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Hepatic encephalopathy: Nomenclature, pathogenesis, therapy

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Budapest, Hungary

2016

Contents

List of abbreviations

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	Serotonin, 5-Hydroxytryptamin
AASLD	American Association for the Study of Liver Disease
ATP	Adenosine triphosphate
BBB	Blood brain barrier
CNS	Central nervous system
cPSS	Congenital portosystemic shunt
EASL	European Association for the Study of Liver disease
Erks	Extracellular signal regulated protein kinases
GABA	Gamma-amino-butyric acid
GLT-1	Glutamate transporter 1
Glu	Glutamic acid
HE	Hepatic encephalopathy
Il	Interleukin
iNOS	Induced nitric oxide synthase
KATP-channel	ATP-sensitive potassium channel
MAO	L-Monoamine oxidases
MAP	mitogen-activated protein
Mn	Manganese
MMP-9	Matrix metalloproteinase-9
MRI	Magnetic resonance imaging
mRNA	messenger Ribonucleic acid
NCC	Non-selective cation channel
NF-kB	Nuclear factor kappa B
NH_3	Ammonia
NH_4^+	Ammonium ion
NKCC-1	Sodium-potassium-chloride-cotransporter-1
NMDA	N-Methyl-D-Aspartate
NO	Nitric oxide
NOX	Nitrogen oxide
PBR	Peripheral type benzodiazepine receptor
PEG	Polyethylene glycol
PSS	Portosystemic shunt
RONS	Reactive oxygen–nitrogen species
SUR-1	Sulfonylurea receptor-1
TNF	Tumour necrosis factor
TGF-β1	Transforming growth factor beta 1

Abstract

The hepatic encephalopathy (HE) is a reversible metabolic syndrome that can appear due to acute liver failure, chronic liver failure or portosystemic shunt. The problem is that several neurotoxic compounds that are absorbed from the gut are not detoxicated by the liver and can reach the central nervous system.

The cytotoxic effect on the astrocytes is a major point of the cerebral pathogenesis, which leads to the swelling of the astrocytes causing the development of brain oedema.

The pathogenesis and the aetiology are not completely understood due to the complexity. In recent years, several theories have been developed about the pathomechanism. These focuses on the main actors: ammonia, gamma-aminobutyric acid, benzodiazepines and false neurotransmitter. New approaches to pathogenesis have also changed the treatment approaches. Concerning the treatment, there are several changes made especially in the dietary aspects.

The exact molecular mechanism of how ammonia contributes to astrocyte swelling and so to brain oedema is not fully explained.

1. Introduction

This thesis will deal with hepatic encephalopathy (HE). This work will be divided into two main parts. The main theme of the first part is the categorisation of the different types of HE, the anatomy of the congenital portosystemic shunt (cPSS) and the most relevant aspects of the pathogenesis. Several mechanisms and factors, those previously existing and those developed in the last years are the ammonia, changing of the GABA-ergic tone, benzodiazepines, manganese (Mn), and aromatic amino acids/false neurotransmitter, etc. The aim will be to discuss the different theories on the basis of the recent studies.

The second main part follows up the numerous treatment possibilities of HE. The focus will be on the medical therapy of this syndrome and not on the surgical intervention. The latter will be discussed only briefly. The different approaches of the medical therapy will be discussed concerning their effectivity.

HE is not a recently discovered syndrome. Already in 1890, first investigations were done by Pavlov. Dogs were used in these investigations. For research purposes, these dogs had a surgical anastomosis, so-called shunt from the portal blood to the *vena cava caudalis*, with the result that the portal blood circulation is connected with the caval circulation. Consequently, detoxification by the liver is limited and nitrogen-rich blood bypasses the liver. Examinations showed an elevated blood and brain ammonia level and changes in the neurological behaviour as well (Tivers et al., 2014). The HE is considered a syndrome of neuropsychiatric disturbances that are associated with acute and chronic liver failure as well as portosystemic shunt (PSS) with the non-occurrence of hepatocellular disease in humans (Aldridge et al., 2015). However, in dogs is liver dysfunction present beside the cPSS and this form is occurring the most in dogs (Lidbury et al., 2016).

The interesting character of this symptom arises from the complex network of interdependent organ systems, the relation mechanisms of which are not fully understood.

2. Nomenclature & Classification system

Due to the broad range of neuropsychiatric abnormalities of different severity, acuity and time course, a precise nomenclature and classification system is needed. To resolve the confusions regarding the nomenclature and classification concerning HE, a conference was held during the 11th World Congress of Gastroenterology in Vienna in 1998. The result of this conference was a multiaxial definition and classification of HE in 2002. In 2014, the AASLD-EASL (American Association for the Study of Liver Disease-European Association for the Study of Liver disease) published new guidelines intended to bring more uniformity in description and categorisation of HE. In this guideline, the *„HE is defined as brain dysfunction caused by liver insufficiency and/or PSS, and manifests as a wide spectrum of neurological/psychiatric abnormalities ranging from subclinical alterations to come "*(Dharel and Bajaj, 2015). This classification system is designed for humans, but the

There are two different categories existing. These are the overt HE (Grade II-IV) and the covert HE (Grade I plus the minimal HE) (Leise et al., 2014).

