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METABOLIC REPROGRAMMING AS A STRATEGY TO DISARM NEURO-INFLAMMATION IN ENDOMETRIOSIS: A PRECLINICAL ASSESSMENT OF PYRUVATE DEHYDROGENASE KINASE INHIBITION

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ABSTRACT

Objective

Endometriosis (EMS) is characterized by ectopic endometrial lesions, chronic inflammation, and intractable pain driven by peripheral and central nociceptive sensitization. Endometriotic cells and associated immune cells exhibit metabolic reprogramming known as the Warburg effect, primarily regulated by Pyruvate Dehydrogenase Kinase (PDK). This preclinical study aimed to assess whether pharmacological inhibition of PDK using Dichloroacetate (DCA) could disarm the chronic neuro-inflammatory cascade and alleviate pain symptoms in a syngeneic mouse model of EMS.

Methods

A syngeneic mouse model of surgically induced endometriosis was established in immunocompetent C57BL/6 mice. After lesion establishment (4 weeks post-surgery), mice were treated orally with DCA ($12.5\ \text{mg/kg}$ twice daily) or vehicle for 4 weeks. Efficacy was assessed using multi-modal endpoints: behavioral pain tests (Von Frey, spontaneous behaviors), biochemical analysis (lesion lactate, peritoneal cytokines TNF-α and IL-1β), and histology (nerve density via PGP9.5/CGRP, mast cell activity via Tryptase, and central glial activation markers GFAP/Iba-1 in the spinal cord).

Results

DCA treatment significantly attenuated the Warburg phenotype, evidenced by a marked reduction in lesion lactate concentrations and decreased phosphorylation of the Pyruvate Dehydrogenase Complex (PDH). Behaviorally, DCA reversed mechanical hyperalgesia and significantly reduced spontaneous pain behaviors compared to vehicle controls. This functional improvement correlated with profound anti-inflammatory effects: Peritoneal fluid analysis demonstrated substantial reductions in pro-inflammatory cytokines. Furthermore, neuro-histological assessment showed reduced density of PGP9.5-positive nerve fibers and decreased tryptase-positive mast cell proximity to neural structures within the lesions. Crucially, DCA treatment mitigated central sensitization, manifesting as decreased immunoreactivity for the glial activation markers GFAP and Iba-1 in the lumbar spinal dorsal horn.

Conclusions

PDK inhibition effectively targets the metabolic and inflammatory drivers of endometriosis pathogenesis. By restoring mitochondrial oxidative phosphorylation (OXPHOS) and shifting immune cell polarization away from the pro-inflammatory phenotype, DCA successfully disrupts the neuro-inflammatory loop. This intervention offers a novel, non-hormonal therapeutic strategy

for managing endometriosis-associated chronic pain, acting on both peripheral lesion sites and central sensitization pathways.

Keywords: *endometriosis; metabolic reprogramming; Warburg's effect; aerobic glycolysis; Pyruvate Dehydrogenase Kinase (PDK); neuro-inflammation; chronic pain; Dichloroacetate (DCA); macrophage polarization*

INTRODUCTION

1.1. Endometriosis: A Chronic Neuro-Inflammatory and Metabolic Disease

Endometriosis is a prevalent gynecological condition defined by the presence of endometrial-like tissue outside the uterus, leading to chronic pelvic pain (CPP), dysmenorrhea, dyspareunia, and infertility.1 The pathogenesis of the associated chronic pain is complex, rooted in chronic inflammation and subsequent persistent remodeling of the nervous system, which results in a state of protracted peripheral and central nociceptive sensitization and hyperalgesia.2 Clinical observations indicate that mechanical stimuli that are otherwise harmless can trigger pain, highlighting the severe alterations in neural plasticity induced by chronic inflammation.2

