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Comparing the antifibrotic effect on the liver of Telmisartan and Pentoxifylline, in a Wistar rat experimental model

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Abstract

Chronic liver diseases are characterized by higher or lower changes of the liver lobe architecture (parenchymatous and vacuolar), the accumulation of inflammatory and collagen infiltrates, mainly in the Kiernan spaces and a progressive evolution to liver cirrhosis. Despite the progresses made in knowing the mechanisms of liver fibrosis and the development of some antiviral drugs with a high potential, that can induce fibrosis regression, there still continues to exist the need for a specific antifibrotic treatment. In our study, we used four groups of Wistar rats: a reference group and three groups that received 40% carbon tetrachloride (CCl_4), intraperitoneally, twice a week, for four weeks; after one week since starting the administration of CCl_4, one of the three groups received, through oral gavage, Telmisartan (TS) 8 mg/kg, and another received Pentoxifylline (PTX) 20 mg/kg, dissolved in saline solution, for four weeks. The antifibrotic action of the two drugs was analyzed by evaluating the histopathological and immunohistochemical changes of hepatocytes, hepatic stellate cells (Ito cells) and macrophages (Kupffer cells). The study highlighted that in the group treated with TS, the process of fibrillogenesis was significantly reduced, in comparison to the group treated with PTX and with the reference group.

Keywords: liver fibrosis, Telmisartan, Pentoxifylline, myofibroblasts, hepatic stellate cells.

Introduction

Liver fibrosis, caused by the lesions induced by viruses, metabolic conditions or alcohol intake, is one of the main causes leading to cirrhosis with severe liver failure and death. Chronic liver diseases, regardless of their cause, are associated to the accumulation of matrix proteins, mainly in the portal spaces, change of the liver and vascular structures architecture (portal veins, liver veins), the result being a non-functional nodular architecture, namely liver cirrhosis [1]. Epidemiological data studied by us showed that in 2013, in the European Union, approximately 29 million people suffered from a chronic liver disease [2].

Until now, there is no pharmacological treatment available for liver fibrosis, and for some patients with liver disease in terminal stage, the only available treatment option remains liver transplant [3]. In order to develop certain antifibrotic treatments, it is important to clarify the mechanisms that are at the basis of the development of liver fibrosis in humans.

There was shown that the macrophages (Kupffer cells) play an extremely important part, both in fibrogenesis and in fibrolysis [4–6]. Kupffer cells initiate the fibrotic response, by recruiting the immune cells, which produce a large number of cytokines, with a proinflammatory or direct action upon the hepatic stellate cells (HSCs) and myofibroblasts, like tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β), respectively [7]. Due to this reason, in our study, we tested the antifibrotic action of Pentoxifylline (PTX), known as the anti-TNF-α agent.

In a previously published paper [8], we showed that we chose to test Telmisartan (TS), an inhibitor of the angiotensin II receptors and an activator of peroxisome proliferator-activated receptor gamma (PPARγ), as liver fibrosis was correlated with the up-regulation of the renin–angiotensin system (RAS), and the depletion of PPARγ activating receptors was associated with the activation of HSCs.

The purpose of our study was to evaluate the process of liver fibrillogenesis in an experimental model, by using 40% carbon tetrachloride as a fibrogenic agent, and to comparatively test the antifibrotic effects of Telmisartan and Pentoxifylline.

Materials and Methods

In our study, we used 65 Wistar rats, from the Animal Facility of the University of Medicine and Pharmacy of Craiova, Romania, who were held in rooms with constant temperature and moisture, in a 12-hour light/dark cycle, receiving plenty of food and water (*ad libitum*). The study

protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova and was performed according to Directive No. 86/609/EEC (November 24, 1986), Ministry Orders No. 143 (April 2, 2002) and No. 400 (May 20, 2002), and the Order of National Veterinary Health and Food Safety (ANSVSA) No. 84 (August 30, 2005) for research animal protection.

The animals were divided into several groups:

Reference group consisted of five Wistar rats, males, aged between 8–10 weeks old, with a body weight of 220 g, held in the above-mentioned conditions and received olive oil by intraperitoneal injection, twice a week, for four weeks, the first dose of 5 mL/kg and the other doses of 3 mL/kg. After four weeks, the rats underwent general anesthesia, by the intraperitoneal injection of Ketamidor 100 mg/mL 20 IU (0.2 mL) and Xylazin Bio 2% 0.3 mL, and were subjected to the liver harvesting procedure, for the histopathological (HP) and immunohistochemical (IHC) study.

Control group was treated with carbon tetrachloride (CCl_4). This group included 20 Wistar rats, males and females, aged between 8–10 weeks old, with a body weight between 200–250 g, who received CCl_4 (40% in olive oil) by intraperitoneal injection, twice a week, for four consecutive weeks. The first dose was of 5 mL/kg and the other doses of 3 mL/kg. Every seven days, we applied the anesthesia procedure and the one of liver harvesting, described below.

Group treated with CCl_4 + Telmisartan (TS) consisted of 20 Wistar rats, females, aged between 15–18 weeks old and a body weight of 300–350 g, to whom there was administered CCl_4 (40% in olive oil) by intraperitoneal injection, twice a week, for four weeks, the first dose of 5 mL/kg and the other doses of 3 mL/kg. Starting with the seventh day of the experiment, we administered Telmisartan (TS), every day, for 21 days, in a dose of 8 mg/kg, through oral gavage. Every day, we prepared the TS solution, with a concentration of 8 mg/mL, by solving TS into 0.9% saline solution. Every seven days since the administration of TS, we applied the anesthesia and liver harvesting procedures, described below.

Group treated with CCl_4 + Pentoxifylline (PTX) consisted of 20 Wistar rats, females, aged between 15–18 weeks, with a body weight of 300–350 g, who received CCl_4 (40% in olive oil) by intraperitoneal injection, twice a week, for four weeks, the first dose of 5 mL/kg and the other doses of 3 mL/kg. From the seventh day of the experiment, we administered through oral gavage Pentoxifylline (PTX), daily, for 21 days, in a dose of 20 mg/kg. The solution was prepared every day, with a concentration of 20 mg/mL, by solving PTX into 0.9% saline solution. Every seven days since the PTX administration, we performed the procedures of anesthesia and liver harvesting.

We decided the administration of TS and PTX seven days after the administration of CCl_4, because, according to our previous observations, this was the moment of first hepatotoxicity elements: granulovacuolar degenerescence and incipient fibrosis in the Kiernan space. In this context, we wanted to establish whether the two drugs slow down or stop the process of fibrillogenesis induced by CCl_4 and to compare their efficiency.

Procedure of liver harvesting. Histological and IHC analysis

After the anesthesia, the animals were fixed on the surgery table, above a plate with a constant temperature; the hair on the abdomen was removed, and the area was disinfected with iodine alcohol. After performing a medial incision, we harvest the liver. After the harvesting, the biological material was immediately passed into glass containers with an adequate volume and size, in a 10% neutral formalin solution, and then they were processed according to the classical histological technique for paraffin inclusion, by performing serial sections with a thickness of 3–5 μm. The sections were stained with Hematoxylin–Eosin (HE) and Masson's trichrome, Goldner–Szekely (GS) technique, then they were examined under the Nikon 55i microscope, attached to a camera and a computer. The representative sections were subjected to the IHC study, analyzing the Ito cells reaction (HSCs) that acquire myofibroblast properties, by using the anti-α-smooth muscle actin (α-SMA) antibody, the macrophage cell reaction (Kupffer cells), by using the anti-CD68 antibody, and the intensity of the inflammatory reaction (T-lymphocytes, B-lymphocytes, mastocytes, plasmocytes).

Results

Histological and IHC observations in the reference group

After the histological analyses performed on the liver tissue samples harvested from the reference group rats, we observed that the liver lobe architecture was normal (Figure 1a). By using the Masson's trichrome staining, GS technique, we observed the absolute normal presence of some collagen fibers in the Kiernan space, without any inflammatory reaction (Figure 1b). The hepatocytes had normal form and size, without any HP changes of the Kiernan space or the central lobular vein. The IHC study with anti-α-SMA antibody (Figure 1c) highlighted a positive reaction of the pericytes in the wall of Kiernan space vessels, the hepatocytes having a negative reaction. By using the anti-CD68 antibody, we highlighted a normal distribution of Kupffer cells in the liver parenchyma (Figure 1d), which shows a normal phagocytic and immunological activity.

