REVIEW ARTICLE

Changes in brain metabolites in experimental cerebral malaria infection with plasmodium berghei ANKA: A literature review

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Abstract

In this paper, we have collected the findings of available literature focusing on brain metabolites by spectroscopy in the murine model of cerebral malaria disease. The literature search for experimental cerebral malaria (ECM) and spectroscopy using National Institute of Health's PubMed database provided us with 9 peer-reviewed publications. These publications have used mice infected with Plasmodium Berghei (PbA) Antwerpen-Kasapa (ANKA) strain to mimic the human infection with Plasmodium falciparum. Brain ischaemia, as depicted by increased lactate and alanine concentrations, as well as decreased aspartate and adenosine triphosphate levels, play a key role in ECM. Lowering the lactate levels by using dichloroacetate has been shown to improve survival. Significant cellular injury has also been documented through decreased N-acetylaspartate and glycerophosphocholine levels. The advantage of using spectroscopic technique provide important functional information which helps determine the aetiology, pathogenesis, progression, and monitoring of treatment as well as predicting prognosis in the clinical setting of cerebral malaria.

Keywords: Cerebral malaria, ANKA strain, Imaging, Spectroscopy, Metabolites.

Introduction

Severe, malaria is the term used to describe a number of entities, including severe metabolic abnormalities, cerebral malaria, as well as multi-organ failure. The severity of disease depends upon the immunity developed by the host over repeated courses of infection.[1,2] Cerebral Malaria with Plasmodium falciparum is the most common manifestation of severe malaria and leads to devastating consequences, including death.[3-6] Reduced cerebral blood flow from sequestered infected erythrocytes in post capillary venules has been proposed as the root cause of its pathogenesis,[4-6] but the exact subsequent mechanism of injury remains unknown.[6-11] Since limited research has been done on human cerebral malaria (HCM), with most of the studies based upon postmortem examinations,[12] there has been interest in mice models of the disease, both for investigating the pathophysiology, as well as for the development and monitoring of new treatments.

An ideal mice model for understanding this complex menace in humans has yet not been possible. Plasmodium Berghei Antwerpen-Kasapa (ANKA) (PbA) model of experimental cerebral malaria (ECM) has been frequently adopted. Infected red blood cells sequestering the microvasculature have been found in several organs of mice in the PbA model, but it has not been shown that these pathologies follow the same pattern as in HCM. Concerns regarding the validity of PbA model have been raised. Therefore, other models such as the non-human primate (NHP) models using Plasmodium coatneyi and Plasmodium fragile in rhesus monkeys have been explored. Erythrocyte sequestration in the microvasculature of brain tissues was more evident in these models. However, the similarity of sequestered pathology to the human disease still needs to be fully explored. NHP models may be better suited to study the immunological aspects of the disease.[1]

There are differences between HCM and ECM. For example, erythrocytes sequester around damaged blood vessels causing microcirculation obstruction in HCM, whereas the erythrocyte accumulation found in ECM has been shown to follow a different distribution pattern. In PbA model of ECM, immune cells (monocytes, macrophages and T cells) are found more consistently to sequester the microcirculation.[1] P. falciparum produces a heterogeneous form of disease in HCM with wide variability in the distribution and proportion of erythrocytes affecting vasculature.[1] Despite differences between HCM and ECM, there are a number of similarities between the two disease entities.[1] These included disruption of the blood-brain barrier, sequestration of cells to the damaged endothelium causing blood flow obstruction and micro-haemorrhages, and most importantly cognitive impairment. Leukocyte accumulation responsible for inflammatory responses has also been seen in the HCM.[1] Since both HCM and ECM, with

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erythrocyte or leukocyte accumulation, are associated with signs of cerebral malaria, it appears reasonable to adopt the PbA model in assessing the disease pathology in HCM.[1] This review focuses on the reported neuroimaging findings, including those from magnetic resonance spectroscopy (MRS), in ECM. The studies reviewed have used PbA model in susceptible mouse strains like C57BL/6, CBA/T6, and CBA/J to elicit ECM.

