

Research article

Electronic Microscopy aspects of experimentally induced chronic arthritis

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Abstract: Introduction: Chronic diseases are responsible for about 38 million deaths annually and represent about 70% of all deaths worldwide. Among these, rheumatoid arthritis is the most common inflammatory rheumatic disease, representing about 10% of all such disorders. Its incidence is approximately 1% of the general population.

Material and method: our study aims to create an experimental model of chronic arthritis on Wistar rats, female sex, to follow the dynamics of joint inflammatory phenomena, the effects of Leflunomide in their evolution and the side effects at cardiac, hepatic and renal level.

Discussions: The group of young experimental animals developed more extensive inflammatory phenomena compared to the group of adult animals. Leflunomide did not significantly improve the evolution and resolution of inflammatory phenomena in either group.

Conclusions: We showed for the first time in the world the effects of Leflunomide on the heart, with the occurrence of myocardial necrosis (myocardial infarction) as its main adverse effect.

Keywords: Arthritis, Leflunomide

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Introduction

Rheumatoid arthritis is a chronic inflammatory disease of multifactorial etiology, predominately affecting the female sex 2:1- 4:1, associated with an arthropathy of unfavorable and destructive evolution, but also multiple systemic manifestations.

The main contributing factors involved in the pathogenesis of PR: the female sex, infectious agents (Epstein-Barr virus, parvoviruses, lentiviruses and measles virus)

Another important element is remote infection, for example in the oral cavity or intestines (porphyromonas gingivalis); (1) antibodies directed against own IgG : ACPA, Anti RA-33 Ac, Anti collagen Ac, Anti stress protein Ac (2,3).

Genetic factors: 11 genes associated with PR have been identified : PTPN 22, STAT4, CTLA4 OR PADI 4 (4,5,6)

Smoking – The association of smoking with the genetic terrain and the presence of ACPA significantly increase the risk of RA. (7,8,9)

Material and methods

Starting from these data, we set out to create an experimental model of chronic arthritis, in which we looked at the influence of age, sex and medication (Leflunomide) on the

evolution of chronic inflammation phenomena in rats, breed Wistav.

The experimental study was carried out over a period of 8 weeks, on young and adult female Wistav rats.

The experimental animals were divided into six groups. The animals received the standard feed and were adequately hydrated.

Batch MT- the control batch, consisting of 10 young animals aged 1.5 months (6 weeks) and weighing 100-150g

Batch MA- control batch, adult consisting of 10 young animals aged 4.5 months (18 weeks) and weighing 300-350g

The AT group- the group of young animals induced with arthritis using 0.02ml carrageenan solution, 1% concentration injected into the tibiofemoral joint 3 times/week for 8 weeks.

Group AA- the group of adult animals that were induced with arthritis with carrageenan solution 0.02 ml concentration 1 % injected into the tibiofemoral joint 3 times/ week for 8 weeks.

Group TT – the group of young animals with arthritis treated, which were induced with 0.02 ml carrageenan solution 1% concentration injected into the tibiofemoral joint, but which were protected by treatment with Leflunomide (Arava) in a dose of 20 mg/ kg body administered by gavage once every 2 days for 8 weeks.

The TA group - the group with adult animals with arthritis treated, which were induced with 0.02 ml carrageenan solution 1% concentration injected into the tibiofemoral joint, but which were protected by treatment with Leflunomide (Arava) in a dose of 20mg/kg body administered by gavage once every 2 days for 8 weeks.

After 8 weeks of monitoring the dynamics of multiple parameters (hematological, biochemical, immunological, osteodensitometric, radiological), at the end of this experiment, we collected a bone fragment from the tibiofemoral joint, respectively a fragment of organs (liver, kidney, heart) for the purpose of evaluating the adverse reactions of the administered substances, respectively the evolution of the inflammatory response in the two animals groups and the influence of Leflunomide in the decline of these phenomena (10,11).

