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Effect of BOTOX on Sex Hormones and Lipid Profile of Females Rats

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Abstract
The present study aimed to investigate the effect of Botox on sex hormones level which including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and progesterone in female rats and measurement of serum lipid profile. Two doses 0.1/ animal and 0.2 ml/ animal from Botox were used and the animals were injected intramuscular of botulinum toxin for 1 day as two dose only. The results showed a significant decrease ($P<0.05$) in the level of FSH and LH, with a significant increase the level of progesterone of the female treated with 0.1 ml and 0.2 ml of Botox compared with the control group. The results showed a significant increase in the level of cholesterol, TG, HDL, LDL and VLDL of the female treated with Botox at dose 0.2 ml when compared with the control group. While, the female treated with Botox at dose 0.1 ml there was no a significant in level of cholesterol compared with control group. Also, there was a significant decrease of HDL of female rats treated with Botox compared with control group. Injections of female rats with botulinum toxin are generally well tolerated and side effects are few. A precise knowledge and understanding of the functional anatomy of the mimetic muscles is absolutely necessary to correctly use botulinum toxins in clinical practice.
Keywords: BOTOX; FSH; LH; Progesterone; Lipid profile

Introduction

Botulinum is a neurotoxin produced by the bacterium Clostridium botulinum, an anaerobic, gram-positive, spore-forming rod commonly found on plants, in soil, water and the intestinal tracts of animals1.There are seven recognized serotype (BoNT/A to /G) with the recent addition of another serotype, BoNT/X2;3.

All serotypes interfere with neural transmission by blocking the release of acetylcholine, the principal neurotransmitter at the neuromuscular junction, causing muscle paralysis2.
BoNT-A is the most commonly used serotype for medical application and was the first to be licensed for medical use. There are several commercially available forms; Botox (Allergan Pharmaceuticals, Parsippany, NJ, USA) is the most widely used and has the most medical applications. Each formulation varies slightly in structure, efficacy, duration, and safety profile4. The effect of BoNT is site specific; it is administered by local injection (subcutaneous or intramuscular) into the targeted area. It can be administered using endoscopic procedure and by injection directly through the skin. Given the high affinity of BoNT to cholinergic neurons, its effects are consistent and, given at a low dose, have limited systemic adverse effects5. Botulinum toxin was first used clinically in the late 1970s in ophthalmology to treat strabismus1 and over the last 20 years has gained widespread use in conditions requiring inhibition of excessive muscle spasm.

The broad range of medical indications for botulinum toxin include treatment of movement disorders (e.g. spasticity, cervical dystonia), urological disorders (e.g. overactive bladder), dermatological conditions (e.g. axillary hyperhidrosis), as well as cosmetic applications. Botulinum toxin A inhibits the release of substance P from the dorsal root ganglia of the rat6 and iris sphincter of the rabbit7. Substance P is believed to sensitize primary afferents and promote local release of histamine and bradykinin, both known to excite

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nociceptors8. Also, BoNT-A was used in medicine in 1977 for the treatment of strabismus in children. Since then, it has been widely used for different conditions and by different specialties. It is best known for its use in the cosmetic industries9. In 2002, the FDA approved the use of Botox (Botulinum toxin-A) for the cosmetic purpose of temporarily reducing glabeller forehead frown lines. The present paper aimed to investigate the effect injection of botulinum toxin on the level of reproductive hormones and lipid profile of females rat.

Materials and Method

Experimental design

The study was carried out on 24 mature female rats (Rattus norvegicus), aged as 10-12 weeks and weighing between 180 - 200 gm were obtained from Department of Biology, College of Science, University of Thi Qar, Iraq. The animals were housed in a well ventilated 12 hrs light and 12 hrs dark cycles. The animals were divided into three equal groups, each group consist of (8) rats:

1-The first group(control group) injected with 0.9% Nacl.
2- The second group was injected with (0.1ml/animal/day) of BOTOX.
3- The third group was injected with (0.2ml / animal/day) of BOTOX.

Blood collection:

After 30 days of treatment, the animals were sacrificed. Blood samples were collected by cardiac puncture, 5mL of blood were drawn from each animal of experimental groups, and put in tubes without EDTA, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20ºC until the time of assay.

Hormone assay:

Serum samples were analyzed for FSH and LH level, through solid phase ELISA based on the principle of competitive binding, using commercial kits from VEDALAB (France), while for measurement of progesterone using kit from Bio Meraux (France).

Measurement of serum lipid profile :

The used reagents were supplied by Biolabo (France), and serum total cholesterol was measured according to10, and serum TG was measured according to11. While, serum HDL was measured according to12. and measurement of LDL and VLDL according to13, LDL and VLDL concentration was measured as follows :

LDL = total cholesterol – (HDL + VLDL)
VLDL = serum TG /5

Statistical analysis:

Standard analysis of the data of different studied groups was performed using the computerized statistical program: The SPSS program (Statistical Program for Social Sciences). The results were expressed as mean± S.E. Analysis of variance (ANOVA) was used to compare the results of different groups. The differences are considered to be significant at the level ($P \leq 0.05$)14.

