

Neuroprotective Effects and Therapeutic Potential of Dichloroacetate: Targeting Metabolic Disorders in Nervous System Diseases

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Abstract: Dichloroacetate (DCA) is an investigational drug used to treat lactic acidosis and malignant tumours. It works by inhibiting pyruvate dehydrogenase kinase and increasing the rate of glucose oxidation. Some studies have documented the neuroprotective benefits of DCA. By reviewing these studies, this paper shows that DCA has multiple pharmacological activities, including regulating metabolism, ameliorating oxidative stress, attenuating neuroinflammation, inhibiting apoptosis, decreasing autophagy, protecting the blood–brain barrier, improving the function of endothelial progenitor cells, improving mitochondrial dynamics, and decreasing amyloid β -protein. In addition, DCA inhibits the enzyme that metabolizes it, which leads to peripheral neurotoxicity due to drug accumulation that may be solved by individualized drug delivery and nanovesicle delivery. In summary, in this review, we analyse the mechanisms of neuroprotection by DCA in different diseases and discuss the causes of and solutions to its adverse effects.

Keywords: dichloroacetate, neuroprotection, mitochondria, energy metabolism, oxidative stress

Introduction

Neurons are necessary for the brain to perform its normal physiological functions. However, both internal and external factors can damage neurons, which ultimately results in their death.¹ Many neurological diseases, such as brain injury and neurodegenerative diseases, cause neuronal damage; therefore, reducing or preventing neuronal damage is especially important in treating or alleviating these diseases.^{2–5} Currently, many neuroprotective drugs have shown an extraordinary ability to prevent neuronal damage by mechanisms such as limiting mitochondrial dysfunction, oxidative stress, neuroinflammation, apoptosis, excitotoxicity, and protein misfolding, which are leading therapeutic strategies for these diseases.^{6–8}

Dichloroacetate (DCA) inhibits all four pyruvate dehydrogenase kinase (PDK) isoforms found in vivo.^{9,10} PDK phosphorylates three serine residues (Ser293, Ser300, and Ser232) of the α -subunit of pyruvate dehydrogenase (PDH) to cause a decrease in PDH activity.^{11,12} PDH is responsible for the irreversible transformation of pyruvate into acetyl coenzyme A, which subsequently engages in the tricarboxylic acid (TCA) cycle to produce energy.¹³ Thus, DCA promotes the mitochondrial oxidation of pyruvate and increases the rate of glucose oxidation, thereby improving tissue energy metabolism (Figure 1).¹⁴ This allows DCA to reverse the Warburg effect in cancer cells, making it a promising anticancer drug that targets cancer cell metabolism.^{15,16} A large number of studies have been conducted to explore the antitumour effects of DCA

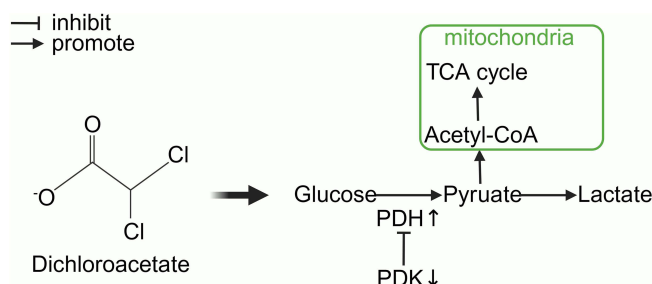


Figure 1 The role of DCA in metabolism.

Notes: DCA enhances PDH indirectly by inhibiting PDK, thereby enhancing mitochondrial oxidation of glucose. Created with BioRender.com.

Abbreviations: DCA, dichloroacetate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; TCA, tricarboxylic acid.

or combination therapies of DCA with radiotherapy, chemotherapy, and immunotherapy, which have been developed in phase II clinical trials, in a variety of cancers.^{17–22} Additionally, DCA has also been used as an investigational drug for lactic acidosis, pulmonary arterial hypertension, sepsis-induced hepatic metabolic dysfunction, and cardiac dysfunction, and a phase III clinical trial involving children with congenital lactic acidosis (NCT00004490) has been completed.^{23–26}

Mitochondria assume a crucial role in upholding cellular physiological activities, encompassing significant functions such as oxidative phosphorylation, phospholipid biogenesis, calcium homeostasis maintenance, and apoptosis regulation.²⁷ Neurons exhibit elevated energy metabolic demands, as the brain accounts for 20% of the body's resting adenosine triphosphate (ATP) production, despite comprising merely 2% of its overall mass.²⁸ If mitochondrial metabolism is disrupted, severe damage to neurons is likely.¹³ Disruption of mitochondrial metabolism is considered an important cause of many neurological diseases, such as stroke and neurodegenerative diseases.^{29–31} Currently, DCA has been shown to modify mitochondrial metabolism in the pathophysiology of several neurological diseases, including ischaemic stroke, perinatal asphyxia-induced hypoxic-ischaemic brain injury, transient cerebral ischaemia, cardiac arrest brain injury, subarachnoid haemorrhage, vascular dementia, hypoglycaemic brain injury, traumatic brain injury, epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, and Parkinson's disease (Table 1). This review comprehensively examines the latest research on the neuroprotective properties of DCA and discusses the molecular mechanisms through which DCA mitigates neuronal death in various neurological illnesses within the context of the corresponding metabolic disorders. Additionally, we discuss some of the newer understandings of the pharmacological effects of DCA. Finally, we discuss the limitations and possible counter-measures of DCA found in clinical trials in other diseases.

Neuroprotective Effects and Mechanisms of Dichloroacetate

The Effects of Dichloroacetate on the Brain in Different Injuries

Ischaemia–Reperfusion Injury

Ischaemia–reperfusion injury (I/RI) is a term used to describe the occurrence of heightened tissue damage and metabolic disturbances following the restoration of blood reperfusion to partially ischaemic tissue.⁵³ Ischaemic stroke, perinatal asphyxia-induced hypoxic-ischaemic brain injury (HIE), cardiac arrest brain injury, and transient cerebral ischaemia are among the diseases in which I/RI plays a role in the pathological processes.⁵⁴ Insufficient oxygen and glucose supply to brain tissue limits aerobic metabolism and ATP production, which leads to lactate accumulation and ion transport dysfunction, triggering cellular and mitochondrial calcium overload and ultimately leading to neuron death.^{54–56} More importantly, PDH activity decreases during I/RI.⁵⁷ DCA has been shown to enhance PDH activity, increase ATP and phosphocreatine levels, and reduce lactate accumulation in the brain, thus reducing lactic acidosis during I/RI.^{32–35} Peeling et al found that administration of DCA (100 mg/kg i.v.) to rats with normal forebrain ischaemia restored brain pH, lactate, ATP, and phosphocreatine levels after reperfusion, thereby reducing hippocampal neuronal damage.³⁶

Oxidative stress is an important cause of I/RI; moreover, reactive oxygen species (ROS) exacerbate mitochondrial dysfunction and impair cellular structure and function, resulting in a vicious cycle.⁵⁸ DCA was shown to reduce oxidative stress in subsequent studies.^{37,38} In middle cerebral artery occlusion mice, the embolus was loosened, and DCA (100 mg/kg, 200 mg/kg) was injected 90 min after embolization, which showed that DCA could activate the nuclear factor erythroid

Table I Neuroprotective effects of Dichloroacetate

Disease	Bioactivity	Mechanism	Animals	The Dosage of DCA	References
Ischaemic Stroke, Perinatal Asphyxia-induced Hypoxic Ischaemic Brain Injury, Transient Ischaemia, Cardiac Arrest Brain Injury	Improve metabolism	Enhance PDH activity, increase adenosine triphosphate and creatine phosphate content in the brain, reduce lactate	Male SD rats	DCA (100 mg/kg i.v.), 30 min before ischaemia, 60 min after ischaemia during reperfusion	[32]
			Male Mongolian gerbils	DCA (2.3 mmol/kg i.p.), 30 min before ischaemia	[33]
			Male SD rats	DCA (5 ml/ kg i.v), 15 min before ischaemia or 1 hour after ischaemia	[34]
			Male rats	DCA (50 mg/kg)	[35]
			Male rats	DCA (100 mg/kg or 10 mg/kg i.v.), 10 min before ischaemia	[36]
			Male C57BL/6 mice	DCA (100 or 200 mg/kg), 60 min after ischaemia during reperfusion	[37]
			Human Brain Microvascular Endothelial Cells	DCA (2.5 or 5 or 10mmol/L) pretreatment for 6h	
			Male rats	DCA (100 mg/kg/d for two days i.p.), after reperfusion	[38]
			Male mice	DCA (100 mg/kg/d for three days i.p.), after hypoxia-ischaemia	[39]
			Brain-derived Endothelial cells (bEnd3)	DCA (30 μ mol/L) pretreatment for 12h	[40]
	Anti-oxidant	Up-regulation Nrf2 signaling pathway	ibid.		[37]
			ibid.		[38]
	Anti-neuroinflammatory	Reduce glial cells activation and production of inflammatory factors (tumor necrosis factor α and interleukin-1 β)	ibid.		[38]
		Upregulate CD206, downregulated inducible nitric oxide synthase and interleukin-6	Male SD rats	DCA (14.4 mg/kg), after the model was established for 2, 4 and 24h	[40]
			Primary mouse microglia	DCA (30 μ mol/L) pretreatment for 3h	
	Anti-apoptosis	Inhibit apoptosis-inducing factor and Caspase-3 activation	ibid.		[37,39]
	Reduce autophagy	Prevent the reduction of Sequestosome 1	ibid.		[39]
	Improve mitochondrial dynamics	Increase Pgc-1 α and nuclear respiratory factor 1 mRNA, but not mitochondrial DNA	ibid.		[39]
	Protect the BBB	Enhance the expression of Zonula Occludens Protein 1 and Occludin	ibid.		[37,38]

(Continued)

Table 1 (Continued).

