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Research Paper

The Effect of Therapeutic Hypothermia on Plasma Levels of Thyroid Hormones During Hemorrhagic Shock in Adult Male Wistar Rats

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ABSTRACT

Background and Aim: Hemorrhagic shock (HS) is one of the most important causes of death. In this study, we investigated the benefits of therapeutic hypothermia (32°C) during HS on blood pressure (BP) and the role of thyroid hormones during HS.

Materials and Methods: Twenty-four male Wistar rats were divided into two normothermic hemorrhagic (NH) and hypothermic hemorrhagic (HH) groups (32°C during shock); the animals were then anesthetized, a microcatheter was inserted into the femoral artery, and one into the femoral vein. The arterial samples were centrifuged, and plasma was isolated to measure thyroid hormones later. The microcatheter was fixed to a physiograph to record BP. Animals were exposed to HS for 90 minutes by withdrawing blood from the femoral vein and BP was assessed during HS.

Results: The BP of HH animals was significantly higher in most times of HS and at 40 minutes (56.8±4.2 mmHg) in comparison to NH rats (45.4±3.8 mmHg) ($P<0.05$). In addition, the amount of BP in HH animals at the end of the shock period at 90 minutes (63.8±5.5 mmHg) was significantly ($P<0.001$) higher in comparison with the NH groups (39±3.2 mmHg). Levels of thyroid hormones T4 and T3 at the end of shock were lower in the HH group compared to the NH group ($P<0.001$).

Conclusion: Therapeutic hypothermia indirectly reduces the level of thyroid hormones and directly reduces the metabolism of non-vital tissues, preserves blood in the central arteries, and increases BP.

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1. Introduction

According to the latest studies, 5.8 million people (9.7% per thousand) die yearly due to trauma issues [1, 2]. Approximately 56% of deaths occurred from hemorrhagic shock (HS) before reaching the hospital and 50% of deaths occurred in the first 24 hours of admission due to the consequences of HS [3]. HS is a type of hypovolemic shock that occurs following the loss of blood in a short time and large volumes and can cause instability of the patient's hemodynamic status, reduced oxygen transfer to tissues, organ damage, and death [4]. Initial treatment for HS is the replacement of lost blood, which in many cases is inadequate, impractical, and has complications. On the one hand, proper blood may not be available, and on the other hand, injecting large volumes of fluids may increase bleeding and cell damage [5]. In addition, the critical time to maintain the brain is less than 5 minutes and to maintain heart function is about 20 minutes [6, 7]. Therefore, even with proper control of bleeding and blood flow, prolonged ischemia, or ischemia-perfusion injury due to inflammatory cytokines produced during tissue ischemia leads to death or long-term complications [7]. In addition, induction of therapeutic hypothermia (in the range of 32-35°C) has been proposed as a potential solution to maintain tissue survival after perfusion [8] in cardiac surgery as well as some diseases such as cardiac arrest, severe brain injury, drowning, spinal cord injury, and some other cases [9]. In hypothermia therapy, while precisely controlling the temperature, sedatives and blockers of nerve and muscle junction are used to prevent severe tremors that increase cellular metabolism and reduce the need for oxygen and cellular metabolism [10].

Hypothermia cell levels can keep cell metabolism at a low level by reducing adenosine triphosphate production by up to 40% in various tissues [11]. Mild hypothermia or therapeutic hypothermia (32-35°C), while maintaining cardiac output and blood flow to the myocardium, lowers the metabolic needs of the heart, delays cardiac arrest, and increases survival after HS [12-16]. On the other hand, thyroid hormones including T4 and T3 play an essential role in regulating cellular metabolism. Given that thyroid hormones cause dilatation of peripheral arteries, they can potentially have detrimental effects during HS [17]. These effects include a lack of blood supply to vital organs such as the brain and heart, resulting in an increased risk of death during HS. Studies show that blood levels of thyroid hormones decrease significantly and unjustifiably with the volume of bleeding twenty minutes after induction of HS so that thyroid hormone levels drop to a minimum within twenty-four hours of hypothermia immediately after hypothermia [18]. Because elevated blood levels of thyroid hormones T4 and T3 can cause a shift in blood from central to peripheral arteries and lower blood pressure (BP), this study investigates the effects of hypothermia on changes in BP and blood levels of T4 and T3 during HS for the first time.

2. Material and Methods

In the present study, adult male Wistar rats born in the animal house of Arak University of Medical Sciences with a weight range of 250-30 g were selected and kept in standard conditions with a temperature of 22±2°C with a light cycle of 12 hours light/dark. Animals had free access to water and food. At first, a special heparin microcatheter was prepared to record BP and take the blood samples. To record the temperature, a microthermometer was prepared to be inserted in the anal part of the rats.