Histological and IHC observations after seven days of CCl_4 administration

In the group receiving only CCl_4 for seven days, there was highlighted a discrete hepatocytolysis and a granulo-vacuolar degenerescence (Figure 2a). In the Kiernan space, there was observed a moderate increase of collagen fibers (Figure 2b) and a lipidic infiltrate in the liver parenchyma. The IHC study, using anti-α-SMA antibody, identified the presence of myofibroblasts in the liver parenchyma, but mainly in the wall of sinusoidal capillaries (Figure 2c). By using the anti-CD68 antibody, there was observed a high activity of Kupffer cells, mainly in the hepatocytolysis areas (Figure 2d).

Histological and IHC observations after 14 days of CCl_4 administration

After two weeks of CCl_4 administration, the IHC study highlighted an intensity increase hepatocyte lesions and

an increase of lesion sizes (Figure 3a). Extended areas of hepatocytolysis and granulovacuolar degenerescence were observed disseminated, around the center lobular vein, where there was also observed an increase of the collagen fibers; moreover, some animals presented areas of necrosis and porto-portal fibrosis (Figure 3b). The IHC study performed with anti-α-SMA antibody revealed an increase of the myofibroblasts in the Kiernan space and the liver parenchyma (Figure 3c). By using the anti-CD68 antibody, there was highlighted an increase in the number of macrophages and in their sizes, especially in the necrosis areas (Figure 3d).

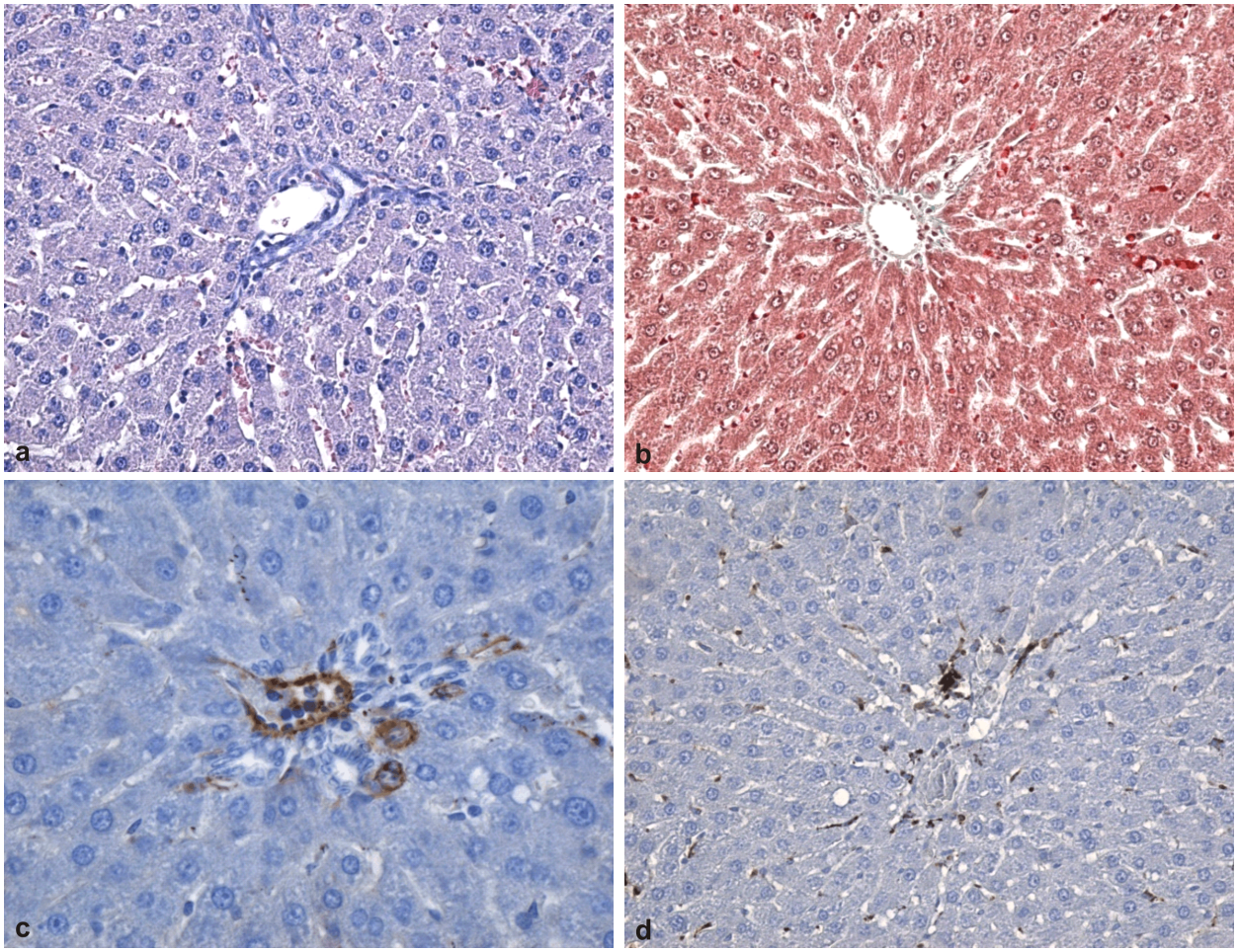

Figure 1 – ***Microscopic image of the liver parenchyma in the reference group: (a) Normal aspect of the liver architecture (HE staining, ×200); (b) In the Kiernan space, there may be observed discrete perivascular and pericanalicular collagen fibers (GS trichrome staining, ×200); (c) In the Kiernan space, there may be observed pericytes positive to α-SMA (Anti-α-SMA antibody immunomarking, ×400); (d) Kupffer cells with a normal distribution in the liver parenchyma (Anti-CD68 antibody immunomarking, ×200).***

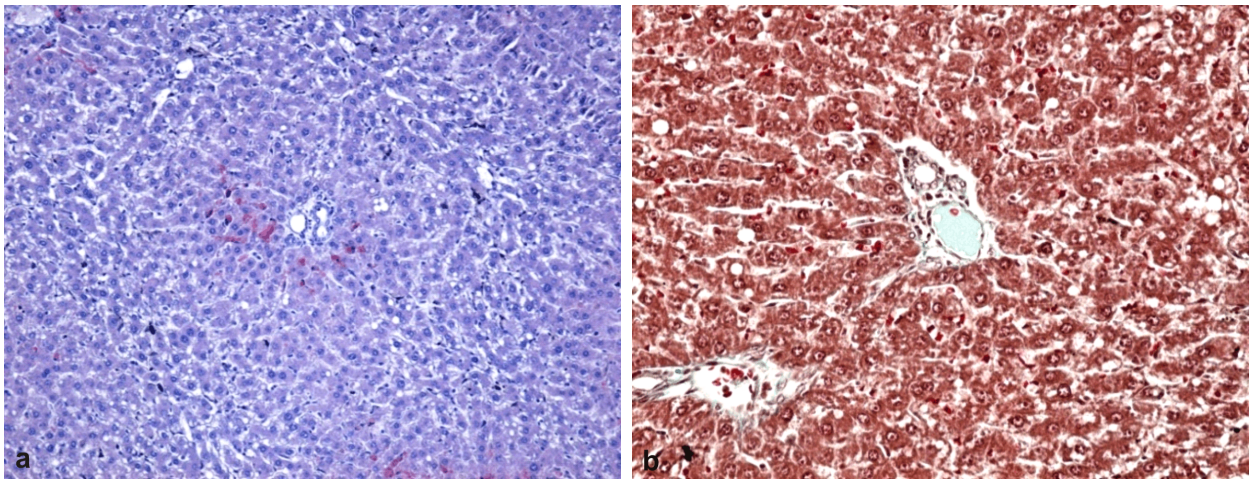

Figure 2 – ***Microscopic image of the liver parenchyma after seven days in the group treated with CCl_4: (a) Lesions of granulovacuolar degenerescence, mainly around the central lobular vein (HE staining, ×100); (b) Granulovacuolar degenerescence, poor lipidic infiltration and moderate fibrosis in the Kiernan space (GS trichrome staining, ×200).***