Discussions

The electron microscopy exam was led by Ms. Academic Prof. Dr. Docent Gioconda Dobrescu.

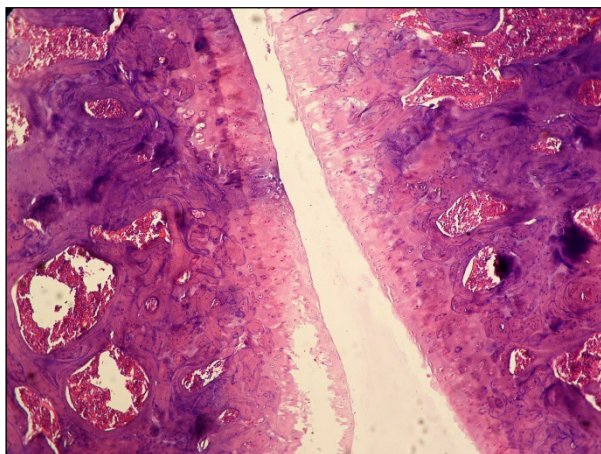


Fig.1: Young control rat: Section through the joint. Col. HE. ob. 20. Free joint space. The two areas of cartilage: non-mineralized and normal-sized mineralized. The subchondral bone plate separates the bone marrow area from the mineralized cartilage.

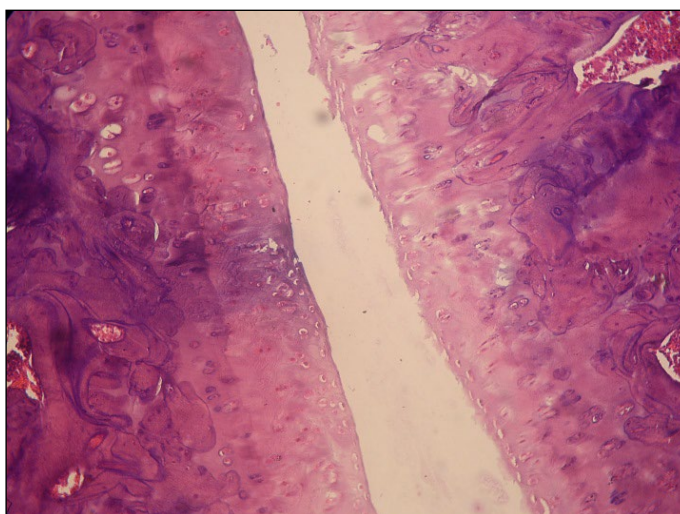


Fig.2: Adult control rat: Section through the joint. Col. HE. ob. 40. detail from the previous figure. The mineralized cartilage in the vicinity of the subchondral bone plate is more intensely colored)