Disease	Bioactivity	Mechanism	Animals	The Dosage of DCA	References
Subarachnoid Hemorrhage	Aggravate oxidative stress and Neuronal death	Inhibit the activity of PDK4, activate reactive oxygen species- apoptosis signal-regulating kinase I/ mitogen-activated protein kinase pathway	Male SD rats	DCA (100mg/kg/d for two days i.p.)	[41]
			Primary neurons	DCA (50 μ mol/L) pretreatment for 2 days.	
Vascular Dementia	Promote angiogenesis and improve endothelial progenitor cell function	Increase protein kinase B expression, decrease glycogen synthase kinase 3 β expression	Male rats	DCA (50 or 100 or 200 mg/kg for 21 days i.g.), 24h after reperfusion	[42]
	Anti-oxidant	Activate Nrf2 pathway			
Hypoglycemic Brain Injury	Improve metabolism	Reduce the level of PDK2, increase the level of PDH, reduce microglia and astrocyte Activation	Male rats	DCA (100 mg/kg/d for two days i.v.), after hypoglycemia	[43]
	Anti-oxidant				
	Anti-neuroinflammatory				
	Protect the BBB				
Traumatic Brain Injury	Improve metabolism	Enhance PDH activity, reduce lactate accumulation, no evaluation of the effects of DCA on brain damage	Male rats	DCA (200 mg/kg i.v.), after traumatic brain injury	[44]
Epilepsy	Improve metabolism	Reduce the level of PDK2, increase the level of PDH, reduce microglia and astrocyte activation	Male CD1 mice	DCA (50 mg/kg/day for 7 days) in drinking water before seizures; DCA (100 mg/kg/day for 14 days) after seizures	[45]
	Anti-oxidant		Male rats	DCA (100 mg/kg/d for one week)	[46]
	Anti-neuroinflammatory				
Anyotrophic Lateral Sclerosis	Reduce toxicity of aberrant glial cells	Improve mitochondrial metabolism, enhance PDH activity, reduce lactate	SOD1 ^{G93A} mice	DCA (100 mg/kg/d), add to drinking water, from 70 days old to death	[47]
			AbGCs (derived from SOD1 ^{G93A} rats)	DCA (0.5–5 mmol/L) pretreatment for 24h	
			Female SOD1 ^{G93A} rats	DCA (100mg/kg/d for 10 days), add to the drinking water	[48]
			AbGCs (isolated from the spinal cords of adult paralytic SOD1 ^{G93A} rats)	DCA (5 mmol/L) pretreatment for 24h	
	Modify metabolism in glycolytic muscles	Reduce the level of PDK4, enhance PDH activity, promote glycolysis and inhibit, inhibit fatty acid β -oxidation, increase Pgc1- α and mitochondrial fusion protein 2 levels	Male SOD1 ^{G86R} mice	DCA (500 mg/kg/d), add to drinking water, from 60 to 95 days of age	[49]
	Anti-oxidant				
	Improve mitochondrial dynamics				

(Continued)

Table 1 (Continued).

Disease	Bioactivity	Mechanism	Animals	The Dosage of DCA	References
Alzheimer's Disease	Reduce amyloid β -protein production	Inhibit amyloidogenic proteolysis of the amyloid precursor protein	Human neuroblastoma cells (SH-SY5Y)	DCA (10 or 20 mmol/L for 24h)	[50]
Huntington's Disease	Alleviate the striatal volume reduction	Enhance PDH activity	N171/82Q mice, R6/2 mice	DCA (100mg/kg/d), add to drinking water, from 4 weeks of age to death	[51]
Parkinson's Disease	Improve mitochondrial dynamics	Improve mitochondrial metabolism	Rat pheochromocytoma cells (PC12)	DCA (10mmol/L for 1h)	[52]

Abbreviations: PDH, pyruvate dehydrogenase; Nrf2, nuclear factor; PDK, pyruvate dehydrogenase kinase; PGC1- α , peroxisome proliferator-activated receptor gamma coactivator 1- α ; BBB, blood-brain barrier; i.v., intravenous injection; i.p., intraperitoneal injection; i.g., gastric lavage.

2-related factor 2 (Nrf2) pathway and reduce ROS production in the brains of mice, reducing the area of cerebral infarction.³⁷ Notably, at low oxidative stress levels, activation of Nrf2 activates downstream antioxidant pathways to exert cytoprotective effects, while excessive activation of Nrf2 stimulates the expression of Kruppel-like factor 9 (Klf9), which, conversely, increases the level of ROS.⁵⁹ Low doses of sulforaphane have been shown to exert antioxidant effects through activation of the Nrf2 pathway, whereas sulforaphane at toxic doses instead overactivates Klf9, leading to increased oxidative damage in cells.⁶⁰ Similarly, it remains to be investigated whether DCA also has the same problem of excessive activation of Nrf2 leading to increased oxidative stress. Indeed, DCA may exacerbate oxidative stress during brain injury in some cases. Interestingly, Gao et al found that DCA (100 mg/kg) inhibited PDK4 activity, enhanced PDH activity, increased ROS production and cell death, and conversely aggravated neurological impairment in rats with early brain injury (EBI) due to subarachnoid haemorrhage (SAH).⁴¹ EBI is a pathophysiological event that occurs within 72 hours after SAH.⁶¹ In contrast to cerebral ischaemia/reperfusion (I/R), cerebral blood flow is suddenly and significantly reduced after SAH, with only a small amount of incomplete reperfusion, and the brain tissue is in a state of persistent hypoxia-ischaemia.^{62–64} Importantly, DCA increases ROS production in all cells.⁶⁵ Even in normal cells, the forward electron transfer of the electron transport chain (ETC) will always cause some of the electrons to leak and generate ROS; when the respiratory chain is damaged or inhibited, it may cause more electrons to leak and generate more ROS. The enhancement of aerobic respiration by DCA increases ROS generated by the forward transfer of electrons.⁶⁶ The cellular shift to glycolysis under sustained hypoxia in EBI is a protective response to alleviate ATP deficiency, and the forced shift from glycolysis to oxidative phosphorylation by DCA in this case is more detrimental to cell survival.⁶⁷ However, the main cause of ROS production during I/R is reverse electron transfer due to a lack of ATP and metabolite accumulation.⁶⁸ The reduction of ATP during ischaemia causes cell membrane pump dysfunction, and calcium ions enter the cell to activate calcium-dependent protease, which converts xanthine dehydrogenase (XD) to xanthine oxidase (XO) in large quantities. When reperfusion occurs, under the influence of a large number of oxygen molecules, XO catalyses the conversion of hypoxanthine to xanthine, generating a large amount of ROS.⁶⁹ On the other hand, the high proton gradient accumulated in the inner mitochondrial membrane and the excessive reduction in coenzyme Q that reverses electron transfer in the ETC also generate large amounts of ROS.⁷⁰ The gradual decrease in the ATP/adenosine diphosphate (ADP) ratio during ischaemia and the degradation of accumulated adenosine monophosphate (AMP) leads to the depletion of the intracellular adenine nucleotide pools, which inhibits ATP production and the accumulation of protons in the inner mitochondrial membrane.⁷¹ In addition, hypoxia leads to a shift in cellular metabolism to glycolysis and β -oxidation, which can result in the accumulation of reduced nicotinamide adenine dinucleotide (NADH), thus causing succinate dehydrogenase to reverse its action to produce succinate.⁷² At the same time, the lack of coenzyme A and guanosine triphosphate (GTP) prevents the conversion of succinate, and the accumulated succinate is rapidly oxidized to fumarate, which leads to an overreduction of the coenzyme Q pool on complex II.^{70,73} A possible explanation for the different effects of DCA on oxidative stress is that DCA attenuates metabolite accumulation and reverse electron transfer in the ETC during I/R to reduce ROS production more strongly than it enhances forward electron transfer in the ETC to produce ROS. Oh et al found that DCA (1 g/l) increased cytochrome C oxidase activity and oxygen consumption of renal tubular cells in the kidneys of cisplatin-treated mice, thereby

attenuating cisplatin-induced acute kidney injury.⁷⁴ Increased cytochrome C oxidase activity may favour the continued forward transfer of electrons from complex II and decrease reverse electron transfer.⁷⁵ Lin et al observed in two human colorectal cancer cell lines that DCA increased the oxidized nicotinamide adenine dinucleotide (NAD)/NADH ratio.⁷⁶ These studies provide indirect evidence for possible mechanisms for the attenuation of oxidative stress during I/R by DCA, but it has not been studied whether DCA improves XO or succinate accumulation. In addition, the different effects of DCA administration at different times after injury need to be further explored, and administration during the ischaemic or reperfusion phase may produce opposite effects.

