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Endometriosis is a chronic pain condition affecting ∼176 million women worldwide. It is defined by the presence of endometrium-like tissue (lesions) outside the uterus, most commonly on the pelvic peritoneum. There is no cure for endometriosis. All endometriosis drug approvals to date have been contraceptive, limiting their use in women of childbearing age. We have shown that human peritoneal mesothelial cells (HPMCs) recovered from the pelvic peritoneum of women with endometriosis exhibit significantly higher glycolysis, lower mitochondrial respiration, decreased enzymatic activity of pyruvate dehydrogenase (PDH), and increased production of lactate compared to HPMCs from women without disease. Transforming growth factor-β1 (TGF-β1) is elevated in the peritoneal fluid from women with endometriosis, and exposure of HPMCs to TGF-β1 exacerbates this abnormal phenotype. Treatment of endometriosis HPMCs with the pyruvate dehydrogenase kinase (PDK) inhibitor/PDH activator dichloroacetate (DCA) normalizes HPMC metabolism, reduces lactate secretion, and abrogates endometrial stromal cell proliferation in a coculture model. Oral DCA reduced peritoneal fluid lactate concentrations and endometriosis lesion size in a mouse model. These findings provide the rationale for targeting metabolic processes as a noncontraceptive treatment for women with endometriosis either as a primary nonhormonal treatment or to prevent recurrence after surgery.

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Endometriosis is a chronic pain condition affecting ∼176 million women worldwide with a high socioeconomic impact. It is defined by the presence of endometrium-like tissue (lesions) outside the uterus, most commonly on the pelvic peritoneum. There is no cure for endometriosis, and it is difficult to treat. Recurrence rates after surgery to remove lesions are as high as 50% after 5 y (1). Current medical treatments are contraceptive, often with unpleasant side effects.

Endometriosis exhibits cancer-like features. For example, tumor cells are programmed by TGF-β1 to use aerobic glycolysis, resulting in increased secretion of lactate (2). TGF-β1 and lactate are both elevated in the peritoneal fluid (PF) of women with endometriosis, and this is paralleled by a switch from normal mitochondrial respiration toward glycolysis in the human peritoneal mesothelial cells (HPMCs) that line the pelvic cavity (3). In tumors, lactate is considered a key factor in driving cell invasion, angiogenesis, and immune suppression (2), changes that are also implicated in the establishment and survival of endometriosis lesions.

In this study, we have demonstrated that we can reverse the aberrantly increased glycolysis of HPMCs with dichloroacetate (DCA) and that oral administration of DCA reduces the size of lesions in a mouse model.

Results

Peritoneal Mesothelial Cells from Women with Endometriosis Have a Glycolytic Phenotype. We documented higher levels of basal glycolysis ($P = 0.0499$), lower mitochondrial respiration (ATP-linked) ($P = 0.0400$), and higher lactate secretion ($P = 0.0004$) in HPMCs from women with endometriosis compared to women without disease (Fig. 1 *A*–*C*). "Endo" HPMCs exhibited decreased enzymatic activity of pyruvate dehydrogenase ($P = 0.0025$) (Fig. 1*D*).

DCA Corrected the Glycolytic Phenotype of Peritoneal Mesothelial Cells from Women with Endometriosis and Decreased Stromal Cell Proliferation In Vitro. Treatment of Endo HPMCs with DCA normalized their metabolic phenotype and increased PDH activity, in the presence or absence of TGF-β1 (Fig. 1 *E*–*H*). In a coculture system (Fig. 1*I*), treatment of endometriosis HPMCs with DCA decreased basal and TGF-β1–stimulated HPMC lactate secretion ($P = 0.0003$) and proliferation of endometrial stromal cells ($P = 0.0002$, Fig. 1 *J* and *K*).

DCA Reduces Lactate Concentrations and Endometriosis Lesion Size in a Mouse Model. In a preclinical mouse model of endometriosis (4), treatment with oral 100 mg/kg DCA for 7 d reduced PF lactate concentrations ($P = 0.0360$) and the size of endometriosis lesions ($P = 0.02$) (Fig. 1 *L*–*N*).