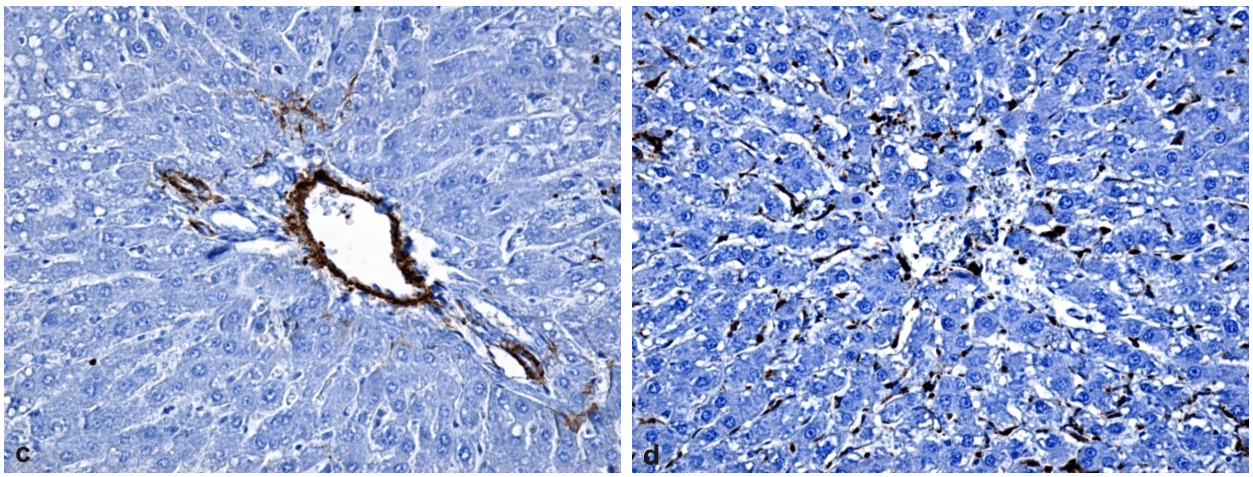

Figure 2 (continued) – ***Microscopic image of the liver parenchyma after seven days in the group treated with CCl_4: (c) Poor anti-α-SMA reaction in the Kiernan space (Anti-α-SMA antibody immunomarking, ×200); (d) Heterogeneous reaction with intense reaction of macrophages, especially in the areas of hepatocytolysis (Anti-CD68 antibody immunomarking, ×200).***

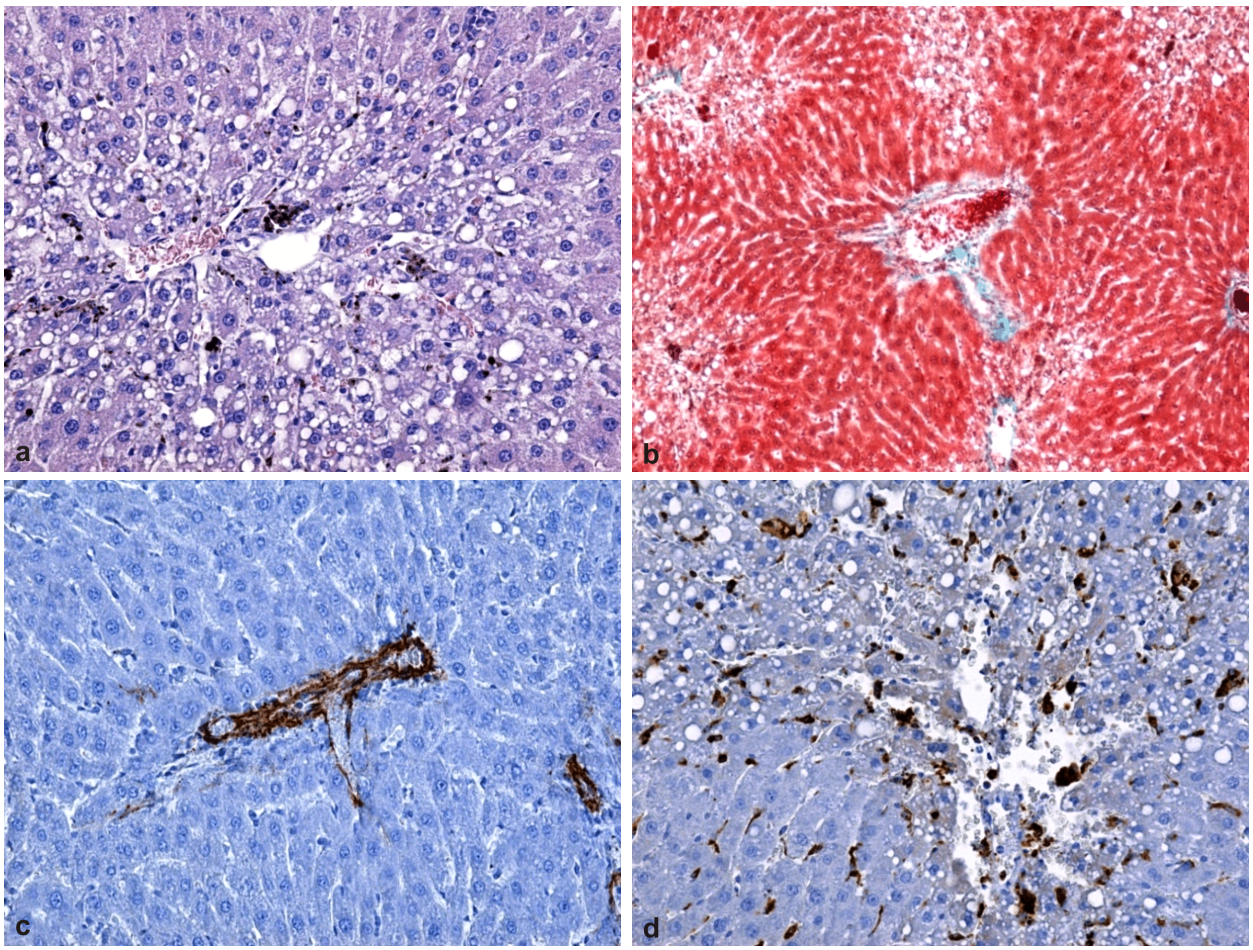

Figure 3 – ***Microscopic image of the liver parenchyma after 14 days in the group treated with CCl_4: (a) Areas of granulo-vacuolar degenerescence and lipidic infiltrate in the whole liver parenchyma (HE staining, ×200); (b) Areas of necrosis and granulovacuolar degenerescence, porto-portal fibrosis (GS trichrome staining, ×100); (c) Positive reaction of α-SMA in the porto-biliary space (Anti-α-SMA antibody immunomarking, ×200); (d) Numerous activated Kupffer cells, arranged mainly in the necrosis areas (Anti-CD68 antibody immunomarking, ×200).***

Histological and IHC observations after 21 days of CCl_4 administration

After 21 days of CCl_4 administration, the HP study showed that the hepatocyte lesions intensified, with an increase of the fibrillogenesis process, especially in the periportal and porto-biliary spaces (Figure 4, a and b). The IHC study with anti-α-SMA antibody highlighted an intense reaction at perivenous level, with activated Ito cells and incipient porto-portal fibrosis (Figure 4c). By using the anti-CD68 antibody, we observed an intense reaction, numerous activated Kupffer cells and heterogeneously arranged macrophages, mainly in the necrosis areas (Figure 4d).

Histological and IHC observations after 28 days of CCl_4 administration

After 28 days of CCl_4 administration, the liver lesions were more intense than in the previous group, which suggests that the severity of the liver lesions is correlated with the duration of the hepatotoxic substance action. There was observed a hepatocytolysis process, on extended areas, with the presence of numerous collagen fibers, which form porto-portal fibrotic bridges (Figure 5, a and b).