Preparing animals

Twenty-four Wistar rats were randomly divided into two hypothermic hemorrhagic (HH) and normothermic hemorrhagic (NH) groups, each group with 12 rats. Animals were anesthetized by intraperitoneal IP injection pentobarbital (50 mg/kg) [19], the femoral region was opened by surgical history, and a microcatheter (size 50) was placed inside their femoral artery and vein. Then, the first heparinized blood sample was withdrawn from the arterial catheter (to measure thyroid hormones later), and a microcatheter attached to the transducer of the physiograph for recording the animal's BP, and then was it was dragged on the calibrated screen of PowerLab device. At the same time, a graduated microtherm was fixed in the anal area of the animal with paper glue. Then, in the HH group, the body temperature was reduced to mild hypothermia (32-35°C) by pouring alcohol on the abdomen, and the body temperature was maintained in the range of 32°C by moving the heater lamp closer to the animal, while body temperature in the NH group was maintained at normal temperature (37°C). Within 15 minutes, 3-5 mL of blood was drawn from the femoral artery to reduce arterial systolic pressure from 120 mmHg to about 50 mmHg, and the animal entered the first phase of HS. The animal in this condition was monitored for 90 minutes without any intervention, and if the animal's death occurred, the case was excluded from the study. The third blood sample was taken at the end of the shock phase. At the end of the HS stage, the animals

were resuscitated and returned to the baseline of the B.P by injecting a lactate ringer via the femoral vein. Finally, after centrifugation of blood samples, their plasma was separated, collected, and stored at -70°C to measure the plasma level of thyroid hormones later.

Laboratory measurement of hormonal variables

Thyroid hormone levels were measured before the shock induction (zero minutes), at the beginning of the shock (fifteen minutes), and after the end of the shock (90 minutes) by laboratory kits. Intra and inter-assay coefficients of variations for the plasma levels of T4 and T3 were 4.4%, 5.6% and 3.5%, 4.9%, respectively

Statistical analysis

The obtained data were analyzed using SPSS software version 25. The data were classified and expressed based on mean and standard error. An independent t-test was used to evaluate the data between groups at the same time, and if significant, the Tukey test was used. In addition, two-way analysis of variance was used to examine the significant differences in the data throughout the study time. In this test, group (hypothermic and normothermic) was considered as an intergroup variable, and time was considered as an intragroup variable (repetitive measures test). The significance level was considered at 0.05.

3. Results

Investigating BP changes during shock with hypothermia and normothermia

The results of the current study showed that BP levels of the hypothermic rats decreased in the first half hour of HS, but the values were not significant. As shown in Figure 1, the BP of the hypothermic group at the end of 90 minutes of HS (63.8±5.5 mmHg) was significantly (P<0.001) higher in the normothermic groups (39±3.2 mmHg). The results of repeated measure analysis of the BP status of the rats represent the higher BP rate in the whole period of HS in the hypothermic group compared to the normothermic group (P<0.001). The BP in hypothermic animals in most of the shock period especially after 40 minutes (56.8±4.2 mmHg for HH group vs 45.4±3.8 mmHg for NH group at the time of 40 minutes) until the end of the shock was significantly higher (P<0.001) in comparison with the normothermic groups.

Results of our study showed that the BP rate at the end of the shock (90th minute) significantly increased compared to BP at the time of the 15th minute (P<0.001) in the hypothermic group. Calculating the percentage of increased BP at the end of shock showed that the BP at the 90th minute increased 1.3% compared to the 15th minute.