Discussion

HPMCs from women with endometriosis synthesize and secrete more lactate than cells recovered from women without lesions. Lactate can stimulate cell migration and invasion, and immune escape during tumorigenesis, with the same processes implicated in the etiology of endometriosis (2). We postulate that increased lactate concentrations in pelvic PF may create an environment that promotes invasion of ectopic endometrial cells into the peritoneum so that they form lesions.

We have shown that Endo HPMCs exhibit a greater dependence on energy production through glycolysis under aerobic conditions than those of women who are disease-free. This abnormal cellular energy state is corrected by treatment with the small-molecule drug DCA. Notably, DCA reduced HPMC lactate release in vitro, and oral dosing of mice reduced endometriosis lesion size in vivo. DCA is a pyruvate dehydrogenase kinase (PDK) inhibitor used to treat cancer (5). Four different isoforms of PDK (PDK1–4) exist with variable expression reported: PDK1 appears to have the highest sensitivity to inhibition by DCA (6). PDK inhibition reduces phosphorylation of PDH, increasing PDH activity leading to lower release of metabolites such as lactate. DCA also inhibits

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Competing interest statement: A.W.H. has received honoraria for consultancy for Ferring, Roche, Nordic Pharma, and Abbvie.

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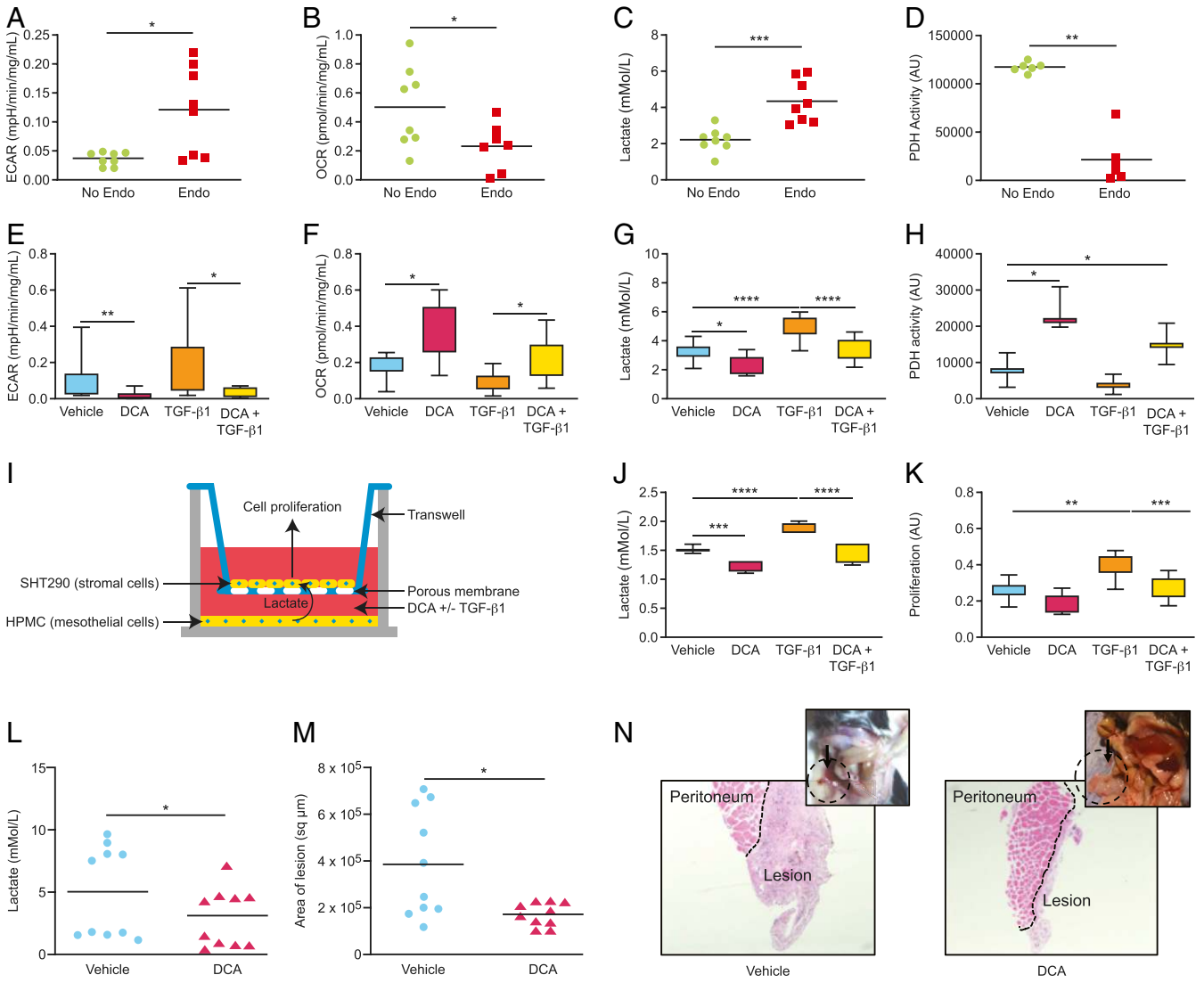

