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Effect of Orlistat on Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) in Wistar Rats

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Abstract

Context: Orlistat is one of the few anti-obesity drugs that have been approved by FDA. It reduces the lipid absorption by 30% in its therapeutic dose by means of reversible inhibition of gastrointestinal lipases.

Objective: Since vitamin K (a lipid soluble vitamin) is important in the synthesis of factors 2, 7, 9 and 10 of the coagulation cascade, we hypothesized that reduction in lipid absorption can cause vitamin K malabsorption and thus coagulation dysfunction.

Subject: 18 Wistar rats divided in three groups. For the purpose of testing our hypothesis, we decided to give Orlistat to Wistar rats (in the form of solution in alcohol), for one week, one month and three months. Thus we made three groups including Control 1 group which only used water, Control 2 group which used water and alcohol and control 3 groups which used water, alcohol and Orlistat as its drink. After measuring PT and PTT of each rat, we used one way analysis of variants for the analysis of the results.

Results: PT and PTT didn't follow a predictable pattern through each series of experiments and therefore comparison wasn't possible between different series of experiments. However, in every three series, both PT and PTT increased as a result of Orlistat consumption and the significancy of difference between control 2 and case group increased as the time of experiments got longer. This mentioned significancy was 0.905, 0.820 and 0.495 for PT, and 0.888, 0.734 and 0.538 for PTT during one week, one month and three months experiment, respectively.

Conclusion: Our results showed that Orlistat didn't have a significant effect on neither PT nor on PTT in the duration of our research (<3 months). However, it can be predicted that longer times of Orlistat consumption may lead to significant effects on PT and PTT.

Keywords: Obesity; Orlistat; Prothrombin time; Partial Thromboplastin time

Introduction

About 2.1 billion people, or almost one-third of the world's population, were overweight or obese in 2013 [1]. Obesity is a risk factor for cardiovascular diseases, musculoskeletal disorders and certain type of cancers (colon, breast and endometrial) [2]. At least 2.8 million people die each year as a result of being overweight or obese [3]. Thus, preventing obesity and treating obese patients can play a vital role in decreasing obesity associated morbidity and mortality. Nowadays, there are several anti-obesity medications which help people not to become obese or obese patients to reduce weight. Among these medications, only a few have been approved by the US food and drug administration (FDA), which Orlistat is one of them.

Orlistat is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl] dodecyl ester. Its empirical formula is $C_{29}H_{53}NO_5$, and its molecular weight is 495.7. It is a single diastereomeric molecule that contains four chiral centers, with a negative optical rotation in ethanol at 529 nm [4]. Orlistat is a reversible inhibitor of gastrointestinal (gastric and pancreatic) lipases. It exerts its therapeutic activity in the lumen of the stomach and small intestine by forming a covalent bond with the active serine residue site of gastric and pancreatic lipases. The inactivated enzymes are thus unavailable to hydrolyze dietary fat in the form of triglycerides into absorbable free fatty acids and monoglycerides. As undigested triglycerides are not absorbed, the resulting caloric deficit may have a positive effect on weight control. Systemic absorption of the drug is therefore not needed for activity. At the recommended therapeutic dose of 120 mg with each meal-3 times a day, Orlistat inhibits dietary fat absorption by approximately 30% [5].

Vitamin K is a lipid soluble vitamin. Dietary vitamin K, is absorbed chemically unchanged from the proximal intestine after solubilisation into mixed micelles composed of bile salts and the products of gastrointestinal lipolysis [6]. In healthy adults the efficiency of absorption of vitamin K in its free form is about 80 percent [6,7]. Within the intestinal mucosa the vitamin is incorporated into chylomicrons, is secreted into the lymph, and enters the blood via the lacteals [7,8]. Vitamin K is essential for synthesis of factors 2, 7, 9 and 10 in the coagulation cascade. Among these four factors, factor 7 is necessary for the extrinsic pathway, factor 9 is essential for intrinsic pathway and factors 2 and 10 are being used in the common pathway of coagulation. Thus, vitamin K deficiency can result in coagulation

problems. Since prothrombin time (PT) and partial thromboplastin time (PTT) are used to assess the extrinsic and intrinsic pathway respectively, these two measures can be helpful in detecting possible problems in the coagulation cascade.

So we hypothesized that Orlistat, by means of inhibiting the absorption of lipids, can potentially reduce the absorption of vitamin K and possibly making some dysfunctions in the coagulation cascade. Therefore, we decided to give Orlistat to Wistar rats and measure their PT and PTT for assessing any possible coagulation dysfunction.

Materials and Methods

54 male Wistar rats, with the mean age of 8 weeks and mean weight of 300 grams were obtained from the animal house of the Shahid Beheshti University of Medical Sciences, Tehran, Iran. The rats were kept at 12 hours of light and 12 hours of darkness each day with the temperature of 20 degrees centigrade. Every three rats were kept at one cage with same source of food and drink. All the steps of this research were in accordance to European convention for the protection of vertebrate animals used for experimental and other scientific purposes.