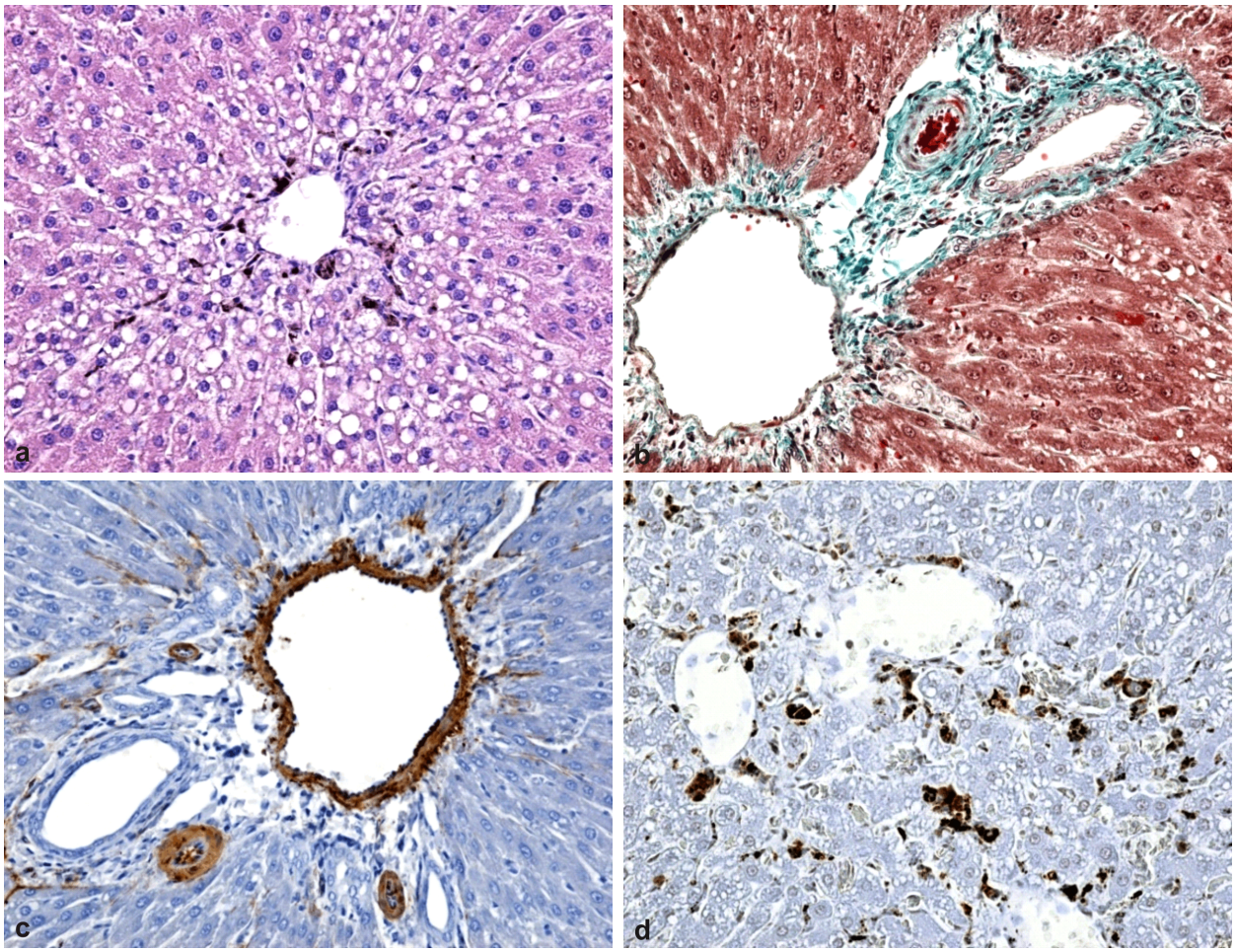

Figure 4 – ***Histopathological changes of the liver parenchyma after 21 days in the group treated with CCl_4: (a) Areas of necrosis and granulovacuolar degenerescence in the whole liver parenchyma (HE staining, ×200); (b) Granulovacuolar degenerescence and porto-portal fibrosis (GS trichrome staining, ×200); (c) Areas of necrosis, granulovacuolar degenerescence and numerous activated Ito cells – intense reaction at perivenous level (Anti-α-SMA antibody immunomarking, ×200); (d) Areas of necrosis, numerous large-sized macrophages and activated Kupffer cells (Anti-CD68 antibody immunomarking, ×200).***

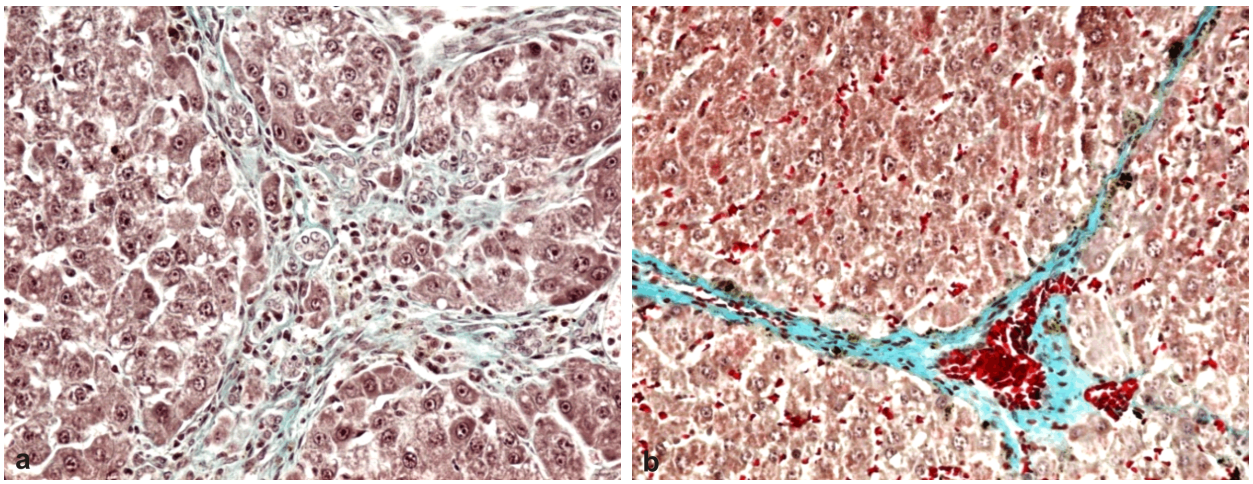

Figure 5 – ***Histopathological changes of the liver parenchyma after 28 days in the group treated with CCl_4: (a) Intense lesions of hepatocytolysis and granulovacuolar degenerescence associated with massive fibrosis in the porto-biliary and intralobular spaces (GS trichrome staining, ×200); (b) Porto-portal fibrotic bridges (GS trichrome staining, ×200).***

The IHC study with anti-α-SMA antibody showed a significant increase of the myofibroblasts, with a massive fibrosis with porto-portal and portal-central bridges, with a tendency of forming cirrhotic nodules (Figure 5c). By using the anti-CD68 antibody, there were highlighted numerous macrophages, of large sizes, especially in the areas with intense hepatocytosis and granulovacuolar degenerescence, an intense reaction of Kupffer cells, in the Kiernan space and around the central lobular vein (Figure 5d).

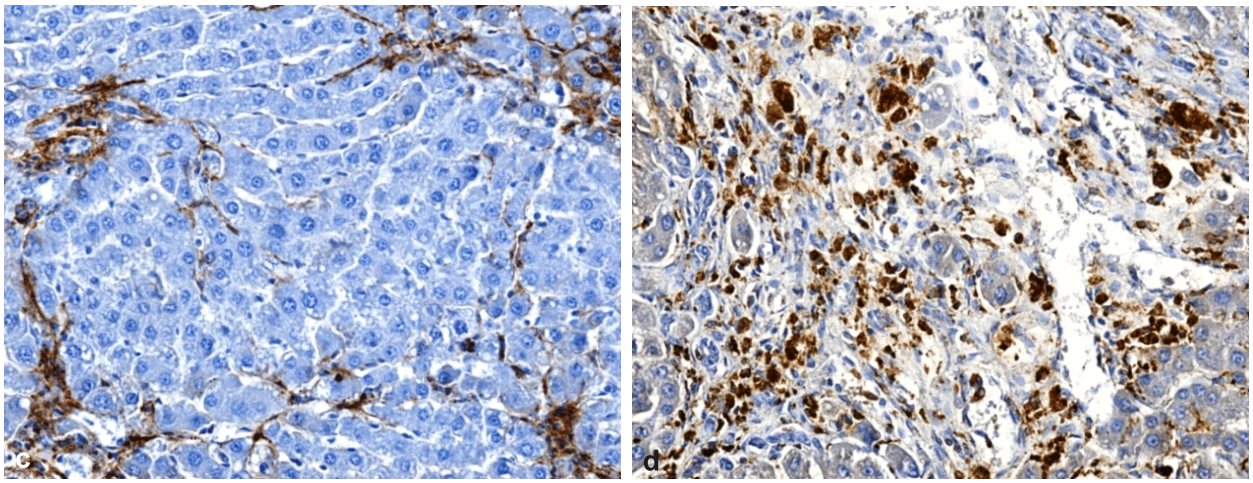

Figure 5 (continued) – ***Histopathological changes of the liver parenchyma after 28 days in the group treated with CCl_4: (c) Intense reaction of Ito cells – massive fibrosis with porto-portal bridges and a tendency to form cirrhotic nodules (Anti-α-SMA antibody immunomarking, ×200); (d) Numerous large macrophages arranged heterogeneously, an intense reaction of Kupffer cells (Anti-CD68 antibody immunomarking, ×200).***

