NAUTILUS
Vol:128; Issue 2(No:1) April 2014: 08-11 ISSN 0028-1344

The Liver Parameters In The Collagen-Induced Arthritis

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ABSTRACT

The living organisms use defensive mechanisms in their struggle to keep the inner homeostasis and potect themselves from the changes induced by external factors and penetrative agents. The manifestation of these changes depends on the character, the intensity and the duration of the agents' activity, and on the physiological characteristics of the organism (gender, age, health condition etc.). The aim of our study was to analyze, the effects of collagen-induced arthritis (that is, the autoimmune reaction and the inflammation) on some liver parameters. We determined the content of proteins, DNA and RNA. Animals with collagen-induced arthritis showed decreased relative content of proteins in liver, compared to controls. On the contrary, the relative content of DNA and RNA were increased in animals treated with collagen.

Keywords: liver parameter, collagen, arthritis.

INTRODUCTION

A common feature of all rheumatic diseases is inflammatory reaction, without pus formation, of the connective tissue, with basic clinical signs, such as inflammation, redness, swelling, local raised temperature, pain and limited motoric movement (Christen 2005). The activation of immunologic composition in such diseases can be seen through the occurrence of auto-antibodies to one or more autoantigenes, and they occur when the immune system loses control of part of the lymphocytes which are specifically auto-reactive to the cells of their own organism (the functional and tissue antigens) i.e. the receptors (Cohen 1993; Banham et al. 2006; Ishii et al. 2006). The inflammation is caused by the organism's immune system that attacks the tissue wrapped around the joints (Kamradt et al. 2004).

There are two hypothetic theories regarding the development of rheumatoid arthritis. According to the first theory, T-lymphocytes, cells that belong to the immune system, come into interaction with yet unknown antigen. According to the second theory, T-lymphocytes are responsible only for triggering the onset of the illness, whereas the chronic inflammation is a result of the activity of the macrophages and the fibroblasts (Bennett et al. 1942; Moritz 1942; De Meyts and Hanoune 1982; Shoenfeld 1989; Michaelsson 1994; Davidson and Diamond 2001)

The impact of rheumatoid arthritis on some liver parameters, especially the activity of hepatic enzymes AST and ALT were the subject of our research interest with the following goals:

- To induce rheumatoid arthritis, by applying collagen, in experiment animals (albino rat of the Wistar strain).
- To determine and compare the AST and ALT activity in serum in healthy population of rat, and enzyme activities in arthrosed group.

METHOD

During the experimental work, albino lab rats of the Wistar strain were used. For the experiment, we chose healthy male animals at the age of around seven weeks. They were placed in cages (Hulskamp Alkamar Holand) under constant control, at the lab animal farm of the Department of Physiology and Bio-chemistry at the Faculty of Natural Sciences and Mathematic in Skopje. They were fed with food produced by Animal Food Factory-Radobor from Bitola (standard food for lab animals). Food and water were given ad libitum.

Around sixty animals were used. All the rats were sacrificed on the 21-st day of the post-immunisation with collagen type-II.

MEASURMENTS

Serum analyses were performed on computerized, automated COBAS Integra bio-chemical analyzer.

Methods for determination of aspartate aminotransferase

The method for determination of aspartate aminotransferase, which is applied by COBAS Integra automatic biochemical analyzer, is an adaptation of the enzymic method, where, AST catalyzes the transamination between aspartic and 2-oxoglutarateacids. At the same time, oxalic etic acid is created from aspartate, which with $NADH_2$ and enzyme malatendehydrogenase(MDH) transfers to malic acid and equivalent quantity of $NADH_2$is oxidized into NAD.

Methods for determination of alanine amino-transferase

The method for determination of alanine amino-transferase is based on ALT catalyzing transamination between L-alanine and 2-oxoglutarate.At the same time, pyruvate is created from alanine, which, with $NADH_2$and the activity of lactate dehydrogenase enzyme (LDH) is converted into lactate (indicator reaction), and equivalent quantity of $NADH_2$is oxidized into NAD.

RESULTS

Average body mass in the control group of experimental animals (n=31) was 127.8 g. Average value of absolute mass of the liver in the control group (n=31) was 6024.5 mg, whereas the value of relative liver mass in control animals was 4.71 mg%. In arthrosed animals (n=30) the average value of body mass was 85.6 g, the average value of absolute mass of the liver in this group was 2416.5 mg, and the value of relative liver mass was 2.82 mg%.

AST activity

Average AST value in control group of animals was 96.8 U/l, and average AST value in arthrosed group of animals was 135.6 U/l. Induced rheumatoid arthritis leads to significant increase of AST activity in the arthrosed, compared to the control group of animals (A:B, $p < .001$). This suggests a possibility that as a consequence of induced arthritis, there is disintegration of one part of the hepatocytes, and due to their disintegration and most probable lesion there is also loss of a part of them, as well as an increased activity of the enzyme.

