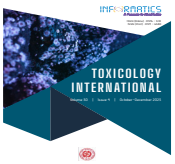
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Chemical Induce Polycystic Ovarian Syndrome-Preclinical Animal Models

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Abstract

Polycystic ovarian syndrome is a complex endocrine disturbance that leads to hyperandrogenism, disruption in the functioning of the Hypothalamic-Pituitary-Ovary (HPO) axis and multiple cysts in ovaries. To understand and study different treatment approaches of polycystic ovarian syndrome, there are several chemical-induced animal models available that mimic polycystic ovarian syndrome. These animal models are designed to closely resemble the characteristic symptoms. Polycystic Ovarian Syndrome's key characteristics are changes in gonadotropin and sex steroid hormone, ovarian morphology, and metabolic characteristics. Direct hormone-regulated animal models are frequently utilized to study PCOS. Rodent animal model is often used which aims to replicate the key feature of human PCOS. Various endocrine-disrupting chemicals also makes a major role in the development of PCOS. In order to bridge the gap between basic research and clinical application in the field of PCOS, PCOS-induced models are essential tools for improving our understanding of the illness and evaluating innovative therapies. The review discusses various animal models used to induced PCOS by various inducers such as aromatase inhibitor inducer (letrozole), androgen excess inducer (dihydrotestosterone, dehydroepiandrosterone, testosterone), estrogen-induced (estradiol valerate), antiprogesterone (mifepristone), monosodium-L-glutamate, bisphenol-A and tributyltin chloride. This article contributed to underlying the current understanding and provides you a complete review that overall covers various aspects, including the impact of chemical-induced models, which also includes changes in the morphology of ovaries, gonadotropin as well as, and alterations in the level of various sex steroid hormone profile. Additionally it explores the metabolic abnormalities caused by various chemical-inducers used to induce PCOS in animal. The objective of this review is to provide a comprehensive review about various chemical inducers which are responsible for the development of PCOS.

Keywords: Gonadotropin, Hyperandrogenism, Metabolic Abnormalities, Ovarian Morphology, Polycystic Ovarian Syndrome, Sex Steroid Hormone Profile

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a prevailing endocrine disturbance within women of reproductive age and a foremost reason of infertility; however, the pathogenesis of this syndrome isn't always fully understood[1].

Animal model studies are crucial to understanding and examining the pathophysiology of PCOS *in vivo*. To take a look at PCOS and higher apprehend its underlying

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mechanisms, researchers frequently use animal models[2]. Those models are helpful in a way that permits scientists to research the improvement, development, and ability treatments for the circumstance. Animal models assist in bridging the distance between fundamental research and clinical packages, offering treasured insights into the human circumstance[3].

The primary characteristics of PCOS that must be demonstrated animal models include hyperandrogenism, and excess of antral follicles, estrous cycle and gonadotropin abnormalities[4]. This heterogeneous ailment is likewise affecting reproductive characteristics; patients regularly show off metabolic abnormalities such as insulin resistance, metabolic syndrome, and obesity[5]. The prevalence of PCOS among women ranges from 8% to 17.8% and is characterized by the formation of cysts in ovaries, hyperandrogenism, and oligo-ovulation[6]. The main purpose of this review of literature is to provide an overview of different types of chemicals induce animal model which helps us to investigate PCOS. Various recent types of treatment involved in PCOS are hormonal birth control, lifestyle changes, anti-androgen medication, metformin, and ovulation induction. Different types of chemical inducers for PCOS are present in Table 1 and the hormonal changes which is caused by the chemical inducer are present in Table 2.

2. Chemical Induce Pre-Clinical Animal Models

2.1 Letrozole Induced PCOS Animal Model

2.1.1 Principle

Letrozole is a non-steroidal aromatase inhibitor. P450 aromatase enzyme converts testosterone and androsterone into estradiol and estrone so a decrease in activity of this enzyme may result in abnormal follicular development and PCOS[7,8].

