DOI: 10.7860/JCDR/2018/36746.11992

Original Article

Pharmacology Section

The Effects of Oxymorphone on Biochemical Parameters of Male Rats

JAMIL KHEIRVARI KHEZERLOO[1], TAHEREH DIANAT[2], MOHSEN TABASI[3], ALI SALIMI[4]

ABSTRACT

Introduction: Oxymorphone is similar in structure to morphine. It is used for the relief of pain and was approved by the Food and Drug Administration (FDA) in 2006. But may have some side effects.

Aim: To illustrate the changes in biochemical parameters in response to the injection of oxymorphone in adult male rats.

Materials and Methods: This research was carried out on 50 male adult Wistar rats which were divided into five groups that included 2, 4 and 6 mg/kg of Oxymorphone received groups, control group and normal saline received group. Injections were administered intraperitoneally via a catheter once a week for 56 days. After eight weeks, blood samples were collected using cardiac puncture method. Following serum preparation, enzymes and hormones levels were quantified using standard automated spectrophotometer especially photometric kinetic methods. All values were presented as mean±SEM. Statistical significance was evaluated by one-way analysis of variance (ANOVA, t-test) using IBM SPSS Statistics 20.0 for Windows.

Results: The results demonstrate that serum levels of Adrenocorticotropic Hormone (ACTH), cortisol, aldosterone and Thyroid-Stimulating Hormone (TSH) increased in all groups which received oxymorphone. The serum levels of Creatine Kinase (CK) had significant decrease in all exposed groups. Oxymorphone had no statistically significant impact on the serum levels of Alkaline Phosphatase (ALP). Serum levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), T3 and T4 had a significant change in group which received 6 mg/kg of oxymorphone. No definite changes in other groups.

Conclusion: Exposure to 2, 4 and 6 mg/kg of oxymorphone may lead to short and long-term side effects. These effects can be therapeutic for a range of patients and can also be very deadly for a group of patients with unbalanced serum levels of special enzymes or hormones.

Keywords: Cortisol, Enzyme, Hormone, Thyroid-Stimulating Hormone

INTRODUCTION

Oxymorphone is a semi-synthetic opioid analgesic used in controlling mild to severe pain, and is similar in structure to morphine and heroin. This chemical substance was developed in Germany and reintroduced in the form of oral formulation in the US in 2006 [1]. Nevertheless, there are few studies available on its pharmacodynamic impacts [2]. In addition, abuse of oxymorphone has been connected to thrombotic microangiopathy [3]. According to the studies, there is an illness related to oxymorphone (intravenous abuse) which is similar to thrombotic thrombocytopenic purpura [4]. Oxymorphone pills must be swallowed whole and are not allowed to be broken, dissolved, chewed or crushed [5]. Endo Pharmaceuticals reformulated tablets of extended release oxymorphone in 2012 [6]. The formulation is mainly composed of high molecular weight polyethylene oxide (HMW PEO or PEO+, ~7,000 kDa) [7]. The FDA ascertained that the tablet with PEO+ also can be readily prepared for intravenous abuse [8]. The comprehensive effects of the PEO+ and the haematotoxic potential of IV PEO+ [7] has remained unclear, and there is no abuse-deterrent labeling on the tablet [9]. And also, an abrupt lethal impact of intravenous PEO+ was detected in animals [10]. Signs of excessive consumption may include severe sleepiness, muscle weakness, confusion, cold and moist skin, needle pupils, Hypopnea, increased heart rate, fatigue, and restlessness [11]. Nevertheless, FDA wanted opioid oxymorphone to be pulled off from markets in June 2017. This is because of some unknown toxicological side effects.

There were reports indicating that long-term treatment with opioids like morphine significantly decreases serum levels of AST, ALT and LDH in male rats [12,13]. Recently, researchers' results show that, oxymorphone injection can increase the serum level of Creatinine which leads to Albuminuria [7]. The study by Guay D, illustrated that, hepatic impairment as well as renal impairment are some of side effects of the oxymorphone [14]. In general, very few studies have been done on the physiological, toxicological or pharmacological effects of this compound.

However, since oxymorphone may play a crucial role in hormonal and enzymatic features of patients, the present study operated to illustrate the changes in several biochemical health parameters in response to the injection of oxymorphone in adult male rats.

