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ARTICLE

Increased oxidation-related glutathionylation and carbonic anhydrase activity in endometriosis

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Abstract This study examined the possible involvement of carbonic anhydrase activation in response to an endometriosis-related increase in oxidative stress. Peripheral blood samples obtained from 27 healthy controls and 30 endometriosis patients, classified as having endometriosis by histological examination of surgical specimens, were analysed by multiple immunoassay and carbonic anhydrase activity assay. Red blood cells (RBC) were analysed for glutathionylated protein (GSSP) content in the membrane, total glutathione (GSH) in the cytosol and carbonic anhydrase concentration and activity. In association with a membrane increase of GSSP and a cytosolic decrease of GSH content in endometriosis patients, carbonic anhydrase significantly increased ($P < 0.0001$) both monomerization and activity compared with controls. This oxidation-induced activation of carbonic anhydrase was positively and significantly correlated with the GSH content of RBC ($r = 0.9735$, $P < 0.001$) and with the amount of the 30-kDa monomer of carbonic anhydrase ($r = 0.9750$, $P < 0.001$). Because carbonic anhydrase activation is implied in many physiological and biochemical processes linked to pathologies such as glaucoma, hypertension, obesity and infections, carbonic anhydrase activity should be closely monitored in endometriosis. These data open promising working perspectives for diagnosis and treatment of endometriosis and hopefully of other oxidative stress-related diseases.

KEYWORDS: carbonic anhydrase, endometriosis, glutathione (GSH), glutathionylation, oxidative stress, red blood cell

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Introduction

Endometriosis is characterized by the presence and growth of endometrial tissue outside the uterine cavity and related local inflammatory processes, and affects 5–15% of women of reproductive age (Olive and Schwartz, 1993; Van Langendonckt et al., 2002). Pain and infertility are the most common gynaecological symptoms of endometriosis and may seriously impair the quality of life.

Many studies have focused on markers of inflammation in an effort to find less invasive methods of diagnosis (Bedaiwy et al., 2002; Darai et al., 2003; Ulukus et al., 2005). One of the causes in the pathogenesis/evolution of endometriosis is oxidative stress, which occurs when reactive oxygen species are produced faster than the endogenous antioxidant defence systems can neutralize them (Agarwal et al., 2012; Ngò et al., 2009). Once produced, reactive oxygen species can alter the morphological and functional properties of endothelial cells, including permeability and adhesion molecule expression, thus contributing to ongoing inflammation (Nagata, 2005). This alteration is the consequence of local macrophage and neutrophil activation in response to the presence of ectopic endometrial tissue reaching the peritoneal cavity through the Fallopian tubes (Jackson et al., 2005; Kyama et al., 2003; Murphy et al., 1998). Although oxidative stress increases locally at sites of endometrial implants, accumulating evidence points to the presence of oxidative stress markers in serum, such as (HSP)70b′ (Lambrinoudaki et al., 2009), and higher concentrations of oxidatively modified lipoproteins (Shanti et al., 1999). Further strengthening the idea of the systemic generalization of oxidative stress is the recent characterization of endometriosis-associated biochemical changes, such as membrane oxidation and glutathione (GSH) reduction, in peripheral circulating red blood cells (RBC; Bordin et al., 2010).

Because of their main cellular functions — delivery of O_2 from lung to tissue and removal of CO_2 from tissue to lung — RBC are particularly exposed to oxidative stress. Carbon dioxide in tissue capillaries diffuses into the cells, where it is rapidly hydrated by the action of cytosolic carbonic anhydrase. Carbonic anhydrase belongs to a family of metalloenzymes, which catalyse the conversion of CO_2 to HCO_3^- and H^+ and which are involved in many physiological processes such as acid–base homeostasis, respiration, carbon dioxide and ion transport, and bone resorption (Henry, 1996; Sly and Hu, 1995). The biological functions of carbonic anhydrases are of great potential interest, as their contribution to the development of complications in some pathologies is not yet completely clarified. Carbonic anhydrase isoform II plays an important role in the production of aqueous humour and much attention has been focused on the synthesis and characterization of isoform II inhibitors, particularly in the treatment of glaucoma (Weiwei and Hu, 2009).

Bicarbonate resulting from carbonic anhydrase action is transported into the plasma in exchange for extracellular chloride by anion exchanger 1 (AE1), the key protein for the blood's capacity to carry CO_2. AEI is particularly sensitive to diamide-induced oxidative stress. Diamide, a mild oxidant, affects the sulphydryl groups of cysteine by inducing disulphide bond formation (Zipser et al., 1997), triggering AEI Tyr-phosphorylation (Bordin et al., 2010, 2005, 2006; Donà et al., 2012a,b) and AEI clustering in a way directly proportional to the oxidation level of cells (Bordin et al., 2005, 2006, 2013; Donà et al., 2012a,b). In addition, in oxidation-related pathological diseases such as polycystic ovary syndrome, diamide treatment has been demonstrated to induce irreversible disulphide bonds between GSH and thiol–protein groups to produce glutathionylated proteins (GSSP) (Donà et al., 2012b) and to reduce total GSH detectable in the cytosol (Bordin et al., 2010; Donà et al., 2012b), without affecting normal subjects.

In continuation and extension of a previous study (Bordin et al., 2010), this study postulated that endometriosis-related inflammatory processes affect the structure and functioning of circulating RBC, detectable by changes in membrane GSSP and carbonic anhydrase location and activity.

Materials and methods

Study population

Between January and December 2012, patients presenting with pelvic pain and ultrasonographically identified adnexal ovarian mass were referred to this endometriosis care unit for laparoscopy. For endometriosis patients, this study included only women classified as having endometriosis by histological examination of surgical specimens (n = 30; age, mean ± standard deviation, 31.2 ± 8.4 years). The endometriosis patients met the following criteria: no hormone therapy for at least 3 months; regular menstruation; nonsmoker; and no signs of other inflammatory disease (as assessed by leukocytes, body temperature or other specific symptoms).

The control group comprised 27 volunteers (age 34.9 ± 9.2 years) whose clinical and ultrasound tests identified them as being healthy.

Clinical data and peripheral blood samples were collected only after explaining the objectives of the study to the patients and obtaining signed informed consent. This study was approved by the University of Padova Ethics Committee on 13 February 2012.

Reagents

Reagents and anti-human haemoglobin antibody were purchased from Sigma (Milan, Italy), and sheep polyclonal antibody to carbonic anhydrase II cross-reacting also with carbonic anhydrase I was obtained from Abcam (Cambridge, UK). Anti-GSH was obtained from Virogen (Watertown, MA, USA). Protease inhibitor cocktail, anti-actin mouse monoclonal antibody, anti-mouse and anti-sheep secondary antibodies conjugated with horseradish peroxidase were obtained from Merck Millipore (Darmstadt, Germany).

Treatment of RBC

Fresh blood was collected from healthy women in the Endocrinology unit, or endometriosis during laparoscopy. RBC were pelleted at 3750***g*** for 3 min and, after removal of supernatant, packed RBC were washed three times at 3750***g*** for 3 min in five volumes of Dulbecco's phosphate-buffered saline (PBS) to avoid contamination by leukocytes and platelets. Packed cells (50 μl) were resuspended to 20%