

Pre-Natal Developmental Toxicity of Oleoylethanolamide (OEA)

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Received: 01 Mar 2024; **Accepted:** 02 Apr 2024; **Published:** 10 Apr 2024

Citation: Narendra S. Deshmukh, Sedratul Muntha, Silma Subah, et al. Pre-Natal Developmental Toxicity of Oleoylethanolamide (OEA). *J Med - Clin Res & Rev.* 2024; 8(4): 1-4.

Keywords

Oleoylethanolamide, Body weights, Obesity Eating disorders.

Introduction

The endocannabinoid system and associated compounds, such as the autacoids anandamide and its mono-unsaturated analogue oleoylethanolamide (OEA) [1,2], constitute a promising target for new therapeutics.

OEA is synthesized from oleic acid and phosphatidylethanolamine in the upper part of small intestine after absorption of lipids from the diet [3-5], and occurs in adipose tissues, neurons and astrocytes [6,7]. While it shares biosynthetic pathways and structural similarities with endocannabinoids, it exerts broadly opposing effects on metabolism. It has no effect at cannabinoid receptors CB1 and CB2 [8] but binds with high affinity to the nuclear receptor PPAR- α through which it regulates the expression of genes concerned with fat absorption and fatty acid metabolism, resulting in enhanced lipolysis and beta oxidation [9].

At the same time, the dual actions of OEA via PPAR- α and an up-regulation of hypothalamic oxytocinergic neurons result in the suppression of appetite [10,11]. The predominant biological functions of OEA are therefore suppression of pro-inflammatory cytokine synthesis via PPAR- α , and regulation of appetite, food intake and overall energy balance.

Pre-clinical studies identified OEA as an endogenous mediator of satiety and a potential treatment for obesity and eating disorders [12-18]. It was also shown to improve alertness and memory and to exert anxiolytic and anti-depressant effects in rodents [19], with analogous effects in humans [20], via mechanisms, which likely include both central actions [16-18] and multiple actions in the

gastrointestinal system [21-25].

OEA activates G protein-coupled receptor-119 (GPR-119) and the transient receptor potential cation channel vanilloid-1 (TRPV1) [2,23]. By binding to GPR119 expressed on enteroendocrine L-cells [22], OEA supports maintenance of normal glucose homeostasis via increasing secretion of the intestinal hormone Glucagon-like Peptide-1 (GLP-1). Activation of TRPV1 is thought to modify gastrointestinal secretions and motility [22,23]. Recent in vitro studies have shown that OEA decreases intestinal epithelial cell permeability via TRPV1 activation [24] and modifies both the intestinal microbiota [9] and the polarization of T_H lymphocytes in Peyer's patches [9]. The overall GI impact may therefore extend to improved colonic epithelial barrier function.

This scenario is lent support by OEA's ability to modulate a number of lymphocyte cytokines and chemokines. Specifically, OEA decreases the release of proinflammatory IFN γ , IL6, IL17, IL4 and the chemokines CXCL1 and CXCL2 which are required for recruiting neutrophils in inflammation [25]. In a mouse model of ulcerative colitis, sub-chronic treatment with OEA ameliorated the inflammatory profile by decreasing systemic and colonic expression of inflammatory cytokines, including the expression of inflammatory cytokines in the mesenteric lymph nodes of diseased mice [26]. The researchers reported that OEA exerted a protective action on gut barrier via restoring mRNA transcription of the tight junctions involved in maintaining colon epithelial barrier function [26].

Due to OEA's ability to cross the blood-brain barrier [27], this fatty acid amide's protective anti-inflammatory and antioxidant properties may eventually find applications in the treatment of neurodegenerative and psychiatric disorders such as depression

and alcohol abuse [19,27-30]. Finally, there is evidence that OEA may be useful in the management of certain viruses such as COVID [32].

