ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prenatal Isotretinoin Exposure Reduces the Neuronal Population of Hippocampus in Rats

Jai Aditya, Sampath Madhyastha*, Sudhanshu Sekhar Sahu, Vasudha Saralaya, Divya Premchandran, Teresa Joy

Department of Anatomy, Kasturba Medical College, Mangalore, Manipal University, INDIA – 575 001

ABSTRACT

Isotretinoin is a drug used in the treatment of acne. Teratogenic effects of isotretinoin are well known. It is causes craniofacial abnormalities like cleft palate in animal model studies. There are very few studies focusing on its effect on the developing brain specially hippocampus concerned with memory. In the present study we investigate teratogenic effect on neuronal population of the hippocampus during postnatal development. Pregnant *Wistar* rats were exposed to either 8 or 16mg/kg dose of body weight of isotretinoin during early or mid-gestation of pregnancy. Pups were sacrificed at postnatal day 7 or day 21, brains were removed and processed for histological studies. Coronal sections o brain were taken and stained with cresyl violet and viable neurons were counted for 250 µm length in different regions of the hippocampus. At postnatal day 7neurons belonging to CA1 region of the hippocampus was severely affected at both the doses tested and also in early & mid-gestation treatment regime. At postnatal day 21, neurons of the CA2 & CA1 regions were severely affected. It is also observed that mid-gestational effect had more severe effect compared to early gestational treatment. This study clearly demonstrates the teratogenic effect of isotretinoin on hippocampal neuronal population of developing brain. Care must be taken while prescribing this drug to women of reproductive age.

Key Words: Hippocampus, Isotretinoin, Prenatal Exposure, Rat brain, Retinoic acid, Vitamin A

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The isotretinoin, a 13-cis-retinoic acid is a derivative of Vitamin A. It is commonly used to treat severe cystic acne. It is also given in few skin conditions like icthyosis, keratosis and xeroderma pigmentosum [1]. It acts by inhibiting the differentiation of sebaceous glands and corrects the defects in keratinisation of the follicle. There is a growing concern with regard to its teratogenicty. It causes various congenital anomalies including craniofacial abnormalities like cleft palate [2], cardiac, thymic and other neural malformations [3]. Though isotretinoin is a known teratogenic agent, it is still being used without adequate surveillance. Unplanned pregnancy during isotretinoin treatment can result in serious complications. Nevertheless, isotretinoin is at present considered to be one of the most widely prescribed teratogenic drug, in USA and Canada [4].Retinoid signaling is required for the development of the neural tube being responsible for differentiation of neurons and neural growth. Evidence suggests that one of the factors responsible for normal functioning of the adult brain is retinoid signaling and the Vitamin A nutritional status of the individual [5].Interruption in this signaling can result in disease states like Alzheimer's, Parkinson's and motor neuron disease [6].

Cell proliferation of neurons is known to occur in restricted regions of the adult brain like the subgranular zone of the hippocampus and the subventricular zone of the forebrain [7].Hence isotretinoin can affect the neuronal population in the hippocampus as well which is essential for the process learning and memory. There are inadequate studies focusing on the effect of prenatal isotretinoin on postnatal neurogenisis which proceeds for sometime during early postnatal development of the brain. Hence in the present study we quantified the neuronal population of the hippocampus.

MATERIALS & METHODS

Animals and housing conditions

In-house bred female albino *Wistar* rats (3-4 months and weight 200-230g) were selected. They were maintained in light and dark cycles of 12 hours each in a controlled environment and fed with standard food pellet and water. Institutional Animal Ethics Committee approval was obtained dated 09/05/2012.

Mating of rats and animal groups

Female rats (n=4) were allowed to mate with one fertile sexually active male rat. After 4 hours, vaginal smears were taken in order to check for the presence of sperms to confirm pregnancy. The rats were then designated as day 0 of pregnancy and were housed individually. One male and female pup from each mother were taken for histological studies (n=8).

Group 1: Control – Pups belonging to pregnant rats who received an equivalent volume of vegetable oil instead of isotretinoin.
Group 2: Pups belonging to pregnant rats who received isotretinoin (8mg/kg body weight dose) during early gestational period (day 1 to 5)

Group 3: Pups belonging to pregnant rats who received isotretinoin (8mg/kg body weight dose) during mid-gestational period (day 6 to 10)
Group 4: Pups belonging to pregnant rats who, received isotretinoin (16mg/kg body weight dose) during early gestational period.
Group 5: Pups belonging to pregnant rats who, received isotretinoin (16mg/kg body weight dose) during mid-gestational period.