Fig. 1. HPMCs from women with peritoneal endometriosis (Endo) had higher basal glycolytic extracellular acidification rates (ECARs) ($P = 0.0499$; $n = 8$) (*A*) and lower ATP-linked respiratory oxygen consumption rates (OCRs) ($P = 0.0400$; $n = 8$) (*B*) than women without endometriosis (No Endo). Consistent with Endo HPMCs having an altered metabolic phenotype, lactate secretion was higher ($P = 0.0004$; $n = 8$) (*C*) and enzymatic activity of pyruvate dehydrogenase (PDH) was lower ($P = 0.$ 0.0025; $n = 6$) (*D*) than in cells from the No-Endo women. Treatment of Endo HPMCs with DCA reduced ECAR ($P = 0.0071$; $n = 8$) (*E*), increased OCR ($P = 0.0140$; $n = 8$) (*F*), suppressed lactate secretion ($P = 0.0104$; $n = 8$) (*G*), and increased PDH enzymatic activity ($P = 0.0373$; $n = 6$) (*H*) in both the presence and absence of TGF-β1. Lesion-in-a-dish coculture system. (*I*) There is no direct contact between HPMCs and stromal cells. Treatment of Endo HPMCs with DCA reduced both basal and TGF-β1–induced lactate secretion ($P = 0.0003$ and $P = 0.0001$, respectively; $n = 3$). (*J*) Treatment of Endo HPMCs with TGF-β1 stimulated proliferation of stromal cells ($P = 0.0044$; $n = 3$), but this did not occur if HPMCs were incubated with TGF-β1+DCA ($P = 0.0002$; $n = 3$). (*K*) In a mouse model, oral DCA reduced PF lactate concentrations ($P = 0.0360$) (*L*) and reduced the size of endometriosis lesions ($P = 0.02$). (*M*) Images of endometriosis lesions from mice treated with vehicle or oral 100 mg/kg DCA daily for 7 d ($n = 10$/group). (*N*) $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

proproliferative and proangiogenic transcription factors such as HIF-1α (5). In a clinical trial in patients with glioblastoma, DCA decreased tumor growth and angiogenesis (7).

While we have focused on HPMCs, an overlooked source of metabolic factors in PF, DCA could also have an impact on other cell types altered in endometriosis patients. Macrophages that are present in PF or lesions of women with endometriosis exhibit a range of phenotypes (8), and treatment with DCA could drive metabolic reprogramming of macrophages toward a prorepair phenotype that could have a positive impact on the pathogenesis of the disease (9). Stromal cells in patient endometrium and lesions exhibit up-regulation of PDK1. If apoptosis of these cells is enhanced by DCA (10), this could contribute to reduced lesion growth.

To translate these results to women, we are using DCA in an exploratory-phase clinical trial (ClinicalTrials.gov identifier NCT04046081). If effectiveness and acceptability are demonstrated, DCA would be a nonhormonal treatment for women with this debilitating condition.