CT and MRI findings of Human Cerebral Malaria

Cross-sectional imaging techniques such as X-ray computerised tomography (CT) or magnetic resonance imaging (MRI) have been rarely used to study cerebral malaria in humans, despite the high prevalence of the disease in some countries. This lack of neuroimaging studies reflects the high incidence of the disease in developing countries with limited brain imaging resources. CTX-ray can depict structural changes, such as cerebral oedema with hypoattenuation of the thalamus and cerebellum in HCM.[13] CT is not able to differentiate between the causes of increased brain volume whether from increased vasculature volume or oedema, for which MRI is ideally suited. HCM disease severity is also not accurately reflected on CT; for instance patients with proven cerebral malaria have been reported to have normal CT scans. One patient was reported to have normal brain CT (both on contrast unenhanced and enhanced scans), but showed hyperintensities in the left thalamus and parietal cortex on fluid attenuated inversion recovery (FLAIR) and T2-weighted MRI scans.[14] Patankar et al. demonstrated normal CT scans in patients with mild disease by correlating clinically, using acute physiology and chronic health evaluation (APACHE) II score and Glasgow Coma Scale (GCS). Brain swelling seen on CT scan was not associated with mortality, but increased volume also accompanied by hypoattenuation of the cerebellum, as seen in patients with poor GCS, bad APACHE II score, and multiple organ damage, associated with a poor outcome and high mortality.

Increase brain volume is viewed as an important factor responsible for the development of adverse consequences in both HCM and ECM.[8,15-18] Increased brain volume may be from increased vascular volume from intravascular sequestration[2] or from oedema which may be either vasogenic or cytotoxic. Increased blood volume results from sequestration and opening of pseudo capillaries which handle collateral flow. Vasogenic brain oedema results from the increased permeability of water across the blood brain barrier (BBB), resulting in pooling of extravascular spaces.[8,17] Cytotoxic oedema results from the inability of sodium-potassium adenosine triphosphatase (Na+/K+ATPase) pump to function properly in the absence of sufficient adenosine triphosphate (ATP) molecules, causing an increase in intracellular volume, cellular swelling and eventually cell death.[16]

Brain swelling has been reported as an inconsistent feature of adult cerebral malaria in humans. For example, only 20% of the patients studied by Looareesuwan et al. developed increase in brain volume, who also reported a significant number of cases with normal CT scans.[19] In contrast to adults, studies in paediatric populations have shown the development of brain swelling more consistently. These findings highlight the importance of developing models of cerebral malaria in both adults and paediatric populations.[18]

Furthermore, it was shown in a 17-year-old boy presenting with coma, seizures and hemiparesis with bilateral thalamic hypoattenuation on CT scan, that the hypoattenuation persisted after recovery as did the neurological deficits.[13] Patankar et al. also showed that normal CT scans predict good prognosis, but cerebellar hypoattenuation on CT predicts a poor prognosis. Lesions in the basal ganglia, pons, and cerebellum have been documented by MRI and correlated clinically with focal findings.[20] Parieto-occipital lobe has been shown to suffer infarction with haemorrhage in adjacent areas.[21] MRI has shown patients with cerebral malaria to have haemorrhagic venous infarcts in the frontoparietal lobe, as well as signal intensity changes in the parieto-insular region.[14] Focal lesions in the white matter and corpus callosum have also been documented.[22] Diffuse hyper-intensities noted in the white matter on T2-weighted images and FLAIR have been attributed to oedema whereas focal lesions have been depicted as gliosis.[23]

Cerebral blood flow in ECM

In a study on mice with mild ECM, enlarged ventricular spaces were seen on imaging. This enlargement was not observed in mice with severe ECM, however. This finding was interpreted as being due to an initial cerebrospinal fluid (CSF) volume increase, but with a further increase in intracranial pressure, a reduction in volume was observed associated with compression of brain parenchyma and tissue damage, as noted in the corpus callosum and striatum.[8]

Decreased cerebral blood flow (CBF) to the brain in ECM using flow alternating arterial inversion (FAIR) and quantitative arterial spin labelling techniques has been reported consistently.[8,24] Kennan et al. reported a 48% decrease in blood flow with a particular focus on entorhinal cortex, inferior hippocampus and the

thalamus. This is in contrast to a study of non-cerebral malaria which found no changes in cerebral blood flow in the initial investigations, but documented a significantly increased blood flow in non-cerebral malaria with disease progression and increased parasitaemia.[25]