2.1.2 Procedure

Normally, six-week-old female rats with a regular estrus cycle of 4 days is taken and Letrozole is administered at a concentration of 0.1 or 0.5 or 1mg/kg orally daily for 21 days. For the duration of this era, vaginal smears had been accrued each day for estrus cycle determination. At the day subsequent to the final letrozole dose administration, rats had been killed; uteri and ovaries were then removed

Table 1. List of chemical inducer animal models for polycystic ovarian syndrome

Sr no.	Inducer	Species	Route of administration	Dose	Age before intervention	Duration of study	Reference
1	Letrozole	Rat Mouse	Oral Oral	1mg/kg/day 3mg/kg/day	6 weeks 6 weeks	21 days 21 days	[8]
2	Testosterone enanthate	Rat	Subcutaneous	1mg/100g	3 weeks	35 days	[41]
3	Testosterone propionate	Rat	Subcutaneous	1mg/100g	3 weeks	35 days	[18, 19]
4	Estradiol valerate	Rat	Intramuscular	2-4mg, single dose	5-weeks	30-32 days	[14]
5	Mifepristone	Rat	Subcutaneous	2-4mg/100g	16-18weeks	9 days	[21,23]
6	Dehydroepiandrosterone	Rat Mouse	Subcutaneous Subcutaneous	6mg/100g 6mg/100g	3 weeks 3-4 weeks	15 days 21 days	[27] [31]
7	5α-Dihydrotestosterone	Rat	Subcutaneous	7.5mg/pellet	3 weeks	90 days	[30]
8	Monosodium-L-glutamate	Rat	Subcutaneous	4g/kg/day	Newly born	Up to Postnatal 10^{th}day^{thth}th[21663]	[34]
9	Bisphenol A	Rat	Oral (mother) Subcutaneous	0.1-1.2mg/kg 50µg/50µ	-	from Postnatal 1 to 10 days	[36]
10	Tributyltin Chloride	Rat	Oral	100ng/kg/day	-	15 days	[40]

Table 2. List of inducers with hormonal changes

Sr. no	Inducer	Estrous cycle	Ovarian cyst	LH level	FSH level	Progesterone level	Testosterone level	Estradiol level	Reference
1	Letrozole	Acyclic (diestrus)	Yes	Increases	Increases	Decrease	Increases	Decreases	[8,9,10]
2	Testosterone enanthate	Irregular	Yes	Increases	Increases	No changes	Increases	No change	[41]
3	Testosterone propionate	Irregular	Yes	Increases	No change	No changes	Increases	No change	[18,19]
4	Estrogen valerate	Acyclic (estrous)	Yes	Decreases	Decreases	Increases	Decreases	Decreases	[15,16]
5	RU486 (Mifepristone)	Acyclic (estrous)	Yes	Increases	Increases	Not reported	Increases	Increases	[23,24]
6	Dehydroepiandrosterone (DHEA)	Irregular	Yes	Increases	Increases	Increases	Increases	No change	[28,29]
7	5α-Dihydrotestosterone (DHT)	Acyclic (diestrus)	Yes	No change	No change	Decreases	Not reported	Not reported	[32]
8	Monosodium-L-glutamate	Irregular	Yes	No change	No change	-	No change	No change	[34,35]
9	Bisphenol A	Irregular	Yes	Decreases	Increases	Decreases	Increases	Increases	[37,36]
10	Tributyltin chloride	Irregular	Yes	Decreases	Not reported	-	Increases	Decreases	[40]

out and weighed. Level of gonadotropin and sex steroids like (Luteinizing Hormone [LH], Follicle-Stimulating Hormone [FSH], estradiol, testosterone and progesterone) and histologic adjustments in ovaries had been tested and compared it with the control group[8].

2.1.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration level of LH, FSH, and testosterone levels were increased and estradiol and progesterone levels were decreased in comparison with the control group of animals[8,9].

2.1.4 Ovarian Morphology

The ovarian morphologic changes of letrozole-induced rats encompass the development of cysts with hyperplasia of internal theca cells and a thickened ovarian capsule. The range of corpora lutea is decreased, indicating oligo-/anovulation. But, unlike human PCOS, the ovarian weight of letrozole-induced rats stays unmodified[8,10].