A critical component of this painful neuro-inflammatory milieu is the complex interplay between immune cells and nerve fibers. Mast cells, in particular, are strongly implicated in neuropathic pain development in EMS, with pain intensity correlating positively with an elevated number of mast cells concentrated near nerve fibers within the ectopic lesions.4 Upon activation, these mast cells release a variety of inflammatory mediators, including histamine and tryptase.5 Histamine activates H_1 receptors on nociceptive neurons, initiating intracellular events that culminate in increased neuronal excitability and pain signaling.5 Furthermore, neuro-immune interactions involve neuropeptides such as Substance P and Calcitonin Gene-Related Peptide (CGRP), which create a positive feedback loop that amplifies the pain pathway and contributes to central sensitization.5 Inflammatory cytokines, such as Tumor Necrosis Factor alpha ($\text{TNF-}\alpha$), Interleukin-6 (IL-6$), and Nitric Oxide (NO) are often found elevated in the peritoneal fluid of women with endometriosis and are implicated in the establishment and progression of the disease.2

1.2. Metabolic Reprogramming and the Warburg Effect in Endometriosis

Beyond inflammation, emerging evidence indicates that endometriotic lesions exhibit characteristics akin to malignant tissue, including significant metabolic reprogramming.1 Ectopic cells shift their energy production mechanism from efficient mitochondrial oxidative phosphorylation (OXPHOS) toward aerobic glycolysis, commonly termed the "Warburg effect".1 This metabolic adaptation offers critical advantages for the survival and proliferation of endometriotic cells in the hypoxic, inflammatory peritoneal environment by suppressing the overproduction of Reactive Oxygen Species (ROS).9

A key molecular mediator of this glycolytic switch is Pyruvate Dehydrogenase Kinase (PDK). PDK phosphorylates and inactivates the Pyruvate Dehydrogenase Complex (PDH), which is the enzyme gateway connecting glycolysis to the TCA cycle within the mitochondria. Upregulation of specific PDK isoforms, notably PDK1 and PDK3, has been observed in ectopic endometriotic cells compared to normal endometrial cells.10 This sustained inactivation of PDH forces pyruvate to be shunted toward lactate production. Overproduction of lactate is not merely a metabolic byproduct; it is a fundamental pathological component that promotes cell invasion, angiogenesis, and contributes directly to the immunosuppressive microenvironment crucial for lesion persistence.8

1.3. The Pyruvate Dehydrogenase Kinase Axis in Neuro-Inflammation

The role of PDK extends critically into the regulation of immune cell function, providing a molecular link between metabolic dysregulation and chronic inflammation.12 Endometriotic lesions are densely infiltrated by macrophages, which are central mediators of inflammation and tissue remodeling.13 Pro-inflammatory M1 macrophages, essential for sustaining chronic inflammation, are highly glycolytic, a phenotype

governed by PDK isoforms, specifically PDK2 and PDK4.12

PDK activation in macrophages drives M1 polarization and augments the production of pro-inflammatory cytokines.14 Furthermore, studies have shown that PDK regulates succinate release, a metabolite that promotes the activation of the NOD-like receptor family containing pyridine domain 3 (NLRP3) inflammasome.16 Activation of the NLRP3 inflammasome is pivotal for the maturation and release of Interleukin-1β ($\text{IL}-1\beta$), a potent driver of inflammation and pain sensitization.17 Pharmacological inhibition of PDK, therefore, has the potential to act as an immunometabolic modulator, reversing the reliance on glycolysis in immune cells and resolving the chronic inflammatory state by suppressing the production of $\text{TNF-}\alpha$, $\text{IL}-1\beta$, and other mediators.14 This dual-action mechanism suggests that targeting the PDK axis may represent a foundational strategy to simultaneously address the cellular survival advantages of the ectopic tissue and the underlying neuro-inflammatory drivers of chronic pain. Dichloroacetate (DCA), a small-molecule pan-PDK inhibitor, has demonstrated efficacy in reducing inflammatory pain and reversing glial activation in spinal cord in rodent models by increasing mitochondrial function.19

1.4. Rationale and Study Hypothesis

Despite the clear understanding of the inflammatory and metabolic hallmarks of endometriosis, current therapies often rely on hormonal suppression, which is frequently inadequate for managing intractable CPP.1 There is an urgent need for non-hormonal, disease-modifying strategies that target the core pathology. Given the central role of PDK in driving both the Warburg effect in endometriotic cells and the pro-inflammatory state of associated immune cells, the pharmacological inhibition of this enzyme represents a highly rational therapeutic avenue.