Histological and IHC observations after seven days of TS *vs.* PTX treatment

In the group treated with CCl_4 and TS, the HP study performed with the HE staining highlighted minimal lesions of granulovacuolar degenerescence, with a localization especially around the central lobular vein (Figure 6a). In the group treated with CCl_4 and PTX, there was also highlighted a microvacuolar steatosis, especially in the hepatocytes situated around the central lobular vein (Figure 6b). With the help of GS trichrome staining, there were highlighted the same minimal granulovacuolar degenerescence lesions and the onset of an incipient fibrosis, in the group treated with TS (Figure 6c). In the group treated with PTX, the fibrous reaction was more intense, and, there was also observed a proliferation of fibroblast and myofibroblast cells (Figure 6d).

The IHC study performed with anti-α-SMA and anti-CD68 antibodies showed a minimal reaction of myofibroblast cells in the liver parenchyma and, also, a poor reaction of Kupffer cells, in the group treated with TS (Figure 6, e and g). In the group treated with PTX, there was highlighted a moderate proliferation of myofibroblasts in the Kiernan space, while in the areas of marked granulovacuolar degenerescence and lipidic infiltration, there was observed an increase of the number of myofibroblasts, possibly by transforming the Ito into myofibroblasts and, also, an increase of macrophages, and an intense reaction of Kupffer cells, respectively (Figure 6, f and h). Moreover, there was observed an increase of macrophage size, thus suggesting an intensification of the phagocytosis process. We mention that we selected the areas with the maximum cellular intensity.

Histological and IHC observations after 14 days of TS *vs.* PTX treatment

In the group treated with CCl_4 and TS, the HP study highlighted a granulovacuolar degenerescence with a steatosis aspect, mainly in the 2–3 segment of the liver lobe (Figure 7a). In the group treated with CCl_4 and PTX, there was observed that there are affected the hepatocytes around the central lobular vein (Figure 7b), while the hepatocytes around the Kiernan space presented big, hypochromic nuclei, with a rough-grained chromatin (signs of liver regeneration). There was observed a heterogeneous damaging of the hepatocytes, from one area to another. GS trichrome staining highlighted a granulovacuolar degenerescence, mainly of the steatosis type, around the central lobular vein, in the group treated with TS (Figure 7c). In the group treated with PTX, there was observed a lipidic infiltrate and an intense fibrous reaction in the Kiernan space (Figure 7d).

The IHC study showed that, in the group treated with TS, the number of activated Ito and Kupffer cells was reduced (Figure 7, e and g), while in the group treated with PTX, there were observed numerous Ito cells, both around the central lobular vein and in the liver parenchyma (Figure 7f), as well as numerous activated Kupffer cells, heterogeneously arranged (Figure 7h), mainly in the areas with intense hepatocellular lesions (central lobular areas). Around the Kiernan space, the reaction of Kupffer cells was low, the cells being in a low number and with smaller sizes.

Histological and IHC observations after 21 days of TS *vs.* PTX treatment

In the group treated with CCl_4 and TS, the HP study highlighted that there were no present signs of hepatocytolysis (Figure 8, a and c), while in the group treated with CCl_4 and PTX there was observed the persistence of microvacuolar steatosis (Figure 8b) and of porto-portal fibrotic bridges (Figure 8d). The IHC study with anti-α-SMA antibody highlighted rare activated Ito cells

(Figure 8e). By using the anti-CD68 antibody, there were observed rare activated Kupffer cells, mainly around the Kiernan space (Figure 8g), in the group treated with CCl_4 and TS. In the group treated with CCl_4 and PTX, there was observed a moderate reaction of myofibroblasts in the granulovacuolar degenerescence areas and necrosis (Figure 8f) and an intense reaction of Kupffer cells, in the central lobular area (Figure 8h).