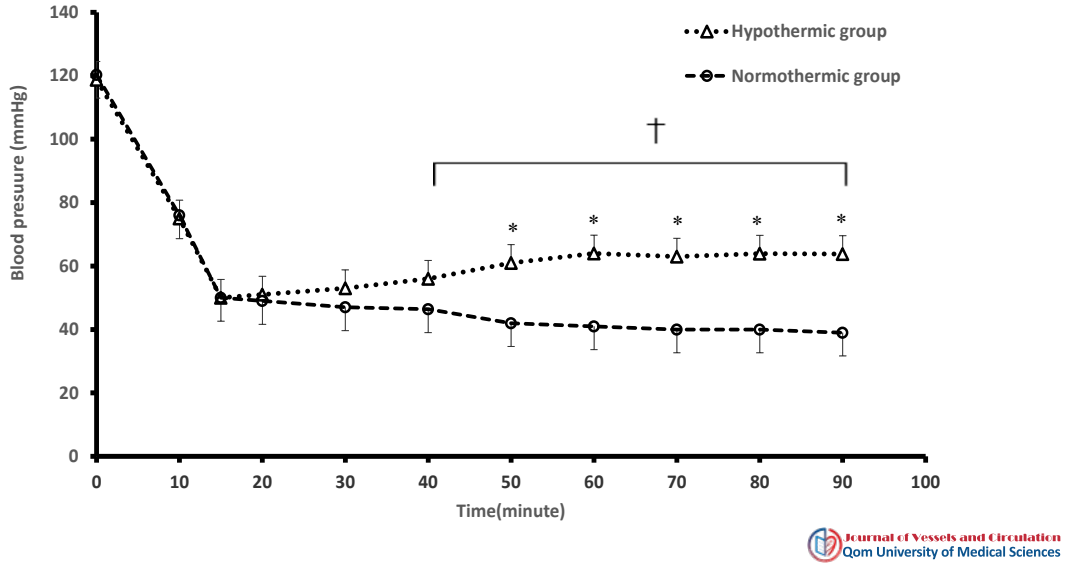

Figure 1. Comparison of Changes of BP at the Beginning, During, and End of HS in Groups Hypothermic and Normothermic (n=12 for each group)

All data are expressed as Mean±SE; * P<0.01, † P<0.001.

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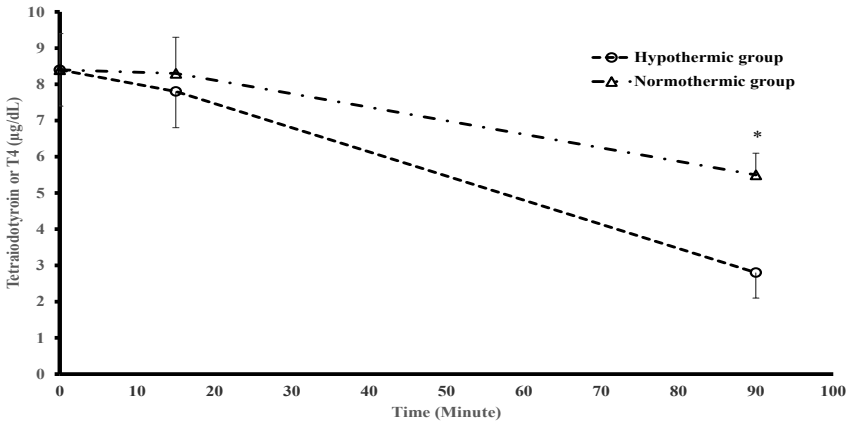

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Figure 2. Comparison of changes of the plasma levels of T4 at the beginning, during, and end of HS in hypothermic and normothermic groups (n=12 for each group)

All data are expressed as Mean±SE, *P<0.01.

On the other hand, the results of our study demonstrated that BP at the end of 90 minutes was significantly reduced compared to zero minutes in the normothermic group (P<0.001). Calculating the percentage of hypertension at the end of shock showed that BP decreased 0.8% at the 90th minute compared to the 15th minute.

Changes in thyroid hormones during shock associated with hypothermia and normothermia

In this study, the level of thyroid hormones, T4 and T3, were measured at the beginning (time of zero), the 15th minute, and at the end (90th minute) of the HS in both normothermic and hypothermic groups. According to the results of this study, as depicted in Figures 2 and 3, levels of thyroid hormones showed a dramatic reduction at the end of the HS in both groups, so that amounts of the T4 decreased significantly (P<0.001 2.92) from 8.3±1.0 µg/dL at zero minutes of HS to 5.5±1.3 µg/dL at 90th minute of HS in the normothermic group. Also, amounts of the T3 decreased significantly (P<0.001) from 2.93 ng/mL±0.3 to 1.38±0.3 ng/mL. In addition, the value of T4 decreased significantly (P<0.001) from 8.2±1.1 µg/dL at zero minutes of HS to 2.8±1.1 µg/dL at 90th minute of HS in the hypothermic group, and also amounts of the T3 decreased significantly (P<0.001) from 2.92 ng/mL±0.4 to 1.01±0.1 ng/mL. Furthermore, the results of this study also showed that this decrease in the plasma T4

