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Evaluation of Relationship Between Cortistatin Administration and Some Hematological Parameters in Acute Inflammation

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ABSTRACT
First identified in the cortical region, Cortistatin (CST) is an endogenous neuropeptide discovered in recent years and its effectiveness continues to be investigated in various conditions including inflammatory, endocrinological and neurophysiological conditions. The present study was conducted to evaluate the relationship between CST and some hematological parameters in acute inflammation induced by turpentine oil in rabbits. In the study, 28 New Zealand male rabbits were used and the animals were divided into control (C), turpentine (T), cortistatin (CST) and turpentine + cortistatin (CST+T) groups. Some hematological parameters such as red blood cells (RBC), White blood cells (WBC), packet cell volume (PCV, Ht) and hemoglobin were examined in the blood samples taken at the 2nd and 6th hours. In addition, Wintrobe red blood cell indices were calculated for each group and sampling time. When the findings are evaluated taking into account the difference between sampling times, no significant difference was observed between the data of groups C and CST in terms of all other parameters examined. Among the parameters examined, only WBC and Hemoglobin levels were found to have statistically significant differences ($P<0.05$) both between groups and between sampling times in the 6-hour data. According to the data obtained as a result of the study, it was determined that although cortistatin application played a suppressive role in inflammation in the acute period, this effect was less significant than the effect observed in conditions such as endotoxemia and sepsis.

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Key Words
Cortistatin, inflammation, turpentine, hematological parameters

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INTRODUCTION

Inflammation is a protective host response that occurs against tissue damage caused by foreign antigen entry or physical or chemical agents and if left untreated, can lead to tissue structure deterioration in addition to loss of function. The self-limitation and self-resolution of many inflammatory processes suggests that there are some endogenous anti-inflammatory or pro-resolution mediators along the inflammatory pathway[1,2].

Cortistatin (CST) is a novel small molecule bioactive peptide containing an FWKT (Phe-Trp-Lys-Thr) tetramer.

Cortistatin (CST), a cyclic neuropeptide discovered in recent years, is mainly expressed in the cortical region and has been reported to suppress cortical activity[3]. While it was initially discovered that its expression was limited to the rat cerebral cortex and hippocampus (Rubinfeld and Shimon, 2007), today we know through studies that it also shows a wide distribution in many peripheral tissues such as the retina, adrenal gland, thyroid, pancreas, testes, liver, stomach, ileum, jejunum, colon, rectum, kidney, pancreas, lung, parathyroid gland and immune system (Baranowska, Marchenko and Strongin. Although it has been suggested that most of its functions described are within the scope of cytokine function, they are generally not given in this classification because they are found in small amounts. Many researchers have described cortistatin as a potent anti-inflammatory agent that can deactivate the inflammatory response in vivo[5,4] and also act as a neuroprotective agent[6]. It has a very high structural homology with somatostatin and has been reported to bind to five cloned somatostatin receptors. It also shares many pharmacological and functional properties with somatostatin. These include depression of neuronal activity and inhibition of cell proliferation[4].

A study by Cassoni and collegues (2002) reported that CST-14 could inhibit proliferation in human thyroid carcinoma cell line similar to SST-14 in cancer cells, which probably indicates the inhibitory role of CST in cancer development.

Differential expression of five somatostatin receptors in human immune cells and tissues has been demonstrated, suggesting that somatostatin may have a functional role in the immune system. However, despite the absence of somatostatin in various human immune tissues, the presence of CST is striking. In order to clarify this, real-time PCR techniques were used to show that CST mRNA is expressed in immune cells, lymphoid tissue and bone marrow and that CST mRNA expression is up-regulated during the differentiation of monocytes into macrophages and dendritic cells. It has also been reported that this upregulation in CST mRNA and SSTR2 expression can be regulated by immune cell stimulators such as lipopolysaccharide and that CST probably has a more important role in the mature immune system. It has also been suggested that CST can replace octreotide, which binds to somatostatin receptor-2 expressing cells with relatively high affinity in human thymic tissues and therefore CST may be a new endogenous ligand of somatostatin receptor-2 in the human immune system instead of somatostatin[7,8].