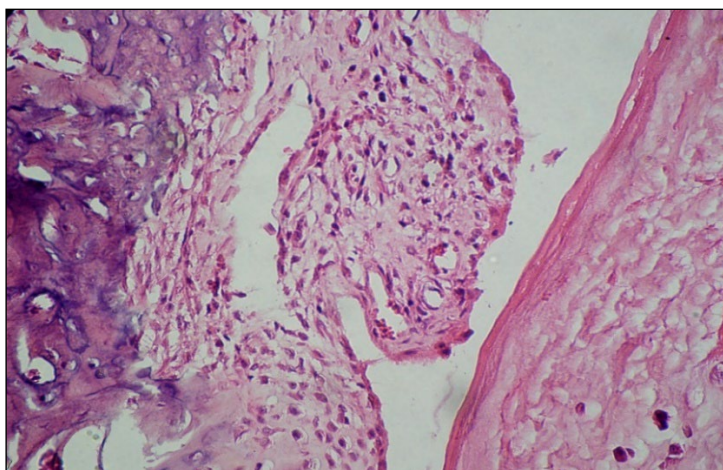


Fig.3: Young rat with arthritis. Section through the joint. Col. HE. ob. 40

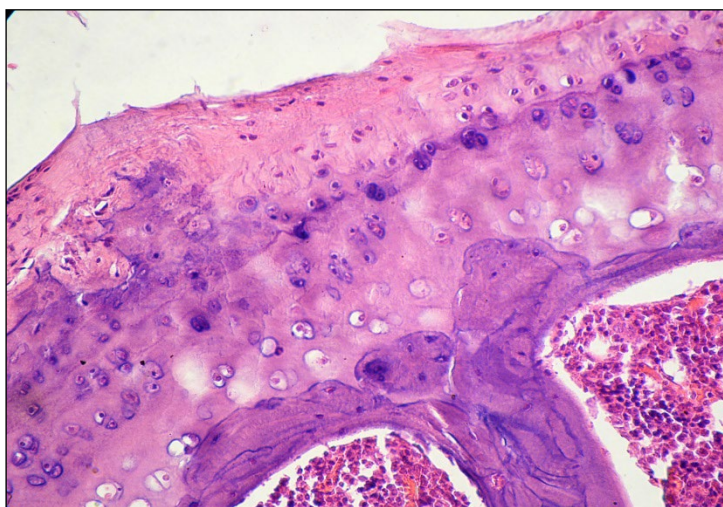


Fig.4.: Adult rat with arthritis; Section through the joint. Col. HE. ob. 40. Detail. Significant disorganization of cartilaginous areas. Reduction of the subchondral bone plate, with areas of resorption

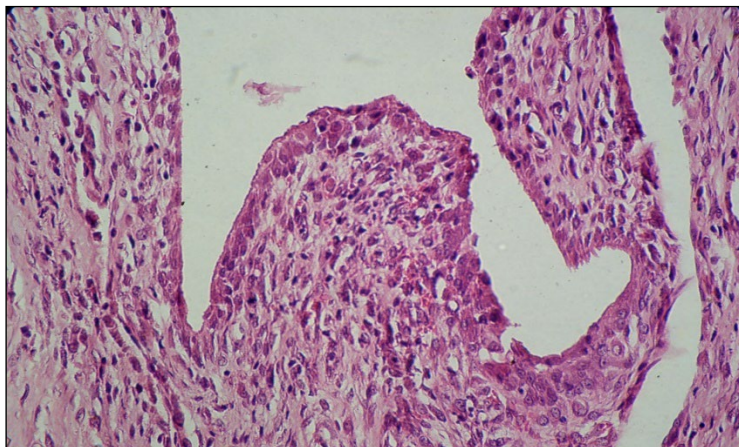


Fig.5: Young rat with arthritis. Section through the joint. Col. HE. ob. 40. thickening and proliferation of the synovium, with an underlying inflammatory reaction.

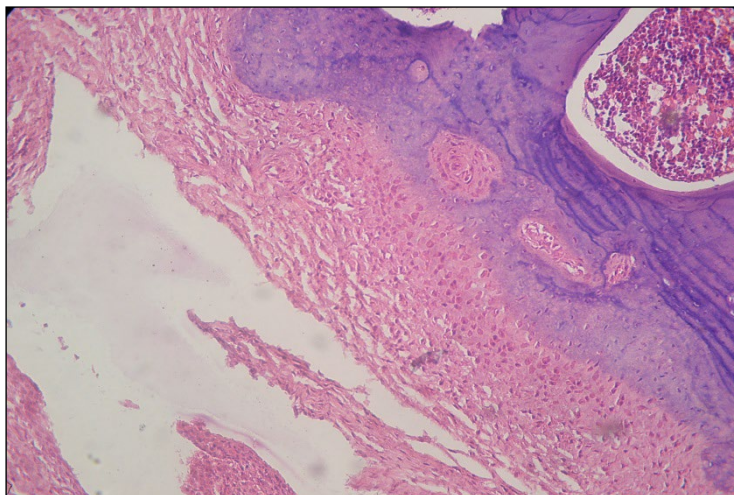


Fig.6.: Young rat with arthritis. Section through the joint. Col. HE. ob. 40. thickening and proliferation of the synovium, with an underlying inflammatory reaction.