The overt HE is the clinical appearing form which can be detected by clinical tests. The diagnosis is based on the combination of the mental status abnormalities on one side and on the other side an impaired neuromotor function like asterixis, hyperreflexia or hypertonicity. Nonetheless other alterations of the mental status must be excluded. The disorders can arise episodically for a few hours per day or as a persistent neuropsychiatric impairment. The so-called *West Haven criteria* were established to categorise the different mental status abnormalities. Grades range from Grade 0 (no abnormalities) up to Grade IV (coma, unable to test mental state). The different grades are based on distinct clinical parameters such as alteration in the level of consciousness, intellectual function, behaviour and occurrence of asterixis. However, the *West Haven criteria* are only used by humans (Dharel and Bajaj, 2015). The different grades of HE are shown in *Table 1* (adapted from N. Dharel, 2015).

The 'minimal HE' is the form that appears subclinical. That is why the old term was 'subclinical HE'. This subclinical impairment of cognition is only detected by specific psychometric or neurophysiological tests. There is neither the appearance of signs nor

symptoms, but it has been shown to impact the quality of life of patients with chronic liver disease (Dharel and Bajaj, 2015). Maybe it is more important in dogs that this minimal HE is predictive of the future onset of an overt HE.

Lastly, there is covert HE, introduced in 2011 by the International Society for Hepatic Encephalopathy and Nitrogen Metabolism and includes the Grade 1 HE and the minimal HE as an umbrella term. All in all, it is defined as psychiatric and neurophysiological abnormalities in the absence of disorientation and asterixis. But it is stated that the *"covert HE is a multifactorial group which requires future validation and study".* Neurocognitive dysfunction appears as a dynamic, continuous process, which constantly moves from one state to another state (Dharel and Bajaj, 2015).

It is questionable if we will be able to diagnose the lower graded HE in dogs by different psychiatric or neurophysiological tests. So far there are no know possibilities for a proper diagnosis in dogs (Lidbury et al., 2016), however this system for the classification and the nomenclature of HE can be used as an orientation for a grading system for dogs with HE.

In 2014, the AASLD and EASL created guidelines for the nomenclature of liver disease. This model is called *the four axis model*, because HE should be classified on the basis of the underlying aetiology, severity of clinical manifestations, the time course and if the precipitating factors are identified or not. These guidelines should help for the management of HE in chronic liver disease, but it can be also applied to HE due to acute liver failure or due to PSS from various aetiologies. Each axis of these four axes has a unique importance in the clinical description, management and prognosis of clients suffering with HE (Dharel and Bajaj, 2015).

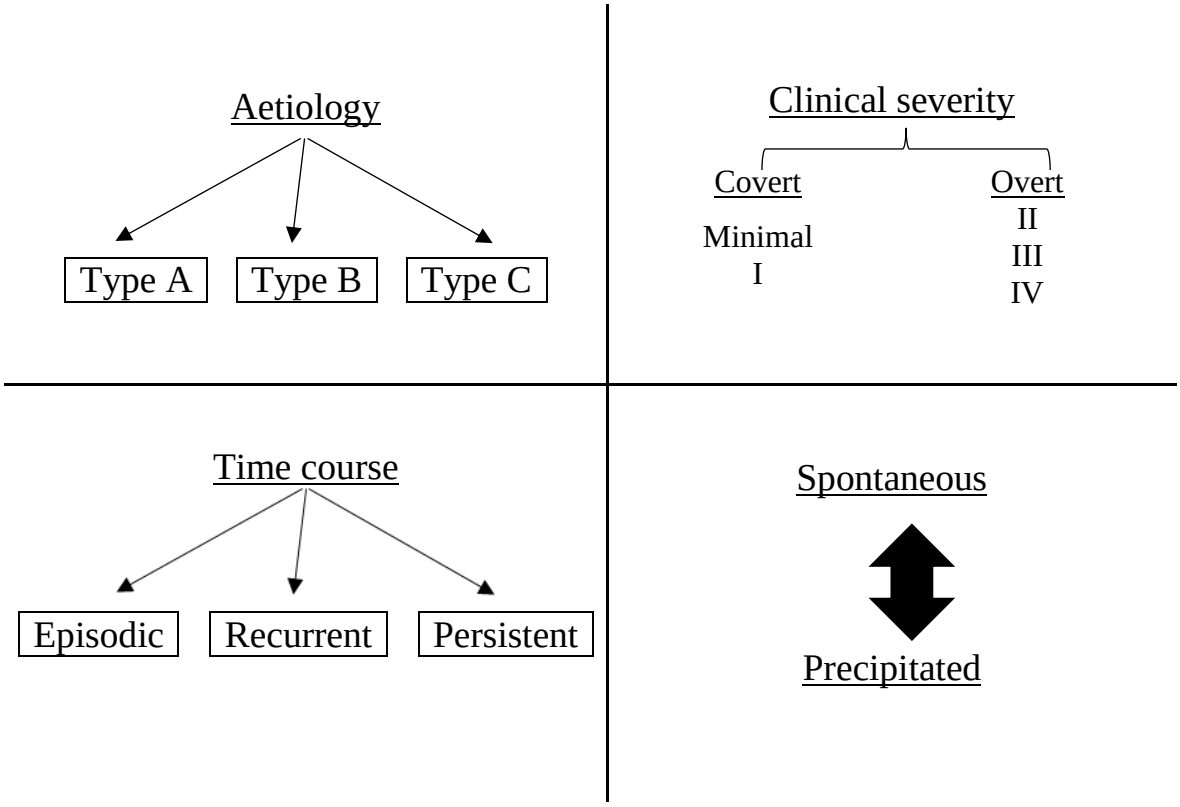

Table 1: The four Axis model of HE

First axis: Aetiology

On the basis of the aetiology, HE is classified into three different types:

Type A: due to acute liver failure

Type B: due predominantly to portosystemic shunting or bypass

Type C: due to cirrhosis

Type B and Type C will be generally similar in the clinical manifestations and management.