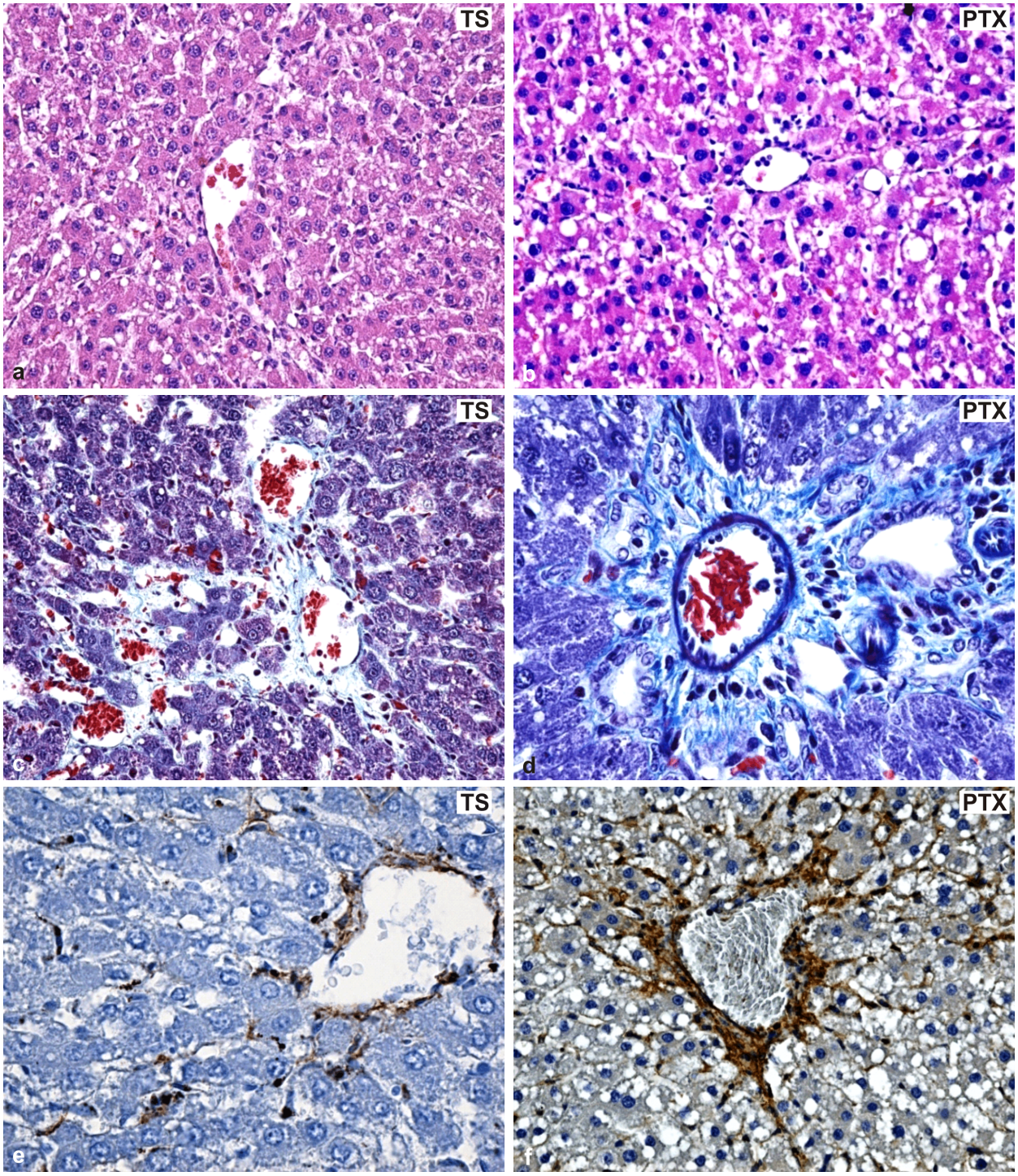

Figure 6 – ***Histopathological aspects of the liver parenchyma after seven days of treatment (TS vs. PTX): (a) Minimal lesions of granulovacuolar degenerescence mainly around the central lobular vein, in the group treated with TS (HE staining, ×200); (b) Granulovacuolar degenerescence and microvacuolar steatosis, mainly in the hepatocytes around the central lobular vein, with incipient fibrosis in the Kiernan space, in the group treated with PTX (HE staining, ×200); (c) Aspect of granulovacuolar degenerescence and incipient fibrous reaction, in the group treated with TS (GS trichrome staining, ×200); (d) Incipient fibrous reaction in the Kiernan space, granulovacuolar degenerescence and proliferation of fibroblasts and myofibroblasts, in the group treated with PTX (GS trichrome staining, ×200); (e) Minimal anti-α-SMA reaction, rare activated Ito cells, in the group treated with TS (Anti-α-SMA antibody immuno-marking, ×400); (f) Highlighted presence of myofibroblasts in the areas of advanced lesions of granulovacuolar degenerescence and lipidic infiltration, in the group treated with PTX (Anti-α-SMA antibody immunomarking, ×200). TS: Telmisartan; PTX: Pentoxifylline.***

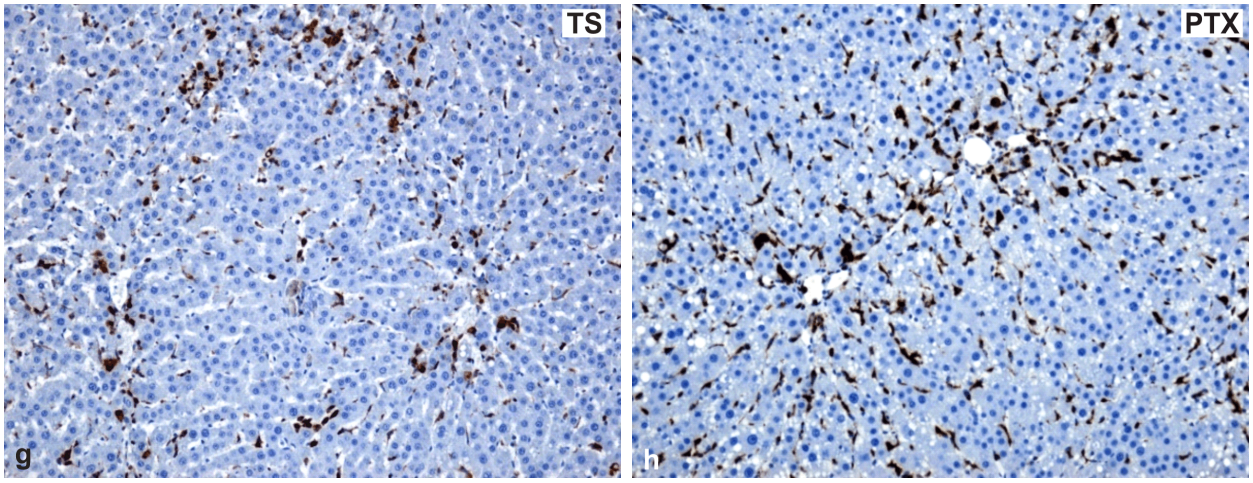

Figure 6 (continued) – ***Histopathological aspects of the liver parenchyma after seven days of treatment (TS vs. PTX): (g) Poor reaction of Kupffer cells, in the group treated with TS (Anti-CD68 antibody immunomarking, ×100; (h) Large activated Kupffer cells, with an intense immunohistochemical reaction in the areas of advanced hepatocellular lesions, in the group treated with PTX (Anti-CD68 antibody immunomarking, ×100). TS: Telmisartan; PTX: Pentoxifylline.***

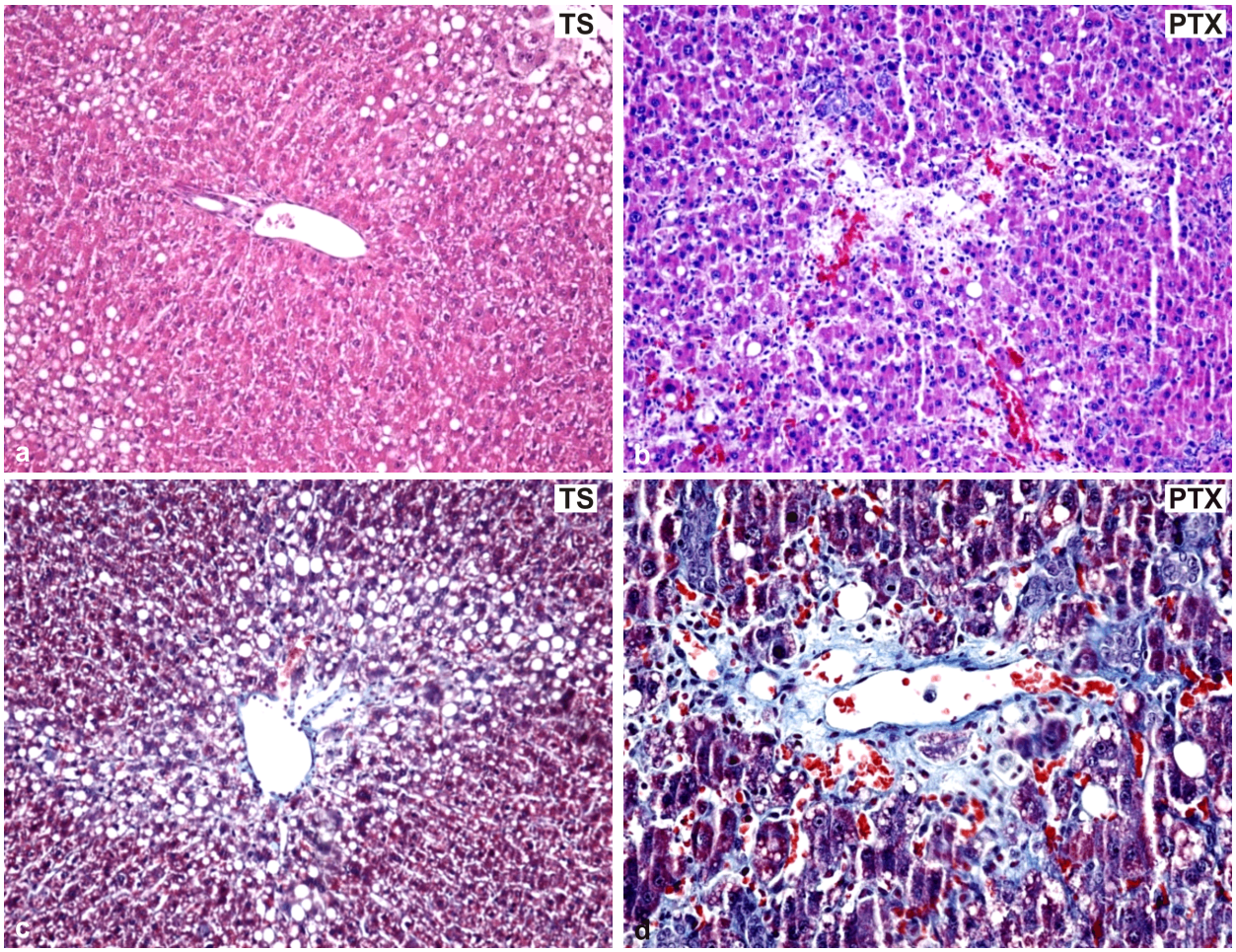

Figure 7 – ***Histopathological aspects of the liver parenchyma after 14 days of treatment (TS vs. PTX): (a) Vacuolar degenerescence, mainly steatosis, around the central lobular vein, in the group treated with TS (HE staining, ×200); (b) Hepatocytes in the Kiernan space present minimal lesions of granulovacuolar degenerescence – there may be observed a heterogeneous damaging of hepatocytes, in the group treated with PTX (HE staining, ×200); (c) Vacuolar degenerescence, mainly of the steatosis type, around the central lobular vein, in the group treated with TS (GS trichrome staining, ×200); (d) Hepatocytes around the central lobular vein present a more intense granulovacuolar degenerescence in comparison to the cells in the Kiernan space, in the group treated with PTX (GS trichrome staining, ×400). TS: Telmisartan; PTX: Pentoxifylline.***