Isotretinoin was administered orally using a metallic oro-pharyngeal cannula. The human dose of the isotretinoin is converted to the rat dose.

Neuronal assay of the hippocampus:

On7thand 21st postnatal day, pups were sacrificed for histological studies. Each rat was anesthetized with a high dose of ether and fixation was performed by trans-cardiac perfusion with 0.9%saline and 10% formalin. The brain was removed and kept in 10% formalin for 2 days post fixation. Paraffin blocks were made and coronal sections of 4-6-µm thickness were cut in the dorsal hippocampus using a rotary microtome. The sections were labeled and mounted onto air dried gelatinised slides. Slides are stained with cresyl violet [8]. In each hippocampal section 250µm lengths of different areas of the cornua amonis, were selected using an ocular-micrometer. The viable neurons were counted using light microscope (40X). Slides were coded to avoid manual bias. The cell counts were expressed as cells/250 µm length [8].

Statistical analysis:

All values were expressed as mean ±SD. The significance of differences among the groups was assessed using ANOVA test followed by Bonferroni's multiple comparison tests. Comparison of data between male and female group was assessed by unpaired “t” test. P values <0.05 were considered as significant.

RESULTS

Isotretinoin treatment (at both dosages-8 &16mg) during day 6 to 10 of pregnancy had 10% mortality at birth and 5% during preweaning period (8mg dose on day 6 to 10 treatment). No sexually dimorphic effect was observed in assessed parameters, hence mean values for both male and female were collapsed together.

Neuronal population of the hippocampus on day 7:

Isotretinoin at 8mg/kg dose (in both early & late gestational treatment) has significantly ($p<0.001$) affected the neuronal populations in all the regions of the hippocampus except in CA4 region ($p>0.05$) when compared to control group. Interestingly isotretinoin at 16mg/kg dose (in both early & mid gestation treatment) did showed any significant ($p>0.05$) effect on all the hippocampal areas quantified except for CA1 region in early gestational treatment ($p<0.01$). These results indicate that isotretinoin will affect the neuronal population of hippocampus especially affecting CA1region at both doses and both treatment regimen followed in this experiment (Fig.1, Fig.3a & 3b).

ISSN: 0975-8585

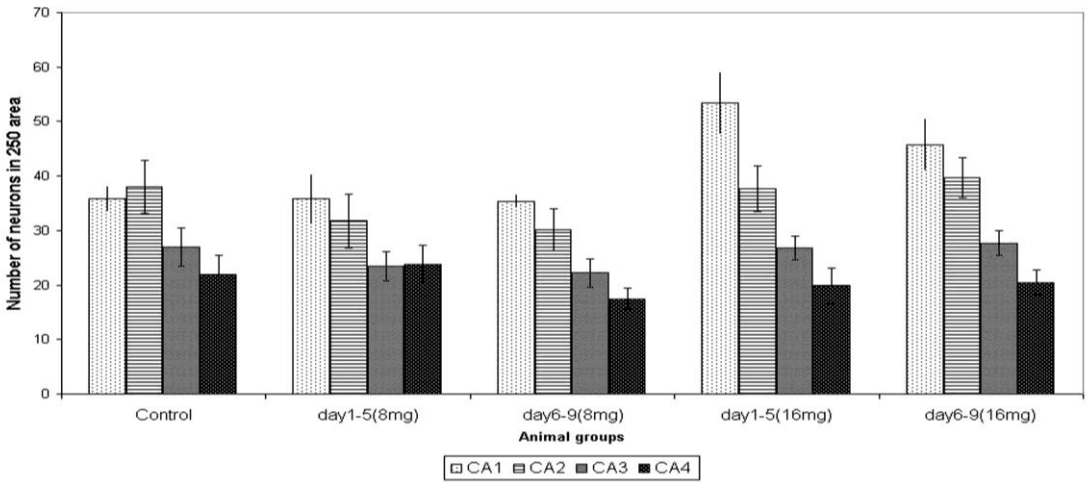

Figure-1: Neuronal population in the different regions of the hippocampus on postnatal day 7 in rats who received isotretinoin at different gestational days. Values are expressed as mean number of cells per 250 µm length. Error bar indicates ±SD (n=8)