The Type C is the most common reason for a HE in humans (Salgado and Cortes, 2013). However, in dogs the most common cause of HE is the Type B, which is very rare in humans (Kanazawa et al., 2015). A big difference between humans and dogs in type B is that there are no hepatocellular damages in humans, but there is liver dysfunction present beside the cPSS in dogs (Lidbury et al., 2016).

Second axis: Clinical severity

Appropriate recognition and correct identification of the severity of the encephalopathy are very important for proper care, therapy and monitoring of the patient.

This axis is subdivided into:

- Covert HE: including minimal HE + West Haven Grade I (absence of disorientation and asterixis)
- Overt HE: including West Haven Grade II-IV HE

Third axis: Time course

The time course of HE may be important in determining prognosis, setting goals of term care and allocations of resources. This category is divided into:

- Episodic (bouts occurring more than 6 months apart)
- Recurrent (bouts recurring within a time frame of 6 months)
- Persistent (patterns of behavioural alterations that are always present interspersed with relapses of overt HE)

Fourth axis: Precipitated or Spontaneous

This axis shows the precipitating factors which needs to be identified and corrected during the management of HE

- Spontaneous (non-precipitated)
- Precipitated (secondary)

The precipitating factors are of special importance because many times several factors are involved simultaneously in episodes of HE. These factors that may cause elevated ammonia level, are the follows:

- Excessive protein intake and fluid restriction (e.g., constipation)
- Gastrointestinal bleeding together with increased production of ammonia, due to intestinal bacteria which are digesting the stored blood in the gastrointestinal tract

- Hypokalaemia plus metabolic alkalosis appearing during the ascites mobilising diuretic therapy, leading to increased renal ammonia production

The last two axes are neither applicable for minimal HE nor covert HE (Dharel and Bajaj, 2015).

The application of such a system of categorisation in veterinary medicine for a better description and categorisation of the diagnosis may improve the quality of care and the result for the patients suffering from HE.

3. The anatomy of portosystemic shunt

A shunt is a local circulation disorder. There are various types of PSS. They can differ in the way in which they develop, as well as their location, number and size. The knowledge of all these parameters are important for the therapy following the diagnosis.

The *portal vein* is the functional vessel of the liver. This vessel brings venous blood from unpaired abdominal organs like the stomach, intestines, pancreas and spleen to the liver. This blood transports components of the digested nutrients which are reabsorbed into the liver. The pressure in the *portal vein* is higher (dog 5,88-7,36 mmHg) than in the *caudal vena cava* (dog 2,2-2,94 mmHg). Due to this the blood, can overcome the resistance of the liver circulation and all the blood will be detoxified by the liver. However, it is possible under physiological conditions in mammals that the blood flows through some veins directly into the *caudal vena cava* by the *oesophageal veins* are located in the cardioesophageal part of the gastrointestinal tract or by the *rectal veins* located in the rectoanal area. The *rectal veins* are connected with the *internal pudenda vein*, which flows into the *caudal vena cava*. This vessel causes a connection to be formed between the portal and the caval blood circulation. A stasis or a change in the blood pressure in the portal circulation can lead to a drainage of blood through these vessels. These kinds of shunts are termed as extrahepatic PSS. This phenomenon at the *oesophageal veins* can lead to oesophageal varices due to local venous dilatation. The major problem is that portocaval shunts pass portal blood by the liver. The portal blood coming from the intestines is rich in protein degradation products and fatty acids, which will have direct access to the caval circulation, because they are not removed by the liver. These substances remaining in the blood may have a neurotoxic effect and the result is the syndrome HE (Salomon et al., 2008).

Another form of the PSS is the intrahepatic shunt. In the embryonic blood circulation, there is an intrahepatic connection between the left *umbilical vein* and *caudal vena cava*, which is called the *ductus venosus* (Arantii). This duct can be found in cats and dogs. This kind of shunt between the portal and the caval blood can also lead to a decreased detoxification by the liver. In the end, this also leads to a situation where toxic substances can enter the body circulation and can have access to the central nervous system (CNS) (Gille, 2008).

In larger dog breeds, the intrahepatic shunt, while in smaller dog breeds extrahepatic shunts are more prevalent (Baumgärtner and Gruber, 2015).

The previous named intra- and extrahepatic PSS are the congenital portosystemic shunts. Another group are the acquired portosystemic shunts. They are collateral vessels which can be the outcome of a portal hypertension (Lidbury et al., 2016).

4. Pathogenesis of Hepatic Encephalopathy

As already mentioned in the introduction, there are different approaches for the pathogenesis. The first published approach was by the physiologist and Noble Prize winner Pavlov. But his results from 1890s showed that an elevated ammonia level is the key in the pathogenesis of HE (Tivers et al., 2014).