Methods

Human Cell Culture. Approval was obtained from the Lothian Research Ethics Committee (LREC 11/AL/0376). Samples were collected from women attending a pain clinic who had a diagnosis of peritoneal endometriosis (Endo group) or women without macroscopic evidence of endometriosis at laparoscopy ("No Endo" group), all of whom had given informed consent. Women were not on hormones and had regular menstrual cycles. HPMCs were cultured in HOSE I media (40% Media 199, 40% IMDM, 15% heat-inactivated FBS, 0.5% penicillin/streptomycin, and 1% L-glutamine). All

cell/tissue samples were deidentified (anonymized but linked) prior to use in the study. Immortalized human endometrial stromal cells [SHT290 (11)] were grown in RPMI 1640 media plus 10% heat-inactivated FBS, 1% penicillin/streptomycin, 1% L-glutamine, and 1% nonessential amino acids. Cells were incubated at 37 °C under 5% CO_2 in air.

Seahorse Analysis. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were determined using a glycolytic stress test kit (102340-100; Agilent) on a Seahorse XFe24 Analyzer. HPMCs were seeded in microplates at a density of 2×10^4 cells per well in 500 μL of HOSE I and incubated for 48 h. Cells were rinsed 3 times with 500 μL of prewarmed Seahorse Assay DMEM (no glucose or pyruvate); 525 μL of same medium was added to wells that were incubated at 37 °C for 30 min. ECAR and OCR measurements were taken simultaneously using an 8-min protocol (3-min mixing, 2-min wait, 3-min measure): 12 measurements were taken from each well; 3 times baseline prior to injection of glucose (10 mM), 3 following glucose and prior to injection of oligomycin (1 μM), 3 following oligomycin and prior to injection of 2-deoxy glucose (100 mM), and 3 following injection of 2-deoxyglucose. Basal glycolysis was calculated by subtracting the average ECARs before, and after, the injection of glucose. ATP-linked mitochondrial respiration was calculated by subtracting the average OCRs before and after the injection of oligomycin. After assay, cells were washed with PBS and protein was quantified to for normalization. Additional experiments used Endo HPMCs cultured for 16 h in HOSE 1, then switched to serum-free HOSE 1 containing vehicle (water), TGF-β1 (2 ng/mL, 240-B-010; R&D Systems), DCA (15 mM, 347795; Sigma), or TGF-β1+DCA, and incubated for 48 h before analysis of ECAR and OCR.

Lactate and PDH Activity. Lactate concentrations were determined using an enzymatic colorimetric kit (Alpha Laboratories) on a Cobas Fara centrifugal analyzer (Roche Diagnostics). PDH enzyme activity was measured using a Dipstick assay kit (ab109882; Abcam): band intensity was analyzed using ImageJ.

"Lesion in a Dish" Coculture System. Endo HPMCs were plated in 24-well plates at 1×10^5 cells per well (lower chamber); SHT290 were plated on Millicell-cell culture inserts (2×10^4 per insert; pore size, 0.4 μm; Millipore) (upper chamber) in serum-free HOSE I and RPMI 1640, respectively. After 16 h, HPMCs were incubated with vehicle (water), DCA (15 mM), TGF-β1 (2 ng/mL), or DCA+TGF-β1 for 48 h. Lactate concentrations were measured, and proliferation of SHT290 cells was determined using CellTiter96 Aqueous-one solution reagent (G3580; Promega).

Mouse Model of Endometriosis. Animal procedures were performed in accordance with UK Home Office regulations (license 70/8945). A preclinical model of endometriosis was as in ref. 4 with the minor modification that recipient mice were not ovariectomized or treated with E2. After 2 wk, mice were randomly assigned to receive vehicle (water) or DCA (100 mg/kg) daily via oral gavage for 7 d (n = 10/group). PF was recovered by lavage (2 mL of ice-cold PBS); endometriosis lesions were fixed in 4% neutral-buffered formalin, lactate was measured in PF, sections of lesions were stained with H&E, and the area was measured using ImageJ software.

Statistical Analysis. Results are expressed as mean ± SEM of a minimum of 3 independent experiments. Statistics were generated using GraphPad PRISM, version 6. Data were analyzed using one-way ANOVA Mann–Whitney U test, 1-way ANOVA Kruskal–Wallis test, and Dunn's multiple-comparisons test, as appropriate.

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