It is now widely recognized that neuronal damage in I/RI is the result of a combination of factors, including excitotoxicity, oxidative stress, apoptosis, neuroinflammation, excessive autophagy, cerebrovascular injury, etc.⁷⁷ Excessive autophagy is a significant cause of neuronal loss during I/RI.^{78,79} ROS are thought to activate autophagy through various pathways, and cells are needed to eliminate damage from excess ROS through autophagy during I/R; however, excessive autophagy can damage neurons.^{80,81} Zou et al found that activation of Nrf2 inhibited autophagy to attenuate limb I/R-induced muscle damage, suggesting that attenuating oxidative stress may inhibit excessive autophagy in I/RI.⁸² Yanyan Sun et al found that DCA (100 mg/kg) prevented an hypoxic-ischaemic-induced decrease in sequestosome 1 (SQSTM1) protein expression, which implies that DCA protects neurons from I/RI by decreasing autophagic activity in the brain.³⁹ During I/R, excess Ca^{2+} concentration, high levels of inorganic phosphate, and ROS lead to mitochondrial membrane potential alterations, opening mitochondrial permeability transition pores and releasing cytochrome C into the cytoplasm, followed by activation of downstream factors, such as caspase-3, causing apoptosis through a cascade reaction.^{83,84} Additionally, noncaspase-dependent apoptosis-inducing factor (AIF)-associated apoptosis has been suggested to be associated with excess ROS.⁸⁵ A study showed that DCA (100 mg/kg) prevented the nuclear translocation of AIF and reduced the activation of caspase-3 after ischaemia and hypoxia in mice, demonstrating that DCA can prevent neuronal apoptosis.³⁹ Injured or dead neurons stimulate the activation of glial cells that release inflammatory factors and cause secondary damage.⁸⁶ Peng et al found that DCA (80 mg/kg) reduced the mRNA expression of tumour necrosis factor (TNF- α) and interleukin-1 β (IL-1 β) in the cerebral cortex and hippocampus of rats resuscitated from cardiac arrest, corroborating the regulatory impact of DCA on inflammation.⁸⁷ Hong et al also found that DCA (100 mg/kg) reduced transient cerebral ischaemia-induced microglial activation.³⁸ Disruption of blood–brain barrier (BBB) integrity is one of the causes of I/RI, which is triggered by ROS through activation of pathways such as matrix metalloproteinases and the breakdown or modification of tight junction proteins (Occludin and ZO-1, among others).^{88,89} In addition, inflammatory cytokines (IL-1 β and TNF- α) also decrease the expression of Occludin and ZO-1 while disrupting the function of the BBB.⁹⁰ DCA has also been shown to increase the expression of ZO-1 and Occludin, thereby decreasing the extravasation of dyes in the cerebral vasculature of mice, suggesting a protective effect of DCA on the BBB.^{37,38} In addition, a study found that DCA increased mRNA levels of peroxisome proliferator-activated receptor- γ coactivator 1 α (Pgc-1 α) and nuclear respiratory factor-1 (Nrf1), two genes that are associated with mitochondrial biogenesis, in the brains of mice after hypoxic-ischaemic treatment, suggesting that DCA may also modulate mitochondrial dynamics, although the exact mechanism remains to be investigated.³⁹

In conclusion, oxidative stress due to impaired energy metabolism can be regarded as an initiating factor in the pathological process of cerebral I/R that triggers subsequent processes. The above preclinical experiments demonstrated the neuroprotective potential of DCA in its ability to ameliorate energy metabolism disorders, oxidative stress, and a series of downstream pathological processes (Figure 2). One study found that DCA coadministered with pyruvate showed more significant neuroprotective effects than low doses of DCA or pyruvate alone.³⁸ Pyruvate, which also acts to energize cells by facilitating the TCA cycle, has been shown to reduce neuronal death in I/RI.^{91,92} Guan et al synthesized the rho-associated coiled-coil containing protein kinase (ROCK) inhibitor fasudil and DCA into a new salt, fasudil dichloroacetate (FDCA), and the results showed that FDCA attenuated neuroinflammation and prevented breakdown of the BBB by inhibiting the activation of ROCK and the activity of PDK1, which reduced brain injury in MCAO mice.⁴⁰ These studies suggest that the combination of DCA with other neuroprotective drugs may be a novel approach to enhance its neuroprotective effects, but the drug ratio and feasible therapeutic window need to be further investigated.

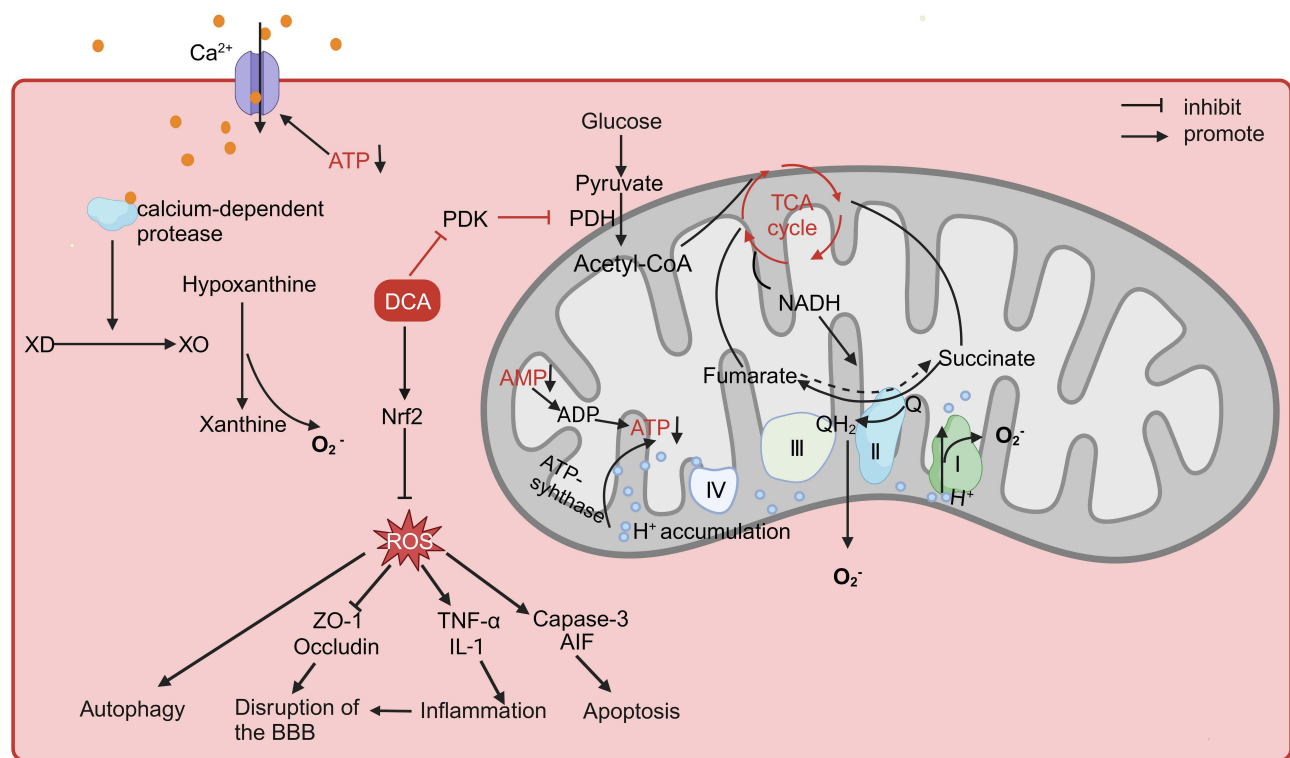


Figure 2 The role of DCA in cerebral ischaemia-reperfusion injury.

Notes: When there is ischaemia-reperfusion injury, ATP deficiency causes the cell membrane ion pump to malfunction and intracellular calcium levels to rise. This triggers the calcium-dependent protease, which in turn catalyzes a sequence of events that result in a significant production of reactive oxygen species. Still, the breakdown of AMP results in the inability to produce new ATP, which causes hydrogen ions to build up in the mitochondrial membrane gap. Similarly, the overabundance of succinate during ischaemia causes the overreduction of CoQ during reperfusion, which causes reverse electron transfer to produce reactive oxygen species. Through the inhibition of PDK activity, DCA regulates mitochondrial metabolism and reduces ROS resulting from reverse electron transfer and ATP deficit. Furthermore, via triggering the Nrf2 pathway, DCA suppresses ROS and lessens ROS-induced cellular autophagy, apoptosis, inflammation, and BBB degradation. Created with BioRender.com.