In recent years, several other approaches have been developed. HE is a pathological alteration in individuals suffering from liver disease. It is defined as a neuropsychiatric syndrome that can be fatal. As of 2014, the full pathogenesis is still not completely understood. Several factors are suggested to play a key mediator role in the pathogenesis such as ammonia, inflammatory cytokines, Mn, gastrointestinal derived endogenous benzodiazepines and aromatic amino acids (Tivers et al., 2014).

There are many similarities between the pathogenesis of HE in dogs with a cPSS and the human variety of HE, however there are also some differences. In dogs and in human is ammonia taking a central role in the pathogenesis, however, in dogs the HE is often connected with a PSS. Most of the knowledges regarding the pathogenesis of HE is based on human-studies, thus this thesis uses as references those papers written about HE occurring in humans due to chronic, acute and cirrhotic liver disease. The cPSS in dogs is a common hereditary abnormality. This abnormality describes a shunting from the portal blood circulation to the caudal cava vein (Tivers et al., 2014).

The clinical signs of HE in dogs are considered to be of neurological impairment, including lethargy, inappropriate behaviour, disorientation, circling, head pressing or seizures (Tivers et al., 2014). Only if one has understood the pathogenesis to its fullest extent, can a responsible therapy of a disease be given.

4.1. Blood-brain barrier permeability

The blood brain barrier (BBB) is a protective wall for the brain. It restricts the free movement of polar and non-polar molecules, water to and from the central nervous system. Concerning the morphology: the barrier consists of endothelial cells that line the lumen of the brain capillaries. A tight junction closes the paracellular space between the cellular edges of its own cells or between two surrounding cells and it rests on the basal lamina, which is engulfed with pericytes, astrocytic endfoods and is encircled by microglial cells and neurons. This together builds up the so-called *neurovascular unit.* (Chen et al., 2013). The permeability of the BBB for a given molecule is directly related to its lipophilicity (Oldendorf, 1974). The molar mass is inversely proportional, therefore the more lipophilic and smaller a compound, the easier it diffuses through the endothelium of the BBB. For the molar mass of a molecule, the limit value is a maximum size of 400 to 500 g·mol-1 (Kaliszan and Markuszewski, 1996). A disturbance of this unit can result in a serious outcome for the CNS function. BBB damage due to a liver failure is connected with changes in the ultrastructural architecture. Dysfunction of the BBB is assumed with injurious factors, neurotoxins and inflammatory cytokines (Chen et al., 2013).

The BBB is highly relevant for the brain microenvironment homeostasis, which is needed for a stable and coordinated neuronal activity. The brain tissue is protected from harmful influences like changes in the blood plasma components, neurotransmitters or invasion of xenobiotics or toxins (Ciecko-Michalska et al., 2012). Furthermore, the BBB makes the selective transport of substances from the blood circulation into the brain possible by diffusion or active transport through the endothelial cells. Another very important role is in providing appropriate nutrients and the removal of waste products (Ciecko-Michalska et al., 2012). Another aspect is that the BBB is influenced by a numerous number of substances, for example, ammonia, serotonin, bradykinin, adenosine purine nucleotides, interleukins, free radicals, nitric oxides or steroids, just to name a few. These substances are involved in the condition of the endothelial function and tightness of the BBB (Ciecko-Michalska et al., 2012).

The BBB prevents neurotoxic metabolites to enter the brain. This is possible because the BBB is not permeable for such neurotoxins in physiological circumstances, but if there is a disturbance, the BBB can become permeable (McMillin et al., 2015).

Ammonia, which plays a major role in the pathogenesis of HE, has a direct effect on the metabolic state and function of the central nerve system and especially on the passage of many different molecules through the BBB. Among these substances branched chain amino acids and aromatic amino acids have an essential role. The increased inflow of aromatic amino acids leads to the formation of glutamine, which is present in large amount during a HE because it is formed by the detoxification of ammonia (Ciecko-Michalska et al., 2012). The higher amino acid inflow influences the brain catecholamine synthesis (serotonin and dopamine) and also the formation of "false neurotransmitters" like octopamine or phenylethylamine (Ciecko-Michalska et al., 2012).

A study has shown that TGFβ1 (Transforming growth factor beta 1) is able to induces the increasing of permeability of BBB. After a liver damage TGFβ1 enters the circulation because it is produced by the liver. The released TGFβ causes an upregulation of MMP9 (Matrix metalloproteinase-9) in endothelial cells of the BBB and afterwards the MMP9 is released into the circulation. TGFβ1 is causing a downregulation of claudin-5 and a disruption of tight junctions. These effects together are able to damage the BBB in such a dramatic level that TGFβ1 and toxins can pass through the BBB and enter afterwards the CNS to worse HE pathology (McMillin et al., 2015).

4.2. Neuroinflammation

Microglia cells are the intrinsic immune cells of the CNS. They are bone-marrow derived cells (Rama Rao et al., 2013). These cells are the constant macrophages of the CNS. They become activated by homeostatic changes in tissue damage, vascular disturbances, pH changes and imminent energy failure (Butterworth, 2015).

Several different stimuli are able to activate the microglia. These stimuli potentiate the nuclear translocation of several transcriptional factors like the nuclear factor-kappa B, which is able to induce genes that can encode several inflammatory factors, for example, NOX, iNOS, cytokines and others.

