Pyruvate dehydrogenase deficiency — morphological and metabolic effects, creation of animal model to study and research for treatment therapy

Authors: A. Ebertowska, B. Ludkiewicz, I. Klejbor, N. Melka, J. Moryś

DOI: 10.5603/FM.a2020.0020

Article type: REVIEW ARTICLES

Submitted: 2019-09-30

Accepted: 2020-02-03

Published online: 2020-02-13

This article has been peer reviewed and published immediately upon acceptance. It is an open access article, which means that it can be downloaded, printed, and distributed freely, provided the work is properly cited. Articles in "Folia Morphologica" are listed in PubMed.
Pyruvate dehydrogenase deficiency — morphological and metabolic effects, creation of animal model to study and research for treatment therapy

A. Ebertowska¹, B. Ludkiewicz¹, I. Klejbor¹,², N. Melka¹, J. Moryś¹
¹Department of Anatomy and Neurobiology, Medical University of Gdansk, Poland
²Department of Clinical Anatomy, Pomeranian University in Slupsk, Poland

Address for correspondence: A. Ebertowska, Department of Anatomy and Neurobiology, Medical University of Gdansk, ul. Dębinki 1, 80–210 Gdańsk, Poland, e-mail: adriana.ebertowska@gumed.edu.pl

ABSTRACT
The main source of energy for brain and other organs is glucose. To obtain an energy for all tissue glucose has to come through glycolysis then as pyruvate is converting to acetyl-CoA due to pyruvate dehydrogenase complex (PDC) and finally join to citric acid cycle. What happens when one of these stages become disturb? Mutation in genes encoding subunits of PDC leads to pyruvate dehydrogenase deficiency. Abnormalities in PDC activity result in severe metabolic and broad brain malformations. For better understanding the development and mechanism of pyruvate dehydrogenase deficiency the murine model of this disease has been created. Studies on a murine model showed similar malformation in brain structures as in the patients suffered from pyruvate dehydrogenase deficiency such as reduced neuronal density, heterotopias of grey matter, reduced size of corpus callosum and pyramids. There is still no effective cure for PDC-deficiency. Promising therapy seemed to be ketogenic diet, which substitutes glucose to ketone bodies as a source of energy. Studies have shown that ketogenic diet decrease lactic acidosis and inhibit brain malformations, but not the mortality in early childhood. The newest reports say that phenylbutyrate increases level of PDC in brain, because reduced level of inactive form of PDH. Experiments on human fibroblast and zebra fish PDC-deficiency model showed that phenylbutyrate is promising cure to PDC-deficiency. This review summarizes the most important findings on the metabolic and morphological effects of PDC-deficiency and research for treatment therapy.

Key words: pyruvate dehydrogenase deficiency, metabolic disorders, Leigh syndrome, phenylbutyrate, ketogenic diet
INTRODUCTION

Glucose is the main source of energy for brain and other organs. To obtain energy for all tissue glucose has to come through glycolysis then as pyruvate is converted to acetyl-CoA due to pyruvate dehydrogenase complex (PDC) and finally join to tricarboxylic acid cycle. Abnormalities in PDC activity results in severe biochemical/cellular, metabolic and morphological/anatomical malformations. The most of study in this field was concerned of molecular/biochemical mechanism, but in this review, authors summarize primarily less numerous findings on the metabolic as well as morphological effects of PDC-deficiency and research for treatment therapy.

PYRUVATE DEHYDROGENASE COMPLEX

The pyruvate dehydrogenase complex (PDC) plays a key role in converting pyruvate to acetyl-CoA. This irreversible oxidative decarboxylation of pyruvate to acetyl-CoA links the glycolytic pathway in the cytosol with the citric acid cycle in the mitochondria [19]. The pyruvate dehydrogenase is a huge complex built of three catalytic enzymes, which collaborate closely with each other. PDC is consist of E1 subunit (PDH) - having pyruvate dehydrogenase activity and catalyze pyruvate decarboxylation, E2 subunit - dihydrolipoamide acetyltransferase, which is responsible for acetylation of coenzyme and the E3 subunit - dihydrolipoamide dehydrogenase - catalyze the regeneration of oxidized lipoamide form [36]. Furthermore, a PDC complex includes protein X, E1- specific kinase and phospo-E1 phosphatase [37].

