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Effect of Chronic Stress on Coagulation Parameters in Warfarin-Treated Male Rats

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Abstract

Objective: Chronic stress affects coagulation system, thus, can potentially interact with oral anticoagulation therapy. Clinically, it can be difficult to induce or quantify stress in human subjects. Therefore, this study proposed to evaluate the effects of chronic stress on coagulation parameters in warfarin-treated rats under controlled conditions.

Materials and Methods: Forty male Wistar rats were divided into unstressed and stressed groups. Rats in unstressed group were assigned to control and normal plus warfarin treatment (N-WT) subgroups (n = 10 each). Rats in stressed group underwent chronic mild stress (CMS) and were assigned to CMS and CMS plus warfarin treatment (CMS-WT) subgroups (n = 10 each).

On the 28th day and after completion of the CMS induction procedure, serum corticosterone was used to confirm CMS induction. Also, the coagulation parameters-prothrombin time (PT), partial prothrombin time (PTT), International normalized ratio (INR) and prothrombinlevels-were assessed by biochemical methods.

Results: Comparison of changes in PT, PTT and INR values between two groups of N-WT and CMS-WT showed significant increase in PT, PTT and INR values in the CMS-WT(PT:6.6 ± 3.23, p<0.001; PTT: 4.4 ± 3.8, p=0.01 and INR: 0.51 ± 0.23, p<0.001).Prothrombin was significantly higher in CMS group that shows hypercoagulable state. Prothrombin was significantly lower in CMS-WT group which can be due to reduced metabolism of warfarin in this group (0.54 ± 0.017, p<0.001).

Conclusion: CMS decreases prothrombin levels and leads to an increase in measures of timed coagulation parameters (PT, PTT and INR), but in no warfarin treated rats leads to hypercoagulable state.

Keywords: *Stress, Coagulation, Prothrombin time, International normalized ratio, Warfarin, Corticosterone.*

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Introduction

Warfarin, a vitamin K antagonist, is among the widely used oral anticoagulants. The drug exerts its therapeutic effects by inhibiting multiple coagulation factors (factor 2, 7, 9 and 10) and is metabolized by cytochrome P450 enzymes (CYP1A2, CYP3A4 and CYP2C9) [1]. Therefore, factors which inhibit or induce each of these enzymes can affect the efficacy and safety of warfarin.

Stress is a biological and psychological reaction to danger perception. Low level of stress is considered physiological and acts as a responder to the environmental conditions. However, chronic stress may increase susceptibility of a person to different illnesses such as heart attacks, gastrointestinal ulcers and psychological disorders [2]. Blombäck et al. [3] found that acute stress conditions are associated with an increase in factor 8, VWF, fibrinogen and tissue plasminogen activator. Chronic

psychological stress (i.e., 77 consecutive hours) result in the reduction of factors 5, 8 and 9 while no increase is observed in fibrinolysis. It seems that stressful conditions are able to affect coagulation and fibrinolysis in part through sympathetic nervous system [4]. All these variations can intervene in coagulation and fibrinolysis procedures by affecting the contributing gene expressions [5]. There is no conclusive evidence regarding the effect of stress on coagulation parameters under controlled conditions. The objective of this study is to investigate the effects of chronic stress on coagulation parameters in rats with or without warfarin treatment.

Materials and Methods

Study design

The study was approved by regional ethic committee of Tabriz University of Medical Sciences. Forty male Wistar rats were divided into unstressed and stressed groups. Rats in unstressed group were assigned to control and normal plus warfarin treatment (N-WT) subgroups (n = 10 each). Rats in stressed group underwent chronic mild stress (CMS) and were assigned to CMS and CMS plus warfarin treatment (CMS-WT) subgroups (n = 10 each).

Chronic mild stress induction

A standard 7-step protocol was used to induce chronic stress [6]: (I) imprisoning the rat in a cage with a 45 degree angle relative to the ground, (II) exposure to a blinking light, (III) exposure to alternate white noise with an intensity of 80 decibels, (IV) moistening the cage, (V) food and water deprivation for 19 hours, (VI) limitations on food and its reduction to 5 grams for each rat, (VII) imprisonment of two rats in a cage and reversing the light-dark cycle. Steps of the scheme were completed in a week and were administered for 4 weeks (Table 1).