Ammonia has a direct effect on the astrocyte swelling, as previously mentioned, but it also has an effect on the microglia cells, causing an inflammatory response, which leads to the formation of the lethal complication, brain oedema (Rama Rao et al., 2013). The activated microglia can release inflammatory cytokines, free radicals, prostaglandins and arachidonic acid and all of them are able to cause a swelling of the astrocytes (Rama Rao et al., 2013). *In vitro* studies showed that microglia treated with ammonia release reactive oxygen and nitrogen species (RONS). This results in astrocyte swelling which can be inhibited by RONS scavengers (Rama Rao et al., 2013). The oxidative stress on the cerebral endothelial cells caused by the hyperammoniaemia can elicit vasogenic brain oedema (Skowrońska et al., 2012).

The close relations between inflammatory cytokines and HE has been shown. A deletion of the coding genes for the TNF-alpha or Il-1beta causes a delay in the onset of encephalopathy and leads to a reduction in the formation of brain oedema. In some studies, there was an activation of microglia parallel with an accumulation of pro-inflammatory cytokines TNF-alpha, Il-1beta and Il-6 in the brain (Butterworth, 2015).

Others have also shown the high correlation between HE and the level of serum cytokines (TNF-alpha, Il-1beta, Il-2R, Il-6 and Il-8), but the severity of this syndrome was only related to TNF-alpha. The relationship was independent from the underlying pathogenetically factors or precipitating factors (Goral et al., 2010).

4.3. Cytotoxic brain oedema

Several studies have shown that the swelling of astrocytes is a key point in the pathogenesis of HE. It is suggested that HE is an implication of a low grade chronic glial oedema with an important impact for glioneuronal communication. There are various osmosignalling pathways which have been perceived. These pathways provide a connection between the cell hydration and the cell function. Consequently, a small increase of the water content in the astrocytes already has a substantial functional impact, even without showing clinical signs of the increase in the intracranial pressure (Häussinger et al., 2002).

However, it is substantial that complex changes in the function of astrocytes function due to the swelling of them, hyperammonemia and other precipitating factors have been perceived. These factors can lead to an activation of osmosignalling cascades, covalent modification of astrocytic proteins and gene expression. In addition, astrocyte swelling can cause the alterations of several signalling pathways (Häussinger et al., 2002).

An increase of the intracellular calcium concentration causes an up-regulation of the peripheral type benzodiazepine receptors (PBR) which affects multiple ion channels and amino acid transport. In addition, the astrocyte swelling causes an increase in the pH of endocytic vesicles by an extracellular signal regulated protein kinases (Erk)-dependent osmosignalling pathway. The low endosomal pH has a significant role for the receptor/ligand sorting. The distinct endosomal alkalinisation due to the astrocyte swelling is believed to influence the receptor densities and neurotransmitter processing. The more the expression of PBR due to the astrocyte swelling is the more the synthesis of neurosteroids is. These neurosteroids are potent modulators of neuronal $GABA_A$ receptor activity. This pathway may explain an increased GABA-ergic tone during a HE. A quick nitration of critical tyrosine residues in astrocyte protein is caused by the astrocyte swelling and ammonia level in HE. This protein tyrosine nitration takes also place *in vivo* after an ammonia injection or surgical portocaval shunt. This NH_3-induced protein tyrosine nitration is a calcium dependent process and involves N-Methyl-D-Aspartate (NMDA) receptor activation, nitric oxide synthase induction. It can be prevented by NMDA-receptor antagonists, methionine sulfoximine and antioxidants (Häussinger et al., 2002).

Patients with acute liver failure has an increased intracranial pressure due to brain edema. One of the main component of the cerebral edema in acute liver failure is the astrocyte swelling and elevated lactate level has been strongly implicated in its formation. (Butterworth, 2015).

Transporters and ion channels are involved in the cell volume regulation. In case of disturbances, cell swelling can be the result. In astrocyte cell culture, pre-treated with ammonia, showed a higher non-selective cation channel (NCCa)-ATP channel activity in the astrocytes. The higher NCCa-ATP channel activity was measured by the sulfonylurea receptor type 1 (SUR-1) protein and mRNA levels. The astrocytes cultures had an increase of SUR-1 protein expression, which is a marker of the NCCa-ATP channel activity (Jayakumar et al., 2014).

As mentioned above, the swelling of the astrocytes is the underlying reason for the formation of brain oedema and it can be a lethal complication. The cytotoxic brain oedema causes an increase in the intracranial pressure and brain herniation. The consequence out of this pathological changes can be coma or death (Rama Rao et al., 2013).

4.4. Circulating neurotoxins

4.4.1. Ammonia neurotoxicity

It is assumed that ammonia plays an important role in the pathogenesis of HE. A healthy organism maintains the balance between production and excretion/detoxification of ammonia (Gerber and Schomerus, 2000). Ammonia is primarily derived from the intestinal bacterial flora, however the intestinal glutaminase activity is also an essential source of ammonia (Tapper, 2015). Ammonia is mainly produced in the *colon* as a by-product of the bacterial metabolism of proteins and urea, but as a by-product of bacterial degradation of glutamine is also produced in the small intestines (Gerber and Schomerus, 2000).

Ammonia is also produced by extra intestinal organs like the muscles and the. The ammonia production of the muscles depends on the muscle work and is proportional to it. During a resting phase of the muscle the production and the breakdown of ammonia is balanced. The kidney produces just a small amount of ammonia, which can be elevated during a diuretic treatment or during hypokalaemia (Gerber and Schomerus, 2000).