We divided these 54 rats into three groups of eighteen rats. After that we put one group for a week, another one for a month and the other one for three months under experiment. Each group were divided into three subgroups of six rats. The first subgroup (control 1) was only given the regular water and food. Since Orlistat isn't soluble in water, we first dissolved it in ethanol and then give the resultant solution to rats. Therefore, the second subgroup (control 2) was given water and ethanol plus the regular food. The third subgroup (case) were given the Orlistat-ethanol solution (the given solution had same amount of alcohol as the control 2 group) plus the regular food. Each cage, depend on what they should have for drink, were given 100 milliliters of water, 1 milliliter of alcohol and 14 milligrams of Orlistat for each day. After every 3 or 4 days we measured the remainder of the drink of each cage and thus calculating the amount of water, alcohol and drug consumption of each cage.

After the time period for each group was overdue, all the rats of each group were first euthanized separately by Carbon Dioxide. Then immediately after the death of each rat, it was beheaded by a guillotine and the blood was collected in a tube which contained Sodium Citrate as coagulation inhibitor. Then the blood was centrifuged with a rate of 4000 rounds per minute and the resultant plasma was obtained with a sampler. PT and PTT were then measured for each rat's plasma using Pacific Hemostasis® PT and PTT reagents.

For the analysis of the results, we used one-way analysis of variants (one-way ANOVA) and Tukey's test in SPSS software. Our confidence interval was 95%.

Results

A one-way analysis of variants (one-way ANOVA) was conducted between groups in the same time period of drug consumption and between case groups in different time periods of drug consumption. Comparison was made using Tukey's test which results can be seen in Table 1 for PT and Table 2 for PTT.

Groups being compared	Mean difference	95% Confidence interval for mean	Significant?
Control 1 vs. Control 2 (One Week)	1.333	-5.543 to 8.210	No
Control 1 vs. Case (One Week)	1.333	-5.543 to 8.210	No
Control 2 vs. Case (One Week)	0.5	-9.569 to 10.57	No
Control 1 vs. Control 2 (One Month)	-4.167	-14.66 to 6.327	No
Control 1 vs. Case (One Month)	-5.833	-21.41 to 9.747	No
Control 2 vs. Case (One Month)	-1.667	-10.24 to 6.907	No
Control 1 vs. Control 2 (Three Months)	-2	-8.083 to 4.083	No
Control 1 vs. Case (Three Months)	-3.5	-9.922 to 2.922	No
Control 2 vs. Case (Three Months)	-1.5	-7.922 to 4.922	No
Case (One Week) vs. Case (One Month)	-7.333	-21.31 to 6.642	No
Case (One Week) vs. Case (Three Months)	-5.167	-11.67 to 1.335	No
Case (One Month) vs. Case (Three Months)	3.167	-20.31 to 26.64	No

Table 1: Results of Tukey's test for PT.

Groups being compared	Mean difference	95% Confidence interval for mean	Siginificant?
Control 1 vs. Control 2 (One Week)	2.5	-9.329 to 14.33	No
Control 1 vs. Case (One Week)	0.8333	-13.88 to 15.55	No
Control 2 vs. Case (One Week)	-1.667	-11.73 to 8.396	No

Control 1 vs. Control 2 (One Month)	0.5	-28.59 to 29.59	No
Control 1 vs. Case (One Month)	-5.333	-54.09 to 43.42	No
Control 2 vs. Case (One Month)	-5.833	-38.39 to 26.72	No
Control 1 vs. Control 2 (Three Months)	4.167	-18.23 to 26.57	No
Control 1 vs. Case (Three Months)	7.833	-4.976 to 20.64	No
Control 2 vs. Case (Three Months)	3.667	-14.70 to 22.03	No
Case (One Week) vs. Case (One Month)	-9.667	-51.40 to 32.07	No
Case (One Week) vs. Case (Three Months)	2.5	-9.902 to 14.90	No
Case (One Month) vs. Case (Three Months)	12.17	-33.55 to 57.88	No

Table 2: Results of Tukey's test for PTT.

Also a comparison was made between means of PT and PTT in different time periods which can be seen in Figures 1 and 2, respectively.