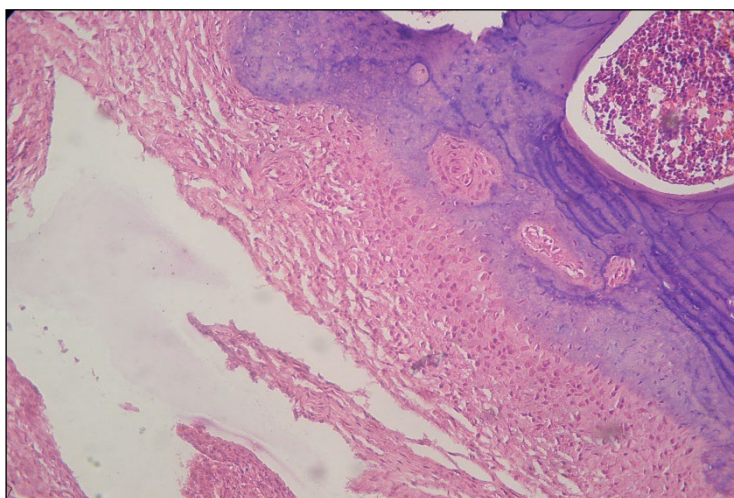


Fig.7: Adult rat with arthritis. Section through the joint. Col. HE. ob. 20. Abundant inflammatory pannus that covers the joint surface and replaces the cartilage area (↑), partially occupying the joint cavity (↑↑).

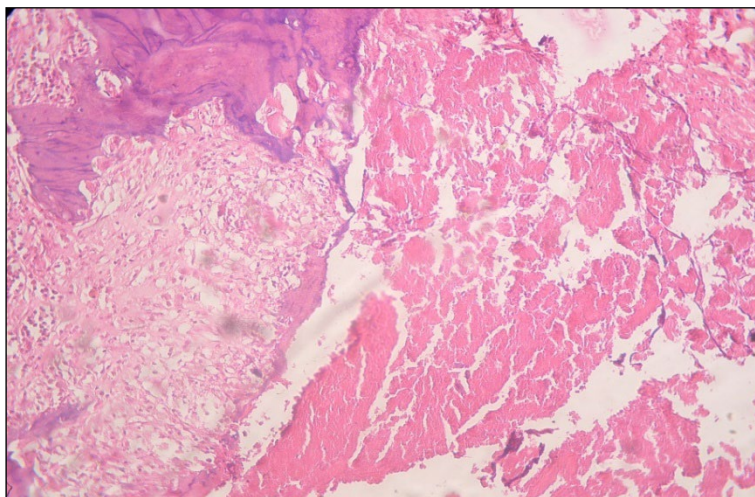


Fig.8: Adult rat with arthritis, treated. Section through the joint. Col. HE. ob. 20. Detail of fibrinoid necrosis at the level of the pannus.

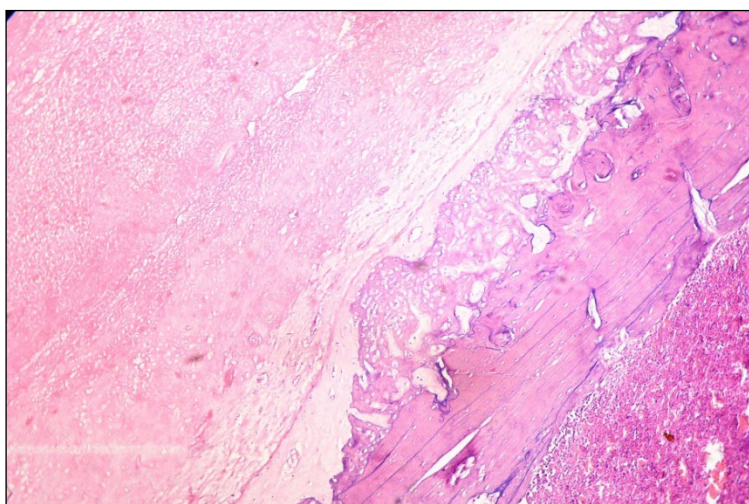


Fig.9: Young rat with arthritis, treated. Section through the joint. Col. HE. ob. 10. The pannus is present, occupies the joint space and covers the cartilage area, which is disorganized.