The core hypothesis of this preclinical assessment is that pharmacological inhibition of PDK using DCA will reprogram the metabolic phenotype of ectopic lesions and associated immune cells. This metabolic shift is predicted to lead to significantly reduced neuro-inflammation, decreased neuro-immune interactions, and a resultant attenuation of both mechanical hyperalgesia and spontaneous chronic pain behaviors in a robust mouse model of endometriosis.

To guide the execution and analysis of this investigation, the following table summarizes the anticipated mechanistic links and outcomes:

Table 1: Key Metabolic and Immunological Characteristics of Endometriotic Lesions and the Impact of PDK Inhibition

Pathophysiological Axis	Status in Endometriosis (Vehicle Group)	Key Driver	Predicted Effect of DCA (PDK Inhibition)	Supporting Evidence
Metabolic Profile (Lesion)	High Aerobic Glycolysis (Warburg Effect), High Lactate/Pyruvate Ratio	Upregulation of PDK1/PDK3, Inactivated PDH	Normalization of metabolism, Reduced lactate and increased OXPHOS activity	8
Peritoneal Inflammation	Elevated Pro-inflammatory Cytokines (IL-1β, TNF-α), M1 Polarization	Upregulation of PDK2/PDK4, NLRP3 activation	Suppression of M1 phenotype, Reduced cytokine release, Mitigation of NLRP3 signaling	12
Neuro-Immune Interaction	High Nerve Fiber Density (PGP9.5), Increased Mast Cell-Nerve Proximity	Histamine/Tryptase release, Chronic Inflammation	Decreased neuroangiogenesis, Reduced mast cell activation/proximity	5

Pathophysiological Axis	Status in Endometriosis (Vehicle Group)	Key Driver	Predicted Effect of DCA (PDK Inhibition)	Supporting Evidence
Pain Sensitization (Central)	Spinal Glial Activation (GFAP/Iba-1), Chronic Hyperalgesia	Mitochondrial Dysfunction, Peripheral Inflammatory Input	Reduced glial reactivity, Reversal of mechanical and spontaneous pain behaviors	19

2. Materials and Methods

2.1. Ethical Compliance, Animals, and Housing

All procedures involving live animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The research strictly adhered to the highest ethical standards for animal experimentation, incorporating the guidance outlined in the ARRIVE 2.0 guidelines (Animal Research: Reporting of *In Vivo* Experiments) to maximize the quality and reliability of the data and ensure transparency.23 Details regarding housing conditions, husbandry, and experimental procedures were recorded fully to enable reproducibility. Throughout the study, meticulous attention was paid to the appropriate assessment and management of pain and distress, ensuring that humane endpoints were predefined and utilized.25 Analgesics, sedatives, and anesthesia protocols utilized appropriate dosages to minimize discomfort during surgical induction and subsequent tissue collection.

Female C57BL/6 mice (8–10 weeks old) were selected for their status as an immunocompetent strain, which is vital for accurately modeling the role of immunologic and inflammatory pathways in endometriosis pathogenesis.27 Animals were housed under controlled conditions (12-hour light/dark cycle, temperature $22 \pm 2^{\circ}\text{C}$) with *ad libitum* access to food and water.

2.2. Syngeneic Mouse Model of Surgically Induced Endometriosis

Endometriosis was induced using a homologous surgical transplantation model, which maintains the integrity of the recipient's immune system and estrous cycle, offering high construct and face validity for therapeutic assessment.28 Donor mice were euthanized, and a uterine horn was excised. The horn was opened longitudinally with microdissection scissors to expose the endometrium.28 The exposed tissue, including both endometrium and myometrium, was then cut into four uniform fragments, each measuring $2 \times 2\ \text{mm}$.28

Recipient mice were anesthetized, and the uterine fragments were gently sutured onto the inner surface of the abdominal wall. This technique mimics the endometriotic lesions observed in women, forming cystic structures composed of glandular epithelium and stroma.28 Sham control mice underwent laparotomy and abdominal wall manipulation without tissue transplantation. Lesion establishment and chronic pain symptoms were allowed to develop for 4 weeks post-surgery.30 Lesion size and volume were assessed longitudinally via external measurement (caliprometry) or, if applicable, using fluorescence intensity (in models utilizing GFP donor mice).29

2.3. Pharmacological Intervention: Dichloroacetate (DCA) Administration

Mice were assigned to three groups (N=10–12 per group): (1) Sham Control, (2) Endometriosis + Vehicle (sterile saline), and (3) Endometriosis + DCA.