Abbreviations: DCA, dichloroacetate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; TCA, tricarboxylic acid; XD, xanthine dehydrogenase; XO, xanthine oxidase; Nrf2, nuclear factor erythroid 2-related factor 2; ATP, adenosine triphosphate; ADP, adenosine diphosphate; ROS, reactive oxygen species; AMP, adenosine monophosphate; NADH, reduced nicotinamide adenine dinucleotide; ZO-1, zonula occludens-1; TNF- α , tumor necrosis factor; IL-1, interleukin-1; AIF, apoptosis-inducing factor.

Vascular Dementia

Vascular dementia (VD) is a neurocognitive dysfunction caused by cerebrovascular diseases such as stroke.⁹³ Bone marrow-derived endothelial progenitor cells (EPCs) have been demonstrated to promote neovascularization and restoration with a protective role in vascular dementia.⁹⁴ In MCAO-induced VD rats, DCA (100 mg/kg/day, 200 mg/kg/day) increased serum levels of vascular endothelial growth factor and promoted cerebral angiogenesis by improving the function of EPCs through the protein kinase B (AKT)/glycogen synthase kinase 3 β /Nrf2 pathway, which suggested that DCA could enhance the function of EPCs, but the exact mechanism of the regulation of the AKT pathway by DCA is unclear.⁴² One possible reason is that ROS generated during hypoxia-ischaemia impairs endothelial cell function, and DCA regulation of the AKT pathway may also be mediated through ROS.⁹⁵ The effects of DCA on vascular endothelial cells in this experiment may provide new potential therapeutic approaches for the treatment of other vascular-related diseases, but the specific mechanisms and efficacy still need more exploration.

Hypoglycaemic Brain Injury, Traumatic Brain Injury, and Epilepsy

The protective effects of DCA on the brain are not only observed in I/R but also in hypoglycaemic brain injury, traumatic brain injury, and brain injury caused by acute seizures. Kho et al used insulin and glucose to cause hypoglycaemia-induced injury in rats and showed that DCA (100 mg/kg) reduced hypoglycaemia-induced oxidative stress, glial cell activation, BBB disruption, and neuron death.⁴³ DeVience et al assessed the extent of traumatic brain injury in rats by magnetic resonance spectroscopy (MRS) of hyperpolarized [1-¹³C] pyruvate and found that DCA (200 mg/kg) increased the bicarbonate/lactate ratio on both sides of the brain and attenuated the inhibition of PDH, but they did not assess whether DCA protects neurons.⁴⁴ DCA (50 and 100 mg/kg/day) was observed to shorten the latency to seizures in a second-hit flurothyl test in the mouse

model.⁴⁵ Lee et al found that DCA (100 mg/kg) and pyruvate (50 mg/kg) improved energy metabolism and attenuated oxidative stress and neuroinflammation, thereby reducing neuronal damage and cognitive dysfunction in seizure rats.⁴⁶

Protective Effects of Dichloroacetate in Neurodegenerative Diseases

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease characterized by motor neuron (MN) degeneration leading to progressive muscle atrophy throughout the body, with most patients progressing to respiratory failure and dying within 3–5 years.⁹⁶ It is currently believed that ALS is caused by the interaction of genetic and environmental factors, and the causative mechanisms include mitochondrial dysfunction, oxidative stress, neuroinflammation, axonal transport disruption, and vesicular transport defects.^{96–100} In ALS caused by mutations, the gene most commonly affected is superoxide dismutase 1 (SOD1).¹⁰¹ Miquel et al found that oral administration of DCA (100 mg/kg for 10 days) to symptomatic SOD1^{G93A} rats reduced MN degeneration in the spinal cord, attenuated the loss of locomotor activity, and increased survival rates, suggesting that DCA has a therapeutic effect in ALS.⁴⁷

Astrocytes are essential in the pathology of ALS, and the presence of aberrant glial cells (AbGCs) leads to the death of motor neurons.^{102,103} AbGCs are a specialized astrocyte A1 phenotype, and it is currently believed that AbGCs cause motor neuronal damage due to the loss of normal astrocyte function and the secretion of some neurotoxic factors.^{103,104} Miquel et al found that DCA (5 mmol/L, 24 h) enhanced PDH activity in AbGCs and increased the survival of neurons cocultured with AbGCs.⁴⁷ This suggests that DCA may reduce motor neuron damage by affecting AbGCs, but the exact mechanism remains to be investigated. Martínez-Palma et al found that DCA (5 mmol/L) improved mitochondrial respiration, reduced lactate production, inhibited the growth of AbGCs isolated from SOD1^{G93A} rats, and increased neuronal survival during coculture of AbGCs with neurons but did not alter the expression of typical phenotypic markers of AbGCs, suggesting that the change in the number of AbGCs during coculture was not significant and that the decrease in the toxicity of AbGCs may be due to DCA-induced shifts in their metabolic phenotype.⁴⁸ SOD1^{G93A} expression in astrocytes leads to a shift in the cellular metabolic phenotype towards glycolysis to produce more lactic acid.¹⁰¹ Lactate released by astrocytes can serve as a metabolic substrate for neurons, and after reaching the neuron, lactate is rapidly metabolized to pyruvate in the cytoplasm and mitochondria, participating in mitochondrial energy metabolism.¹⁰⁵ Jia et al found that inhibition of PDH in Schwann cells leads to a sustained and massive accumulation of lactate, which drives a sustained increase in neuronal mitochondrial activity and an increase in ROS, leading to neuronal oxidative stress and axonal destruction.¹⁰⁶ Additionally, excessive accumulation of lactic acid can lead to cellular acidosis and neuronal damage.¹⁰⁷ This suggests that DCA may protect neurons by reducing lactate production from AbGCs. Another question that remains to be addressed is why DCA inhibits AbGC growth. Mutations in SOD1 result in low glutathione levels and high NO levels, which can lead to oxidative stress and promote the production of peroxynitrite in cells, damaging the ETC.^{108,109} In addition, aberrant expression of SOD1 disrupts the connection between cytochrome C and the inner mitochondrial membrane, directly leading to ETC damage.¹¹⁰ Inadequate ATP production due to ROS damage to the ETC may be responsible for the forced shift in the metabolic phenotype of AbGCs to glycolysis.¹¹¹ Stockwin et al found that DCA forces cells to switch to defective oxidative phosphorylation, causing insufficient ATP production and leading to metabolic stress and cell death in ETC-deficient cells.⁶⁵ Therefore, DCA may inhibit AbGC growth by stimulating the damaged ETC and generating excess ROS, similar to its inhibitory effect on cancer cells.¹¹²

Studies in SOD1^{G86R} model mice have shown that altered metabolism of glycolytic muscles may predispose the organism to motor neuron loss.^{113–115} In the physiological state, both glucose and fatty acids can be used as metabolic raw materials for glycolytic muscles, in which glucose is converted to pyruvate by glycolysis and then enters the TCA cycle or is converted to lactate.^{116,117} Fatty acids are then converted to lipoyl coenzyme A by lipoyl coenzyme A synthetase and transported to mitochondria via carnitine palmitoyltransferase for β -oxidation.¹¹⁸ However, the production of lipid byproducts from fatty acid β -oxidation increases ROS production.¹¹⁹ In ALS, instability of the neuromuscular junction induces elevated PDK4 expression, which prevents the entry of pyruvate into the TCA cycle.^{120,121} Energy deficiency leads to more fatty acids entering the glycolytic muscle, which further induces the transcription of the nuclear hormone receptor peroxisome proliferator-activated receptor β/δ (PPAR β/δ) to stimulate forkhead box transcription factor O1 (FOXO1), and high levels of FOXO1, as well as ATP and NADH generated by β -oxidation, can activate the expression of PDK4, which further inhibits glucose oxidation and creates a vicious cycle, with increased oxidative stress leading to neuronal death.^{49,122,123} Palamiuc et al

found that DCA (500 mg/kg) decreased the mRNA expression of PDK4, FOXO1, and glutathione peroxidase 1 and increased the mRNA expression of PPAR β/δ and phosphofructokinase 1 (PFK1) in SOD1G86 mice, suggesting that DCA relieves the inhibition of PFK1 by excess pyruvate through the inhibition of PDK4 activity and the enhancement of mitochondrial oxidation of pyruvate, which enhances glycolysis and reduces oxidative stress by decreasing β -oxidation of fatty acids, thereby reducing motor neuron death and improving locomotor activity and survival rates in mice.⁴⁹ The results also showed that DCA can increase the mRNA expression of Pgc-1 α and mitochondrial fusion protein 2 (Mfn2), which may have a role in improving mitochondrial dynamics.⁴⁹ Although it was described above that DCA enhances ROS production in all cells, the reason for the attenuation of oxidative stress here may be that on the one hand, glycolytic muscles themselves have a low ETC metabolic flux, and on the other hand, the effect of DCA on attenuating ROS production from β -oxidation is greater than that produced by its increase in ETC function.⁶⁵

In summary, in ALS, DCA regulates the metabolism of both AbGCs and glycolytic muscles to reduce motor neuron death, but the effects of DCA on motor neurons themselves have not been explored (Figure 3). In addition, an animal model with a single mutation is not fully representative of the complete pathology of ALS; therefore, further preclinical and clinical studies are still necessary to explore the efficacy of DCA.