OEA's many biological functions in different domains make it a promising candidate for the clinical management of metabolic, inflammatory and neuroinflammatory conditions. A robust safety profile for this metabolite, however, does not yet exist. This paper aims to contribute to the debate by providing a prenatal / developmental safety profile of OEA. The study was designed to evaluate the effect of OEA administered daily to pregnant female rats at 3 different dose levels, from the beginning of the gestation period until the expected day of parturition. The primary probes in this study included the standard indicators of maternal toxicity and foetal survival, external anomalies and altered patterns of growth.

Methods

Groups of ten '0-day pregnant' female Wistar rats were given oleoylethanolamide by oral gavage, at the doses of 1000 mg/kg, 2000 mg/kg or 3000 mg/kg body weight/day, for 19 consecutive days. Control group female rats were administered with the vehicle only i.e. analytical grade water with Tween 80 (1% w/v) in a similar manner.

Maternal and foetal observations were recorded. The dams were observed for signs of systemic toxicity, body weight and food intake. They were sacrificed on day 20 of gestation and subjected to necropsy examination including examination of their ovaries and uterine contents and litters. External examination, body weight and AGD of foetuses were recorded for abnormalities.

Results of Pre-natal developmental toxicity of OEA Maternal Clinical Observations and Pregnancy Data

No deaths occurred among any of the dams during the period of the study. No abnormal clinical signs indicative of systemic toxicity were observed during the study period.

Table 1: Summary of Clinical observation during gestation – Dams.

SUMMARY OF CLINICAL OBSERVATIONS DURING GESTATION - DAMS

Group	Ref: Appendix. 1			
	G1	G2	G3	G4
Dose (mg/kg bw/d)	Control	1000	2000	3000
Number of Animals	10	10	10	10
No abnormality detected	10	10	10	10

Body weight

At the end of the treatment period, the average weight gain by dams treated at 1000, 2000 and 3000 mg/kg between gestation days 0-20 was respectively 15%, 23% and 19% lower than that by the control group dams. These changes seem consistent with known OEA pharmacology, and although they did not reach statistical significance ($P > 0.05$) they are in line with reduced food intake (below).

The average food intake by treatment group female rats remained slightly lower than Control group during the period of gestation, and the differences were statistically significant ($P < 0.05$) at certain intervals. The mean food intake by dams treated at 1000, 2000 and 3000 mg/kg between gestation days 0-20 was respectively 11%, 11% and 16% lower than that of the control group dams.