It has been suggested that cortistatin may function as a major endogenous immune system regulatory factor in the immune system and may also bind to the receptor of anti-inflammatory mediator hormones such as ghrelin, a growth hormone secretagogue[9].

Although there are numerous publications on hematological and biochemical parameters that change during various inflammatory conditions, since there is insufficient literature information on the changes in these parameters after cortistatin application, the relationship between cortistatin application and hematological parameters is examined in this section. In this study., after chronic aseptic inflammation is created, cortistatin is applied and hematological parameters such as RBC, WBC, Hb and Ht (Packet Cell Volume, PCV) will be measured and the effect of cortistatin, whose effect on hematological parameters has not been known before, will be determined.

Inflammation, defined as a large and complex reaction of the body to tissue damage against infections, involves the collection and activation of leukocytes and plasma proteins at the site of infection to eliminate infectious agents[10].

Since injections of turpentine oil, an oleoresin of Pinus, Pinaceae, are widely used as an inducer of acute phase response in laboratory animals[11]. Therefore in the present study, turpentine oil, which was selected to create acute inflammation, is widely used to induce acute or chronic systemic inflammation with a single application at various doses or multiple applications over a long period of time.

In the light of literature, the present study aimed to evaluate the relationship between cortistatin application and some hematological parameters in inflammation created by subcutaneous turpentine injection.

MATERIALS AND METHODS

The chemicals used in the study are listed below: Cortistatin-29 (rat) Trifluoroacetate, (H-6444, Bachem AG) was supplied by Bachem (since it is structurally homologous to rat, human and rabbit cortistatins) and Turpentin (Oil of turpentine, 24245-Aldrich) was supplied by Sigma-Aldrich.

In the study, 28 male, healthy, white New Zealand rabbits, aged 8-12 months, weighing between 1.5-2 kg, were used. Before starting the study, an ethics committee approval was obtained for the intervention to be performed on the animals. The research animals were provided by Manisa Neky Cage and Poultry Co. Ltd. The animals were weighed and randomly assigned to 4 groups, namely Control Group (C), Cortistatin Group (CST), Turpentine Group (T) and Cortistatin + Turpentine Group (CST+T), with their group average weights close to each other.

The experimental animal unit was used to house the animals. Individual rabbit cages made of stainless steel with fixed feeders, waterers and grills were used in the environment. After the transportation process, the animals were subjected to a general health check and were allowed to adapt to the environment for 10 days before starting work. The ambient temperature was adjusted between 10-20ºC with the air conditioning system, ensuring that the temperature and humidity remained constant.

The animals included in the study were fed ad libitum with commercial rabbit feed (MBD Feed), the composition of which was prepared in accordance with the values specified by the National Research Council Nutrient Requirements of Rabbits (1977), while clean water was available to them at all times. The composition of the feed is presented in Table 1:

Blood samples were taken from rabbits in all groups at the beginning of the study, at the 2nd and 6th hours, into K EDTA blood collection tubes in accordance with the measurement purposes. Samples taken at the intermediate time intervals were taken from the auricular vein and the last blood sample was taken from the heart via intracardiac puncture. The indicated measurements were made from the collected samples without wasting time.

Hemoglobin level was determined spectrophotometrically in blood samples taken in EDTA tubes, erythrocyte count(RBC) and leukocyte count (WBC) were counted on Thoma slide and hematocrit value was measured with Hettich brand microhematocrit centrifuge.

In the statistical evaluation of the data obtained from all groups, the data obtained as a result of the was statistically analyzed in IBM SPSS v25statistical program with General Linear Model Univariate. Accordingly, in determining the significance of the differences between the groups belonging to the same sampling time of all findings, variance analysis was performed and Duncan's Multiple Range test was used and in determining the significance of the differences between the sampling times, Student t-test was used. In the comparisons, a difference of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

The data obtained as a result of the measurements are presented in Table 2 below.

AB: The difference between sampling times carrying different letters in the same row is significant ($P<0.05$)

ab: Differences between groups with different letters in the same column are significant ($P<0.05$)

There was no change in RBC levels between groups and between sampling times (Table 2). As an indicator of the onset of inflammation, WBC count increased in the T group at the 6th hour as expected ($p<0.05$). The fact that CST, whose protective effect was tested, decreased the WBC count in the CST+T group at the 6th hour compared to the value in the T group at the same sampling time was attributed to its suppressive effect on inflammation. However, this decrease still did not reach the value in the K group.