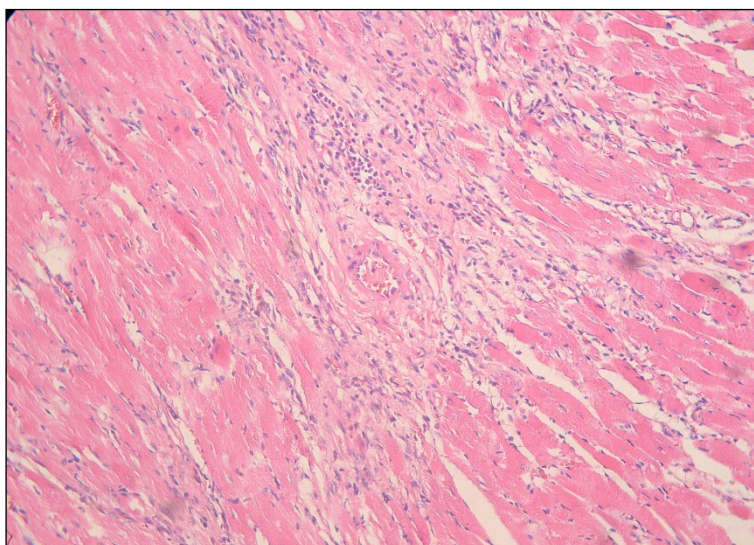


Fig.10: Adult rat with arthritis, treated. Heart. Col. HE. ob. 20. Interstitial and perivascular inflammatory infiltrate. Early fibrosis.

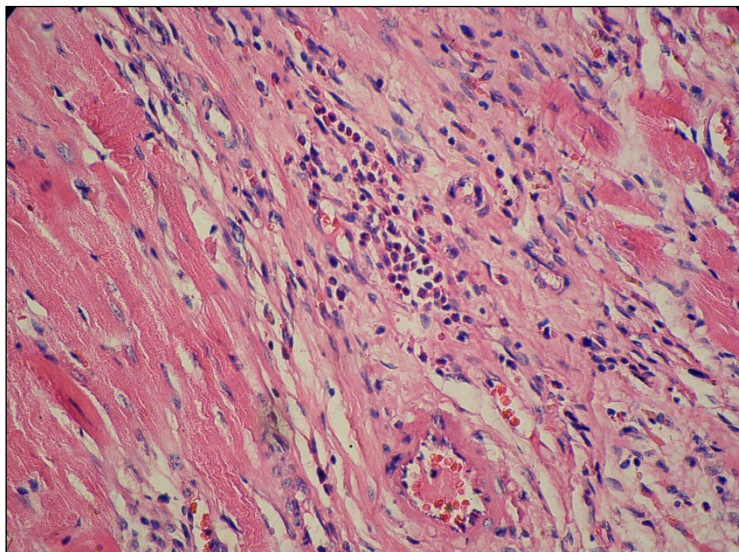


Fig.11: Adult rat with arthritis, treated. Heart. Col. HE. ob. 40. Detail from the previous figure.