MATERIALS AND METHODS

This prospective interventional study was carried out on 50 male adult Wistar rats with a weight of 200±20 gm which were obtained from Pasteur Institute of Iran. This study was carried out between October 2017 and January 2018. The average weight of each cage was kept similar. The temperature was at 22±2°C and animals were kept under the condition of half light and half darkness (light on at 08:00 am). Animals had free access to water and standard laboratory chow. The protocol was approved by Tehran University of Medical Sciences Ethics Committee which is registered under the registration number IR.TUMS.VCR.REC.1397.186, and it conformed to the provisions of the World Medical Association's Declaration of Helsinki.

Animals were randomly assigned to five groups with 10 rats in each group according to the study design below; Group 1, comprised the control group maintained under experimental conditions, without any injection. Group 2, received normal saline, to provide the same psychological conditions with control group, both groups were injected simultaneously. Based on animal physiological guidelines, three groups received 2, 4 and 6 mg/kg of oxymorphone, which contains the suitable drug dose for injection [12,15]. The bulk oxymorphone (Extended release) powder was dissolved in 900 mL

phosphate buffered saline1X (PBS) by heating (45ºC with gentle agitation) to achieve different concentrations of stock solution. Bolus solution was centrifuged (10,000 gm for 25 minutes) to remove large undissolved aggregates. During the experiment the animals were healthy and were repeatedly screened. Injections were administered intraperitoneally via a catheter once a week for 56 days. After eight weeks, blood samples were collected using cardiac puncture method. Sera were collected and quantified for enzymes including CK, ALP, AST and ALT levels using standard automated spectrophotometer especially photometric kinetic methods based on the German Biochemical Society (Beckman DU-6, Fuller-ton, CA) [16].

All the chemicals had the highest purity (95%). Experiments were performed at 37ºC, and as time required evaluating reaction rates must be atleast three times, the enzymatic and hormonal OD at 1, 2 and 3 minutes was recorded. Hormones including ACTH, TSH, T3, T4, Cortisol and Aldosterone levels were quantified using the enzyme-linked immunosorbent assay (ELISA). ELISA kits for ACTH, TSH, T3, T4, TSH and Cortisol were purchased from Sigma- Aldrich (St. Louis. Missouri). ELISA kit for Aldosterone was purchased from Cayman (Cayman Chemicals, Ann Arbor, MI).

STATISTICAL ANALYSIS

All values are presented as mean±SEM. Statistical significance were evaluated by one-way analysis of variance (ANOVA, t-test) using IBM SPSS Statistics 20.0 for Windows. Differences with $p<0.05$ were considered as significant.

RESULTS

The results demonstrate that serum levels of ACTH increased in all groups which received oxymorphone. These changes were more significant than control group (p-value <0.001) as well as groups that received saline (p-value <0.01).

As the results in [Table/Fig-1], the higher amount of oxymorphone leads to the greater quantity of ACTH.

The [Table/Fig-1] illustrates that, in all groups that received oxymorphone, the cortisol levels significantly increased, which were near 10 times more than control group ($p<0.001$) and normal saline group ($p<0.001$). Thus, the higher amount of oxymorphone may lead to increased serum levels of cortisol.

With the treatment of oxymorphone in different doses, the serum levels of aldosterone raised up. The groups that received 2 mg/kg and 4 mg/kg of oxymorphone had significantly increased Aldosterone in comparison to control and normal saline group ($p<0.001$). The last group which received 6 mg/kg of oxymorphone also had a significant increase in aldosterone serum levels ($p<0.01$) [Table/Fig-1].

The mean serum levels of CK in the control group were 427±30.5. In contrast, the mean serum levels of CK in experimental groups were 299.8±28.57 (group treated with 2 mg per kg of oxymorphone, $p<0.05$ in comparison with control group), 134.2±12.44 (4 mg/kg, $p<0.001$ in comparison to normal saline received group) and 201±17.39 (6 mg/kg, $p<0.01$ compared with normal saline group) which had significant decrease in all exposed groups in comparison to control or normal saline groups [Table/Fig-1].

Results illustrated that, treatment by oxymorphone significantly increased serum levels of TSH in all three groups compared to control group ($p<0.05$). The statistical details of the serum levels of TSH are shown in [Table/Fig-1]. Oxymorphone had no statistically significant impact on the serum levels of ALP. Serum levels of ALT had a significant change in group which received 6 mg/kg of oxymorphone ($p<0.01$). Other groups did not have a definite change. Serum levels of AST had a significant increase in 6 mg/kg oxymorphone received group ($p<0.05$). Rest of groups were unchanged. The statistical details for this section are presented in [Table/Fig-2].