2.1.5 Metabolic Abnormalities

After continuous treatment of letrozole from puberty to adulthood no aberration in metabolic parameters was observed inclusive of insulin sensitivity, dyslipidemia, and adiposity[11].

2.1.6 Advantages

a) The route of administration can be both orally and subcutaneously, Oral route is quite convenient for the administration of the drug to rats.
b) Most commonly used induction model in PCOS.
c) Rats when treated with letrozole show an acyclic diestrus cycle.

2.1.7 Disadvantages

a) No metabolic aberration is observed.
b) Postnatal administration of letrozole is generally used to generate PCOS animal models.
c) Duration of study is longer than other endocrine-disrupting chemical

2.2 Estrogen-Induced PCOS Animal Model

2.2.1 Principle

The abnormal function or expression of estrogen and estrogen receptors can cause PCOS[12]. Estradiol valerate is protracted estrogen that causes sudden disturbance in hypothalamic-pituitary regulation of gonadotropic hormone, resulting in fallacious release and storage of LH. This also plays a key role in the development of PCOS[13].

2.2.2 Procedure

Normally, 5-week female rats in estrus are divided into two group from which one group is served as control, while the other receives a single dose of estradiol valerate 2-4 mg dissolved in 0.5ml sesame oil intramuscular. At the last day of the experiment (30-32 days), rats were sacrificed under anaesthesia, oviducts are removed and examined[14]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals.

2.2.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration of LH, in basal serum is lower than in the control group of animals after 8 weeks of treatment after 22 weeks the level of LH is gradually increased and FSH, testosterone levels, and estradiol levels were also decreased in plasma and progesterone levels were increased in estrogen valerate induced animals[15,16].

2.2.4 Ovarian Morphology

Granulosa cellular layers and hyperthecosis is decreased ovaries of Estradiol valerate-induced rats that are shrunk as compared with controls and are characterized with the aid of big cystic follicles with. Ovaries from Estradiol valerate brought about rats additionally develop an excessive prevalence of atretic/degenerative secondary follicles. The size of ovaries is reduced as compared to a control group of animals[11,13].

2.2.5 Metabolic Abnormalities

The Hypothalamic-Pituitary-Adrenal axis and sympathetic activity increase as the development of hypertension shown in the estradiol valerate induces rats. Although, it fails to develop insulin resistance and adiposity in the estradiol-induced animal which are the common characteristics of PCOS[16,17].

2.2.6 Advantages

a) Single dose of estrogen valerate is enough to induce PCOS in rats.

b) The number of atretic follicles was higher.
c) Rats when treated with estrogen valerate show acyclic estrous cycle

2.2.7 Disadvantages

a) Hormone and metabolic disruption levels were not the same as women with PCOS when exposed to estrogen
a) When the dose is increased to 4mg the level of testosterone decreases and the level of LH increases.

2.3 Testosterone Induces PCOS Animal Model

2.3.1 Principle

Hyperandrogenemia can be induced by testosterone. Generally, testosterone enanthate and testosterone propionate were administered to induce PCOS in rats.

2.3.2 Procedure

Female rats aged of 21 years old are taken and divided into two groups. One group is exposed to 1mg/100kg of testosterone propionate dissolved in propylene glycol (0.1ml) subcutaneously for 35 days and another group of rats is treated with only propylene glycol (0.1ml) alone for an equivalent duration of time and serves as a control group of animals[18,19]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals.

2.3.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration level of LH and FSH level is higher in testosterone propionate induced animal as compared to a control group of animals in serum, whereas the level the level of testosterone is higher but progesterone and estradiol is decreased[20].

2.3.4 Ovarian Morphology

A higher number of the antral and prenatal follicles. Ovarian cysts are in large number and decrease in corpus luteum cells in the ovaries of rats that were exposed to testosterone propionate in comparison with a control group of animals[19,20].