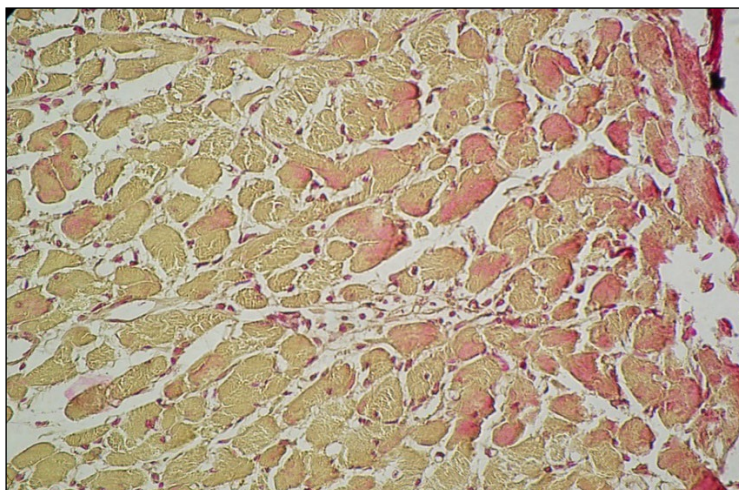


Fig.12: Cord, adult rat with arthritis, treated. Lie coloring. Necrotic myocardial fibers, Lie+.

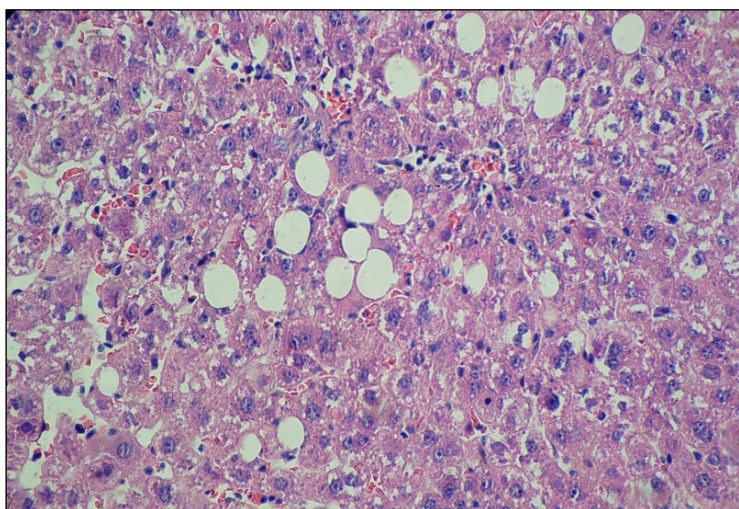


Fig.13: Liver, adult rat with arthritis. Col. HE. ob. 40. Patchy micro and macro vesicular lipid dystrophy.

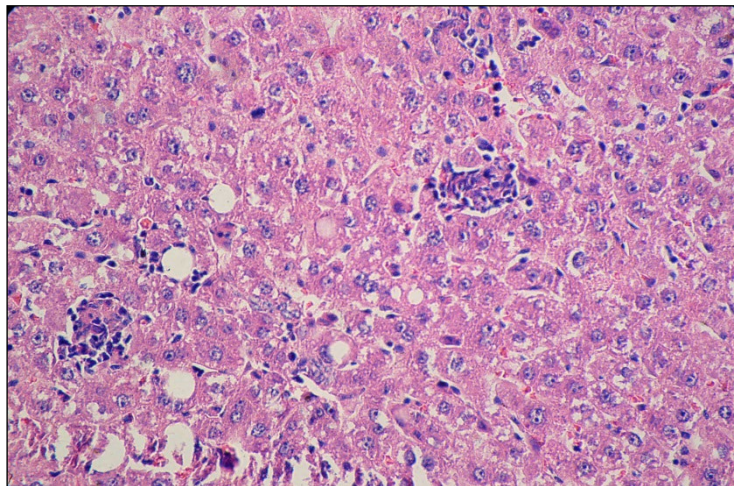


Fig. 14. : Liver, adult rat with arthritis. Col. HE. ob. 40. Patchy lipid dystrophy, moderate inflammatory infiltrate in the port space.