Serum level of T3 was influenced by 6 mg/kg of oxymorphone, which was significantly decreased in comparison to control group with $p<0.01$. Other groups didn't indicate meaningful change [Table/Fig-3]. Whereas, serum levels of T4 were affected by 4 and 6 mg/kg of oxymorphone treatment compared with control group ($p<0.01$). However, 2 mg/kg oxymorphone received group had no significant change [Table/Fig-3].

DISCUSSION

In the present study, the changes in biochemical parameters in response to the injection of oxymorphone in adult male rats, which could cause harmful effects on the involved organs were evaluated.

It was observed that, significant elevation in the quantity of ACTH, cortisol and aldosterone in rats serum exposed to different

Parameters	Control	Normal saline	Group 1	Group 2	Group 3
ACTH (pg/mL)	1.23±0.14	1.35±0.26	**3.45±0.42 ***	**3.83±0.35 ***	**4.73±0.3 *++**
Cortisol (pg/mL)	0.85±0.12	0.42±0.18	**5.53±0.39 *+**	**6.24±0.88 *+**	**7.71±0.46 *+**
Aldosterone (pg/mL)	1297±55.83	1382±56.32	**1626±61.25 *+**	**1690±62.19 *+**	**1635±60.82 *++**
CK (IU/L)	427±30.5	493.5±34.21	**299.8±28.57 ****	**134.2±12.44 +**	**201±17.39 ++**
TSH (µmoL/mL)	0.146±0.08	0.16±0.1	**0.201±0.23 ****	**0.2±0.24 ****	**0.199±0.19 ****

[Table/Fig-1]: Serum levels of ACTH, cortisol, creatine kinase and TSH.
Groups 1, 2 and 3 received 2, 4, 6 mg/kg of Oxymorphone, respectively.
* Indicates significant change in comparison of Control group ($p<0.001$).
** Indicates significant change in comparison of Control group ($p<0.05$).
+ Indicates significant change in compare with Saline received group ($p<0.001$).
++ Indicates significant change in compare with Saline received group ($p<0.01$).
Values = Mean±SEM (Standard error of mean)
One-way ANOVA,Student's t-test; $p<0.05$=significant

Parameters	Control	Normal saline	Group 1	Group 2	Group 3
ALP (IU/L)	302.6±13.7	302.±13.8	257.8±25.9	368.2±31.1	291.8±34.8
ALT (U/L)	89±1.48	88±2.57	90.1±5.69	90.9±5.84	**67.18±3.47 ***
AST (U/L)	200.33±8.1	251.68±2.8	279.8±35.9	240±37.7	**285.18±13.5 ****

[Table/Fig-2]: Serum levels of ALP, ALT and AST after injection of oxymorphone.
Groups 1, 2 and 3 received 2, 4, 6 mg/kg of Oxymorphone, respectively.
* Indicates significant change in comparison of Control group ($p<0.01$).
** Indicates significant change in comparison of Control group ($p<0.05$).
Values = Mean±SEM (Standard error of mean)
One-way ANOVA, t-test; $p<0.05$=significant

Parameters	Control	Normal saline	Group 1	Group 2	Group 3
T3 (mmol/L)	2.6±0.31	2.7±0.46	2.86±0.37	3.3±0.28	**2.1±0.22** *
T4 (mmol/L)	8.76±0.35	7.98±0.42	8.82±0.25	**7.1±0.61** *	**7.23±0.39** *

[Table/Fig-3]: Serum levels of T3 and T4 after treatment by different dosages of oxymorphone.
Groups 1, 2 and 3 received 2, 4, 6 mg/kg of Oxymorphone, respectively.
* Indicates significant change in comparison of Control group (p<0.01).
Values = Mean±SEM (Standard error of mean)
One-way ANOVA, t-test; p<0.05=significant

concentrations of oxymorphone. Increase in amount of ACTH in the plasma may represents a disorder in the pituitary gland function because ACTH is a tropic peptide hormone secreted from the anterior pituitary gland [7], and also elevation in the quantity of ACTH has a subsequent effect on the increase of cortisol hormone, because the role of ACTH in the hypothalamo-pituitary axis includes stimulating the release of cortisol from the adrenal glands (the HPA system) [17,18], and also the increase in ACTH in the serum cause the increase in aldosterone, because ACTH is one of the principal regulators of adrenal aldosterone biosynthesis [19]. One of the other reasons for increased amount of cortisol in plasma could be abnormality in the adrenal gland function; hyperaldosteronism being associated with renal failure [20]. The significant changes in the activity of these enzymes in blood plasma may indicate side effects of oxymorphone on renal, pituitary and adrenal gland functions or enzymes secreted by these organs. Therefore, these patients are more vulnerable to taking oxymorphone. Patients with Cushing's syndrome, who also experience increased cortisol levels [21], should stop using oxymorphone drugs because excessive cortisol increases digestive problems and causes heart disease. And also, in people with hyperpituitarism [22] or pulmonary tumours [23] taking an oxymorphone pill is not recommended because of an increase in ACTH serum levels.