Alzheimer's Disease

Amyloid β -protein (A β) deposition in Alzheimer's disease (AD) is one of the important pathogenic mechanisms of the disease.¹²⁴ Amyloid precursor protein (APP) is cleaved by β -site APP cleavage enzyme (BACE1) to generate soluble amyloid precursor protein β (sAPP β) and β C-terminal fragment (β CTF); β CTF is then cleaved by γ -secretase to generate A β .¹²⁵ In addition, APP can generate neuroprotective soluble amyloid precursor protein α (sAPP α) via metalloproteinase 10 (ADAM10), and this competitive process prevents the cleavage of APP via BACE1 and γ -secretase.¹²⁶ Parkin et al found in SH-SY5Y cells that DCA (10 and 20 mmol/L) increased the level of sAPP α and decreased the levels of sAPP β and A β but did not change the activity and mRNA expression of ADAM10 and BACE1, which proved that DCA could reduce the generation of A β .⁵⁰ However, since the effect of DCA on the protein expression level of BACE1 was not measured, the

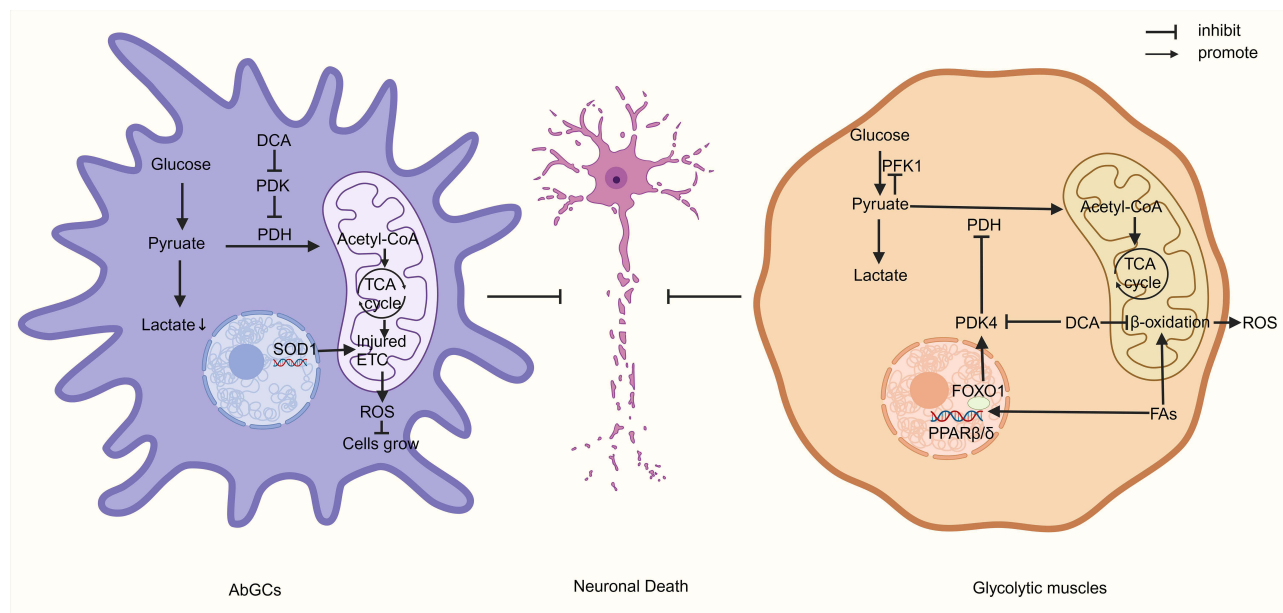


Figure 3 DCA attenuates neuronal death in ALS by targeting AbGCs and glycolytic muscles.

Notes: In AbGCs, DCA increased PDH activity to cause a change in cellular metabolism to mitochondrial metabolism. This prevented lactate from being harmful to neurons and decreased the amount of lactate produced. Furthermore, DCA stimulated the disrupted electron transport chain in the mitochondria of AbGCs, which raised the degree of oxidative stress and prevented AbGC development. When cellular metabolism is disrupted in glycolytic muscles, fatty acids β oxidation provides cells with additional energy. This process activates PPAR β/δ with FOXO1 and inhibits PDH. By increasing PDH activity, DCA lessens the inhibition of glycolysis caused by pyruvate accumulation and increases energy availability, which lowers the metabolism of fatty acids and the generation of reactive oxygen species. Created with BioRender.com.

Abbreviations: DCA, dichloroacetate; PDH, pyruvate dehydrogenase; SOD1, superoxide dismutase 1; ROS, reactive oxygen species; FAS, fatty acids; PFK1, phosphofructokinase 1; PDK, pyruvate dehydrogenase kinase; FOXO1, forkhead box protein O1; PPAR β/δ , peroxisome proliferators-activated receptors β/δ .

mechanism by which DCA affects the generation of A β remains to be explored. Velliquette et al found that using drugs (insulin, 2-deoxyglucose, 3-nitropropionic acid, and kainic acid) to inhibit energy metabolism in APP transgenic mice increased BACE1, sAPP β , and A β 40 levels, suggesting that reduced energy metabolism increases A β production by increasing BACE1 expression levels.¹²⁷ O'Connor et al demonstrated that energy deprivation leads to phosphorylation of the translation initiation factor eIF2 α , which increases BACE1 expression by regulating BACE1 translation rather than transcription, which could also explain why Edward T. Parkin et al detected no change in BACE1 mRNA levels in their experiments.¹²⁸ In addition, mitochondrial dysfunction, oxidative stress, neuroinflammation, and metabolic alterations contribute to the pathophysiology of AD.^{129,130} Some drugs targeting mitochondrial metabolism have been found to have therapeutic potential in AD; for example, a phase 2 clinical trial showed that a combination of metabolic activators (L-serine, nicotinamide ribose, N-acetyl-L-cysteine, and L-carnitine tartrate) improved cognitive function in patients with AD.¹³¹ DCA, as a regulator of energy metabolism, may also have therapeutic potential for AD due to its ability to reduce A β production by affecting BACE1 expression. In addition, the ability of DCA to modulate oxidative stress, inflammation, and mitochondrial dynamics may also contribute to the reduction of A β production, as these factors have all been found to be associated with BACE1.^{132–134} In conclusion, the current evidence suggests that DCA may have a therapeutic effect on AD, but the pathogenesis of AD is affected by many factors, and further experiments are needed to prove the effects and mechanisms of DCA on AD.^{135,136}

Huntington's Disease and Parkinson's Disease

Huntington's disease (HD) and Parkinson's disease (PD) are also neurodegenerative diseases, and mitochondrial dysfunction is an important factor in the pathogenesis of both diseases.^{137,138} DCA has been demonstrated to exert a therapeutic effect in Huntington's disease animals and Parkinson's disease cells in vitro. The addition of DCA (100 mg/kg) to drinking water in mice improved body weight, locomotion, and mean survival time in R6/2 versus N171/82Q mice; in R6/2 mice, DCA improved striatal volume reduction and neuronal area reduction.⁵¹ In 1-methyl-4-phenyl-pyridinium ion (MPP)-treated PC12 cells, the use of DCA (100 μ mol/L) improved mitochondrial dynamics, but the decreased cell viability, increased ROS, altered mitochondrial membrane potential, and reduced oxygen flux after MPP treatment were not reversed.⁵²

DCA and PGC-1 α

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) is a transcription factor that is an essential node linking cellular metabolism, mitochondrial dynamics, oxidative stress, and inflammation regulation and has been recognized as an important target for pharmacological intervention in many cardiovascular, metabolic, and neurodegenerative diseases.^{139–142} The transcriptional activity of PGC-1 α is regulated by AMP-activated protein kinase (AMPK) via phosphorylation.¹⁴³ AMPK is the most direct energy receptor, which directly senses changes in the intracellular AMP/ADP ratio and accordingly regulates mitochondrial function to adapt to energy changes.¹⁴⁴ Li et al found that DCA activated the AMPK pathway and ameliorated oxidative stress injury in a mouse model of cardiac ischaemia–reperfusion injury, providing indirect evidence that DCA activates PGC-1 α via AMPK.²⁵ In addition, PGC-1 α activity is regulated by acetylation, and sirtuin 1 (SIRT1) activates PGC-1 α through deacetylation.¹⁴⁵ An increase in the NAD/NADH ratio triggers the activation of SIRT1, which activates PGC-1 α .¹⁴⁶ Cells with abnormal mitochondrial metabolism are forced to undergo glycolysis and fatty acid β -oxidation to supplement ATP production, but it results in the accumulation of NADH.^{147,148} Lin et al found that DCA increased the NAD/NADH ratio in human colorectal cancer cell lines.⁷⁶ However, due to different cellular metabolic phenotypes, the changes in the intracellular AMP/ADP and NAD/NADH ratios induced by DCA under different pathological conditions need to be further investigated. Interestingly, the regulation of the aforementioned Nrf2 pathway, inflammation, apoptosis, mitochondrial dynamics, and A β by DCA can also be explained by PGC-1 α . Lee et al showed that treatment of dorsal skin cells of nude mice with Galangal activates PGC-1 α and further enhances the expression of Nrf2 for antioxidant effects.¹⁴⁹ PGC-1 α was also found to block the transcription of nuclear factor-kappaB and its downstream genes encoding proinflammatory cytokines, which demonstrated the regulation of anti-inflammation by PGC-1 α .¹⁵⁰ In addition, Niel et al observed in a mitochondrial creatine kinase knockout model of ageing mice that PGC-1 α may affect mitochondrial dynamics by regulating Mfn2.¹⁵¹ Li et al also showed in their study that PGC-1 α regulates mitochondrial function through activation of

the Nrf1 pathway.¹⁵² Liu et al observed that PGC-1 α inhibited apoptosis by suppressing caspase activation in heat shock protein 12A knockout mice.¹⁵³ Wang et al found that PGC-1 α directly binds to the promoter of the BACE gene and represses the transcription of BACE1 mRNA, revealing the regulatory role of PGC-1 α in A β generation.¹⁵⁴ In summary, the modulation of PGC-1 α by DCA may be a key factor in its multiple pharmacological effects, and mediating the importance of PGC-1 α , this hypothesis also opens up pharmacological possibilities for a wider application of DCA (Figure 4).