The E1 component is a heterotetramer consisting of two subunits- α and β. Its activity depends on its phosphorylated or dephosphorylated form [28]. The dephosphorylation converts E1 subunit to an active form and is regulated by specific phosphatase, which is one of components of PDC. Consequently, regulation of E1 subunit impacts the activity of whole complex. The organ where the accumulation of active form of PDC is the highest is the brain [[45]. In mammals PDHα component is known in two isoforms, which are encoded in two ways - PDHA1 gene in human’s somatic cells (Pdha1 in mouse) is located in a X chromosome and PDHA2 (Pdha2 in mouse) localized in chromosome 4 and expressed only in testis. The cognizance about E1α genetic record is very useful, due to many primary defects occurring in this region are the main reasons a PDC deficiency [8,29].
PYRUVATE DEHYDROGENASE DEFICIENCY AND ITS COROLLARY

Pyruvate dehydrogenase deficiency is a congenital, metabolic disorder caused by incorrect version of gene encoding respective subunits of PDC. The faulty genes pertain to each of the complex subunits, but the E1α deficiency is the most frequent [35]. Within mutation of E1α some missense/nonsense as well as deletions and insertions were noticed. Lissens and coworkers studied a group of 130 mutations. They discovered that insertions and deletions occurred on the exons 10 and 11 the most frequent and the missense/nonsense mutation appeared at all exons. There were also some sex differences, namely they found the missense/nonsense mutation in different exons in male and female. Furthermore, frequency of missense/nonsense mutation was lower in female [27]. Interestingly, the highest amount of mutation in E3 subdivision was observed in Ashkenazi Jews population [2]. There was also known case of missense mutation in gene encoding E2 component of PDC, which resulted in Paroxymal Exercise-Induced Dyskinesia [14].

Glucose is the primary source of energy for every cell in the body. Because the brain is the most energy-demanding organ (brain glucose utilization accounts for approximately 80% of whole-body glucose disposal in humans), impaired glucose metabolism results mainly in morphological and hence functional changes of nervous structure. Commonly there were known two forms of pyruvate dehydrogenase disorder symptoms - metabolic (severe neonatal lactic acidosis, hyperlactacidemia, hyperpurvic acidemia) and neurological (congenital brain malformations like microcephaly, ventricular enlargement, periventricular leukomalacia, agenesis or absence of pons, corpus callosum, pyramid, likewise neurodevelopmental delay is observed). There were also some characteristic features in appearance noticed [6,9,21,57]. Barnerias et al. presented four neurological types comprising (1) malformations in corpus callosum and cortex that occur with early encephalopathy, (2) basal ganglia abnormalities and dysfunction of brainstem, (3) congenital motor disorders with paroxysmal dystonia or neuropathy and (4) chronic axonal neuropathy [3].

The disease, related with an abnormal activity of pyruvate dehydrogenase is the Leigh syndrome (subacute necrotizing encephalomyelopathy). Symptoms of this neurodegenerative congenital disorder were described by the first time in 1951 by Denis Leigh based on case of 7-month old boy who died due to numerous cerebral abnormalities [26]. The subacute necrotizing encephalomyelopathy is the most frequent phenotype of mitochondrial diseases in children, affect in 1:40 000 live births [15,46]. Symptoms of this disorder occur in infancy or early childhood (3-12 months, 2nd year of life - the latest) [43,46]. Leigh syndrome was shown primarily in a neurological lesion in brain structures like ventriculomegaly, encephalopathy,
changes in the grey matter, changes in basal ganglia; in the research of Lee and his teamwork
the putamen was the most lesioned structure among the basal ganglia [13,25]. There were also
pathological changes observed in medulla oblongata and midbrain, particularly in substantia
nigra and red nucleus. Lesions in other brain areas like thalamus and cerebellum were also
noticed. In the cerebellum atrophy as well as in MRI studies T2 hyperintensity was observed in
hemispheres as well as in vermis

Therefore, as a conclusion, this is important to mention that among morphological effects
of an abnormal activity of pyruvate dehydrogenase a particularly large number applies of
brain structures involved in motor function (basal ganglia, cerebellum, red nucleus, pyramids)
and cognitive function (cerebral cortex, dentate gyrus, corpus callosum) are involved.
Whether is this associated with a higher energy demand for cells in these structures remains
an open question and requires further research.

MURINE MODEL OF PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY

The pyruvate dehydrogenase complex deficiency and its consequences are neither
completely understood nor no fully effective medicine is discovered. For this reason,
scientists from Buffalo University developed murine model of pyruvate dehydrogenase
complex deficiency with deletion of Pdha1 gene. To make it happen they used Cre-loxP
recombination to delete Pdha1 gene encoding alfa subunit of E1 component [17,22,31].
Amount of alive progeny were reduced, furthermore only female pups survived. The null
mutation was lethal for male progeny [41,42]. Obtained animal model of PDC deficiency
showed similar features as cases of human PDC deficiency described in other publication,
therefore it became a promising tool in PDC deficiency studies.