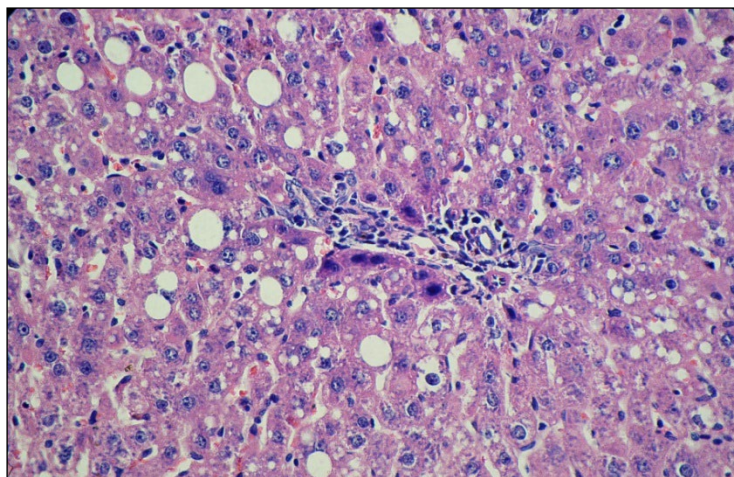


Fig.15: Adult rat with arthritis, treated. Liver. Col. HE. ob. 40. Lipid dystrophy, cells in apoptosis, inflammatory infiltrate in portal spaces.

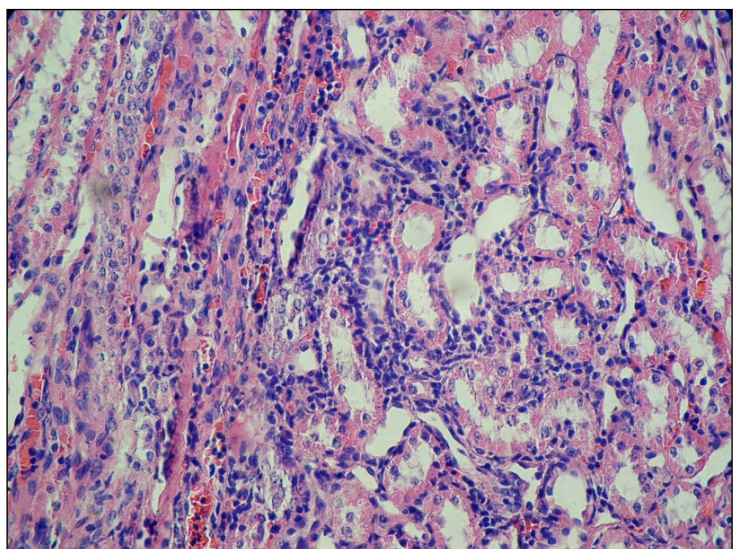


Fig.16: Kidney, adult rat with arthritis. Col. HE. ob. 40. Interstitial inflammatory infiltrate.

In the adult animals that developed arthritis, the morphological lesions were characteristic of chronic arthritis and showcased disorganization of the cartilaginous area, its replacement and covering with inflammatory pannus.

Compared to the young animals, the intra-articular pannus was abundant, showing either internal areas of fibrinoid necrosis or important inflammatory infiltrates, and cartilaginous disorganization and bone resorption were much more evident than in adult animals.

Proof of the toxicity of carrageenan, respectively Leflunomide was evident in the organic lesions of the liver, kidneys and, especially, those of the heart.

Conclusions

- The group of young animals developed inflammatory phenomena with much more severe joint destruction compared to the group of adult animals
- Leflunomide did not clearly act in the process of reducing joint pannus in young animals compared to adults.
- Lipid dystrophy, apoptotic cells and inflammatory infiltrates in the liver, heart and kidneys demonstrated the toxic role of the two substances.
- We demonstrated for the first time in the world the role of Leflunomide in the occurrence of necrotic lesions (myocardial infarction) in experimental animals.

Author Contributions: M.I.M acquired, analyzed and interpreted all the data and information. M.C contributed to the conception and design of the presentation. All the authors contributed equally to the analysis and interpretation of the references. All the authors have read and agreed to the published version of the research article.

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