Barriers to the Clinical Application of DCA and Possible Coping Strategies

Pharmacokinetics

Oral DCA is quickly absorbed, providing a bioavailability comparable to that of parenteral administration.^{9,155} Due to the high fat solubility of DCA, it is distributed in the liver, muscle, skin, intestines, kidneys, lungs, heart, brain, and other tissues and organs.^{156,157} DCA crosses the cell membrane via the monocarboxylate transporter and then through the mitochondrial membrane via the pyruvate transporter, thus entering the mitochondrial matrix to exert its role.¹⁵⁸ In vivo, DCA is metabolized mainly by the zeta-1 family isoform of glutathione transferase (GSTZ1).¹⁵⁹ GSTZ1 is distributed in tissues such as the liver, kidney, testis, heart, and brain, but the amount in the liver is much higher than that in other organs, so the liver is the main metabolizing organ of DCA.¹⁶⁰ Further studies showed that 86% of GSTZ1 in rat liver was in the cytoplasm and 14% in mitochondria.¹⁶¹

As early as the last century, it was discovered that DCA has nonlinear kinetics at single injection doses ≥ 35 mg/kg.¹⁶² This is because DCA limits its own metabolism by inhibiting GSTZ1, and the plasma clearance of the drug is reduced after multiple

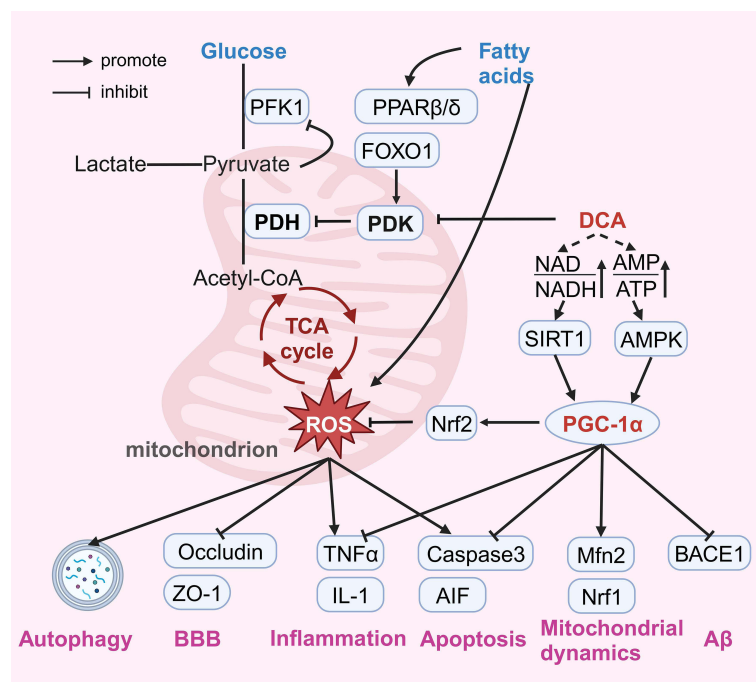


Figure 4 Possible mechanisms of neuroprotective effects of Dichloroacetate.

Notes: DCA inhibits PDK activity, thus enhancing PDH activity. This allows more glucose to enter the mitochondria for oxidation and reduces fatty acids β -oxidation, which corrects the disturbance of energy metabolism and reduces oxidative stress caused by insufficient ATP production. DCA exerts protective effects by attenuating multiple damages caused by oxidative stress, including: attenuating excessive autophagy, attenuating neuroinflammation, protecting the BBB, and attenuating apoptosis. DCA may increase the intracellular NAD/NADH AMP/ATP ratio by regulating metabolism and thus activating PGC-1 α . Activation of PGC-1 α has multiple protective effects including: attenuating oxidative stress by activating Nrf2, decreasing the release of inflammatory factors (TNF α , IL-1 β), attenuating caspase 3 and AIF-mediated apoptosis, activating Mfn2 and Nrf1 to improve mitochondrial dynamics, and inhibiting BACE1 to reduce A β production. Created with BioRender.com.

Abbreviations: DCA, dichloroacetate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK1, phosphofructokinase 1; PPAR β/δ , nuclear hormone receptor peroxisome proliferator-activated receptor β/δ ; FOXO1, forkhead box transcription factor O1; TCA, tricarboxylic acid; ROS, reactive oxygen species; NAD, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; AMP, adenosine monophosphate; ATP, adenosine triphosphate; SIRT1, sirtuin 1; AMPK, AMP-activated protein kinase; PGC1- α , peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Nrf2, nuclear factor erythroid 2-related factor 2; ZO-1, zonula occludens protein 1; BBB, blood-brain barrier; TNF α , tumor necrosis factor α ; IL-1 β , interleukin-1 β ; AIF, apoptosis-inducing factor; Mfn2, mitochondrial fusion protein 2; Nrf1, nuclear respiratory factor 1; BACE1, β -site amyloid precursor protein cleavage enzyme; A β , amyloid β -protein.

repeated administrations.¹⁶³ More importantly, the activity of GSTZ1 and the time to recovery of activity were related to the duration of DCA administration and the dose.¹⁰ After a single intraperitoneal injection of DCA (45 mg/kg) in male Fischer-344 rats, there was a significant loss of GSTZ1 protein and activity within 12 hours, with a minimum value of less than 40% of the initial value, which was not restored until 10 to 12 days after the administration of the drug.¹⁶³ Li et al found that GSTZ1 in rat mitochondria had a 2.5-fold higher apparent K_m for glutathione than cytoplasmic GSTZ1, while DCA had the same apparent K_m values, which suggests that there may be a difference between cytoplasmic GSTZ1 and mitochondrial GSTZ1 activity.¹⁶⁴ However, Smeltz et al found that when adult male rats were orally administered DCA (100 mg/kg), with DCA acting as a substrate together with glutathione (GSH), the half-life of GSTZ1 in adult rat cytoplasm was 0.82 ± 0.02 hr and that in mitochondria was 0.54 ± 0.05 hr, which suggests that mitochondrial GSTZ1 is more susceptible to DCA inhibition.¹⁶⁵ Zhong et al found that although the contribution of mitochondrial GSTZ1 to total GSTZ1 increased with age, the contribution of mitochondrial GSTZ1 was still inferior to that of cytoplasmic GSTZ1 when repeated DCA treatments were administered.¹⁶⁶ Although there are differences in the kinetics of mitochondrial and cytoplasmic GSTZ1, cytoplasmic GSTZ1 remains a key area of interest for studying DCA metabolism, considering the long-term clinical use of the drug.¹⁶⁷

In addition to inhibition by DCA, GSTZ1 activity is affected by age.¹⁵⁵ After treatment with DCA (25 mg/kg/day) for 6 months, the $t_{1/2}$ for DCA was 6.4 ± 3.4 hours in the paediatric group (mean 5.2 ± 1.8 years) and 21 ± 5.8 hours in the adult group.¹⁵⁸ The total activity of GSTZ1 in hepatocyte lysates of rats after DCA (100 mg/kg) administration for 8 days was 113 ± 37 and 115 ± 33 nmol glyoxylate/min/kg in young male and female rats, respectively, compared with 44.7 ± 13.4 nmol glyoxylate/min/kg in 52-week-old female rats.¹⁶¹ This may be because children have a higher liver/body weight ratio, or it may be the result of the effect of chloride ions.¹⁰ Jahn et al found that chloride ion concentrations in human liver from donors aged 1 day to 84 years decreased with age, consistent with age-related changes in GSTZ1, and the chloride ion concentrations of the cytoplasm (mean 105 mmol/L) were much larger than those of mitochondria (mean 4.2 mmol/L), which could also explain the greater susceptibility of mitochondrial GSTZ1 to inactivation compared to that in the cytoplasm.¹⁶⁸ Zhong et al found that chloride, bromide, iodide, and sulfite attenuated the loss of GSTZ1 activity in human hepatocyte cytosol in a concentration-dependent manner after 2 h of DCA (0.5 mmol/L) incubation, but the mechanism by which chloride ions and other ions affect the loss of GSTZ1 activity is still unclear.¹⁶⁹ In addition, Jahn et al showed that miR-376c-3p prevents the expression of GSTZ1 by repressing translation, and the expression of this miRNA is lower in adults than in children, which provides new ideas for studying the factors that influence GSTZ1.¹⁷⁰