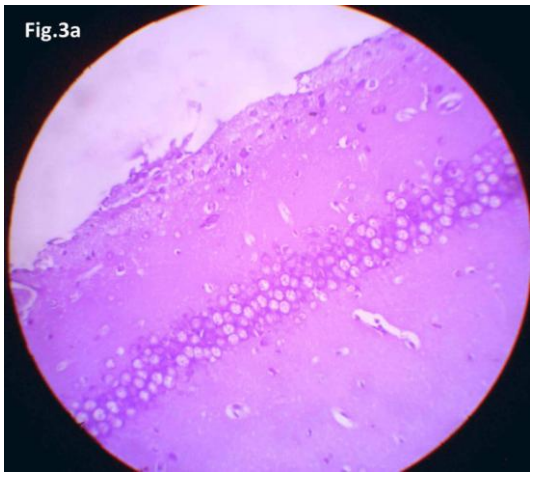

Figure-3a: Histomicrographic picture showing coronal section of 7 day old control rat's CA1 region of the hippocampus (all the remaining pictures are also stained with cresyl violet under 40X

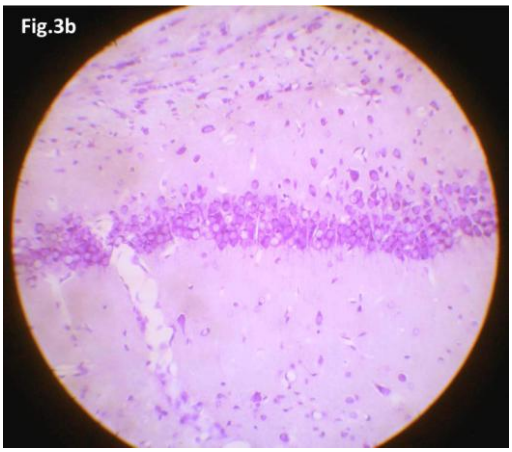

Figure-3b: Histomicrographic picture showing coronal section of 7 day old rat's CA1 region of the hippocampus, who received 8mg/kg dose of prenatal isotretinoin

ISSN: 0975-8585

Comparison between early and mid gestation treatment at 8mg/kg doses showed a significant effect in onlyCA4 region ($p<0.001$) but not in the remaining regions of the hippocampus. Similar comparison at 16mg/kg doses showed a significant ($p<0.001$) effect only in CA1 region. These results indicate that mid gestational treatment will have significantly reduced the neuronal population of hippocampus in CA4 &CA1 regions.

Comparison between 8 and 16mg/kg do seat early gestation treatment showed adecline in neuronal population in CA1 ($p<0.001$), CA2 ($p<0.05$) andCA3 ($p<0.05$) but not in CA4 region at 8mg/kg dose group. Similar comparison at mid gestation treatment, only CA1 neurons showed a significant ($p<0.001$) decline in neuronal number at 8mg/kg dose. These results demonstrate that the 8mg/kg dose has significant effect (decline in the neuronal population) at early gestational treatment.

Neuronal population of hippocampus on day 21:

Isotretinoin at 8mg/kg dose has significantly affected the neuronal population ofCA2 neurons in early ($p<0.01$) & mid gestational treatment ($p<0.001$) and CA1 neurons ($p<0.05$) at only late gestational treatment compared to the control group. Isotretinoin treatment at early gestation at 16mg/kg dose has significantly ($p<0.001$) affected all the regions of the hippocampus except forCA4 region compared to control. Similar results were obtained at 16mg/kg dose at mid gestational treatment except for CA3 &CA4 regions. From these results it is clear that prenatal isotretinoin will decline the neuronal population even at 21 day especially the CA2 region at both doses as well as two treatment regimen followed in this experiment (Fig.2, 4a & 4b).