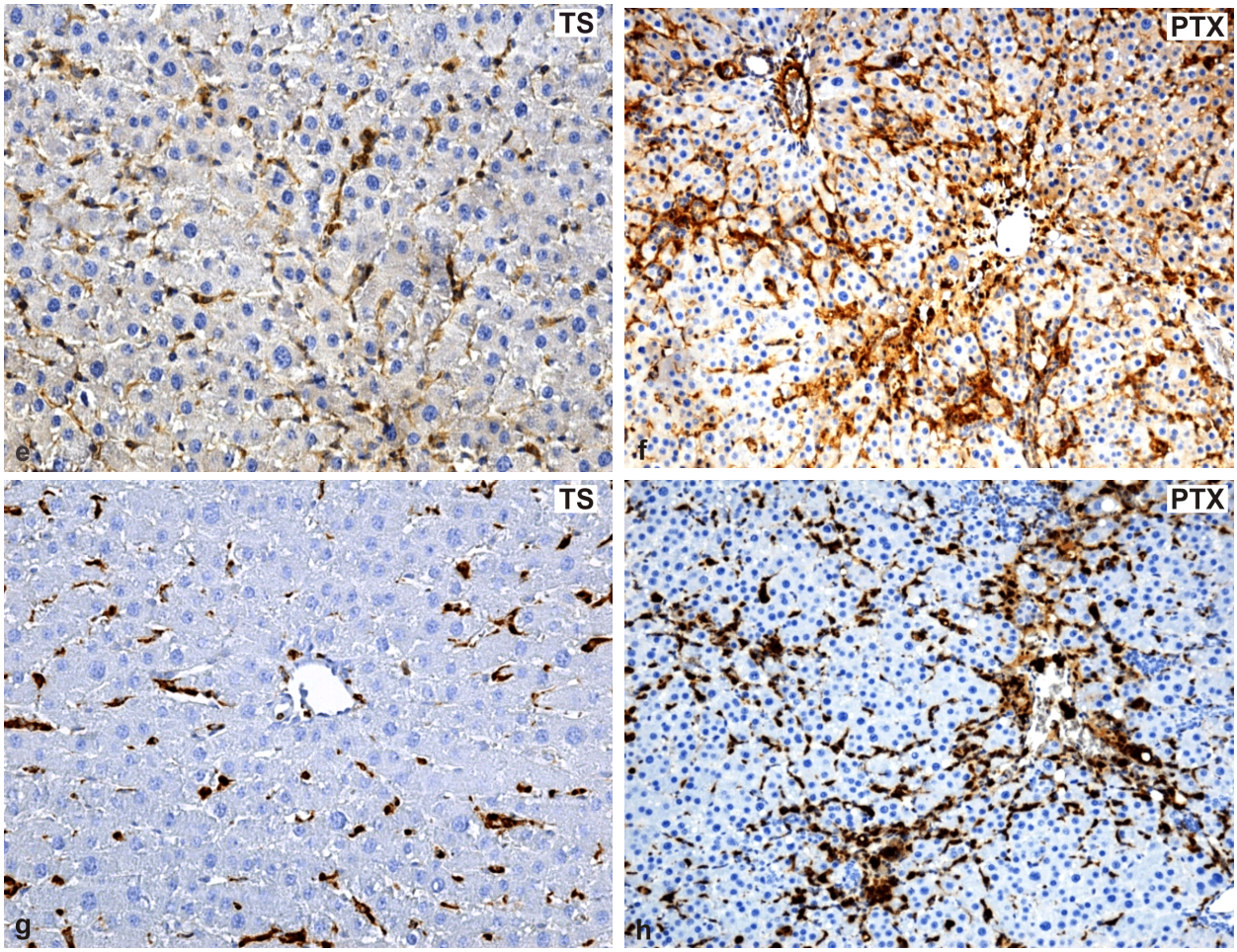

Figure 7 (continued) – ***Histopathological aspects of the liver parenchyma after 14 days of treatment (TS vs. PTX): (e) Rare activated Ito cells and persistent steatosis, in the group treated with TS (Anti-α-SMA antibody immunomarking, ×200); (f) There may be observed numerous Ito cells (myofibroblasts), both around the central lobular vein and in the liver parenchyma, in the group treated with PTX (Anti-α-SMA antibody immunomarking, ×100); (g) Rare activated Kupffer cells around the porto-biliary Kiernan space, in the group treated with TS (Anti-CD68 antibody immunomarking, ×200); (h) Numerous activated Kupffer cells, unevenly distributed, mainly in the central lobular areas (Anti-CD68 antibody immunomarking, ×100). TS: Telmisartan; PTX: Pentoxifylline.***

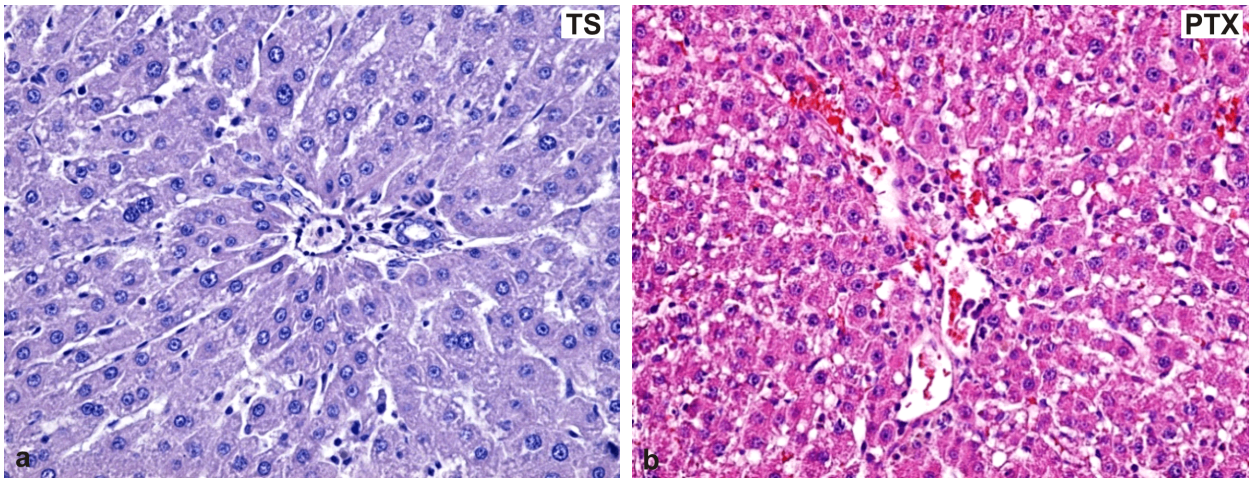

Figure 8 – ***Histopathological aspects of the liver parenchyma after 21 days of treatment (TS vs. PTX): (a) There are no signs of hepatocytolysis, in the group treated with TS (HE staining, ×200); (b) Granulovacuolar degenerescence and microvacuolar steatosis, mainly in the hepatocytes around the central lobular vein, in the group treated with PTX (HE staining, ×200). TS: Telmisartan; PTX: Pentoxifylline.***