Corticosterone levels

On the 28th day and after the completion of the CMS induction procedure, animals (n=3) were anaesthetized with a mixture of xylazine (10 mg/kg, intraperitoneally) and ketamine (90 mg/kg, i.p.). After deep anesthetization, blood samples were obtained and centrifuged at 1000 rpm for 10 min at 4°C for obtaining of serum. Corticosterone levels were measured using a specific kit developed for this purpose to verify the effect of stress induction.

Warfarin administration

In warfarin treated groups, warfarin was administered in dosages of 0.1 mg/kg through oral rout in the last 5 days of chronic stress induction [7]. The current dose was based on previous pilot study which found 0.1 mg/kg as an effective dose without compromising safety [8]. Normal saline (through gavage) was used as placebo. All solutions were prepared and administered in the volume of 1 ml/kg.

Coagulation parameters assessment

Finally, rats were anesthetized and blood samples were obtained and centrifuged and serum separated [9,10]. All samples were

Table1. Timetable for the infliction of chronic stress

Days of the week	Time	Description
Saturday	14:30	Administration of food and water, inclination of the cage in a 45-degree angle
Sunday	10:00	Exposure to a blinking lamp in a dark room (300 flashes per minute), cage in a horizontal position
Sunday	16:00	Turning of the blinking lamp and turning the light on along the day, moistening the cage with 200cc water and initiation of food and water deprivation
Monday	11:00	The cage is dried and a bottle of water is provided
Monday	12:00	Provision of enough food, taking away the bottle of water and exposure to a white noise with an intensity of 80 decibels
Monday	15:00	Turning of the white noise
Tuesday	10:00	Provision of empty bottle of water
Tuesday	11:00	Provision of full bottle of water
Tuesday	17:00	Exposure to a blinking lamp (300 flashes per minute) with the lights off during the night
Wednesday	10:30	Blinking lamp is turned off, food deprivation is initiated and the cage is positioned in a 45-degree angle
Wednesday	15:30	Putting two mice in a cage, Exposure to a blinking lamp (300 flashes per minute) and positioning the cage in a horizontal state
Wednesday	22:30	White noise is turned off
Thursday	10:00	Each mouse is put in a separate cage and scarce food is provided
Thursday	12:00	Adequate food is provided for the mouse
Friday	19:00	Lights are turned on during the day and food and water deprivation is initiated

processed and measurements were performed within 6 hours.

To evaluate PT, tissue thromboplastin and plasma of rats were incubated for 5 minutes in 37°C temperature. Plasma of the rat was reclassified after the addition of 30 mM calcium chloride and clotting time was registered with a stopwatch [9]. In order to assess PTT, phospholipid and citrated plasma of a rat (citrated with 3.2% sodium citrate) were incubated for 5 minutes. In this case, ratio of the coagulant (i.e., sodium citrate) to the gross blood of a rat should be 1/9. Thereafter, calcium chloride was added to complete the reclassification process and the clotting time was registered with a stopwatch. This test is commonly known as the PTT test but it is actually an activated PTT test because its indicators have a surface with a negative charge which accelerate the reaction [12]. In order to evaluation of prothrombin, ELISA kit (ZellBIO, Germany) with a sensitivity of 0.02 ng/ml and assay range of 0.5-0.16 ng/ml was used.

Statistical analysis

Statistical analysis was carried out in SPSS software version 21 (vendor). Demographic data were provided in frequency percentages, graphs and frequency tables. Two-step (two-way) variance method was used to analyze the data. The p-value of > or =0.05 was considered significant.

Results

Serum corticosterone levels

The serum corticosterone levels increased in Serum corticosterone levels were increased in CMS and CMS-WT groups when compared with control and N-WT rats ($p<0.001$) (Table 2), suggesting induction of stress.

Coagulation parameters levels

There was a significant difference in PT variations between the N-WT and CMS-WT groups ($p<0.001$). PT level was notably higher in CMS-WT rats compared to N-WT. Therefore, chronic stress significantly contributes to higher PT values ($p<0.001$).