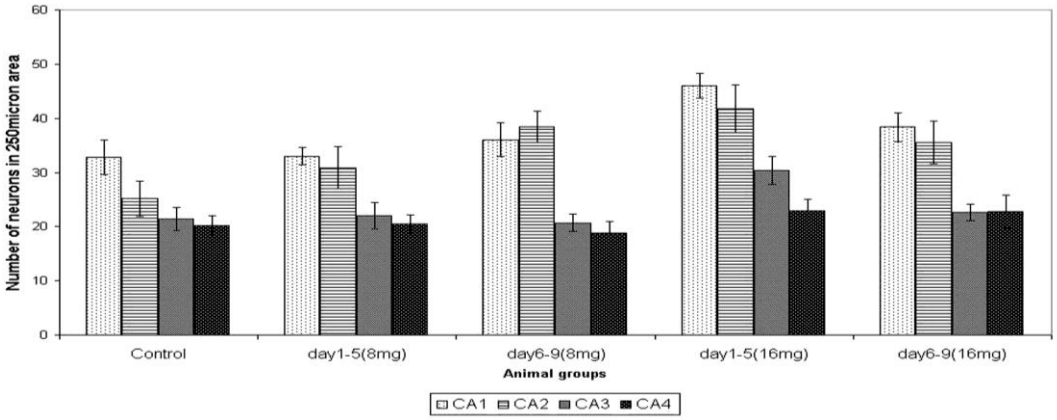

Figure-2: Neuronal population in the different regions of the hippocampus on postnatal day 21 in rats who received isotretinoin at different gestational days. Values are expressed as mean number of cells per 250 µm length. Error bar indicates ±SD (n=8)

ISSN: 0975-8585

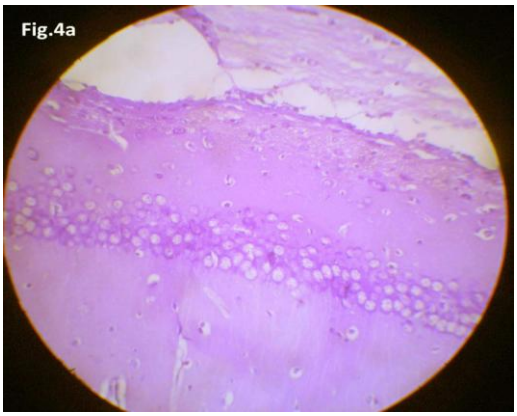

Figure-4a: Histomicrographic picture showing coronal section of 21 day old control rat's CA1 region of the hippocampus, who received 8mg/kg dose of prenatal isotretinoin

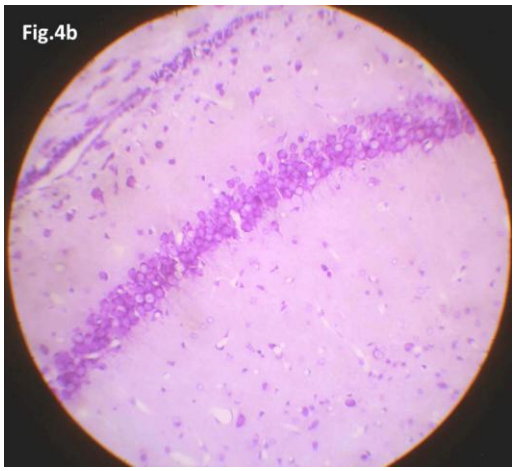

Figure-4b: Histomicrographic picture showing coronal section of 21 day old rat's CA2 region of the hippocampus in coronal section stained with cresyl violet under 40X, who received 8mg/kg dose of prenatal isotretinoin

Comparison between early and mid-gestation treatment at 8mg/kg dose, showed a significant ($p<0.001$) loss of neurons in CA2 region (in early gestational treatment) but this effect was not seen in the remaining regions. Similar comparison at 16mg/kg doses showed a significant decline in neuronal number in CA1region ($p<0.001$), CA2 region ($p<0.01$) and CA3 region ($p<0.001$) but not in CA4 region. Interestingly this decline was seen in mid gestational treatment. These results indicate that early gestational effect was inCA1 region and mid gestational treatment effect in CA1, CA2 &CA3 regions.CA1 neurons are affected at both day7 and day21. Comparison between 8 and 16mg/kg dose at early gestation treatment showed a significant decline ($p<0.001$) in neuronal population at 8mg/kg dose in all the regions of the hippocampus except in the CA4 region. Similar comparison at mid-gestation treatment did not showed any decline in neuronal number in the hippocampus. These results demonstrate that the 8mg/kg dose has significant effect (decline in the neuronal population) at early gestational treatment.

