterior hypothalamus, or the midbrain tegmentum was observed.[8,9] Studies conducted on pregnant rats and rabbits showed developmental toxicity at clinically significant drug exposures.

Orally administered Modafinil to pregnant rats showed an increase in resorptions and increased incidence of visceral and skeletal variations in offspring at higher doses. Similar study in rabbits showed an increase in fetal structure alterations and fetal mortality at higher dose.[10]

Modafinil interaction with genes

In various studies, Modafinil was found to regulate dopamine transporters (DAT) which may help in regulation of wakefulness and alertness.[11] The gene *slc6a3* is said to be responsible for encoding the production of DAT which plays a major role in cognition, motivation, behavior, and control of movement.[12] A study found polymorphism of the gene *slc6a3* in genetically modified animals leads to reduced responsiveness to Modafinil, suggesting the drug response is affected by gene modification.[13]

The COMT gene is responsible for the methylation of catecholamines such as dopamine, norepinephrine and epinephrine.[14,15] A study showed that the polymorphism of COMT modulates the effects of Modafinil, showing pharmacological interference with dopaminergic and adrenergic neurotransmission.[16] Another study found drug interaction of Modafinil and

FOS gene in mice, suggesting change in expression of the gene when the drug was introduced.[17]

It is possible that Armodafinil, an R-enantiomer of Modafinil, can induce teratogenesis and otherbirth complications during pregnancy.

Armodafinil can cause intrauterine growth restriction and also lead to spontaneous abortion.[18]

This paper deals with the potential effects of Modafinil on early embryonic development usingchick embryos as model organisms.

MATERIALS AND METHODS

Fertilized and pre-incubated eggs (HH stage 0-1 (0hr+), 12-13 (48h)) of ***Gallus gallus domesticus*** were obtained from Venkateshwara Hatcheries Pvt Ltd, Pune. Freshly prepared solution of 10mM and 100mM Modafinil (Sun Pharma Laboratories Ltd.) were used for treatment by *In-ovo* technique (air sac route).

Control and treated embryos (Modafinil 10mM and 100mM respectively) were incubated for 24h - 48h at 37.8°C and relative humidity of 70-80%in a BOD incubator.

Post-treatment embryos were harvested in 1X chilled PBS (pH 7.4) and observed for drug-induced anomalies. Observations were recorded under a dissecting binocular Stereo Zoom microscope (Magnus MAZ Series). Permanent slides of treated and control embryos were prepared by the standard microtechnique procedure for detailed observations.

DISCUSSION

Set 1 (HH 0-1, 0hr, 24hr incubation)

In embryos treated with 10mM Modafinil (Fig. 1.B), Accelerated embryonic development, optic cups well developed, **somitogenesis** +++ (doubling of somites), wavy neural tube (NTD), normal heart development was observed. In 100mM Modafinil treated embryos (Fig. 1.C), severe teratogenesis w.r.t. cephalization, brain vesicles inseparably fused, **somitogenesis** +++ **(**doubling of somites), premature and abnormal bending of embryo (abnormal torsion and flexion), extreme CNS anomalies, CVS anomalies (fused cardiac vesicles) were observed.

Set 2.1 (HH 12-13, 48hr, 24hr incubation)

After 24h incubation, embryos treated with 10mM Modafinil (Fig. 2.B), had an enlarged Telencephalic hemisphere and enlarged Mesencephalon, Isthmus was more prominent. Embryos treated with 100mMModafinil showed (Fig. 2.C), enlarged brain, fused neuromeres or holoprosencephaly, and hypertrophy of anterior brain structures. Isthmus was undifferentiatedwhile the Telencephalic-Mesencephalic boundary wasnot clearly visible. The embryonic axis was highly curved as compared to the control embryo. These Observations were consistent with other isolated embryos.

Set 2.2 (HH 12-13,48hr, 48 incubation)

To check for duration of drug exposure dependent teratogenesis, another replicate of set2 was cultured, and was isolated after 48h incubation post-treatment. In embryos treated with 10mM Modafinil and 48h incubation following observations were noted (Fig. 3.B) severe haematoma throughout the embryo, hemorrhage, normal limb buds, well-developed extra-embryonic membranes, enlarged brain.

Thus the degree of teratogenesis was directly proportional to dose concentration and drug exposure time. 48h (HH 12-13) embryo with 100mM Modafinil treatment after 48hr incubation, failed to survive.

RESULTS

Our studies showed that Modafinil induced teratogenesis in the developing embryos, and the degree of teratogenesis was proportional to the drug concentration and duration of drug exposure. Eggs treated with 10mM Modafinil with 24h incubation, the degree of teratogenesis was moderate, and gradually increased as incubation time and drug exposure time was increased. Eggs treated with 100mM Modafinil, 24h incubation showed severe teratogenesis.

