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INTRODUCTION

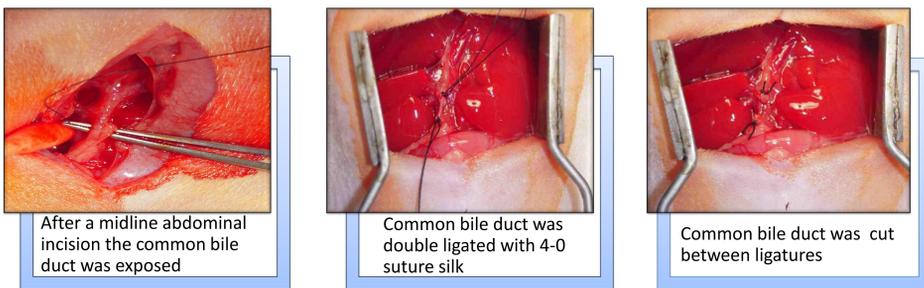
Human placenta at term, which is usually discarded as medical waste, has attracted much attention as a source of tissues and stem / progenitor cells for research and potential clinical applications [1]. The anti-inflammatory, antiscarring, and wound healing properties of amniotic membrane (AM) could make her useful for development of new therapeutic approaches in regenerative medicine and warrant studies of their effects in animal models of inflammatory and fibrotic pathologies [2,3]. Biliary fibrosis and resultant cirrhosis are among the most common outcome of chronic liver disease. Currently, liver transplantation remains the only effective treatment. We have recently shown that AM used as a patch onto liver surface at the same time of fibrosis induction reduces significantly the progression and severity of biliary fibrosis [4].

Here, we investigated if the intact human AM has the capacity to reduce a well-established liver fibrosis induced in rat by bile duct ligation (BDL) model.

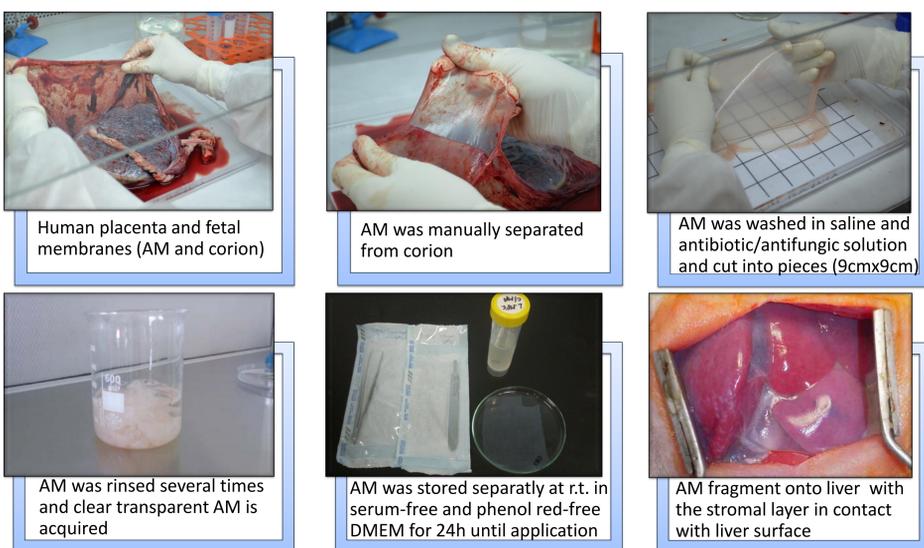
METHODS

The human term placentas were obtained from caesarean sections with maternal consent and according to the Ethical Committee of Hospital Municipal in São José dos Campos, SP, Brazil and approved by the Research Ethics Committee. Twenty male Wistar rats weighing 200-220g were submitted to BDL to induce biliary fibrosis. After 2 weeks from BDL, the liver was covered with a fragment of AM (BDL+AM group), or left untreated (BDL group). Six weeks later, liver samples were histologically processed for Sirius red for collagen analysis or for immunohistochemical stainings to identify biliary structures, myofibroblasts and Transforming Growth Factor (TGF)- β 1. The fibrosis was first assessed by semiquantitative scoring systems and, thereafter, by digital image analysis using the CellProfiler software [5] to quantify the area occupied by collagen deposition, ductular reaction, myofibroblasts, and by TGF- β 1.