When Table 2 above are examined, at the 2nd hour sampling time, no statistically significant difference was observed for all the parameters examined. It was considered quite normal that such major changes that could be reflected in the statistics in the early stages of inflammation were not observed.

Since no statistically significant difference was determined in terms of erythrocyte counts and hematocrit values both between groups and between sampling times, naturally no statistically significant difference was observed in the MCV value of Wintrobe red blood cell indexes between groups and between sampling times.

In terms of its immunosuppressive role, it has been emphasized that in combination with vasoactive intestinal peptide, another anti-inflammatory endogenous peptide, it significantly prevents lethality induced by high dose lipopolysaccharide and thus the therapeutic dose can be reduced with combined applications. This finding is consistent with its suppressive role on the WBC count that increases with inflammation in our study.

Unfortunately, due to our limited working conditions and regional technical possibilities, other parameters that could be included in this study and strengthen the study could not be measured. An advanced study is planned to be conducted on this promising subject, to examine the molecular dimension of the event and other processes that will provide support.

Tous[12] injected a turpentine-olive oil mixture (1:1) into the hind legs twice a week for 12 weeks to create a chronic aseptic inflammation model in mice with turpentine. They found increased TNF-a and serum amyloid A (SAA) concentrations, which are indicators

Table 1. Composition and analysis of commercial rabbit feed

Content	Percentage rate
Dry matter	%89,4
Crude protein	%18,0
Crude fiber	%20,0
Raw ash	%6,45
Crude oil	%4,5
Calcium	%1,0
Total phosphorus	%0,59
Lysine	%1,0
Methionine	%0,60
Methionine-cystine	%0,90
Sodium	%0,27
Linoleic acid	%1,26
Metabolic energy	2650 calories/kg

Table 2. Results of hematological parameters determined after cortistatin administration (X±SEM, n=7)

Grps	N (28)	RBC (x106/mm3)		WBC (x103/mm3)		PCV (Ht) (%)		Hb (g/dl)	
		2nd hour	6th hour	2nd hour	6th hour	2nd hour	6th hour	2nd hour	6th hour
C	7	6.040±0.43	6.01±0.13	6.09±0.27	6.30±0.53 a	41.23±0.53	40.00±0.06	13,21±0.53	13,71±0.64a
T	7	6.021±0.11	5.92±0.89	6.13±0.27 A	7.2±0.42 Bb	45.54±0.12	41.02±0.89 A	12,25±0.01	7,21±0.53 Bb
CST	7	6.101±0.65	6.204±0.78	6.24±0.42	6.35±1.02 a	40.45±0.52	38.23±0.43 A	12,36±0.05	14.01±0.30A Ba
CST+T	7	6.0263±0.76	6.432±0.87	6.41±0.36	6.82±0.52 ab	42.98±0.78	37.23±0.98	11,30±0.90	12,99±0.77 a

of the systemic inflammatory response, in mice treated with turpentine. Although cytokine levels were not measured in our study, the significant increase in WBC count at the 6th hour in the T group is an indication of the onset of an inflammatory response.

Again, no difference was observed between the groups and between the sampling times in terms of erythrocyte count and hematocrit value. Since there was no hemolytic condition that could be evaluated as erythrocyte destruction, this is also considered normal considering the short duration of the study.

CONCLUSION

Interest in approaches to the use of certain endogenous substances known to be synthesized in the body or naturally found in other living species (plants and animals) rather than synthetic substances as preventive or therapeutic against any local or systemic or acute or chronic inflammation is increasing. Cortistatin, which is the focus of this study, is an endogenous peptide isolated for the first time from the cerebral cortex. Although the focus was on its interaction with the nervous system and the endocrine system, with which it is anatomically closely related, there are now studies on its effects on the immune system.

In conclusion, it can be said that cortistatin injection in rabbits with acute aseptic inflammation exhibited its protective effect only on some hematological data, and this was probably due to the fact that the experimental period was terminated at the 6th hour.

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