2.3.5 Metabolic Abnormalities

In testosterone propionate-exposed rats, the level of insulin is increased and fasting glucose level is decreased and there is no alteration in body weight as compared with the control group of animals[11].

2.3.6 Advantages

a) A lower dose is required to induce PCOS in rats than other inducer except tributyltin chloride.
b) 64% of rats induce anovulation when testosterone propionate is exposed to rodent fetuses by intra-amniotic route of administration.
c) After birth when rats are exposed to 1mg testosterone propionate shoes immediately continuous anovulatory estrous cycle.

2.3.7 Disadvantages

a) Lack of metabolic disruption.
b) Low-density lipoprotein increases and high-density lipoprotein decreases which can lead to risk of obesity
c) Testosterone propionate when administered to rats between 21-25 days did not show ovarian phenotype.

2.4 Anti-Progesterone Induces PCOS Animal Model

2.4.1 Principle

Mifepristone is an artificial steroid that blocks the activity of progesterone. Progesterone plays an important role in regulating ovulation, gonadotropin hormone secretion, and in follicular development[21]. When RU486 is given to the rats the concentration of progesterone is decreases and the GnRH pulsatility is increased and producing ovulatory failure and enlarged ovaries containing follicular cyst[22].

2.4.2 Procedure

Adult Wistar rats were taken showing 4 4-day estrous cycle. Mifepristone 2-4 mg/100g dissolved in oil and administered for 8 days continuously subcutaneously and the level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals[23].

2.4.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration level of LH level is higher in Mifepristone-induced animals as compared to a control group of animals in serum, whereas the level of FSH is decreased. The level of testosterone and estradiol is also increased in Mifepristone-treated rats[23,24].

2.4.4 Ovarian Morphology

The frequency of large atretic follicular cysts increases and enlarges when treated with Mifepristone compared with the control group of animals[24].

2.4.5 Metabolic Abnormalities

Elevated insulin level was found in the rats treated with Mifepristone and may be correlated with ovarian abnormalities, although there are no changes in body weight and blood pressure[11].

2.4.6 Advantages

a) Arrest in follicular growth.
b) Stop the opposing action of progesterone and estrogen which leads to PRL hypersecretion.
c) Induction of hyperprolactinemia leads to multiple cystic conditions.

2.4.7 Disadvantages

a) There is no effect in body weight and serum FSH.
b) Limited study which related to risk of metabolic and cardiovascular diseases.
c) Subcutaneously route of administration is quite tricky to administer than oral route.

2.5 Dehydroepiandrosterone (DHEA) Induced Polycystic Ovarian Syndrome Animal Model

2.5.1 Principle

First androgen to arise in female prepubescent DHEA act as a precursor for testosterone hormone and the most abundant steroid hormone. It is used as an inducer for polycystic ovarian syndrome in rodents because the level of DHEA is very high in the women suffering PCOS[25,26].

2.5.2 Procedure

Sprague–Dawley female rats aged approximately 21 days were divided into two groups. One group of rats treated with 6mg/100kg DHEA dissolved in sesame oil (0.2ml) subcutaneously for 15-20 days and another group of rats is treated with only sesame oil (0.2ml) alone for the equivalent duration of time serve as a control group of animals[27]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals.

2.5.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration level of LH and FSH levels is higher in DHEA-induced animals as compared to a control group of animals in serum, whereas the level the level of testosterone progesterone and estradiol is also increased[28].

2.5.4 Ovarian Morphology

The granulose cell layer was tinner and the theca cell layer were thicker and multiple ovarian cysts were observed in the ovaries of DHEA-induced rat than the control group. The weight of the ovaries also increased in comparison with the control group[29].

2.5.5 Metabolic Abnormalities

In DHEA-exposed rats the level of insulin, glucose, and body weight was significantly higher in comparison with the control group of animals[11].

2.5.6 Advantages

a) Irregular estrous cycle was observed in DHEA-induced rats.
b) DHEA leads to hyperandrogenic, anovulation, and cystic conditions.
c) Mostly postnatal female rats exposed to DHEA and mimic the condition of PCOS.