MURINE MODEL OF PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY
IN RESEARCHES

Pyruvate dehydrogenase complex is an important factor in a glucose homeostasis and
glucose is the main energy substrate for a brain [10]. Therefore, as was mentioned above, the
malformations caused by deficiency in PDC occur mostly in a brain. The experiments on a
murine model of PDC demonstrated a numerous deformities and abnormalities in brain
structures in comparison to control animals. Researches of Buffalo University used the MR
imaging, spectroscopy studies and ADC mapping to evaluate the lesions. The analysis of
brain structures in experimental animals showed plenty of morphological and histological
changes. Thickness of neocortex was decreased as well as pyriform cortex; density of
pyramidal neurons was dramatically reduced. Heterotopias in forebrain particularly in caudate nucleus/putamen, changes in architecture of thalamus- reduced neuronal density in paraventricular, anterodorsal, lateral and medial nuclei, reduced thickness of corpus callosum, reduced size of anterior commissure and lateral olfactory tract and enlarged lateral ventricle were observed. Some structures of forebrain were underdeveloped as well. They also observed disorganization of neuronal axons in pyramidal decussation, anterior commissure, trigeminal lemniscus, dorsal tegmental decussation, rubrospinal tract and lateral olfactory tract. Some malformations in cerebellum also were observed - the most conspicuous feature was the general size of this structure which were smaller than in control group also reduced size of cerebellar hemisphere cortex, particularly density of Purkinje and granular neurons as well as cerebellar peduncles. In molecular layer they observed reduced amount of fibers so its general size was decreased. ADC analysis confirm significant cell loss and changes in brain structures. As expected, observed malformations correspond with altered brain tissue observed in the human suffered from PDC deficiency [41,42]. The important aspects of PDC deficiency were also biochemical parameters, particularly activity of PDC. Results of this analysis showed that transgenic female mice had wild-type as well as PDC-deficient cells in the brain. The activity of active form and total PDC was reduced by 25% compared with control female mice. Homogenates of brains from PDC-deficient demonstrated reduced level of α and β subunits of PDH in PDC-deficient animals [42].

Transgenic animals were examined on the different stages during development. Previous studies reported that developing brain demands a lot of energy sources right after birth and a PDC level significantly increases in this period [20,51,54]. Developmental studies demonstrated that the disturbances in PDC activity impairs differentiation and proliferation of neuron cells in both prenatal and postnatal periods. Analysis of brain structures confirmed previous observation like reduction of white matter structures, thickness of neocortex and heterotopias in grey matter. [41,42]. Furthermore, they observed gliosis and significant decrease of neuron number. In a given work were behavioral tests performed as well. Obtained results demonstrated that mice with PDC-deficiency suffered from neurological disorders [39].

Brain was not the only structure investigated in mice with PDC-deficiency. Because of important role of PDC in glucose homeostasis and lipid synthesis also liver seemed to be important subject to researches. Choi et al. [7] developed PDC-deficiency mice model with deletion of PDC specific for liver. As a result, they observed reduced body mass in transgenic mice. These studies were based on observation of the carbon movement via PDC and
investigated its role in biosynthesis of lipids and generation an energy. The sensitivity of insulin was also examined: the results showed that in liver and in other tissues the sensitivity of insulin was enhanced. It was suspected that improved sensitivity arises out of the need to keep the normal rates of oxidative phosphorylation, which demand increased usage of free fatty acid [7]. To take a closer look at insulin management in PDC-deficiency one more specific murine model was created. Result showed that acetylo-CoA, which come from pyruvate oxidation due a PDC was very important factor participating in processes involved in insulin secretion from β-cells [48].