Rat model of liver fibrosis-BDL



Amniotic membrane processing and application



CONCLUSION

The fresh human amniotic membrane when applied as a patch onto the liver surface is useful for treating well-established cholestatic fibrosis and the mechanism was partly by, means of downregulating the profibrotic factor TGF- β 1.

REFERENCES

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RESULTS

The results of semiquantitative evaluation of the degree of fibrosis in rat liver are demonstrated in Figure 1. This analysis allows a staging of fibrotic lesions, by considering the pathologic alterations of liver tissue.

Figure 2 shows photomicrographs of rat liver sections marked with Sirius red for collagen (A-B) and antibodies against CK19 (D-E), α -SMA (G-H) and TGF- β 1 (J-L) respectively.

Sirius Red staining, CK19, α -SMA and TGF- β 1 immunostainings were quantitatively evaluated by computing-assisted image analysis (Figures 2C, 2F, 2I, 2M). The liver area occupied by collagen deposition, ductular reaction, myofibroblasts (the three main parameters specifically involved progression of biliary fibrosis), and TGF- β 1 (the main profibrogenic factor in chronic liver disease), were all reduced in AM-treated rats to about 50% of levels observed in BDL-only rats.

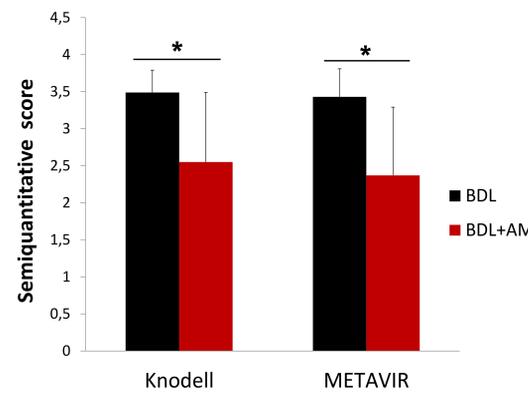


Figure 1: Semiquantitative evaluation of the degree of fibrosis in liver tissue by application of the Knodell and METAVIR scoring systems. The severity of fibrosis was lower in rats treated with AM than in the BDL group. Means \pm SD of the fibrosis score evaluated in 8 rats is represented.