Increasing incubation period upto 48h showeddegenerated embryos.

A **B** **C**

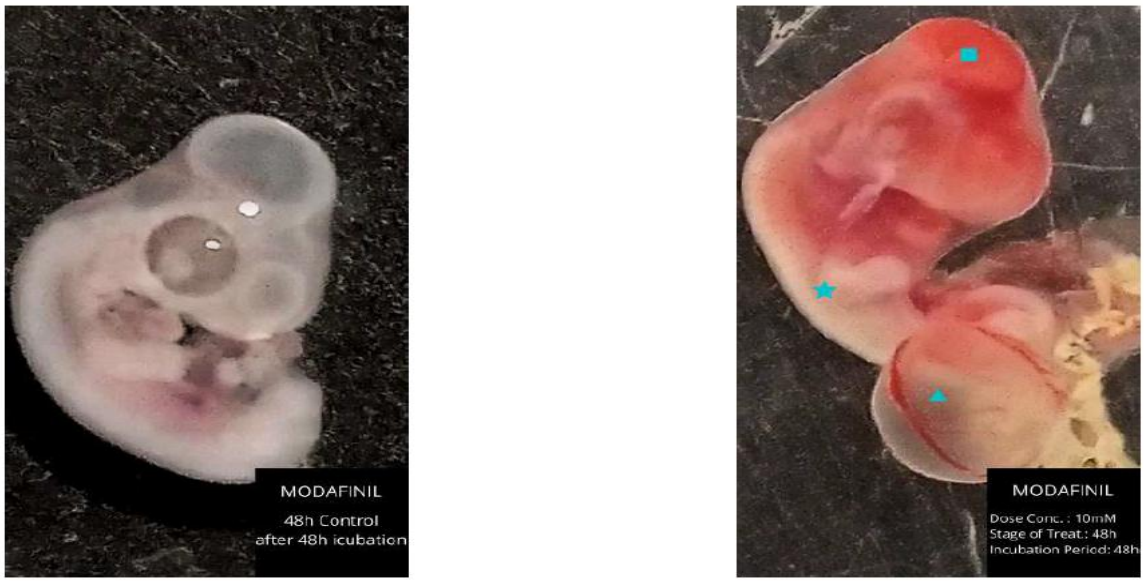
Figure 1: Set 1 (HH 0-1, 0hr, 24hr incubation)

Fig. 1: A shows Control embryo of	**Fig. 1: B Shows Accelerated embry-**	**Fig. 1: C shows severe teratogenesis**
0hr after 24h incubation	**onic development, optic cups well**	**w.r.t. cephalization, brain vesicles**
	developed, somitogenesis +++ do-	**inseparably fused, Somitogenesis+++**
	ubling of somites, wavy neural tube	**doubling of somites, premature and**
	(NTD), heart development normal.	**abnormal bending of embryo**
		(abnormal torsion and flexion),
		Extreme CNS anomalies,
		Fused cardiac vesicles (CVS
		anomalies), heart inconspicuous.

A **B** **C**

Figure 2: Set 2.1 (HH 12-13, 48hr, 24hr incubation).

Fig. 2: A shows Control embryo of 48h after 24h incubation.	**Fig. 2: B shows an enlarged telen- cephalic hemisphere(■) and enlarged Mesencephalon(□). Isthmus was deep(★) and cardiac anomaly(▲).**	**Fig. 2: C shows severe deformit- ies in the brain(★), like the brain was enlarged, fused neuromeres or holoprosencephaly, and hyper- trophy of anterior brain structures Isthmus was undifferentiated while Telencephalic - Mesenceph- alic boundary was not clearly visible. Cardiac anomaly (▲)Embryonic axis was highly curved (■).**



A **B**

Figure 3: Set 2.2 (HH 12-13, 48hr, 48 incubation).

Fig. 3: A shows 48h control embryo after 48h incubation.	**Fig. 3: B shows severe hematoma throughout embryo, hemorrhage, Limb buds normal (★), extra-em- bryonic membranes well develop- ed(▲) and enlarged brain (■).**

CONCLUSION

The above studies showed that Modafinil induces embryonic malformations and is embryotoxic in a dose-dependent manner. The possible mechanisms by which Modafinil induces teratogenesis in developing vertebrate embryos needs to be studied in extensive detail at cellular and molecular level and these studiescan be used to extrapolate Modafinil toxicity on developing human embryos and whether the drug crosses the placental barrier to the developing human embryo is not yet known. Also, whether Modafinil changes embryonic

brain structure and affects synaptogenesis, neuroplasticity, behavior, memory formation need to be investigated.

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