We also observed significant decrease in Creatine Kinase (CK) in the serum from all groups which received injection oxymorphone. CK is an enzyme found in the heart, brain, skeletal muscle, and other tissues, and decrease in CK may be found as a consequence of diminished efflux of the muscle enzyme into serum from reduced physical activity caused by illness or advanced age [24], in pregnant women (in the first months of pregnancy) [17], or in some disease such as polymyositis and dermatomyositis [18], Rheumatoid arthritis (RF) [19], and also liver diseases caused by alcohol [20]. From this, it can be concluded that, taking of oxymorphone pill puts these people at risk.

In the present study, the liver enzyme activities (serum aminotransferases, including ALT and AST) that are critical in the diagnosis and assessment of liver disease, ALT significantly decreased and AST significantly increased in 6 mg/kg oxymorphone received group. These results indicate possible liver dysfunction as a result of given maximum dose of oxymorphone, which reduces the amount of liver enzymes. Patients with liver dysfunction and patients who use cholesterol-lowering statin drugs such as lipitor, pravachol and mevacor the oxymorphone drug metabolism may be altered [25]. Given the fact that alcohol abusers, patients with non-alcoholic liver disease, hepatitis, cirrhosis, dermatomyositis, cytomegalovirus infection, and celiac patients are at increased serum AST levels, the use of oxymorphone for these groups might be dangerous [26].

The serum levels of TSH significantly increased in oxymorphone treated rats; TSH induces the thyroid gland to produce T3 and T4. In these conditions, which was similar to the early hypothyroidism (thyroid dysfunction), the low levels of T3 and T4 are the main trigger for TSH secretion [27]. These two hormones are involved in wide range of metabolic activities influencing the growth and development of organisms, and primarily involved in energy production by increasing the metabolic rate [21-23]. So, disruption of these hormones indicates a thyroid dysfunction and has harmful effects on the growth and development. Thus, it was concluded that although oxymorphone seems to be effective in pain control, their toxic side effects should be considered.

LIMITATION

Although this research was carefully prepared, certain limitations were inevitable. These include time and finance. It is suggested that future studies should be done for a longer time, with higher doses. Moreover, the study could be done on histological characteristics to assess the effect of this drug.

CONCLUSION

In this study, various biochemical parameters were assessed after oxymorphone injection in rats by using photometric kinetic methods that included ELISA and spectrophotometry. Serum levels of ACTH, cortisol and aldosterone had significant changes in all groups which received oxymorphone. With the treatment of oxymorphone at 6 mg/kg done, the serum levels of ALT, AST and T3 were found raised. In conclusion, oxymorphone injection has a significant impact on biochemical parameters. Therefore, three groups of patients, including patients with liver disorders, alcoholism, and people who suffer from heart failure are more likely vulnerable and it's better to stop taking oxymorphone pill. However, more human studies should be done in this regard to confirm this finding.

ACKNOWLEDGEMENTS

This research has been done with the financial support of Baqiyatallah University of Medical Sciences, Tehran, Iran. Additionally authors are grateful to all who helped them to excute the present study, especially Dr. Marzieh Ghollasi and Mr. Mohammadreza Yazdan nasab for their technical assistance.