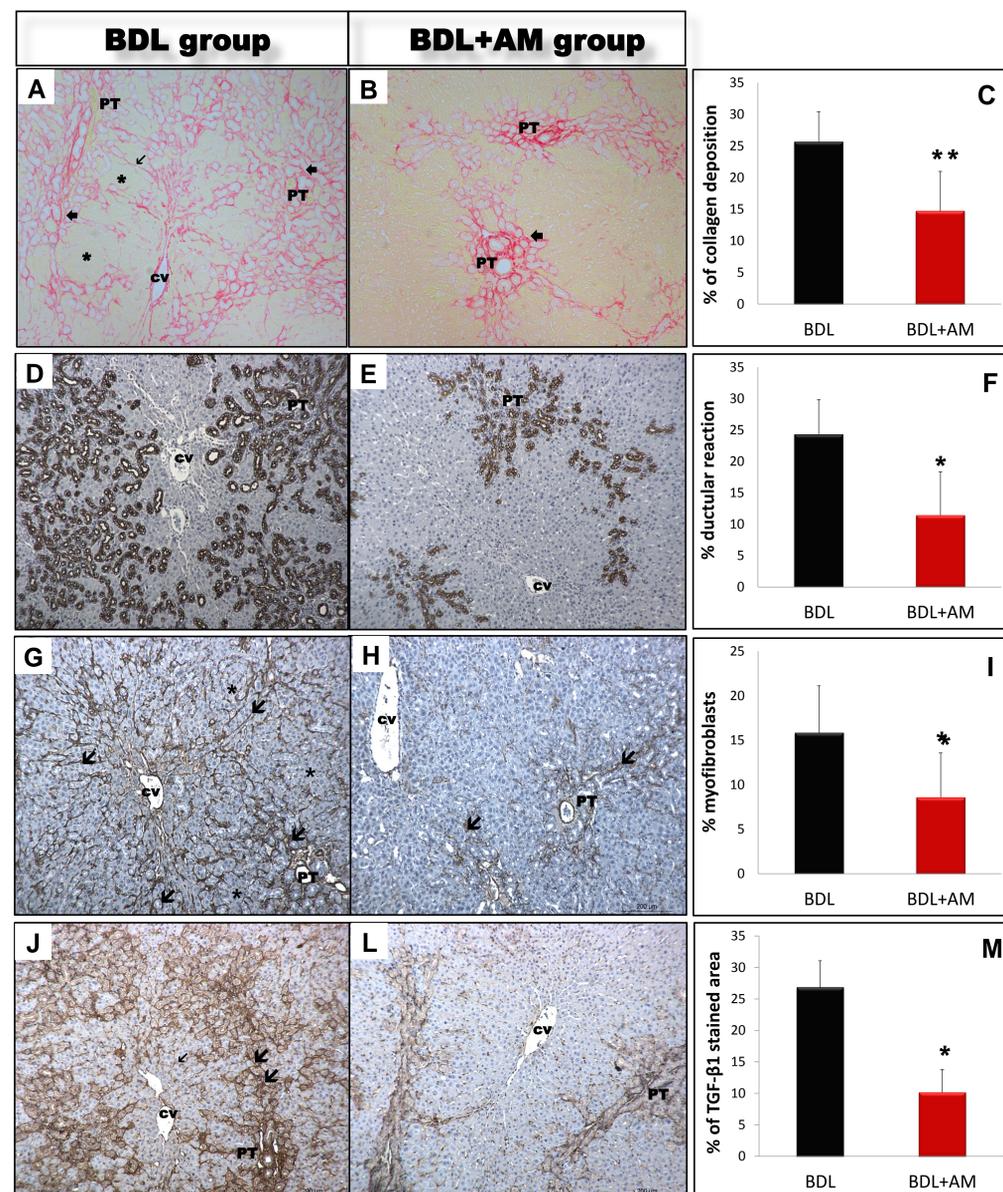


Figure 2: Immunohistochemical and quantitative evaluation. Sirius Red staining (A-B): intense periductular fibrosis with bridging septa (\blacktriangleleft) between portal tracts and interstitial collagen (\blacktriangleleft) dividing parenchyma in cirrhotic nodules (*). In the AM-treated group, the architecture of hepatic parenchyma is more preserved. CK19 immunostaining (D-E): CK19-positive biliary structures expand and connect adjacent portal tracts and finally isolate small nodules of hepatocytes (*). In AM-treated group, the CK19-positive biliary structures is lower than in the BDL group. α -SMA immunostaining (J-H): in the BDL group the α -SMA-positive myofibroblasts (\blacktriangleleft) surround all biliary structures and are along the perisinusoidal spaces. In AM-treated group, the myofibroblasts (\blacktriangleleft) are lower than in the BDL group and not all biliary structures are surrounded by the α -SMA-positive cells (inbox). TGF- β 1 immunostaining (J-L): in the BDL group the expression of TGF- β 1 was stronger than AM-treated group and located in connective tissue of PT and mainly around biliary structures (\blacktriangleleft) and perisinusoidal spaces (\blacktriangleleft). In the AM-treated group the integrity of hepatic parenchyma was more preserved. Central vein (CV); Portal tract (PT). Magnification \times 100. Values of collagen deposition (C), ductular reaction (F), myofibroblasts (I), and TGF- β 1 (H) in different groups: Means \pm SD of 8 independent experiments are represented as bars. * p <0.05, ** p <0.01 versus BDL group.