Under physiological conditions the detoxification of ammonia occurs in the hepatocytes during the urea cycle mainly but also during the formation of glutamine. In the case of the urea cycle, ammonia is after excreted from the body as urea. In the situation when the liver function is impaired, the first-pass hepatic extraction can decrease dramatically and the brain can be exposed to excessive amounts of ammonia. This can happen in situations like, hepatocellular failure and/or portosystemic shunting (Williams, 2007).

There are numerous effects of ammonia in the CNS function. This also includes the direct effect of ammonium ions (NH_4^+) on the excitatory and inhibitory neurotransmission, inhibition of glucose (pyruvate) oxidation and stimulation of glycolysis, altered mitochondrial function and impairment of key cellular transport system (Butterworth, 2014).

Ammonia is able to pass the BBB and can be detoxificated in the brain in the astrocytes. The astrocytes are the only cells in the CNS which are able to metabolise ammonia (Williams,

2007). The enzyme needed for this reaction is the glutamine-synthetase. In this reaction, ammonia and glutamate is converted to glutamine. The circumstances of an excessive concentration of ammonia leads to a high accumulation of glutamine in the astrocytes which cause water influx in the cells (Butterworth, 2014).

Another important theory for the astrocyte swelling has been described as the so-called "The Trojan Horse Hypothesis" which will be discussed in details in the part "Glutamine theory". The detoxification of ammonia to glutamine is an ATP-dependent process. Hyperammonemia results in high glutamine production and also in higher energy consumption. The so created neurotoxicity of ammonia is leading to neuropsychiatric symptoms in the patient (Williams, 2007).

4.4.2. Manganese neurotoxicity

Several factors are involved in the swelling of the astrocytes like transporters, exchangers, and ion channels, which are attributable to the swelling as a result of the deregulation of the cell volume. Products of oxidation and nitration are involved in the pathophysiology of the astrocyte swelling. A study in which primary astrocyte cultures of rats were used to investigate *"the activation of Na-K-Cl Cotransporter-1 (NKCC-1) a downstream mechanism for free radical induced astrocyte swelling as a result of Mn toxicity".* In the end, it was proven that Mn, oxidants and NO donors are potent inducers of oxidation and nitration of the NKCC-1. Furthermore, the study has shown that Mn increased the total protein, phosphorylation, the activity of NKCC-1 and the cell volume of the astrocytes (Alahmari et al., 2015).

Mn is an essential trace mineral, which is essential in all known living organisms. It has several functions including a key component of several enzymes, which are needed for the synthesis of proteins, carbohydrates and lipids (Aschner et al., 2005). Furthermore, this cofactor is needed for the mitochondrial superoxide dismutase in the detoxification of superoxide free radicals. Non-experimental deficiencies in humans or in animals are uncommon because Mn is present in most foods (Finley et al., 1999). Several oxidation states of Mn are known, but only Mn^{2+} and Mn^{3+} are common in biological systems. The predominant species is Mn^{2+} (Roth et al., 2006). Only small quantities (~5%) of the orally ingested Mn is absorbed by the gastrointestinal tract (small intestines) (Davis et al., 1993). For the absorption, divalent metal transporter 1 (DMT1) and ferroportin are needed. After absorption, Mn is bound to a protein in the blood circulation. These transport proteins are mainly ɣ-globulin and albumin. The removal is mainly done by conjugation and excretion with the bile. The excretion via kidney and urine is low. With approximately 98% of the absorbed Mn, a so-called substantial first-pass effect occurs – so it is excreted before it can reach the circulation of the body. The distribution is fairly homogenous throughout the tissues. An increase in the concentration of Mn in the body organs is found in cells with a high number of mitochondria and pigmentation together with bone, pancreas, liver and kidney. The Mn concentration considered to be normal in a human brain is reported to be 0.26 µg/g wet weight. In dog a reference range between 0.3 and 0.99 µg/g wet weight is given. Due to a tightly controlled regulation of absorption and excretion of Mn, its level in

the tissue of an adult human is generally stable. Thus, it is not influenced by the intake but Mn toxicity or manganism has been reported in humans and experimentally in animals (Gow et al., 2010).

Reasons for an elevated Mn concentration in the body can be chronic exposure to Mn due to contaminated drinking water (Kondakis et al., 1989) and a lower excretion of Mn can be the result of any liver damage and other alterations like a PSS which leads to a decrease detoxification of Mn by the liver. Furthermore, Mn is hepatotoxic so it can worst an existing liver damage (Gow et al., 2010). Manganism, which is a Mn intoxication, manifests itself as a psychiatric disturbance, gait abnormalities, and cognitive deficits (Gow et al., 2010). Mn is deposited in the body in specific regions of the brain. MRI studies have shown that the regions with the basal ganglia are mostly affected (Rose et al., 1999; Ciecko-Michalska et al., 2012).