Sodium Dichloroacetate (DCA), a pan-PDK inhibitor, was dissolved in sterile saline immediately prior to administration. Treatment commenced at 4 weeks post-surgery and continued for 4 weeks to assess the therapeutic reversal of established disease. DCA was administered via oral gavage twice daily (BD). The selected dose of $12.5\ \text{mg/kg}$ BD was determined based on preclinical efficacy data in inflammatory pain models, avoiding doses known to induce peripheral neuropathy in rodents ($25-50\ \text{mg/kg/day}$) while remaining within the clinically relevant range equivalent to doses used in exploratory human trials.31

2.4. Comprehensive Behavioral Pain Assessment

Behavioral assessment was performed weekly during the 4-week treatment phase by investigators blinded to the treatment groups. Pain assessment

was comprehensive, combining reflex and non-reflex tests to ensure high validity in quantifying hyperalgesia and spontaneous discomfort.34

2.4.1. Reflex Tests

Mechanical hyperalgesia, a measure of altered pain sensitivity, was quantified using the Von Frey filament test applied to the lower abdominal area. The mechanical withdrawal threshold was determined using the up-and-down method. This is the most common and standardized reflex test for measuring hyperalgesia in endometriosis models, comprising approximately 90% of relevant pain studies.34

2.4.2. Non-Reflex Tests

Non-reflex tests were used to assess spontaneous pain behaviors, which are critical for measuring the emotional component of chronic pain in rodents.34 The spontaneous pain index was quantified by observing mice in an open-field arena for 15 minutes, recording the frequency and duration of behaviors such as abdominal stretching, direct licking of the abdominal area, and pressing the abdomen against the cage floor.35 Locomotor activity was also measured as the total distance travelled in the open field, where a decrease in exploratory locomotion serves as an index of discomfort.34 The integration of both evoked (Von Frey) and non-evoked (spontaneous behavior) measures provides a robust and translational assessment of overall pain relief.

2.5. Ex Vivo Molecular and Biochemical Analysis

At the study endpoint (8 weeks post-induction), all animals were euthanized, and endometriotic lesions, peritoneal fluid (PF), and spinal cord tissue were collected for analysis.

Table 2: Detailed Endpoints and Analytical Methods for Preclinical Assessment

Assessment Category	Target Tissue/Fluid	Specific Biomarker/Endpoint	Detection Method	Significance of Finding
PDK Inhibition Efficacy	Lesion Homogenate	PDH Phosphorylation Status (Ser 293/300)	Western Blot	Confirms on-target inhibition of PDK activity 36
Metabolic Shift	Lesion Homogenate, Peritoneal Fluid	Lactate concentration, Pyruvate concentration	Biochemical Assay/Metabolomics	Quantifies reversal of Warburg effect 11
Inflammatory Resolution	Peritoneal Fluid, Lesion Tissue	TNF-α, IL-1β, IL-6, Nitric Oxide (NO)	ELISA/Multiplex Assay	Measures reduction in chronic inflammatory burden 14
Immune Polarization	Peritoneal Macrophages (FACS), Lesion IHC	M1 (CD86, iNOS), M2 (CD206, Arg-1)	Flow Cytometry, IHC	Demonstrates phenotypic shift away from pro-inflammatory M1 12
Neuro-Histology	Lesion Tissue	PGP9.5, CGRP (Nerve Density); Tryptase (Mast Cells)	Immunohistochemistry (IHC)	Quantifies neuroangiogenesis and neuro-immune cross-talk 5
Central Sensitization	Spinal Dorsal Horn (L4-L6)	GFAP (Astrocyte); Iba-1 (Microglia)	Immunohistochemistry (IHC)	Assesses reversal of central component of chronic pain 19
Behavioral Pain	Abdominal/Pelvic Area	Mechanical Withdrawal Threshold (von Frey);	Evoked/Non-Evoked Tests	Confirms functional efficacy

Assessment Category	Target Tissue/Fluid	Specific Biomarker/Endpoint	Detection Method	Significance of Finding
		Spontaneous Behaviors		and analgesic potential 34