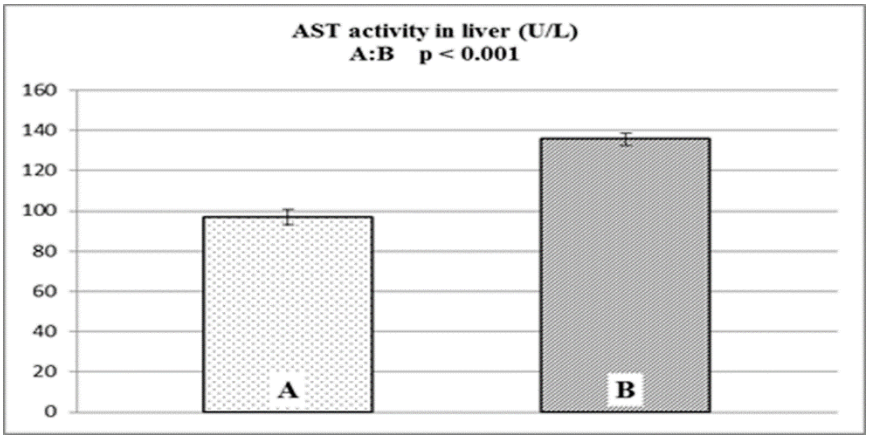

Figure 1. Level of AST activity in liver (U/l) in control and arthrosed groups of animals (means±SE).

- control, group A, (animals from this group during the whole experiment period were on ad libitum food and water diet,with no treatment and in standard breeding conditions; (n=31)- at around fifty days of age at the beginning of the experiment).

- animals with collagen type-II induced arthritis, group B, (the animals from this group were also on ad libitum food and water diet, but at the beginning of the experiment we induced arthritis in them; (n=30) at around fifty days of age at the beginning of the experiment).

ALT activity

The effect of induced arthritis on the ALT activity in liver in control and arthrosed groups of animals is graphically shown in Figure 2. It can be seen from the charts that the level of "normal" ALT and the level of "arthrosed" ALT differ. It can also be seen that, for the most part, there is a significant deviation, although it is not that high and characteristic as for AST. Average ALT value in control group of animals is 69.15 U/l, and average ALT value in arthrosed group of animals is 78.11 U/l (Fig. 2, A:B, p< .005).

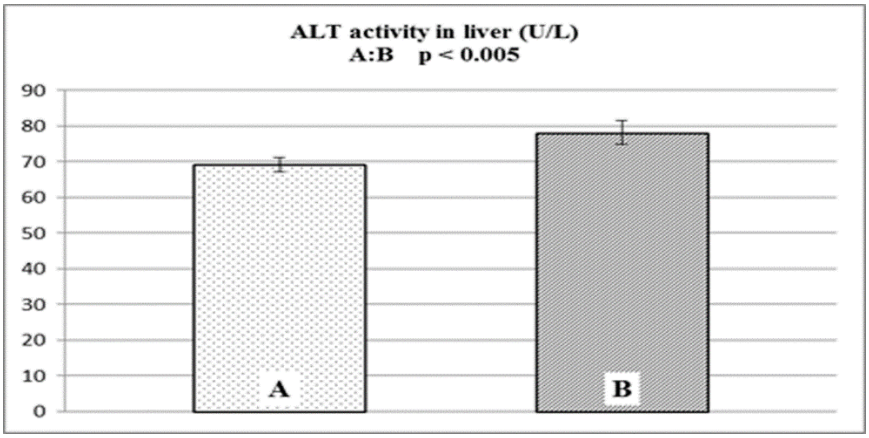

Figure 2. Level of ALT activity in liver (U/l) in control and arthrosed groups of animals (means±SE). (The legend is the same as in Figure 1).

DISCUSSION

Collagen-induced arthritis caused reaction in almost 100% of the immunized rats. The results from our examinations on average absolute values, and especially on average relative values, suggest that the effect of induced rheumatoid arthritis is manifested through significant decrease of absolute and relative liver mass in arthrosed animals in relation to control animals.

The effect of induced rheumatoid arthritis, according to the results we obtained, upon AST activity in control and arthrosed groups of animals show that the level of enzyme activity in arthrosed group of rats, has been increased significantly (A:B,p<.001). The enzyme activity in the treated group of animals is 135.6 U/l, which compared to the 96.8 U/l, measured in control group, is by 40 % higher value of enzyme activity. It may be noticed from the results on the ALT that the level of "normal" ALT and the level of "arthrosed" ALT differ, and in the most part there is significant deviation. This may be asserted from obtained values from our examinations in which average ALT value in control group of animals amounts 69.15U/l, whereas average ALT value in arthrosed group of animal amounts 78.11 U/l, which is by 13 % higher value. It may be concluded that there has also been an impact of induced rheumatoid arthritis on the activity of ALT (A:B, p<.005). In processes in which there is lesion of cells and tissues rich with AST, the enzyme transfers into the circulation and this results in his increased activity in blood serum (Wroblewski 1958; Christen 2005; Banham et all. 2006). Since ALT is typical cytoplasmic enzyme, during small tissue damage or change in cell membrane's permeability, it exits into the intercellular space, i.e. into the circulation, which increases its serum activity (Shoenfeld 1989; Young et all. 2000).

CONCLUSION

Our examinations on the impact of collagen-induced arthritis in albino lab rats, lead us to the following conclusions:

- Induced rheumatoid arthritis causes decrease of liver mass in treated animals in comparison with control rats.
- It can be clearly seen that AST activity in collagen treated, i.e. arthrosed group of animals, in comparison to the control group, is significantly higher.

- ALT activity in arthrosed group is significantly higher compared to the ALT activity in the control group of animals.

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