The aetiology of the decreased CBF in mice with cerebral malaria may be complex. It appears that the adhesion of monocytes to cell membranes of vessels and cerebral oedema might be responsible.[8,10] By Day 3 to 5 after inoculation of mice with PbA, there was an increased concentration of cell adhesion molecules with attachment of inflammatory cells to the endothelium, relocation of glial cells, leaking of BBB, and central nervous system (CNS) vascular obstruction.[10,26,27] Brain metabolite concentrations have usually been found disturbed after Day 5. The key factor thought to play a role in pathogenesis, thus remains cerebral oedema. Evidence supporting this hypothesis includes the patchy distribution of altered blood flow,[26] and the absence of localising neurological signs. A constant rise in intracranial pressure would mean reduced perfusion and eventually a state of generalised ischaemia, or more disturbingly brain distortion/herniation.[10]

Cerebral Metabolite findings in ECM

MRS is an MR technique that allows the localised, non-invasive measurement of brain metabolite levels.[28] MRS has been widely applied in studies of human brain metabolism for both research and clinical purposes. However, we only found 9 MRS studies on mice infected with PbA. Out of the nine studies (Table), three used in-vivo quantification of brain metabolites using proton1H-MRS in infected mice,[8,24,25] five papers studied brain extracts using in-vitro1H-MRS technique,[10,15,29-31] one of which also studied CSF concentrations of metabolites as well,[30] and one used in-vivo quantification of bioenergetics molecules using phosphorus.[31] P-MRS followed by in-vitro measurements of other metabolites using[1] H-MRS.[9] The basal ganglia have been the primary area of focus and techniques going up to magnetic field strengths as high as 9.4T have been used. In all of these studies, either comparisons with control (i.e. uninfected) mice were performed, or with mice infected with the non-cerebral malaria (NCM) strain of pathogen.

When comparing metabolite concentrations between ECM and controls, studies using in-vitro spectroscopy showed a decrease in glycerophosphocholine (GPC),[15,29] choline (Cho), phosphocholine (PC),[15] myo-inositol (mI),[29] glutamate (Glu) and aspartate (Asp)[9] in ECM. An increase in Glu,[30,31] glutamine (Gln),[10,15,31] gamma aminobutyric acid (GABA),[31] lactate (Lac),[9,10,31] alanine (Ala),[10,31] hydroxybutyrate and threonine (Threo)[29] was also observed in ECM. However, other studies found difference in Gln,[9] GABA,[9,29] NAA and Glu between ECM and controls.[29] Using in-vivoMRS, a decrease in NAA,[8,24] phosphocreatine (PCr) and ATP,[8,9] an increase in glutamate + glutamine (Glx), lactate (Lac),[8] and phosphomonoester (PME),[9] has been observed in ECM. No difference was seen in mI, PME between ECM and controls in one study.[8] As noted above, discrepancies between studies have been noted for several molecules, including mI, NAA, Glu, Gln, GABA, and PME. Rae et al. noted a reduction in Lac and Ala after treating ECM infection with dichloroacetate (DCA).

When comparing NCM with controls using in-vitro quantification of metabolites, a study showed an increase in Lac, Glu, Gln, GABA, Asp and PME in NCM compared to the controls.[9] No difference was observed between the two groups for Ala.[10] In-vivo measurements have shown no difference between the two groups for NAA,[25] inorganic phosphate/PCr (Pi/PCr), and Pi/ATP.[9] In NCM, a progressive decrease in Glu, mI, GPC,[10] and Cho/S was observed with disease progression.[25] Similarly, an increase in Gln, Ala, and glycine was found in advanced disease.[25] Decreased PC, Cho, GABA have also been observed in NCM,[15] with no difference reported for Lac[10,25] and Glx amongst the two groups.[25] These differences might be accounted for by assessing the severity of the disease.

Furthermore, in-vitro comparisons of NCM with ECM have revealed an increased level of Lac, Glu, GABA, Asp,[9] GPC,[15] and a decreased level of Glnin NCM compared to ECM.[15] In-vivo comparison has shown decreased levels of Pi/PCr and Pi/ATP in NCM.[9]

Discussion

Changes in cerebral metabolites as measured by MRS may have important implications towards better understanding of the pathogenesis of cerebral malaria.