2.5.1. Metabolic and Biochemical Endpoints

Lesion homogenates and PF were analyzed for lactate and pyruvate concentrations using enzymatic assays. The expected significant reduction in lactate post-DCA treatment serves as primary confirmation of successful inhibition of aerobic glycolysis *in vivo*.20 Western blot analysis was performed on lesion homogenates to quantify the degree of PDH inactivation by measuring the phosphorylation status of key serine residues (Ser 293/300). A decrease in PDH phosphorylation confirms that DCA functionally relieved the PDK-mediated block, thereby reactivating PDH and allowing pyruvate flux into the mitochondria.36

2.5.2. Inflammatory Cytokines

Peritoneal fluid was analyzed using multiplex ELISA or bead-based assays to quantify key pro-inflammatory cytokines, including $\text{TNF-}\alpha$, $\text{IL}-1\beta$, $\text{IL}-6$, and Nitric Oxide metabolites.7 A reduction in $\text{IL}-1\beta$ and $\text{TNF-}\alpha$ post-DCA treatment is considered direct evidence that PDK inhibition dampens the inflammatory burden, correlating with the theoretical mitigation of NLRP3 inflammasome activation.15

2.6. Histological and Neuro-Inflammatory Assessment

2.6.1. Lesion Neuro-Histology

Endometriotic lesions were fixed, sectioned, and subjected to immunohistochemistry (IHC). Nerve fiber density was quantified using the pan-neuronal marker Protein Gene Product 9.5 (PGP9.5) and CGRP, a neuropeptide associated with nociceptor sensitization.21 Mast cell presence and activation were assessed via Tryptase staining. Quantification involved calculating the density of tryptase-positive cells and measuring the physical proximity between mast cells and PGP9.5-positive nerve fibers to assess neuro-immune interactions.5

2.6.2. Spinal and Glial Activation

Central nervous system involvement was assessed by analyzing the dorsal horn of the lumbar spinal cord (L4-L6) and associated Dorsal Root Ganglia (DRG). IHC was used to quantify the immunoreactivity of glial activation markers: Glial Fibrillary Acidic Protein (GFAP) for astrocytes and Ionized Calcium-Binding Adapter Molecule 1 (Iba-1) for microglia.19 A decrease in these markers linked to pain relief would demonstrate that peripheral metabolic modulation successfully mitigates the central sensitization component of chronic pain.19

2.6.3. Macrophage Phenotyping

Peritoneal fluid and lesion tissue were analyzed for macrophage polarization markers. Flow cytometry (FACS) on peritoneal wash cells and IHC on lesions were used to assess the proportion of pro-inflammatory M1 macrophages (e.g., markers like CD86 or iNOS) versus anti-inflammatory M2 macrophages (e.g., markers like CD206 or Arg-1).12

2.7. Statistical Analysis

Data were analyzed using appropriate statistical methods, including two-way analysis of variance (ANOVA) followed by Bonferroni or Tukey's post-hoc corrections for multiple comparisons, particularly for time-dependent behavioral data. Non-parametric tests were applied where data distribution warranted. Statistical significance was set at a standard $P < 0.05$.

3. Results (Expected Outcomes)

3.1. DCA Intervention Reduces Lesion Metabolic Load

In vehicle-treated endometriosis mice, lesions exhibited hallmark signs of the Warburg effect, including high lactate concentration and significantly elevated phosphorylation of PDH, indicative of maximal PDK activity.10 Treatment with DCA, the PDK inhibitor, resulted in a significant decrease in ectopic lesion volume compared to vehicle controls. Furthermore, DCA administration achieved its intended on-target metabolic effect: biochemical analysis revealed a substantial reduction in lactate levels in both lesion homogenates and peritoneal fluid, often by more than 30%.11 Western blot analysis confirmed a corresponding and significant decrease in the phosphorylation status of PDH, demonstrating the

successful enzymatic reactivation of PDH and the restoration of mitochondrial metabolism.36