DISCUSSION

The result of the present study demonstrates that isotretinoin has a teratogenic

effect by affecting the neuronal population of the hippocampus in early as well as mid gestational treatment (CA1at day7and CA2 at day21). In adult rat brain therapeutic dose of isotretinoin has influenced regions where there is neurogenesis throughout the life. The neuronal outgrowth in the hippocampus was found to be inhibited.

The report by Crandallet al [9] concluded that 13-cis-retinoic acid in mice reduces the cellular proliferation in the adult brain. It also stated that regions of the brain with ongoing cellular proliferation were disrupted by treatment with isotretinoin.

A study by Cocco et al [10] revealed that rats that were treated with a nutritionally deficient diet of Vitamin A caused a reduced size of hippocampal neurons at CA1 region. But there were no reports on teratogenic effect of isotretinoin on hippocampal neurons.

Suppression of hippocampal neurogenesis can disrupt the capacity to learn and recollect. The present study clearly demonstrates that isotretinoin effects the neuronal population of the hippocampus.

Ritchie and Webster [11] revealed that fibroblast growth factor and retinoic acid receptors of neural crest cells play a major role in the development of skeletal portions of the cranio-facial region and altered level of retinoic acid receptors by isotretinoin was attributed the cause for cleft palate. Though the present study does not focus on migration of neural crest cells, it demonstrates that isotretinoin affect the neuroepithelial cells, as confirmed by neuronal loss in the hippocampus.

In the present study, treatment at mid gestation had a greater effect as compared to early gestation. There are no convincing reports on the timings of intrauterine neurogenesis and their migration; hence it would be difficult to point out why isotretinoin has induced greater teratogenic effect during mid-gestational treatment period.

A study conducted by Divya et al [12], demonstrated that isotretinoin has a teratogenic effect by declining the neuronal population of the prefrontal cortex on postnatal day 7 and 21 in rats with more severe effect with early gestational treatment. From these two studies, it can be concluded that early and mid-gestational treatment may have different toxic effects on different areas of the developing brain.

Though this preliminary study clearly demonstrates the teratogenic effect of isotretinoin, but it cannot answer many of the results observed especially with respect to the dose effects. More sensitive study models using specific markers for adult neurogenesis in this respect would be ideal to continue this work.

CONCLUSION

In this animal model study, isotretinoin has proved to be teratogenic at both early and mid- gestational consumption. As hippocampus and the dentate gyrus plays a major role in memory and emotional activities in both animals and humans, consumption of isotretinoin during pregnancy must be avoided.

ISSN: 0975-8585

ACKNOWLEDGEMENT

We would like to thank Indian Council of Medical Research (ICMR), New Delhi, for funding the present work under STS programme. (Ref: 2012-03622). All the authors declare no conflict of interest.

REFERENCES

[1] Strauss J, Krowchuk D, Leyden J, Lucky A, Shalita A; Siegfried, E, et al. J American Acad Dermatol 2007; 56: 651–63.
[2] Hendrick AG, Silverman S, Pellegrini M, Steffek AJ. Teratol 1980; 22:13-22.
[3] Sladden MJ, Harman KE. Arch Dermatol 2007; 143:1187-88.
[4] Moskop JC, Smith ML, De Ville K. J Clin Ethics 1997; 8:264-78.
[5] O'Reilly KC, Shumake J, Gonzalez-Lima F, Lane MA, Bailey SJ. Neuro Psychopharmacol 2006; 3:1919-27.
[6] Malcolm Maden. Nat Rev Neurosci 11/2007; 8:755-6.
[7] Gage FH. J Neurosci 2002; 22: 612–13.
[8] Madhyastha S, Bairy KL, Nalini K, Somayaji SN. Canadian J Physiol Pharmacol 2002; 80: 1076-84.
[9] Crandall J, Sakai Y, Zhang J, Koul O, Mineur Y, Crusio WE et al. Proc Nat Acad Sci USA 2004; 101:5111-16.
[10] Cocco S, Diaz G, Stancampiano R, Diana A, Carta M, Curreli R et al. Neurosci 2002; 115: 475-82.
[11] Ritchie H and Webster WS. Teratol 1991; 43: 71-81.
[12] Divya P, Sampath MV, Jai A, Teresa J, Sudhanshu S. Res J Pharm Biol Chem Sci 2013; 4(1): 902- 11.