**COULD WE FIND A CURE?**

The oldest applied therapy in PDC deficiency was the ketogenic diet. In the early 20th century the ketogenic diet was used as a treatment for epilepsy and uncontrolled seizures but afterward it was superseded by antiepileptic drugs. These days the diet is still in use, but mostly in drug-resistant epilepsy [1,33,55]. The aim of ketogenic diet is to substitute the fuel source from carbohydrates to ketone bodies and get effect similar to long-term fasting. In normal condition the energy in human organism is acquired from glucose. During ketogenic diet energy is derived from fatty acid oxidation [4,18]. Several studies reported that ketone bodies have neuroprotective effect as they influence to mitochondrial activity improved ATP production and decrease production of free radicals. The influence of ketogenic diet to nervous system is also significant for GABA signaling in neurons as well as supervise the work of neuronal synapses [30,50,56]. Ketogenic diet could be also applied in diverse neurological disorders such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, strokes and other mitochondrial disorders [50]. The abnormality in PDC deficiency arises from incorrect converting pyruvate, which is a product of glycolysis to acetylo-CoA, which is a substrate in tricarboxylic acid cycle [34]. In case of disorder connected with a carbohydrate metabolism ketogenic diet seems to be a perfect solution to reinstate an energy balance. But what the results have shown. Sofou and coworkers examined a group of 19 PDC-deficient children of different age. Results of ketogenic diet treatment showed positive effect on lactic acidosis, because the level of lactate significantly decreased. In pediatric patients with epileptic seizures reduced frequency or even completely suspension were observed. Improvement was noticed also in neurocognitive functioning as well as motor function. However, the ketogenic diet therapy did not significantly affected life expectancy (median in this study was 6 years) [47]. The beneficial effects like stabilization and inhibition of expending the brain abnormalities were also observed in other studies [38,53]. The effects
of ketogenic diet had been also investigated in mice model of PDC-deficiency with the
difference that, the special diet was applied to pregnant animals and then during lactation.
Therefore, they examined effects of treatment on the progeny. The brain structures in studied
progeny (with feeding fat-rich diet) were much better improved than in control group,
especially in neocortex, organization of neurons fibers and cerebellar structures. Furthermore,
no structural abnormalities in forebrain and brainstem structures were observed. This study
concluded that ketogenic diet during prenatal and early postnatal period was gainful for PDC-
deficient mice progeny [40].

The administration of thiamine pyrophosphate (TPP) as an additional treatment can
potentially be effective, given its role as an obligate cofactor for the E1 component of the
PDC complex. Many cases of PDC deficiency involved a pathological mutation affecting the
thiamine pyrophosphate binding site of E1, supplementation with high doses of thiamine
seems justified. The TPP treatment showed improved the clinical outcome like suspension of
ataxia episodes and/or reduced lactate acidosis. These results were confirmed by in vitro
studies showing a thiamine-responsive functional defect in the activities of PDHC and E1 in
the lymphoblastoid cells cultured as well as in muscles and fresh mononuclear cells. Results
showed that measurement of TPP is essential in low as well as high activity of PDHC to
determine the thiamine responsiveness. [3,16,24,44]

Dichloroacetate (DCA) stimulates the activity of the PDC by inhibiting the
phosphorylation (and inactivation) of the E1α subunit of PDC complex. In some patients with
E1α deficiency, DCA may also stabilize the enzyme and decrease its rate of turnover.
Several studies had reported that the chronic, oral administration of dichloroacetate has been
beneficial for patients with PDC deficiency or some patients with respiratory chain defects.
DCA treatment was effective in reducing blood, CSF, or brain lactate concentrations and in
improving quality of live. However, cases of peripheral neuropathy and hepatocellular
toxicity has been noted [5,23,32,49]

Phenylbutyrate seems to be a promising therapy in PDC-deficiency. This compound
is well known and applied in other therapies such as urea cycle disorder, so the activity and
effects on healthy tissue is well-known [52]. Experiments tested in human fibroblasts and
zebra model of PDC-deficiency showed that phenylbutyrate increased activity of PDC in
brain and other organs. This is because the phenylbutyrate affect the PDC Kinase and inhibits
it - in consequences the phosphorylated (inactive) form of PDH is reduced, whereas the
dephosphorylated form (active) was predominant. Furthermore, the systemic lactic acidosis
was reduced [12]. Interestingly, phenylbutyrate in combination with dichloroacetate is much
more effective in increasing the activity of PDC in mice. However, the toxicity of these drugs combination is unexplored and demand further tests [11]. The impact of phenylbutyrate on PDC complex activity and its result in brain and other tissue is still examined.

CONCLUSIONS

Pyruvate dehydrogenase deficiency is a serve metabolic and neurological disorder. Despite many promising therapies applied to patients no one is completely effective. Ideally, ketogenic diet should be applied to pregnant mothers and to infants immediately after birth, unfortunately the knowledge about disorder comes too late. Phenylbutyrate, which reduced level of inactive form of PDH is safe for healthy tissue and seems to be hopeful weapon against PDC-deficiency. This therapy however, demands further examination.

REFERENCES