2.5.7 Disadvantages

a) May not fully replicate the variability and complexity of PCOS in humans.
b) FSH level was found to be decreased.

c) The route of administration is difficult compared to the oral route of administration of letrozole.

2.6 5α-Dihydrotestosterone (DHT) Induced PCOS Animal Model

2.6.1 Principle

DHT is an androgenic hormone which are non-aromatizable and cannot converted into estradiol. It can produce irregular cyclicity in ovaries which can lead to PCOS[30,31].

2.6.2 Procedure

Sprague–Dawley female rats aged approximately 21 days were divided into two groups. One group is exposed to 7.5mg/pellet of DHT for a duration of 90 days[30]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals.

2.6.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration level of LH and FSH levels is higher in DHT-induced animals as compared to a control group of animals in serum, whereas the level of testosterone and estradiol shows no changes. Although, the concentration of progesterone is decreased[32].

2.6.4 Ovarian Morphology

The granulose cell layer was tinner and the theca cell layer was thicker and multiple ovarian cysts were observed in the ovaries of DHT-induced rats than in the control group. The weight of the ovaries also decreased in comparison with the control group[32].

2.6.5 Metabolic Abnormalities

In DHT-exposed rats the level of insulin sensitivity decreases and body weight is higher in comparison with the control group of animals[11].

2.6.6 Advantages

a) Both prenatal and postnatal female rats exposed to DHT and mimic the condition of PCOS.
b) Fewer antral preovulatory and corpus lutea.
c) Used to study the altered hormone mechanism and ovarian changes.

2.6.7 Disadvantages

a) Duration of study is longer than other inducers.
b) Show no changes in the level of LH and FSH.
c) The level of testosterone and estradiol was not reported.

2.7 Monosodium-L-Glutamate-Induced PCOS Animal Model

2.7.1 Principle

Obesity on reproductive biology is induced by monosodium-L-glutamate and it also cause metabolic syndrome which causes disturbance in the Hypothalamic-Pituitary Gonads (HPG) axis and alters gonadotropic hormone level which is a common reason for producing PCOS in rats[33].

2.7.2 Procedure

Newly born female rats were exposed to 4g/kg/day monosodium-L-glutamate and the control group of rats received equiosmolar saline alternatively up to postnatal day 10. The estrous cycle is evaluated by taking a vaginal smear twice a day for 15 days[34]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals.

2.7.3 Gonadotropin and Sex Steroid Hormone Profile

The concentration level of LH, FSH, testosterone, and estradiol show no alteration in rats that were exposed to monosodium-L-glutamate when compared with the control group of animal[35].

2.7.4 Ovarian Morphology

The ovarian follicle cyst numbers are higher in monosodium-L-glutamate-induced rats when compared with the control group of animals. Additionally, the number of primordial and atretic follicles was increased. Ovaries were lighter in weight than a control group of animals[34].

2.7.5 Metabolic Abnormalities

Body weight is increased in monosodium-L-glutamate-induced rats with higher levels of triglyceride and lower of glycemia. The insulin level was high in monosodium-L-

glutamate-induced rats when compared with the control group of animals[34].

2.7.6 Advantages

a) Produces irregular estrous cycle which indicates PCOS in rats.
b) Through this model we can able to understand the pathogenesis of PCOS affected by metabolic syndrome.
c) Monosodium-L-glutamate-induced obesity results in early development of PCOS.

2.7.7 Disadvantages

a) Do not produce any hormonal changes in rats when they are exposed to monosodium-L-glutamate.
b) Monosodium-L-glutamate's continued use can damage several brain cells.
c) The dose of monosodium-L-glutamate is very high to induce PCOS in animals.

2.8 Bisphenol-A Induced Polycystic Ovarian Syndrome Animal Model

2.8.1 Principle

Bisphenol-A induces PCOS by altering 17β-hydroxylase which helps in the synthesis of gonadal steroid hormone and also increases the level of 17-hydroxyprogesterone level which causes increases in androgen production by ovaries. It also leads to stimulating theca cells and the production of androgen hormone is increased[36].