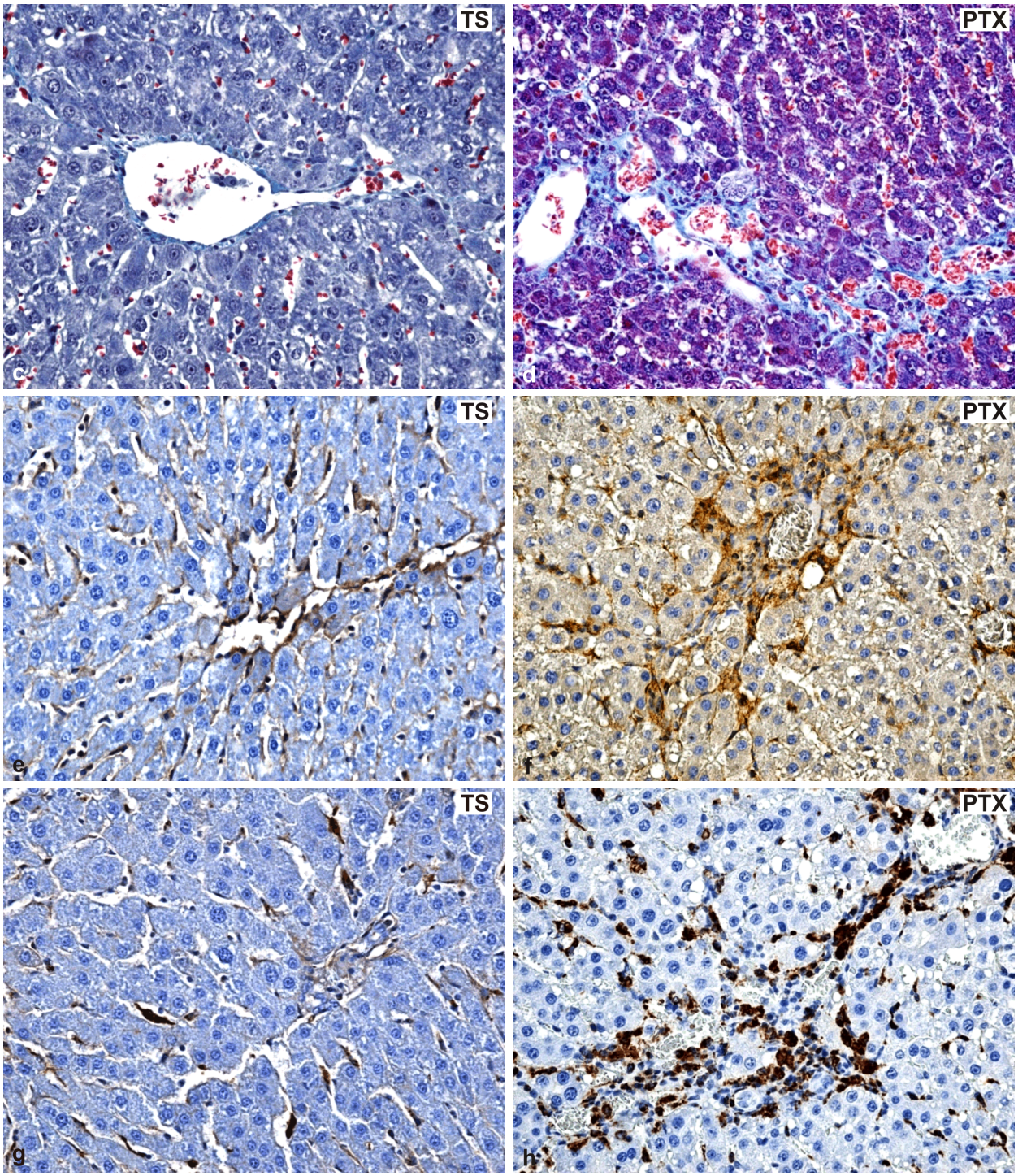

Figure 8 (continued) – ***Histopathological aspects of the liver parenchyma after 21 days of treatment (TS vs. PTX): (c) There are not highlighted any signs of hepatocytolysis in the group treated with TS (GS trichrome staining, ×200); (d) Porto-portal fibrosis bridges, fibrotic lesions in the porto-biliary space, with fine intralobular and porto-portal septa, in the group treated with PTX (GS trichrome staining, ×200); (e) Rare activated Ito cells, in the group treated with TS (Anti-α-SMA antibody immunomarking, ×200); (f) Moderate reaction of myofibroblasts in the areas of granulovacuolar degenerescence and necrosis, in the group treated with PTX (Anti-α-SMA antibody immunomarking, ×200); (g) Rare activated Kupffer cells around the porto-biliary Kiernan space, in the group treated with TS (Anti-CD68 antibody immunomarking, ×200); (h) Intense reaction of Kupffer cells in the central lobular area, in the group treated with PTX (Anti-CD68 antibody immunomarking, ×200). TS: Telmisartan; PTX: Pentoxifylline.***

Discussion

Carbon tetrachloride is an organic solvent largely used in industry and a well-known hepatotoxic xenobiotic, frequently used for inducing liver fibrosis, in experimental models. The progression and regression of fibrosis is a complex process involving the parenchymatous and non-parenchymatous liver cells, as well as the immune cells. Hepatocytolysis induces the activation of the inflammatory and pro-fibrogenic ways in the non-parenchymatous and infiltrated cells, these triggering the initiation and progression of fibrosis, but they may also contribute to

the regression of fibrosis [9, 10]. Fibrosis results from the excessive accumulation of the extracellular matrix (ECM), produced mainly by the myofibroblasts, leading to severe architectural changes of liver cirrhosis and hepatocellular carcinoma [10, 11]. Myofibroblasts are present only in the lesional liver, and their main source is constituted by the HSCs, whose depletion was shown to significantly reduce liver fibrosis, in the experimental model of liver fibrosis induced with CCl_4 [12]. The regression of liver fibrosis is characterized by the decrease of inflammatory and fibrogenic cytokines, the increase of collagenase activity and the disparity of myofibroblasts and fibrous lesions [13].

Pentoxifylline (PTX), a methylxanthine derivate, a non-selective inhibitor of phosphodiesterase, is largely used as a peripheral vasodilator, in vascular perfusion disorders. PTX is an inhibitor of the pro inflammatory cytokine production, mainly TNF-α [14]. Some studies showed that the antifibrotic effect may be due to TNF-α inhibiting, which stimulated the fibroblast activation, increase of collagenase activity and, also, by down-regulating profibrinogenic cytokines and the procollagen expression I [15, 16].

The effects of the axis angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/receptors 1 of angiotensin II (AT1) in liver diseases are largely described in specialty papers. Both ACE genes, and the AT1 receptors are up-regulated in the areas with active liver fibrinogenesis [17], and the activation of the AT1 receptor by Ang II induces the contraction and proliferation of HSCs [18, 19]. As a result, it is expected that the use of angiotensin receptor blockers should lead to a decrease of inflammation, and thus reducing the liver fibrosis [20].

In our study, we decided the initiation of the treatment with TS and PTX after seven days of CCl_4 administration, because, at that moment, we observed an increase of the number of fibroblasts and collagen fibers in the Kiernan space.

After seven days of TS or PTX treatment and 14 days of administering CCl_4, the comparative analysis of the liver HP study in the rats that received only CCl_4 and in those who received CCl_4 together with TS or PTX, highlighted that the lesions of granulovacuolar degenerescence and fibrosis in the portal space, present in the group that received only CCl_4, were significantly reduced in the animals treated with TS, in comparison to the group treated with PTX.

After 14 days of TS or PTX treatment and 21 days of administering CCl_4, the HP examination showed that numerous activated Kupffer cells, especially in the necrosis areas and the intense process of fibrillogenesis recorded in the group receiving CCl_4, were reduced significantly in the group treated with TS, and in the group treated with PTX there were observed numerous myofibroblasts and activated Kupffer cells, mainly around the central lobular vein.

After 21 days of TS and PTX treatment and 28 days of CCl_4 administration, the severity of hepatocytolysis and granulovacuolar degenerescence (Figure 5, a and b), observed in the group treated exclusively with CCl_4, as well as the massive presence of fibrosis, were practically inexistent in the group treated with CCl_4 and TS (Figure 8, a and c), while in the group treated with PTX there was observed the persistence of porto-portal fibrotic bridges and an intense reaction of Kupffer cells in the central lobular area.

Our observation is different from other studies, which showed that PTX indicated remarkable results regarding the degree of hepatocellular damaging induced by CCl_4 or the occlusion of the gallbladder canal [21, 22].

Conclusions

In this study, the treatment with Telmisartan was shown to determine a significant decrease in the number of myofibroblasts and Kupffer cells, slowing down the fibrillogenesis process, without any other severe liver lesions, during all the testing periods, as they were observed in the untreated group. By comparison, Pentoxifylline highlighted a poor hepatoprotective action, as there persisted the porto-portal fibrotic bridges, even after 21 days of treatment. Our study highlighted that Telmisartan had an antifibrotic action highly superior to Pentoxifylline, in the experimental model of liver fibrosis induced with carbon tetrachloride.

Conflict of interest

The authors declare that they have no conflict of interests.

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