GSTZ1 genotypes are also important in influencing the metabolism of DCA.¹⁷¹ There are five common haplotypes based on the three common asynchronous single-nucleotide polymorphisms (rs7975, rs7972, rs1046428) in the GSTZ1 gene coding region, which, in order of population frequency, are EGT (GSTZ1C, 50%), KGT (GSTZ1B, 28%), EGM (GSTZ1D, 15%), KRT (GSTZ1A, 7%), and KGM (GSTZ1F, 0.4%).^{172,173} The residual activity of the variants was different for each variant in a single DCA-induced inactivation: 1A-1A (12%) > 1B-1B \approx 1C-1C \approx 1D-1D (<5%).¹⁷⁴ In vitro studies on human hepatocyte cytoplasm showed that GSTZ1A homozygous or heterozygous samples had 3 times higher DCA dechlorination activity than those carrying other alleles.¹⁷⁵ However, with multiple administrations, after 5 or more days of DCA treatment (2.5 μ g/kg/day, once or twice), those with one copy of the GSTZ1C variant had a lower reduction in DCA clearance than those without the GSTZ1C variant, implying that the GSTZ1 enzyme activity of those with a copy of the GSTZ1C variant is less affected by multiple DCA administrations.¹⁷⁶ James and Stacpoole classified individuals into fast-metabolizing (at least one GSTZ1C copy) and slow-metabolizing (no GSTZ1C copy) phenotypes based on the level of GSTZ1 inactivation following multiple doses of DCA.¹⁶⁷ Notably, the chloride concentrations that provided 50% inactivation protection (EC50) in the EGT and EGM homozygotes were similar; however, the EC50 concentration of chloride in the EGT/KRT haplotype was approximately 2.5-fold higher than that of the EGT/EGT and EGM/EGM haplotypes.¹⁶⁹ This is consistent with the faster inactivation of GSTZ1 in individuals carrying the GSTZ1A variant and suggests that the mechanism of the protective effect of chloride ions may involve binding to a specific regulatory site on GSTZ1, such that different GSTZ1 variants have different affinities for chloride ions. Interestingly, in the study by Shroads et al, patients received oral DCA (12.5 mg/kg/12 h) or placebo for 6 months, after which subjects in the placebo group were switched to the DCA group (patients were treated for a total of 30 months), and the results showed that although the plasma trough concentration of DCA was lower in EGT carriers than in non-EGT carriers, none of the genotypes showed a gradual accumulation of plasma drug concentrations, and the differences in kinetic parameters stabilized.¹⁷¹ Therefore, it is theoretically possible to achieve controllable blood concentrations by administering

different doses of DCA according to GSTZ1 haplotypes at the time of treatment, and it is also valuable to further develop models for the nonlinear pharmacokinetics of DCA according to age and GSTZ1 genotype.¹⁷⁷

Adverse Reactions

The toxicity of DCA, as a byproduct of water chlorination disinfection, has been an area of concern. In animal experiments, it was found that long-term use at high doses could induce liver cancer. The addition of DCA to drinking water (3.5 g/L for 93 weeks) increased the incidence of liver tumours in mice.^{178,179} However, epidemiological evidence does not demonstrate a direct carcinogenic effect of DCA in humans, and long-term use of DCA has been found to have no haematological, hepatic, or renal toxicity.^{10,180} For example, Abdelmalak et al followed eight patients with congenital lactic acidosis who received oral DCA (5.12 mg/kg/9 h) for 7.16 to 5.1 years, and their renal, liver, electrolyte, and hepatic status remained stable.¹⁸¹

The biggest limiting issue found with DCA in clinical trials is reversible peripheral neuropathy, with dose dependence and individual variability.¹⁸² DCA is catalysed by GSTZ1 and converted to glyoxalate in a reaction that needs but does not consume GSH.¹⁸³ The intermediates of tyrosine catabolism, maleoylacetate and maleoylacetone, are physiological substrates of GSTZ1, which converts them to fumarylacetoacetate and fumarylacetone.¹⁸⁴ Fumarylacetoacetate hydrolase (FAH) converts fumarylacetoacetate to two nontoxic substances, fumarate and acetoacetate.¹⁸⁵ Maleoylacetate, maleoylacetone, fumarylacetoacetate, and fumarylacetone are considered to be toxic.¹⁷⁴ The accumulation of maleoylacetone and fumarylacetone inhibits the conversion of δ -aminolaevulinic acid (δ -ALA) to porphobilinogen by ALA dehydratase, leading to the accumulation of δ -ALA, a neurotoxic substance.^{186,187} Patients with hereditary tyrosinemia caused by abnormal FAH function develop symptoms of hepatic, neurologic, and renal system damage, including hepatocellular carcinoma and peripheral neuropathy, as a result of the accumulation of these toxic substances.¹⁸⁸ Toxic metabolites as well as δ -ALA have been found in the urine of adults and children treated with DCA, and the accumulation of toxic products has been correlated with the GSTZ1 genotype, suggesting that DCA produces many neurotoxic metabolites due to the inhibition of GSTZ1 (Figure 5).¹⁷⁶ The metabolite of DCA, glyoxalate, is further metabolized by lactate dehydrogenase (LDH) to oxalate, carbon dioxide, and oxalate.^{189,190} Oxalate is considered relevant to peripheral neuropathy, and its metabolism consumes thiamine.^{191,192} In addition, thiamine is a cofactor of pyruvate dehydrogenase.¹⁹³ After 7 weeks of DCA (1.1 g/kg) treatment in rats, oxalate in the urine was 86% higher in rats treated with DCA than in controls but was only 28% higher in those treated with DCA plus thiamine than in controls; thus, DCA may lead to peripheral neuropathy due to thiamine deficiency and oxalate accumulation.¹⁸² A trial of DCA in combination with thiamine in a 13-year-old girl with complex I deficiency found that the patient still had peripheral neuropathy; however, the study had an inadequate sample size and did not consider the effect of the GSTZ1 genotype.¹⁹⁴ More experimental validation is needed to verify whether thiamine can alleviate DCA-induced peripheral neuropathy. Thiamine is used to treat mitochondrial diseases as a drug that enhances pyruvate dehydrogenase, similar to DCA, and the combination may result in better efficacy.¹⁹⁵ Broxton found that DCA (25 mmol/L) with thiamine (25

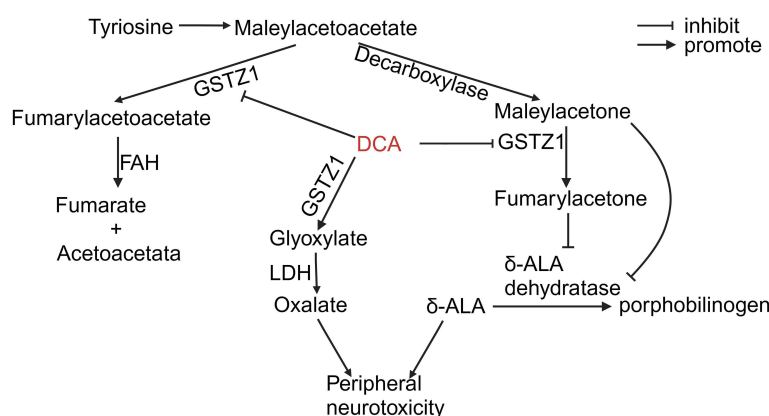


Figure 5 Metabolism of DCA and related neurotoxic products.

Note: Created with BioRender.com.