2.8.2 Procedure

Sprague Dawley newly born rats are taken and divided into two groups. One group is exposed to Bisphenol A 50μg/50μl and the dose range 2.5 to 6.5mg\kg dissolved in castor oil weeks and another group of rats is treated with only castor oil as serve as control[37]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and progesterone) is measured and compared with the control group of animals.

2.8.3 Gonadotropin and Sex Steroid Hormone Profile

The level of testosterone, FSH and estradiol was found to be increased and the level of LH and progesterone was decreased in Bisphenol-A-induced animals when compared with the control group of animals[37].

2.8.4 Ovarian Morphology

The ovarian follicle cyst numbers are higher in Bisphenol-A-induced rats when compared with the control group of animals. Additionally, decreased in number of corpora lutea and atretic follicles number were increased. Ovaries were lighter in weight and a thin layer of granulosa cells was observed and compared with the control group of animals[36].

2.8.5 Metabolic Abnormalities

Bisphenol-A-induced rats show insulin resistance and promote inflammatory conditions. The body weight remains the same when compared with the control group of animals[38].

2.8.6 Advantages

a) Bisphenol-A have a high affinity for both estrogenic and androgenic receptor.
b) Bisphenol-A can mimic the PCOS phenotype in rats as it shows endocrine abnormalities and disturbance in metabolic pathways.

2.8.7 Disadvantages

a) Increased level of Bisphenol-A in maternal circulation shows deformities in the offspring.
b) Longer time exposure to Bisphenol-A causes brain disorder.

2.9 Tributyltin Chloride-Induced PCOS Animal Model

2.9.1 Principle

Tributyltin chloride is a subclass endocrine-disrupting chemical and acts as an obesogen. Exposure to Tributyltin chloride can cause alteration in the estrous cycle, and ovulation, and a higher number of ovarian cysts were observed[33,39].

2.9.2 Procedure

Adult female rats aged approximately 15-40 days were divided into two groups. One group of rats administered orally with 100ng/kg/day tributyltin chloride for 15 days and another group is served as a control group of animals[40]. The level of various gonadotropin and sex steroids like (LH, FSH, estradiol, testosterone, and

progesterone) is measured and compared with the control group of animals.

2.9.3 Gonadotropin and Sex Steroid Hormone Profile

The level of testosterone increased and estradiol was found to decrease and level of LH was also decreased in tributyltin chloride-induced animals when compared with the control group of animals[40].

2.9.4 Ovarian Morphology

The ovarian follicle cyst numbers are higher in tributyltin chloride-induced rats when compared with the control group of animals. Additionally, decreased in number of corpora lutea and atretic follicles number were increased. Ovaries were lighter in weight and thin layer of granulosa cell was observed and compared with the control group of animals[40].

2.9.5 Metabolic Abnormalities

Tributyltin chloride-induced rats show obesity, insulin resistance, hepatic steatosis and also show abnormal vascular function and abnormal lipid profile.

2.9.6 Advantages

a) Duration of induction pf PCOS in rats is shorter than letrozole.
b) The dose level is comparatively the lowest to induce PCOS in animals.
c) Oral route of administration of tributyltin chloride is easy.

2.9.7 Disadvantages

a) A higher dose of tributyltin chloride can be teratogenic.
b) Tributyltin chloride administration causes dyslipidemia and vascular dysfunction.
c) Progesterone level is not reported in the tributyltin chloride-induced PCOS rat model.

3. Conclusion

Rodents are widely used animals to study polycystic ovarian syndrome. The chemical-induced pre-clinical model which are chosen to study about polycystic ovarian syndrome and describe about changes in different parameters that ensure PCOS in rats like changes in the estrous cycle, gonadotropin and sex steroid hormone, ovarian morphology, and metabolic characteristics. Findings from this research the use of these novel chemical-induced preclinical animal models can offer important mechanisms underlying PCOS as well as to understand the pathophysiology and provide better development of new interventions and control for this complex endocrine disorder.

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