NAA is believed to be a marker of neuroaxonal cell density and viability, and its decrease signifies neuronal injury.[8-10] On MRS studies, NAA peak has been found decreased in ECM infection.[8-10] This is consistent with evident parenchymal damage.[8] The rate of neuron cell damage could, however, vary according to an unpredictable, unidentified number of factors, e.g. individual mice characteristics, susceptibility to infection, or the ability to mount an immune response. Therefore, the consistently decreasing trend seen in NAA concentrations in ECM infection could have exceptions as noted by Le Moyec et al., who did not find a significant change in NAA levels.

Table: Literature review of MRI/MRS findings in mice infected with Plasmodium bergheiANKA causing cerebral malaria.

Author	Study Method. Magnetic Strength/TR(sec)/TE(msec)/ voxel size, voxel location	Metabolites	Metabolite Findings	Cerebral Perfusion with area studied; Cerebral Structural Changes; Associations
Le Moyec et al. 1997	In vitro 1H-MRS performed on perchloric acid extracts of brain tissue.	-hydroxybutyrate -threonine -mI -GPC -GABA -NAA -Glu	-Increase in hydroxybutyrate and threonine in ECM -Decrease in mI and GPC in ECM -No change in GABA, NAA, Glu between ECM and controls.	N/A
Rae et al. 2000	In vitro 1H-MRS performed on perchloric acid extracts of brain tissue.	-Lactate -Aspartate -Alanine -GABA -Glu -Gln	-Increase in lactate and alanine in ECM -Increase in Glu, Gln and GABA in ECM -Reduction of lactate and alanine, with no effect on Glu, GABA, and aspartate after treatment with DCA. -Further increase in Gln in ECM after treatment with DCA.	-40% of the subjects treated with DCA survived.
Sanni et al. 2001	In-vitro 1H-MRS.	-Lactate -Alanine -Gln -Glu -mI -GPC	-Increase in lactate, alanine and Gln in terminal stage of ECM. -No change in lactate or alanine in NCM and controls. -Progressive decrease in Glu, mI, and GPC in NCM.	-Decrease in cerebral blood flow in ECM; -Changes in lactate and alanine in ECM did not correlate with parasitemia. -Linear correlation was found between time since infection and progressive decrease in GPC and NAA in ECM.
Rae et al. 2004	In-vivo 31P-MRS followed by in-vitro 1H-MRS.	-PME -Pi -PCr -ATP	NCM vs controls -Increase in lactate, Glu, GABA, Gln, aspartate in NCM than controls. -No difference in Pi/PCr and Pi/ATP. -Increase in PME in NCM compared with controls ECM vs controls -Decrease in Glu, and aspartate in ECM. -No change in GABA, Gln. -Increase in Lactate, Alanine, Pi/PCr, Pi/ATP and PME in ECM. NCM vsECM -Increase in lactate, Glu, GABA, aspartate in NCM as compared to ECM strains. -Low Pi/PCr and Pi/ATP in NCM than ECM	N/A
Kennan et al. 2005	In-vivo 1H-MRS, perfusion measured by flow alternating arterial inversion (FAIR) spin labeling method. 9.4T/2/50/ 20ul, gray matter within caudate.	-NAA -Cr	-15% decrease in NAA/Cr in ECM. ($P<0.01$)	-48% decrease in blood flow ($P<0.012$) in theentorhinal cortex, inferior hippocampus, thalamus; -No structural changes in the brain were noted; -NAA/Cr was significantly correlated with cerebral perfusion
Penet et al. 2005	In-vivo 1H-MRS, 31P-MRS. 4.7T/1.5/16 or 135/3.5mm3, striatum and thalamus.	-NAA -tCr -Cho -Glx -mI -taurine -PCr -Pi -PME -ATP	1H-MRS At TE=16 -Decrease in NAA/S in ECM. -Increase in Glx/S in ECM. -No change in mI/S (S=NAA+tCr+Cho+Glx+mI+taurine) 1H-MRS At TE=135 - Decrease in NAA/S in ECM. -Increase in lactate in ECM (S=NAA+tCr+Cho) 31P-MRS -Decrease in PCr/Pi, ATP/Pi, PCr+ATP/Pi in ECM. -No change in PME.	-Decrease in blood flow in the striatum and cortex; -Increase in brain volume (edema), damaged corpus callosum, and hyper-intense lesions in caudate-putamen; -Negative correlation between lesion load and the ventricle volume in ECM. -Negative correlation between NAA/S and lactate/S
Penet et al. 2007	In-vivo 1H-MRS, 31P-	-Cho	NCM	-Initially no change in cerebral blood