REFERENCES

[1] Davis MP, Glare PA, Quigley C, Hardy J. Opioids in cancer pain. 2nd edition. OUP; 2009.
[2] Shusterman N, Diana F, Ciliberto C, Xiang Q, Jobes J, Kelsh D, et al. Evaluation of the clinical abuse potential of Opana ER versus oxymorphone HCl powder. J Pain. 2016;17(4):S85.
[3] Thakur K, Agrawal V, Kass A, Dimarino LM, Dorion RP, Vadakara J. Thrombotic Microangiopathy Secondary to Intravenous Abuse of Opana® ER. Case Rep Hematol. 2017;2017: 1623907.
[4] Centers for disease control and prevention (CDC). Thrombotic thrombocytopenic purpura (TTP)-like illness associated with intravenous Opana ER abuse-Tennessee, 2012. MMWR. Morb Mortal Wkly Rep. 2013;62(1):01-04.
[5] Shah RJ, Cherney EF. Diffuse retinal ischemia following intravenous crushed oxymorphone abuse. JAMA ophthalmol. 2014;132(6):780-81.
[6] Bartholomaeus JH, Arkenau-Marić E, Galia E. Opioid extended-release tablets with improved tamper-resistant properties. Expert Opin Drug Deliv. 2012;9(8):879-91.
[7] Hunt R, Yalamanoglu A, Tumlin J, Schiller T, Baek JH, Wu A, et al. A mechanistic investigation of thrombotic microangiopathy associated with IV abuse of Opana ER. Blood. 2017;129(7):896-905.
[8] Rudd RA, Aleshire N, Zibbell JE, Matthew Gladden R. Increases in drug and opioid overdose deaths—United States, 2000-2014. Am J Transplant. 2016;64(50);1378-1382.
[9] Alexander L, Mannion RO, Weingarten B, Fanelli RJ, Stiles GL. Development and impact of prescription opioid abuse deterrent formulation technologies. Drug Alcohol Depend. 2014;138:01-06.
[10] Smyth Jr H, Weil C, Woodside M, Knaak J, Sullivan L, Carpenter C. Experimental toxicity of a high molecular weight poly (ethylene oxide). Toxicol Appl Pharmacol. 1970;16(2):442-45.
[11] Schuster M, Bayer O, Heid F, Laufenberg-Feldmann R. Opioid rotation in cancer pain treatment: a systematic review. Dtsch Arztebl Int. 2018;115(9):135-42.
[12] Samarghandian S, Afshari R, Farkhondeh T. Effect of long-term treatment of morphine on enzymes, oxidative stress indices and antioxidant status in male rat liver. Int J Clin Exp Med. 2014;7(5):1449.
[13] Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral U. Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. J Biosci.

2005;30(2):245-52.
[14] Guay D. Use of oral oxymorphone in the elderly. Consult Pharm. 2007;22(5):417-30.
[15] Shuey DL, Woodland C, Tremblay C, Gregson R, Gerson RJ. Oxymorphone hydrochloride, a potent opioid analgesic, is not carcinogenic in rats or mice. Toxicol Sci. 2006;96(1):162-73.
[16] Huang X-J, Choi Y-K, Im H-S, Yarimaga O, Yoon E, Kim H-S. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors. 2006;6(7):756-82.
[17] Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy: review articles. Cardiovasc J Afr. 2016;27(2):89-94.
[18] Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet. 2003;362(9388):971-82.
[19] McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakova O, Ford I, et al. Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet. 2004;363(9426):2015-21.
[20] Athyros VG, Tziomalos K, Katsiki N, Doumas M, Karagiannis A, Mikhailidis DP. Cardiovascular risk across the histological spectrum and the clinical manifestations of non-alcoholic fatty liver disease: An update. World J Gastroenterol. 2015;21(22):6820.
[21] Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008;93(5):1526-40.
[22] Ragel BT, Couldwell WT. Pituitary carcinoma: a review of the literature. Neurosurg Focus. 2004;16(4):01-09.
[23] Beckles MA, Spiro SG, Colice GL, Rudd RM. Initial evaluation of the patient with lung cancer: symptoms, signs, laboratory tests, and paraneoplastic syndromes. Chest. 2003;123(1):97S-104S.
[24] Rosalki SB. Low serum creatine kinase activity. Clin Chem. 1998;44(5):939-43.
[25] Ekstedt M, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S. Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. J Hepatol. 2007;47(1):135-41.
[26] Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003;98(5):960-67.
[27] Szkudlinski MW, Fremont V, Ronin C, Weintraub BD. Thyroid-stimulating hormone and thyroid-stimulating hormone receptor structure-function relationships. Physiol rev. 2002;82(2):473-502.

PARTICULARS OF CONTRIBUTORS:
1. MSc Student, Young Researchers and Elite Club, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.
2. PhD Student, Department of Genetic Biology, Faculty of science, Yazd University, Yazd, Iran.
3. PhD Candidate, Legal Medicine Research Centre, Legal Medicine Organization, Tehran, Iran.
4. Professor, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Ali Salimi,
Professor, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
E-mail: salimibio@gmail.com, Salimiali@bmsu.ac.ir

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 11, 2018**
Date of Peer Review: **Apr 24, 2018**
Date of Acceptance: **May 28, 2018**
Date of Publishing: **Sep 01, 2018**