A direct relationship in humans between the blood Mn concentration, MRI hyperintensity consistent with Mn deposition and severity of encephalopathic score has been identified (Layrargues et al., 1998). The MRI investigation of 13 dogs having a cPSS showed hyperintense focal areas in the *lentiform nuclei* that were considered to be consistent with Mn deposition (Gow et al., 2010). MRI investigation on chronic liver failure patients shows a bilateral signal hypersensitivities in g*lobus pallidus* of T1-weightes images in 80–90% of the cases (Spahr et al., 1996). This bilateral signal hyperintensities are seen in patients with chronic liver failure and not in acute liver failure. It is believed that the BBB permeability to Mn may be selectively increased in the chronic condition. At an autopsy of a patient who died in hepatic coma, up to sevenfold increase was manifest in the concentration of Mn in the *globus pallidus*. In the same patient, the brain-copper level was increased by twofold. Due to the neurotoxic properties of Mn (Cu), the astrocyte swelling may be a consequence of metal poisoning. Dopaminergic neurons are especially sensitive to Mn and the alterations of dopaminergic parameters can be seen in the brain of chronic liver failure results from the Mn toxicity (Butterworth, 2003).

4.4.3. Benzodiazepine-like compounds theory

Several studies are debating about the role of endogenous benzodiazepine-like substances in the pathogenesis of HE. It is believed that benzodiazepine-like compounds are implicated in the neuropsychiatric symptoms of HE (Williams, 2007). A crucial factor in the pathogenesis of HE is the imbalance between inhibitory and excitatory neurotransmission. There are two important factors of the syndrome that are the increased tone of the inhibitory GABA receptor system and the elevated NH_3 level (Venturini et al., 2001). Endogenous benzodiazepine-like compounds are positive allosteric modulators of the GABA-A receptor (Venturini et al., 2001). Under normal physiological circumstances, bacteria of the intestines produce GABA. Then it is metabolised in the liver after the arrival via the portal blood vessels. Only trace amounts of benzodiazepines-like compounds are found in healthy animals and humans, but an increase has been noticed in diseased patients (Williams, 2007). An upregulation of peripheral benzodiazepine receptor cause an increased cholesterol uptake and neurosteroids synthesis. The neurosteriods have potent positive allosteric modulator properties on the GABA-A receptors which could the reason for the neural inhibition in HE (Poh and Chang, 2012). The benzodiazepine theory appears as attractive because in animals and humans with HE there is an elevated level of endogenous benzodiazepine-like substances noticed (Odeh, 2007). In case of a diseased liver or a PSS, GABA has the chance to pass by the liver and to go into the systemic circulation. Thus, a higher amount of GABA is not metabolised in the liver and able to have access to the CNS (Dragonjic et al., 2013).

An increased level of benzodiazepines is found in the brain and blood of patients suffering from HE in 60% of the cases (Venturini et al., 2001).

The role of benzodiazepine-like compounds in the pathogenesis of HE is regarded as controversial. An increase in the benzodiazepines-like compounds concentration in the blood circulation is also associated with an impaired hepatic cleaning effect. The benzodiazepine-like compounds have been identified as a potential precipitating factor for the HE (Williams, 2007). It is further believed that benzodiazepine-like compounds pathogenic mechanism involves an impaired astrocyte function and disorders in the GABA-ergic neurotransmission. The sources of these benzodiazepine-like compounds have different origins including vegetables and other plants in the nutrition, endogenous

biosynthesis and synthesis by the intestinal flora from some amino acids. The amino acids phenylalanine and methionine are responsible for this (Williams, 2007).

There are two different types of benzodiazepine receptors: the central type and the peripheral type. The central type forms a part of the GABA-benzodiazepine receptor complex. The peripheral type was originally found in the periphery as the name suggests. But they are mainly found in the mitochondrial membrane of the astrocytes in the brain. The two different receptor types differ in their pharmacological specificity, anatomical and their subcellular distribution. A higher densities of peripheral type benzodiazepine receptor was demonstrated in the brains of animals having an experimental HE. Considering the maintenance of the brain energy metabolism due to the mitochondrial function, the increased densities of peripheral type benzodiazepine receptor can have a pathophysiological significance in HE (Odeh, 2007). However, researches with rats showed that there is not always an increased expression of peripheral type of benzodiazepine receptors when cognitive or motor deficits were present or when some inflammatory makers were increased (Agusti et al., 2014).

Nonetheless, another factor is GABA, that takes the major role in the inhibition of neurotransmission. It is synthetized by the glutamate-dehydrogenase from glutamate in the presynaptic neurons as well and then is stored in vesicles. It can be released due to the activation of a specific GABA receptor in the postsynaptic membrane. This receptor is a part of a larger receptor complex, which is necessary for the binding of barbiturates and benzodiazepines (Dragonjic et al., 2013). The binding results in opening of the chloride ion channel, which results in an influx of chloride. A hyperstimulation of the postsynaptic membrane happens and this results in neuroinhibition. An increase in this event can lead to a lowered motor function and decreased consciousness. Both can be recognised in HE and can be characteristic for this syndrome (Dragonjic et al., 2013).

GABA increases inhibitory neurotransmission by several mechanisms. These are the increase of endogenous benzodiazepines, increased availability of GABA at GABA-A receptors due to enhanced synaptic release of the amino acid, immediate interaction of an elevated ammonia level with the GABA-A-benzodiazepine receptor complex and the upregulation of astrocytic peripheral benzodiazepine receptors which is induced by ammonia (Ciecko-Michalska et al., 2012).

Another hypothesis associates HE with an inhibition of the complex GABA-benzodiazepine receptor. With the use of flumazenil, which is a competitive antagonist of benzodiazepine receptors, a significant improvement of the clinical status in patients was achieved. The latter is taken as confirmation of this theory (Ciecko-Michalska et al., 2012), however the effect of flumazenil is controversial (Venturini et al., 2001).