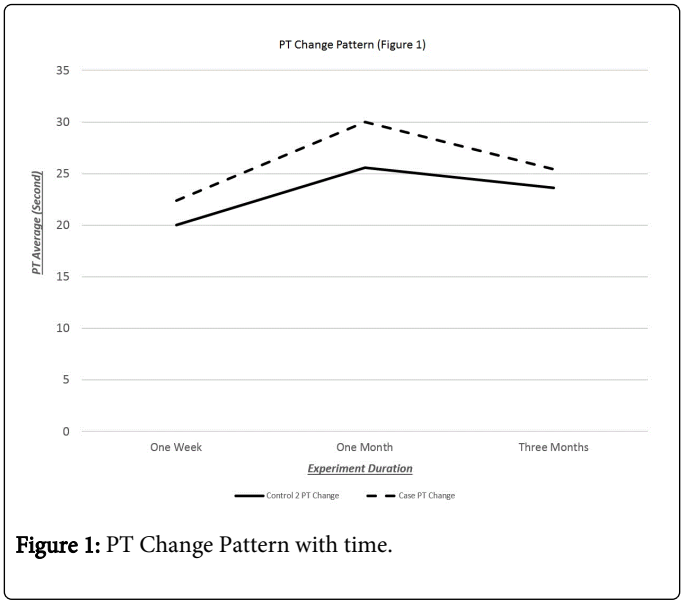

Figure 1: PT Change Pattern with time.

Discussion and Conclusion

Our research shows that Orlistat increases PT in every three experiments, whereas alcohol decreased PT in the one week experiment and increased PT in the one month and three months experiments. The pattern of PT change with alcohol consumption didn't follow a predictable pattern through time, thus PTs of the case groups didn't follow a predictable pattern, too (Figure 1). Therefore, comparison between prothrombin times in groups with different times of Orlistat consumption was not possible. However, the amount of significancy of differences between control 2 and case groups in three given times were comparable (Figure 2).

Our research also shows that Orlistat increased PTT and alcohol decreased PTT in every three experiments, but the increases were stronger than the decreases. The pattern of PTT change with alcohol consumption didn't follow a predictable pattern through time, thus PTTs of the case groups didn't follow a predictable pattern, too. Therefore, comparison between partial thromboplastin times in groups with different times of Orlistat consumption was not possible.

However, the amount of significancy of differences between control 2 and case groups in three given times were comparable.

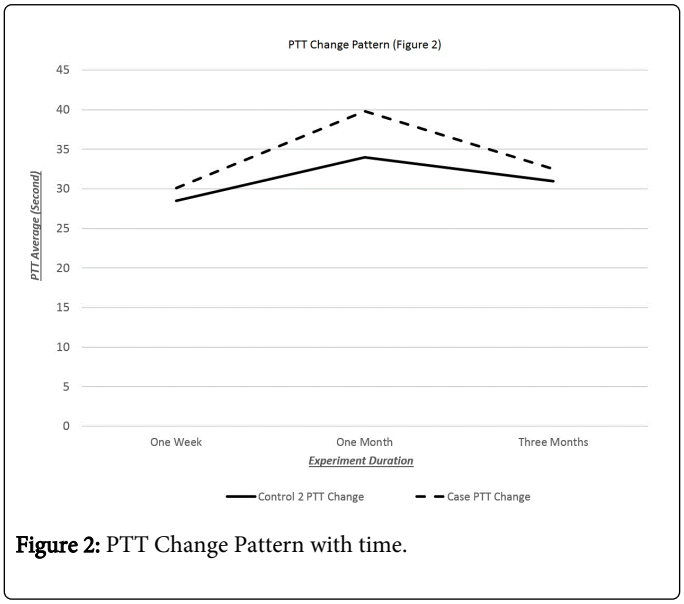

Figure 2: PTT Change Pattern with time.

There was a study on effects of Orlistat on fat-soluble vitamins in obese adolescents which showed that Orlistat causes non-significant reduction in vitamin K absorption. This study also showed that Orlistat causes a non-significant increase in prothrombin time (PT) [9]. However we think that this research has some problems:

Orlistat is approved by FDA for adults over 18 years old [10], but this study used adolescents with average age of 14.8 years old. Therefore results of this study can't be distributed to the group of people who take Orlistat.

This study used a sample of only 17 adolescents, which included 8 Caucasians and 9 African Americans. The small Size of the sample (knowing that there are substantial genetic differences among humans) in addition to the difference among the race of the participants can make the results doubtful.

So because of the reasons mentioned above, we decided to test our hypothesis on Wistar rats. These rats have less genetic variability among their population, so the size of the sample doesn't need to be large. Also, the results of this experiment are fairly distributable to the human population since 90% of the rat genome is similar to humans [11].

During the time period of our study (3 months), we couldn't find any significant increase in PT or PTT due to Orlistat consumption. However, the significancy of PT and PTT change, increased more as the time of Orlistat consumption got longer. These results make the probability those longer times.

(>3 months) of Orlistat usage may lead to significant increase in PT and PTT.

The mentioned evidences suggest that Orlistat as an FDA approved anti-obesity drug doesn't cause any serious coagulopathies (if any coagulopathy happens), although theoretically it can cause vitamin K malabsorption and thus causing increases in both PT and PTT. But it should be taken to account that people who use other vitamin K inhibitors such as Warfarin, should use Orlistat with care.

Ethics

All the steps of this research were in accordance to European convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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