Results

The results showed a significant decrease ($p<0.05$) in the level of FSH and LH of the female treated with BOTOX at dose 0.1 and o.2 ml when compared with the control group. While the results showed a significant increase ($p<0.05$) in the level of progesterone of the female treated with BOTOX at dose 0.1and 0.2 ml when compared with the control group(table1). The results showed a significant increase ($p<0.05$) in the level of TG, , LDL and VLDL of the female treated with Botox when compared with the control group, while, the female treated with Botox at dose 0.1 ml there was no a significant in level of cholesterol compared with control group. Also, there was a significant decrease of HDL of female rats

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treated with Botox compared with control group. (table2).

Table 1: Effect of BOTOX on sex hormones levels of female rats

Animal groups	FSH (mg/dL)	LH(mg/dL)	Progesterone(mg/dL)
First group	3.33± 0.14[a]	4.06± 0.19[a]	22.59± 0.17[c]
Second group	1.32± 0.33[c]	1.45± 0.12[c]	34.56± 0.64[a]
Third group	2.34± 0.49[b]	3.45± 0.02[b]	31.11± 0.51[b]
LSD	1.0	0.6	3.0

Values are means ± S.E.
Different letters refer to a significant differences at ($p<0.05$).
Same letters refer to no significant differences at ($p<0.05$).

Table 2: Effect of BOTOX on lipid profile of female rats

Animal groups	Cholesterol Mg/dl	T.G Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl
First group	97.45±0.56[b]	63.00±0.34[b]	52.23±0.12[b]	27.34±0.23[b]	15.34±0.34[c]
Second group	110.66±1.77[b]	77.00 ±0.65[a]	43.34±1.12[a]	48.45±0.87[a]	20.34±0.56[b]
Third group	140.78±2.78[a]	80.56±0.55[a]	41.23±0.34[a]	51.16±0.34[a]	28.45±0.43[a]
LSD	21.0	8.0	3.0	5.0	4.0

Values are means ± S.E.
Different letters refer to a significant differences at ($p<0.05$).
Same letters refer to no significant differences at ($p<0.05$).

Discussion

The present study indicated the effect of Botox on sex hormones female rats by decreasing level of FSH and LH and increasing level of progesterone level compared with the control group. The indications for Botox have evolved beyond cosmetic use to urinary incontinence and muscle spasms. With this popularity come more potential adverse effects, which are known to be short-lived and involve general or extremity weakness and pain. Researcher findings present the first reported case of a potential severe adverse side effect affecting the pituitary gland and persisting over a year after the injection.

These results are in line with the findings of other studies which found that (Botox), Botulinum toxin inhibits neurotransmitter release by cleaving SNAP-25 and SNARE proteins15. These proteins are necessary for vesicular exocytosis and have been implicated in the release of hormones from the anterior pituitary. Similarly, Botox could have caused inhibition of ACTH release resulting in central adrenal insufficiency for patient. Central adrenal insufficiency is a severe but treatable condition. Being aware of this potential adverse event and further researching its mechanism can help diagnose and treat affected patients promptly. This mechanism for GH and prolactin release has made Botox a targeted secretion inhibitor to treat prolactinomas and acromegaly. In our results showed a significant decrease

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in the level of FSH and LH and a significant increase in the level of progesterone of the female treated with BOTOX when compared with control group, as this side effect was documented when used difference of doses .While the side effect profile of long-term botulinum toxin injections has been well documented, especially in individuals with dystonia and spasticity16;17.

Although commercially available preparations of BoNT have an excellent safety profile, especially for cosmetic purposes. Also in our result we showed higher significant in lipid profile in animals groups injection with Botox, but, no significant in level of cholesterol in rats treated with 0.1 ml of Botox compared with control group and there was a significant decrease of HDL of female rats treated with Botox compared with control group. This result accept with several studies have reported that following multiple and higher doses of BoNT injections, there is evidence of inter amuscular lipid accumulation as a pathological response18;19. Although the mechanisms are not known, several factors such as activation of satellite cells or alteration of muscle ultrastructure could promote this lipid accumulation dysferlin18;19, an important muscle membrane protein, is deficient in limb girdle muscular dystrophies which leads to intramuscular lipid accumulation20;21. Additionally, treatment with acetylcholinesterase inhibitors such as pyridostigmine can restore synaptic function and aid in muscle strength recovery. As discussed above, after BoNT injections muscles have the propensity to accumulate lipid which may lead to underestimation of atrophy in these muscles22.In present study the lipid accumulation leads to increased cholesterol and lipid profile in the blood ,the reason back to Dysferlin, an important muscle membrane protein, is deficient in limb girdle muscular dystrophies which leads to intramuscular lipid accumulation20;21,this side effect was not documented. Some animal studies have focused on the atrophy-inducing effects of BoNTs; however, in this study showed when injection with high doses from Botox induced side effect on level sex hormones and lipid profile.

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