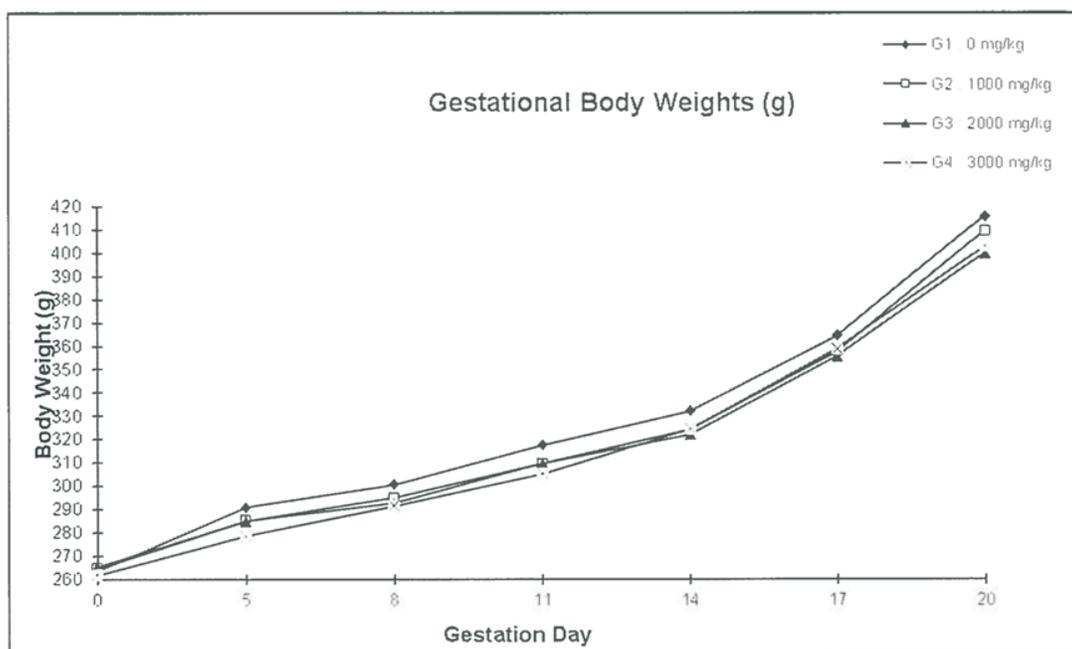


Figure 1: Gestational Body Weights.

Table 2: Summary of food consumption during gestation (g/rat/day).

Group & Dose mg/kg bw/d		Feed Consumption During Gestation Days						
		0-5	5-8	8-11	11-14	14-17	17-20	0-20
G1 0	Mean	21.18	27.22	26.33	27.74	28.41	30.63	26.34
	SD	5.05	13.21	2.83	2.13	3.93	3.46	2.58
	n	9	9	9	9	9	9	9
G2 1000	Mean	20.44	22.26	22.00^{s-}	24.26^{s-}	25.93	27.00^{s-}	23.33^{s-}
	SD	3.39	3.74	3.71	2.66	2.27	1.97	2.08
	n	9	9	9	9	9	9	9
G3 2000	Mean	20.05	21.29	24.04	23.54	27.21	26.88^{s-}	23.46^{s-}
	SD	2.70	3.65	3.74	3.10	2.44	3.65	2.28
	n	8	8	8	8	8	8	8
G4 3000	Mean	16.91^{s-}	21.59	21.70^{s-}	23.74^{s-}	27.44	25.52^{s-}	22.23^{s-}
	SD	1.82	2.17	2.76	3.34	2.09	2.32	1.81
	n	9	9	9	9	9	9	9

Note: n=number of females pregnant at term

S-: Group mean values of the treatment groups significantly lower (P<0.05) from those of the control group at 5% level of significance.

Non-pregnant females have been excluded from statistical computation where appropriate.

These changes were not associated with pathology signals; none of the dams exhibited any gross abnormalities in any of their tissue/organs during their necropsy examinations.

Various pregnancy related parameters of the vehicle control group rats and those treated with oleoylethanolamide were compared. With regard to numbers of females with confirmed pregnancy, litters available for external evaluation, live implants, dead implants, resorptions and females with resorptions, there were no significant differences between the vehicle control group and the treated groups. No adverse effects of oleoylethanolamide were noted.

Maternal Uterine Observations

On examination, the gravid uteri of females sacrificed on day 20 of gestation did not reveal any adverse effects of oleoylethanolamide. The observed parameters included absolute and relative uterus weight, number of corpora lutea, number of implantations, number of live and dead implants, number of early and late resorptions and pre and post implantation losses (%). Group mean values of these parameters in females treated with oleoylethanolamide were comparable to those of the control group.

Foetal Examination and Litter Data

On examination, the litters of females sacrificed on day 20 of gestation did not reveal any adverse effects of oleoylethanolamide. The observed parameters included litter size, number of foetuses, sex ratios of the foetuses, anogenital distance in male and female pups and foetal weights. Group mean values of these parameters in females treated with oleoylethanolamide were comparable to those of the control group. No external developmental anomalies were observed in any of the litters from the treated and control groups

Discussion

OEA is a simple derivative of oleic acid with promising potential in anti-obesity, anti-inflammatory, psychiatric and immune-therapeutics [27]. Its safety profile is expected to be benign, but is

not yet well documented. This study focused on one of the most sensitive areas, namely developmental toxicity [33-35].

Based upon the results of this study, oleoylethanolamide at doses of up to 3000 mg/kg body weight/day did not induce any noticeable toxicity. OEA did not increase mortality, abnormal clinical signs or gross pathological alterations in the dams, and showed no signs of developmental toxicity in the litters.

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