Efficacy and underling mechanisms of D-Chiro Inositol in a mouse model of endometriosis

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Endometriosis, a disease affecting 5-10% of women of reproductive age, is characterized by the spread of endometrial-like tissue outside the uterine cavity that produces ectopic endometriotic lesions causing pain and infertility. Surgical intervention and hormonal treatments have no long-term effects. The sensitivity of endometriosis to estrogens is a characteristic that can be used for therapeutic purposes. D-Chiro Inositol (DCI), one of the nine isomers of inositol, is known to decrease the CYP19A1 aromatase gene expression in granulosa cells. Based on these premises, it was suggested that treatment with DCI may counteract endometriosis. To address the study question, a mouse model of endometriosis was generated. Out of 20 CD1 mice, 4 mice were randomly selected as donors of uterine fragments, and the remaining 16 were recipient mice. The first day after transplantation, mice were randomly assigned to four experimental group which received for 28 days 2ml water containing: none (CTRL); DCI 0.4 mg (DCI 0.4); DCI 0.2mg and Dienogest 0.33ng (DCI 0.2+DG 0.33); DG 0.67ng (DG 0.67). Uterine horns were removed from donor mice at the diestrous stage of the reproductive cycle. The tissue cut into fragments was inoculated in recipient mice by intraperitoneal injection. Four weeks after induction, all mice were sacrificed and the endometriotic lesions were excised, measured by number, size and examined for the presence of blood vessels vascularization under stereomicroscope. Then, lesions were processed for immunohistochemical analysis. Endometriotic lesions developed in recipient mice met all criteria for endometriosis, including the presence of endometrial epithelial and stromal cells, and encapsulation in neighboring tissues or organs. The lesions number was reduced in all the treatment groups when compared to CTRL and the rate of vascularized lesions was lower in the treated groups, with more pronounced effect in the DCI group where no vascularized lesions were observed. The histological analysis revealed a marked reduction of endometriotic foci in all groups. The lesions were then subjected to immunohistochemical analysis aimed at evaluating the proliferative and vascularization status using two specific markers, PCNA and CD34, respectively. PCNA-positive epithelial and stromal cells were detected in the lesions; in the DCI group a decrease in the expression of this marker is observed. CD34 labeling showed similar levels in CTRL compared to DCI+DG and DG treated groups; the DCI group presents a reduction in the expression of this marker. Searching for mechanisms underlying DCI effects, the expression of SIRT1 and E-cadherin as markers of epithelial-mesenchymal transition (EMT) involved in endometriotic lesion development, revealed that DCI negatively affects this process. CYP19A1 transcripts in the ovaries decreased in all groups, especially in the DCI group. This work has made it possible to validate an easily reproducible, minimally invasive model of endometriosis in CD1 mice. Based on this model, we demonstrated that DCI reduces endometriotic lesions development probably by interfering with EMT progression and reducing ovarian estrogens production by aromatase inhibition. In conclusion, the administration of DCI, both alone and in combination with DG, shows promising results in the treatment of endometriosis and opens the doors to further investigations aimed at introducing this substance into clinical practice.