Based on findings concerning the comparison of PTT variations in different groups and the control group, no difference was observed between the N-WT and control group. Furthermore, higher PTT values were observed in CMS-WT group in comparison to N-WT group which clearly highlights the effect of chronic stress on increasing the PTT ($p<0.01$).

Similarly, variations of INR values for both N-WT and CMS-WT groups were higher than that of the control group ($p<0.001$). Comparison of variations in INR values in two groups of N-WT and CMS-WT shows relatively higher values in the latter group ($p<0.01$).

With regard to the variations in prothrombin coagulation parameters, the respective values for CMS rats were higher than control group ($p<0.001$). Similar comparison between two groups, N-WT and CMS-WT shows noticeable lower values of prothrombin in the latter group ($p<0.001$). Therefore, chronic stress leads to an increase of prothrombin in rats not prescribed with warfarin and leads to a decrease in warfarin-treated rats. It should be noted that prothrombin levels for the N-WT rats were lower than that of the control group which shows the efficacy of warfarin ($p<0.05$) (Table 3).

Table 2. Comparison of serum corticosterone levels in each group.

Mean SD			
Control	**CMS**	**N-WT**	**CMS-WT**
28.769.06	99.7914.15**	60.438.82	96.2626.11**

**p<0.01 in comparison to control and N-WT groups.

Discussion

This study investigated the effects of chronic stress on coagulation parameters in Wistar rats. Our results suggest that chronic stress in warfarin-treated animals decreases prothrombin and prolongs the coagulation times. The changes could predispose warfarin-treated rats to warfarin toxicity. On the contrary, chronic stress in warfarin non-exposed rats increases hypercoagulability and might render rats prone to vascular disease.

Studies in healthy people suggest that acute psychological stress especially, depression and anger might cause hypercoagulation through various mechanisms including neuroendocrine activation and hemoconcentration [11,12]. Accordingly, mental stress is a condition that needs to be considered prior to the coagulation laboratory testing [3]. Various factors may cause sudden changes in INR, for example, abrupt changes in diet, long-term alcohol misuse, drugs affecting the metabolism of warfarin and irregular use of anticoagulant edibles [13]. Psychological stress and distress should be considered as additional factors which affect the INR values in patients treated with oral anticoagulants. In this regard, three studies have shown that psychological stress in healthy people reduce PT [11,14,15]

Table 3. Results of coagulation parameters in investigated groups.

Coagulation parameter	Mean SD			
	Control	**CMS**	**N-WT**	**CMS-WT**
Prothrombin time (s)	13.600.84	15.100.56	22.203.08***	28.803.08###
Partial prothrombin time (s)	23.801.87	25.402.54	25.803.08	30.203.82##
International normalized ratio	1.040.06	1.160.04	1.700.23***	2.210.23###
Prothrombin level (ng/ml)	2.130.179	2.950.335$	1.780.188*	1.240.205###

$p<0.05, *p<0.05 and***p<0.001 in comparison to control group; ##p<0.01 and ###p<0.001 in comparison to N-WT group.

In another clinical study, no significant variations were observed in PT values of people in stressful job conditions [16]. In the current study, which was carried out under controlled laboratory conditions, no significant variations were observed in PT, PTT, and INR values of healthy rats inflicted with chronic stress. It is noteworthy that none of the above-mentioned studies have investigated the effect of warfarin. In a study by Hawk et al. two patients were treated with warfarin, it was concluded that physical and psychological stress leads to an increase in INR [5]. Authors speculated that the increase in INR due to stress could be attributed to decrease in the metabolism of warfarin.

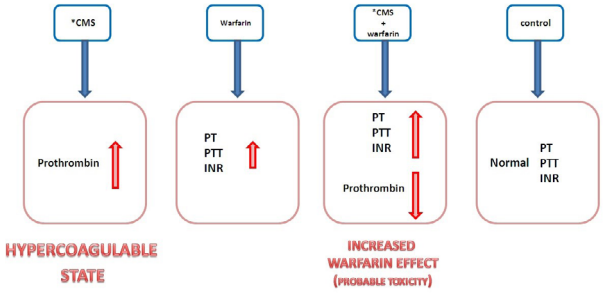
Acute psychological stress and chronic stressful conditions such as stressful jobs and/or bad economic conditions could stimulate the coagulation system in elderly people [15].