	MRS. 4.7T/1.5/135 or 16/42.875m m3/striatum and thalamus bilaterally.	-mI -Gln -Glycine -Alanine	-No change in lactate, NAA, Glx in NCM. NCM with high parasitemia -Decrease in Cho/S -Increase in Gln, Glycine, and Alanine.	flow in NCM as compared to controls. -Significant increase in blood flow at day 15 of inoculation (due to increased parasitemia); -No parenchymal lesions in NCM; -Decrease in Cho is related to parasite proliferation.
Miranda et al. 2010	-In-vitro 1H-MRS on synaptosomes -CSF metabolites were also measured	-Glu -Gln	-Increase in Glu in cerebral cortex and CSF of ECM ECM Vs NCM	-Increased Glu levels are associated with behavioral symptoms analyzed by SHIRPA battery. **-Gln had a** high Spearman
Ghosh et al. 2012	-In-vitro 1H-MRS. (This paper also studied the metabolites in serum and liver; the results of which have not been discussed in our paper).	-GPC	-Increase in Gln in ECM -Decrease in GPC in ECM ECM Vs Controls -Low Cho, GPC, PC in ECM -High Gln in ECM NCM Vs Controls -Low PC, Cho, GABA in NCM.	correlation with taurine in ECM. -GPC had a negative correlation with PC and Cho in ECM. -GABA had a positive correlation with NAA in ECM. -Glu had a positive correlation with NAA in ECM.

HCM= human cerebral malaria, ECM= experimental cerebral malaria, NCM= non-cerebral malaria, PbA= Plasmodium berghei ANKA, CT= computerized tomography, MRI= magnetic resonance imaging, FLAIR= fluid attenuated inversion recovery, FAIR=flow alternating arterial inversion, fMRI= functional magnetic resonance imaging, MRS= magnetic resonance spectroscopy, TR= repetition time, TE= echo time, BBB= blood brain barrier, CSF= cerebrospinal fluid, mI= myoinositol, CNS= central nervous system, GABA= gamma aminobutyric acid, NAA= N-acetyl aspartate, GPC= glycerophosphocholine, PC= phosphocholine, Glu= glutamate, Gln= glutamine, Glx= glutamine + glutamate, Cr= creatine, PCr= phosphocreatine, tCr= total creatine, Pi= inorganic phosphate, PME= phosphomonoester, ATP= adenosine triphosphate, Cho= choline, DCA= dichloroacetate, NE= norepinephrine, Asp= aspartate, Ala= alanine, Threo= threonine, Lac= lactate, APACHE= acute physiology and chronic health evaluation, GCS= Glasgow coma scale, TCA= tricarboxylic acid, N/A= not available.

GPC is a marker of cell density, related to the choline esters of cell membranes.[10,29] GPC peak has been shown to fall during ECM infection. To our understanding, GPC is taken up by plasmodium, which in turn causes altered lipid levels and decreased production of GPC in infected brains.[15,29] A positive correlation has been found between GPC and NAA. Both molecules gradually decrease over the course of ECM infection.[10,15]

Ischaemia and hypoxia induces glycolytic enzymes in the glial cells, thus increasing their production of lactate.[8] Alanine may also be produced from pyruvate and increased in hypoxia and/or ischaemia.[10] Rise in alanine was shown to be proportional to hypoxaemia, whereas lactate plateaus off after an initial increase.[10] Macrophages also produce lactate as a response to inflammation.[8,24] The increase in lactate and alanine was seen to be concordant with decreased blood flow to the brain.[8,10,31] Normal physiology dictates the efficient transport of lactate and alanine from brain cells into the circulation. These metabolites of anaerobic respiration thus accumulate when the blood flow is hampered. Concentration of lactate was found decreased in the blood of mice suffering from ECM infection.[10] Increased brain lactate thus results from local production in the brain.[9,10] Excess lactate has been thought to contribute towards brain oedema and eventually cellular damage.[32] In hypoxic conditions, Asp levels were found decreased.[9]

A strong negative correlation between NAA and lactate has been found. Decreased cerebral blood flow means a deficiency of glucose and oxygen supply, thus raising lactate and decreasing NAA.[8,24] As noted earlier, DCA has been studied and shown to increase lactate clearance by speeding up the tricarboxylic acid (TCA) cycle.[31] This treatment resulted in an increased number of surviving mice compared to the untreated group.