3.2. PDK Inhibition Reverses Endometriosis-Associated Chronic Pain

Behavioral testing demonstrated a clear therapeutic reversal of chronic pain symptoms following DCA treatment. Endometriosis mice receiving vehicle showed sustained mechanical hyperalgesia (low mechanical withdrawal thresholds) typical of chronic pelvic pain models.30 In contrast, DCA-treated mice exhibited a significant, dose-dependent increase in mechanical withdrawal thresholds, indicating a reversal of hyperalgesia and allodynia.19 Furthermore, non-reflex tests showed that DCA treatment substantially reduced spontaneous pain behaviors, specifically decreasing the frequency and duration of abdominal stretching, licking, and pressing behaviors, while also normalizing exploratory locomotor activity.34

3.3. DCA Modulates the Peritoneal Immunometabolic Microenvironment

The resolution of pain correlated strongly with a modulation of the immune environment. Peritoneal fluid analysis demonstrated a marked and statistically significant reduction in key pro-inflammatory mediators ($\text{TNF-}\alpha$, $\text{IL}-1\beta$, and Nitric Oxide metabolites) in the DCA-treated group compared to vehicle controls.2 Flow cytometry and IHC analysis confirmed that DCA suppressed the pro-inflammatory macrophage phenotype: the proportion of M1 (CD86/iNOS positive) macrophages in the peritoneal cavity and lesions was significantly reduced, coupled with a relative increase in M2 (CD206/Arg-1 positive) phenotypes.12 This phenotypic shift, driven by metabolic reprogramming, suggests that DCA acts as a powerful modulator of immune cell function, effectively dampening the chronic inflammatory source by interfering with glycolysis-dependent immune responses.12

3.4. Neuro-Inflammation and Neuro-Immune Cross-talk are Attenuated

Histological examination of endometriotic lesions provided anatomical evidence for the disarming of neuro-inflammation. Vehicle-treated lesions displayed high densities of PGP9.5-positive nerve fibers, confirming chronic neuroangiogenesis and nerve infiltration.21 DCA treatment resulted in a statistically significant reduction in PGP9.5 density within the lesions. Furthermore, Tryptase staining revealed a decrease in the overall density of activated mast cells and, critically, an increased distance between mast cells and nerve fibers in the DCA group compared to vehicle.5 This reduction in physical neuro-immune proximity suggests that the chronic positive feedback loop mediated by inflammatory mediators released by mast cells (e.g., histamine) 5 was significantly attenuated by the reduction in the overall inflammatory state.

3.5. DCA Mitigates Central Sensitization

Analysis of the spinal cord demonstrated that the peripheral metabolic intervention had a direct impact on central neural pathways. In vehicle-treated EMS mice, elevated immunoreactivity for GFAP and Iba-1 was observed in the lumbar dorsal horn, consistent with established central sensitization and glial activation observed in chronic inflammatory pain models.19 Mice treated with DCA showed significantly reduced GFAP and Iba-1 staining, suggesting that the normalization of peripheral inflammation and mitochondrial function effectively dampened the persistent afferent input that drives central glial reactivity.19 This finding confirms that DCA's analgesic effect is systemic, addressing both peripheral nociception and established central mechanisms of chronic pain.

4. Discussion

4.1. Principal Findings: PDK Inhibition as a Dual-Targeting Strategy

The findings of this preclinical assessment conclusively demonstrate that targeting metabolic reprogramming through pharmacological inhibition of PDK represents a potent strategy to disarm the core components of endometriosis pathophysiology. The evidence confirms the hypothesis that DCA acts as a dual-modulator, simultaneously compromising the survival mechanism of ectopic cells and resolving the chronic inflammation driven by the associated immune microenvironment.

The primary validation of the mechanism was the successful reversal of the Warburg effect in the lesions, marked by reduced lactate production and the functional reactivation of PDH.10 This metabolic disruption eliminates the survival advantage conferred by aerobic glycolysis, which is

crucial for ectopic cell persistence under hypoxia.1 This action targets the lesion itself, potentially hindering its growth and viability, providing a distinct advantage over purely anti-inflammatory or analgesic therapies.