In several studies, the effect of stress on coagulation factors were investigated. It has been demonstrated that the coagulation factors including factor 7, 8, VWF and fibrinogen activity were increased, when encountered with stressful conditions [3].

Therefore, it is observed that chronic stressful conditions result in the hypercoagulation state by increasing the procoagulation molecules (such as fibrinogen or factor 7) and/or by decreasing the fibrinolytic capacity. In this study, rats inflicted by mild chronic stress exhibited higher prothrombin levels than that of the control group. Furthermore, prothrombin levels were significantly lower in CMS-WT group when compared with the N-WT group. Consequently, chronic stress causes the increase in prothrombin level in non-warfarin treated rats and leads to a decrease in warfarin-treated rats.

Conclusions

The present study demonstrates that chronic stress in warfarin-treated animals decreases prothrombin and prolongs coagulation times of PT, PTT, and INR (Figure 1).The overall findings suggest that chronic stress could predispose warfarin-treated rats to warfarin toxicity. Additionally, chronic stress in healthy rats (non-treated) increases hypercoagulability and might render rats prone



*CMS:Chronic Mild Stress

Figure 1. Effect of chronic stress and warfarin in coagulation system of rat

to vascular diseases.

Conflict of interest

The authors declare that this manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

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References

1. Mega JL, Simon T. Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments. The Lancet. 2015;386(9990):281-91.
2. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiological reviews. 2007;87(3):873-904.
3. Blombäck M, Konkle B, Manco-Johnson M, Bremme K, Hellgren M, Kaaja R, et al. Preanalytical conditions that affect coagulation testing, including hormonal status and therapy. Journal of Thrombosis and Haemostasis. 2007;5(4):855-8.
4. von Känel R. Acute mental stress and hemostasis: when physiology becomes vascular harm. Thrombosis research. 2015;135:S52-S5.
5. Matchar DB, Love SR, Jacobson AK, Edson R, Uyeda L, Phibbs CS, et al. The impact of frequency of patient self-testing of prothrombin time on time in target range within VA Cooperative Study# 481: The Home INR Study (THINRS), a randomized, controlled trial. Journal of thrombosis and thrombolysis. 2015;40(1):17-25.
6. Ceriello A, Giacomello R, Stel G, Motz E, Taboga C, Tonutti L, et al. Hyperglycemia-induced thrombin formation in diabetes: the possible role of oxidative stress. Diabetes. 1995;44(8):924-8.
7. Zaghloul IY, Aly ZH, Radwan MA. Effect of ciprofloxacin on the hypoprothrombinemic activity of warfarin in rats. International journal of pharmaceutics. 1996;131(2):131-6.
8. Mule JB, Lafond DJ, Hoffmann R. 1963 Prothrombin time test survey, College of American pathologists, standards Committee, subcommittee on coagulation. American journal of clinical pathology. 1967;47(1):40-59.
9. Friedman M, Rosenman RH, Carroll V, Tat RJ. Changes in the serum cholesterol and blood clotting time in men subjected to cyclic variation of occupational stress. Circulation. 1958;17(5):852-61.
10. Thrall G, Lane D, Carroll D, Lip GY. A systematic review of the effects of acute psychological stress and physical activity on haemorheology, coagulation, fibrinolysis and platelet reactivity: Implications for the pathogenesis of acute coronary syndromes. Thrombosis research. 2007;120(6):819-47.
11. Austin AW, Patterson SM, von Känel R. Hemoconcentration and hemostasis during acute stress: interacting and independent effects. Annals of behavioral medicine. 2011;42(2):153-73.
12. Hjemdahl P, von Känel R. Haemostatic effects of stress. Stress and Cardiovascular Disease: Springer; 2011. p. 89-110.
13. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2008;133(6):160S-98S.
14. De Boer D, Ring C, Wood M, Ford C, Jessney N, McIntyre D, et al. Time course and mechanisms of mental stress-induced changes and their recovery: Hematocrit, colloid osmotic pressure, whole