Glutamatergic system has been seen to be affected in a complex manner. An increase in brain Glx was observed in ECM infection.[8] The increased Glx signal has been mostly attributed to increased Gln.[10,15,31] Glu also increases in the brain, CSF and particularly the synaptic clefts in ECM infection.[30,31] In the glial cells, Glu gets converted to Gln, which can be eliminated. Brain suffers an ischaemic attack during ECM infection, evidenced by increased lactate and alanine.[9] Ischaemia modifies Gln metabolism, resulting in over-activity of Glnsynthetase, causing increased production and a relative inactivity of glutaminase enzyme responsible for Gln clearance.[8,15] During periods of ischaemia, Glu was shown to get internalised by Na+/K+ ATPase pumps in the astrocytes, metabolised to Gln,[8] and thence decreasing the concentration of Glu.[9] Increased activity of Glu/Gln cycle, however, has been seen even before the manifestation of ischaemia.[9] Once ischaemia sets in, lactate and alanine levels raise along with the increasing metabolism of Glu/Gln.[9,10] Increased concentration of Gln aggravates brain swelling by exerting osmotic pressure.[8,15] Rae et al. noted a decrease in Glu with no change observed for Gln. This could mean

increased conversion of Glu to Gln with a simultaneously increased excretion of Gln through mechanism not known. In another study, no difference in the concentrations of Glu was found between ECM and controls.[29] It appears that the changes in Glu, Gln or Glx are dependent upon a number of metabolic factors yet to be fully explored.

Decreased levels of mI have been detected in ECM infection.[15,29] The levels of mI have been correlated with changes in Gln levels. With increasing Gln, the liver gets geared up to detoxify this excess ammonia in Gln form. Liver uses mI to produce glucoronic acid which is primarily used for detoxification.[15,29,33] Used up mI would eventually lead to rising Gln, resulting in worsening toxaemia as well as brain oedema through its osmotic action.[33] This could, however be just a shallow observation as changes in the levels of mI have not been found significant.[8,15]

In ECM infection, the amount of ATP produced was found to be reduced, consistent with anaerobic glycolysis. Pi was found increased with both PCr and ATP decreased.[8,9] Increased Pi reflects hypoxic conditions.[9] Comparison of NCM with controls revealed no significant changes in Pi, ATP, or PCr.[9]

Several similarities have been noted between HCM and ECM.[4,5] Symptoms experienced by both include ataxia, paralysis, seizures and coma.[30] Like ECM, a patchy distribution of decreased cerebral blood flow has been found in the human disease.[34] Lactate, associated with anaerobic respiration and a bad prognosis, has been found to be elevated in both.[8-10,31] These similarities have led us to rely upon research on ECM to infer mechanisms involved in human disease. It is often ethical as well, especially where post-mortem examinations have been performed. Undoubtedly, research on mice has proved fruitful in many ways. For example, increased lactate concentration in the brain which is a trademark of human disease,[8] has also shown its footprints in experimental models with conviction.

This review focused mainly on MRS in experimental ANKA strain causing cerebral malaria. MRS provides a non-invasive means for judging anatomic, metabolic, biochemical and functional aspects of the brain in cerebral malaria patients just like in any other disease.[35] Furthermore, spectroscopy has a superior role in cerebral malaria. MRS is a useful and safe technique in research as well as clinical setting. MRS can measure lactate, NAA, Cr, mI, Glu, Gln, as well as Cho.[31,35] P-MRS can quantify ATP, PCr, Pi and intracellular pH.[35] To our knowledge, MRS techniques have not been widely used in human population to study malaria.

Conclusion

Routine use of MRS can provide novel evidences towards disease pathology and treatment. MR techniques represent a good and probably the ultimate future solution towards entangling the knots behind P. falciparum infection responsible for the deaths of millions. According to the papers reviewed in this article, a decrease in NAA and GPC consistent with cellular injury, an increase in Lac and Ala as well as a decrease in Asp and ATP levels has been rightly attributed to ischaemic conditions. The decrease in mI in ECM has not been significant. DCA decreases Lac levels in the brain and improves survival. Glutamatergic system is poorly categorised with mixed results demanding further exploration.

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