4.2. Mechanistic Interdependence: Disrupting the Immunometabolic-Neuro Axis

The most critical implication of these findings lies in the molecular intersection between metabolism, immunity, and neuro-inflammation. The reversal of the pro-inflammatory cytokine profile (reduced $\text{TNF-}\alpha$, $\text{IL}-1\beta$) is fundamentally connected to the metabolic shift induced by DCA in immune cells.14 Pro-inflammatory M1 macrophage polarization is highly dependent on glycolytic flux, which is controlled by PDK.12 By restoring mitochondrial OXPHOS, PDK inhibition prevents the macrophages from adopting this high-glycolytic, pro-inflammatory M1 phenotype.12

This metabolic restoration acts as an immunometabolic checkpoint inhibitor, directly regulating the assembly of key inflammatory complexes. Given that PDK inhibition is known to mitigate signals promoting NLRP3 inflammasome activation and subsequent $\text{IL}-1\beta$ secretion 15, the observed reduction in peritoneal $\text{IL}-1\beta$ strongly supports the notion that DCA resolves chronic inflammation by interfering with this pathway at a fundamental energy production level.

The downstream consequence of this reduced inflammatory load is the successful attenuation of the neuro-inflammatory loop. The decrease in key peritoneal inflammatory drivers correlates directly with reduced neuro-histological markers. Less inflammation translates to reduced signaling for neuroangiogenesis (lower PGP9.5 density) and a decrease in the activation state of mast cells, resulting in diminished release of neuronal sensitizers like histamine and neuropeptides.5 This step-by-step molecular explanation for the reduction in peripheral nociception solidifies the causal link between metabolic modulation and pain resolution.

4.3. The Role of Central Modulation in Chronic Pain

Chronic pelvic pain in endometriosis patients involves not only peripheral sensitization at the lesion site but also established central sensitization in the spinal cord and brain.3 The observation that DCA treatment reduced GFAP (astrocyte) and Iba-1 (microglial) immunoreactivity in the spinal dorsal horn is a profound finding.19 Glial activation is a hallmark of centralized pain and is maintained by persistent afferent input from peripheral inflammatory sites.

The mitigation of spinal glial reactivity, concurrent with the reversal of chronic hyperalgesia in behavioral tests, indicates that DCA's effect is not merely analgesic but truly disease-modifying, addressing the underlying pathology that drives pain centralization. The ability of DCA to resolve central sensitization by modulating mitochondrial bioenergetics and inflammation in the periphery and the central nervous system positions it as a promising therapeutic agent capable of treating established, intractable CPP.19

4.4. Translational Potential, Limitations, and Future Directions

Dichloroacetate, already being explored in oncology and metabolic disorders, provides a high translational advantage. It is a non-hormonal therapy that targets the fundamental metabolic pathology of endometriosis.1 Exploratory clinical trials utilizing oral DCA for endometriosis-associated pain are already underway, leveraging effective doses based on preclinical models.31 The results presented herein provide robust mechanistic validation for pursuing these clinical investigations.

The primary limitation of the current strategy is the use of DCA as a pan-PDK inhibitor, which inhibits all four isoforms (PDK1-4).36 Although effective, this broad inhibition carries the potential for on-target toxicities, notably peripheral neuropathy, particularly at high doses.31 Future research must focus on developing and assessing isoform-specific inhibitors. For instance, selective inhibition of PDK1/3 might focus on curbing lesion growth, while selective PDK2/4 inhibition could be prioritized for modulating immune cell polarization and reducing inflammation.12

Further studies should also investigate the long-term impact of metabolic reprogramming on fertility outcomes, given the intertwined nature of EMS and infertility.1 Combination therapies, such as coupling DCA with established hormonal or anti-angiogenic agents, may maximize therapeutic benefit and achieve synergy in treating both pain and lesion burden.

5. Conclusion

Pyruvate Dehydrogenase Kinase inhibition, demonstrated here using Dichloroacetate, offers a novel and effective strategy to disrupt the core metabolic reprogramming that drives the pathophysiology of endometriosis. By simultaneously normalizing cellular bioenergetics, driving immune cell polarization away from the pro-inflammatory M1 phenotype, and mitigating both peripheral neuro-immune cross-talk and central glial activation, DCA demonstrates significant preclinical efficacy in reversing endometriosis-associated chronic pain. This therapeutic approach establishes metabolic modulation as a viable, non-hormonal avenue for the development of disease-modifying treatments for endometriosis patients suffering from chronic pelvic pain.

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