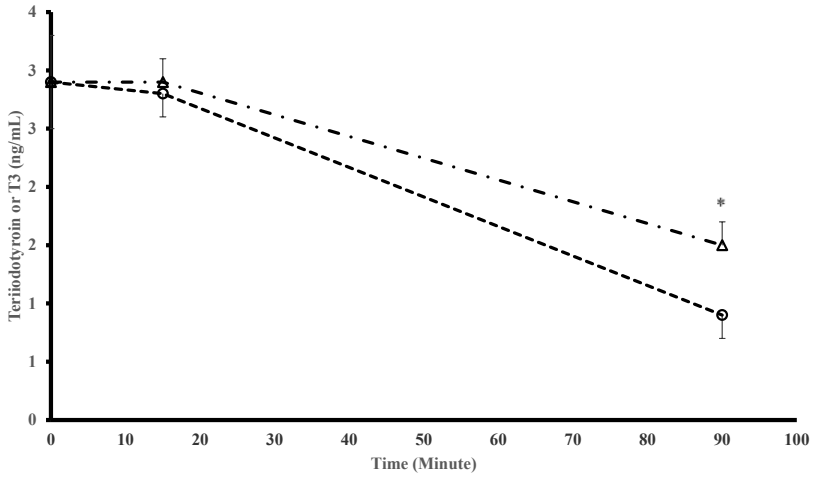

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Figure 3. Comparison of Changes of the Plasma Levels of T3 at the Beginning, During, and End of HS in hypothermic and normothermic groups (n=12 for each group),

All data are expressed as Mean±SE; * P<0.001.

and T3 levels was much greater in the hypothermic group than in the normothermic group. The level of T4 at the end of the shock in the hypothermic group was 35% of the baseline, while in the normothermic group, the amount of T4 at the end of the shock was 66% of its initial value (P<0.05). On the other hand, the level of T3 hormone in the hypothermic group reached 34% after induction of HS, but this ratio was 47% in the normothermic group (P<0.05).

4. Discussion

The present study showed that HS in animals significantly reduces the level of thyroid hormones. In addition, the simultaneous induction of mild hypothermia (TH) with HS exacerbates this reduction largely (Figures 1 and 2). These findings are in line with Vladimir et al. [20] and also show a significant decrease in thyroid hormones in response to HS. Their study on dogs showed that at the end of the bleeding shock, T4 decreased by 42% and T3 by 17% compared to baseline values. In the present study, in the normothermic group, this decrease rate was 66% for T4 but 49% for T3. On the other hand, the decrease in total levels of thyroid hormones in the hypothermic group in our study was much greater in comparison to the normothermic group. This concept suggests that the induction of mild hypothermia during HS can further reduce thyroid hormone levels. According to the results of the current study, at the end of HS in the hypothermic group, T4 reached 35% of baseline, and T3 reached 37% of baseline. A similar study by Shirpour et al. [21] showed a decrease in thyroid hormone levels during hypothermia induction. According to their study, the T4 level was 4.87±0.59 µg/dL at the beginning of induction of hypothermia, which reached 3.25±0.25 µg/dL at the end of two hours, equivalent to 66% of the baseline [21].

Based on our study, changes in thyroid hormones in response to HS associated with hypothermia exacerbates largely. Winfred et al. [22] reported that HS reduces metabolism mainly caused by a decrease in the level of thyroid hormones, especially T3, which is also reduced in the later stages of T4. Given that, thyroid hormones reduce peripheral vascular resistance and shift blood to non-vital organs such as the skin and gastrointestinal tract by increasing tissue metabolism [11-13]. Hypothermia induces blood lowering thyroid hormone levels for maintaining central BP and preventing damage to vital organs such as the heart and brain.

The results of the present study showed that at the end of the bleeding shock (after 90 minutes) in the hypothermic group, BP increased significantly compared to the beginning of the shock. However, BP in the normothermic group decreased significantly at the end of the shock compared to the beginning of the shock. Therefore, the BP of the HH group was higher than the NH group during the whole HS, that is, inducing hypothermia can prevent dropping BP during HS or even increase BP.

A study by Jiang et al. on the induction of hypothermia during HS in rabbits showed that induction of hypothermia improves cardiac cytological function and pulmonary function in shock and increases the chances of survival [23]. Takasu et al. also proved the beneficence of mild hypothermia in the resuscitation of injured patients [24] which is in line with the results of the current study. It is essential to mention that the current emergency procedure is to warm HS patients with a blanket. Using a blanket in the emergency ward should be considered with caution, as hypothermia after hemorrhagia is a physiologic response of the body to preserve BP inside the vital central arteries of the body, brain, and heart. The current study showed that hypothermia can independently help animals maintain central BP during HS. Hypothermia treatment, by reducing metabolism in non-vital tissues, prevents blood shifting from vital organs (brain and heart) to non-vital organs, which increases the chances of survival of the injured.

5. Conclusion

Therapeutic hypothermia increases the survival of patients involved in HS in several ways such as increasing central BP toward the brain and heart and decreasing blood circulation of the non-vital peripheral organs of the body. In addition, hypothermia therapy reduces thyroidal hormone effects. One reason for the highest BP in hypothermic animals at the end of the shock is the decreased plasma thyroid hormone levels and tissue metabolism of vital and non-vital organs.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were performed in this study following instructions of NIH. The ethical approval code of IR.MUQ.REC.1398.043 was taken.

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Authors' contributions

All authors equally contributed to preparing this study.

Conflict of interest

The authors declared no conflict of interest.

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