Abbreviations: DCA, dichloroacetate; GSTZ1, glutathione transferase zeta 1; GSH, glutathione; δ -ALA, δ -aminolaevulinic acid; FAH, fumarylacetoacetate hydrolase; LDH, lactate dehydrogenase.

mmol/L) showed isolated or synergistic therapeutic effects in pyruvate dehydrogenase complex-deficient *Cryptobacterium hidradii* nematodes.¹⁹⁶ In a randomized controlled trial of patients with MELAS and A3243G mutations, DCA (25 mg/kg/day) was discontinued due to strong peripheral neurotoxicity.¹⁹⁷ Several other clinical trials in solid tumours and congenital lactic acidosis have shown that DCA-induced peripheral neurotoxicity at therapeutic doses is tolerable.^{198–200} For example, a study of patients with advanced solid malignancies found that DCA (6.25 mg/kg/12 h) was associated with fatigue, neuropathy, and nausea in only a subset of patients and that the response to DCA varied among patients.²⁰¹ More importantly, adults and children do not respond consistently to DCA, children tolerate DCA better than adults, and the use of DCA for several years in children and adolescents to treat primary mitochondrial disease is considered to have an excellent long-term safety record.^{181,202} Therefore, the clinical side effects of DCA may be due to the different abilities of different patients to metabolize DCA, which is also affected by age and GSTZ1 genotype, as mentioned above. Tian et al found in six adult patients treated with DCA (mean 25 mg/kg/day) that a patient without the EGT variant had a higher neuropathy score on Day 84 of treatment compared with other patients carrying the EGT variant and that this patient's plasma trough concentrations of DCA on Day 56 and Day 84 were 2- to 3-fold higher than the mean values of the other patients, suggesting that peripheral neuropathy caused by DCA is related to the accumulation of DCA resulting from GSTZ1 suppression.²⁰³ Therefore, individualized dose design is one way to address the peripheral neurotoxicity of DCA.¹⁶⁷ In a study of 15 adults with recurrent gliomas or intracranial metastases, Dunbar et al stratified the administration of DCA according to whether the patient carried the EGT variant: patients carrying the EGT variant were administered DCA (8.0 mg/kg/12 h, 12.5 mg/kg/12 h, 5.0 mg/kg/12 h), and patients without the EGT variant were administered a dose of 4.0 mg/kg/12 h; no patients discontinued the drug due to adverse effects of DCA.²⁰⁰ This suggests that it is feasible to group patients by whether they carry the EGT variant and administer different concentrations of DCA individually. Dunbar et al concluded that the starting oral dose for patients who do not carry the variant should be 5 mg/kg/12 h, which can be increased appropriately based on the absence of peripheral neuropathy, and that subjects carrying the EGT variant should be able to tolerate at least 6.25 mg/kg/h.²⁰⁰ However, because of the differences in the metabolism of DCA between children and adults, this finding may apply only to adults, and there are currently no clinical trials in children designed to administer DCA based on the GSTZ1 genotype.^{181,202} Mangal et al developed a population pharmacokinetic model for DCA based on data from a randomized controlled trial of DCA in children with congenital lactic acidosis and recommended optimal DCA dosages of 12.5 and 10.6 mg/kg twice daily for children who are EGT carriers and non-EGT carriers, respectively.^{23,204} More data based on GSTZ1 genotypic dosing are needed in all age groups to determine the dose of DCA in clinical therapy.

Various synthetic nanocarriers and cell-derived nanovesicles now offer new possibilities for drug delivery.^{205,206} They can improve drug absorption, enable precise drug delivery and reduce side effects and have been used as a potential therapeutic option for cancer, diabetes and other diseases.^{207–210} In tumour therapy, to enhance drug delivery targeting and reduce side effects, DCA is commonly delivered in metal-organic frameworks.^{211–215} For example, Lázaro et al functionalized DCA using zirconium (Zr) terephthalate (UiO-66) nanoparticles to achieve cytotoxicity and selectivity against different cancer cell lines.²¹⁶ For drug delivery in the nervous system, cell-derived exosomes can cross the BBB and exhibit high biocompatibility, good stability, low accumulation, and ease of modification, which show more advantages than traditional synthetic delivery carriers and have been widely used in therapeutic research on neurological diseases.^{217,218} There have been no studies using nanovesicles to deliver DCA for the treatment of brain diseases. DCA, as a small-molecule lipid-soluble compound, binds itself passively to the lipid bilayer of exosomes; therefore, utilizing exosomes and delivering DCA to the brain through passive or active targeting strategies may allow for the administration of controlled concentrations of DCA to avoid its side effects.^{218,219} In addition, multitarget drug delivery utilizing specific markers of disease pathophysiological processes, such as ROS, inflammatory factors, and pH, is another method to enhance drug targeting.²²⁰ In a study on AD, Hu et al designed nanoparticles based on the specific response of Congo red to amyloid plaques and the redox sensitivity of boronate ester bonds, using Congo red/boronate ester bonds as the ligands, and realized the targeted delivery of AD therapeutic agents and controlled release by H₂O₂.²²¹ Qiao et al designed nanoparticles based on poly[(2-angiopep)ethyl(p-boronic acid benzyl) diethylammonium bromide] (BAP)'s ability to respond to ROS, using angiopep-2/zwitterionic lipid distearoyl phosphoethanol-aminepolycarboxybetaine/BAP as a ligand to design the nanoparticle, thereby enabling the drug to overcome the limitations of the BBB, specifically target glioma cells, escape endosomes/lysosomes, and release the drug in response to ROS.²²² Goyal et al exploited the characteristics of pH and redox sensitivity of Lf (part of the transferrin family)-conjugated

aggregates in the treatment of amnesia to design Lf/disulfide linkage nanoparticles, thus enabling drug-targeted delivery by detecting the pH microenvironment.²²³ Modulation of metabolism is the commonality of DCA's pharmacological effects in the aforementioned neurological disorders; metabolic disorders in the brain are associated with decreased pH and increased oxidative stress, and in some diseases, there is an accumulation of specific markers such as A β . These existing studies may provide ideas for the future targeting of ROS, pH, and disease-specific markers for the delivery of DCA.

Conclusion and Prospects

In this review, we first summarized the current studies on the treatment and mechanisms of DCA in some neurological disorders. Second, we analysed the limitations of the clinical application of DCA. Finally, possible coping strategies were proposed based on its limitations.

Current studies on the neuroprotective effects of DCA involve a variety of diseases (Table 1). The neuroprotective mechanisms of DCA include regulating metabolism, improving oxidative stress, reducing neuroinflammation, reducing apoptosis, reducing autophagy in the brain, protecting the BBB, improving endothelial progenitor cell function, improving mitochondrial dynamics, and reducing A β protein production. Studies have focused on cerebral I/RI-related disorders, and these studies have shown that DCA reduces excess ROS production primarily by improving metabolism, thereby reversing the subsequent cascade of damage caused by reperfusion. In neurodegenerative diseases, major studies have found that DCA attenuates motor neuron death in ALS model animals and the production of A β protein in AD model cells by modulating metabolism. In ALS, DCA reduced the toxicity of AbGCs to neurons, which may be related to the attenuation of lactic acid toxicity and the inhibition of AbGC growth and proliferation. On the other hand, DCA favours motor neuron survival by regulating glycolytic flux in glycolytic muscles. Additionally, a study in VD animals found that DCA improves brain function by enhancing the function of endothelial progenitor cells. These studies suggest that DCA has the potential to be beneficial when it acts on different cells, but these studies are limited, and further exploration of the effects of DCA on different cells is needed. Notably, the exertion of all these pharmacological effects seems to be related to the regulation of PGC-1 α , which senses changes in cellular energy metabolism and is the connection point for multiple pathways of metabolism, oxidation, inflammation, the production of A β and mitochondrial dynamics. However, due to the complex metabolic profile of cells *in vivo*, the specific mechanisms by which DCA regulates PGC-1 α remain to be explored, and the changes in the AMP/ADP and NAD/NADH ratios induced by DCA may be different in different situations. It is now well established that DCA enhances mitochondrial respiration and enhances ROS production in all cells, but this is not sufficient to affect normal cells under normal conditions.⁶⁵ Moreover, PDH in normal neurons maintains a low level of phosphorylation, close to maximal activity.²²⁴ The role of DCA needs to be specifically analysed in different pathological situations. In cells with a severely impaired or inhibited ETC, for example, the application of DCA to tumour cells, AbGCs in ALS, and neurons in hypoxia may disrupt the protective process of cellular switching to glycolysis, resulting in accelerated death due to cellular energy depletion. In other cases, such as cerebral I/RI, the enhancement of mitochondrial metabolism by DCA may be accompanied by some ROS damage to the cell, but it attenuates the more serious consequences of insufficient energy metabolism and protects neurons overall. In addition, current studies on the neuroprotective effects of DCA are only at the cellular or animal level. DCA is metabolized primarily in the cytoplasm of hepatocytes by the enzyme GSTZ1, which can be inhibited by DCA itself. The activity of GSTZ1 is affected by age, and in general, the older the person is, the lower the GSTZ1 activity. Furthermore, the activity of GSTZ1 and the extent to which DCA inhibits it after multiple administrations varies between different GSTZ1 genotypes. Therefore, during clinical use, peripheral neurotoxicity often results from inhibition of GSTZ1 activity. Accumulation of the toxic product δ -ALA due to GSTZ1 inhibition and accumulation of oxalate, DCA's own metabolite, may be responsible for its peripheral neurotoxicity. Coadministration of thiamine with DCA may alleviate oxalate accumulation and reduce peripheral neurotoxicity. Alternatively, individualized dosing by dividing patients into populations with different metabolic rates based on their age and GSTZ1 genotype or targeted delivery of DCA using nanovesicle-targeted dosing may be possible strategies to enhance its therapeutic efficacy and address its accumulation and peripheral neurotoxicity.

In conclusion, current animal and cellular experiments suggest that DCA is a promising neuroprotective drug with multiple pharmacological activities. Since energy metabolism disorders and mitochondrial dysfunction are important pathologic mechanisms in a variety of neurological disorders, further preclinical and clinical studies of this drug are warranted.

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Disclosure

The authors report no conflicts of interest in this work.

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