A synergistic effect of ammonia and benzodiazepines in the pathogenesis of HE cannot be demonstrated because there is a lack of it (Venturini et al., 2001). Furthermore, it has shown that the ammonia, GABA level and the degree of HE does not correlate with each other. On the other hand, the receptor complex indicates a correlation between the benzodiazepines activity in the plasma and the severity of HE (Dragonjic et al., 2013). Studies in cirrhotic patients showed that the level of benzodiazepines just rise in proportional relation to the liver damage but neither to the degree of HE nor to the level of ammonia (Venturini et al., 2001).

4.4.4. False neurotransmitter hypothesis

Changes of plasma amino acids may alleviate some symptoms of HE by alteration in the profile of the brain neurotransmitter due to a shift in the uptake mechanism of the CNS of the different neutral amino acids (Jellinger et al., 1978). A shift in the quantitative ratio between branched and aromatic amino acids may become very problematic for the synthesis of the neurotransmitter in the CNS. The amount of aromatic amino acids (phenylalanine, tyrosine, tryptophan) is rising and the branched amino acids (valine, isoleucine, leucine) are decreasing (Gerber and Schomerus, 2000). The so-called Fischer-Index expresses this ratio (Val+Ile+Leu)/(Phe+Tyr). A higher proportion of aromatic amino acids enters the CNS and a disequilibrium in the synthesis of dopamine, noradrenaline and serotonin is the result and the synthesis of octopamine, phenylethanolamine, and tyramine is up-regulated. The latter are termed: “false neurotransmitters” (Holecek, 2015).

A hypothesis declares that aromatic amino acids and tryptophan will become elevated in the CNS during a decrease of branched chain amino acids. The two-different amino acid types

compete for the same carrier system at the BBB. Due to a reduced transfer of branched chain amino acids through the BBB, the transfer of aromatic amino acids is increased (Gerber and Schomerus, 2000).

The aromatic amino acids are the precursors for the synthesis of neurotransmitter. The oversupply of precursors leads to an elevated synthesis of false neurotransmitters (tyramine, octopamine and phenyl-ethanolamine). Octopamine can be also produced by the intestinal flora, leading to a further elevation of the systemic level. The false neurotransmitter may compete with the normal neurotransmitters at the same receptor. This competition is culminating in the neurological symptoms of the HE (Gerber and Schomerus, 2000).

4.4.5. Glutamine theory

There are several different assumptions about the role of glutamine in the pathogenesis of HE. Concerning the glutamine concentration in the brain of HE patients, biochemical investigations and 1H-magnetic resonance spectroscopy showed a significant increase. It was suggested that there is a relation between the accumulation of glutamine in the brain and the development of encephalopathy and brain oedema, however other studies showed that there is no significant correlation between the accumulation of glutamine and the severity of encephalopathy or the presence of brain oedema. This finding suggest that glutamine cannot be taken as a major reason for neurological disturbances (Butterworth, 2015).

It is also proposed that the signal which leads to an increase in the cerebral blood flow occurs after the generation of glutamine in the astrocytes. However, another theory explains the neurotoxic role of glutamine by its transamination to alpha-ketoglutaramate, a neurotoxic metabolite (Butterworth, 2015).

“The Trojan Horse Hypothesis” is the term of a theory in which glutamine (the Trojan horse) acts as a shuttle for the ammonia transport to the inside of the mitochondria of the astrocytes. The ammonia production continues by the degradation of glutamine which results in a mitochondrial energy failure (Butterworth, 2015). Studies showed that glutamine leads to

Ca^{2+}-dependent mitochondrial swelling. The glutamine is transported into the mitochondria and hydrolysed by phosphate-activated glutaminase to NH_3 and glutamate. The increase of neurotoxic NH_3 in the CNS results in harmful processes including oxidative/nitrative stress and mitochondrial permeability. This theory has been proven with a study in which L-histidine, was used as an inhibitor of the transport of glutamine into the mitochondria. A significant down-regulation of the ammonia-induced mitochondrial abnormalities and oxidative stress was the result. The use of 6-diazo-5-oxo-norleucine in a study blocked the mitochondrial abnormalities and the production of free radicals by NH_3 because it is able to inhibit the phosphate-activated glutaminase. This underlines the role of glutamine as a Trojan horse for the NH_3 to transport it to the inside of the mitochondria (Rao and Norenberg, 2014).

4.5. Neurophysiological level

4.5.1. Brain monoamines / Serotonergic neurotransmission

It is suggested that impaired serotonergic neurotransmission is important in the neurophysiological background of HE leading to behavioural changes in the patients. Monoamine neurotransmitter disorders are very similar in the symptoms of neurological disorders. Therefore, a high risk of misdiagnosis is existing (Kurian et al., 2011).

Serotonin is a widely distributed neurotransmitter in the brain. It has a key effect in the regulation of several physiological behaviours and functions, and it is involved in the pathogenesis of various pathological processes. Around 90% of serotonin is present in the enterochromaffin cells of the intestinal mucosa. Only around 1–2% of serotonin is found in the CNS, localised in the serotonergic neurons. The precursor amino acid of this neurotransmitter is tryptophan (Lozeva-Thomas, 2004).

In the CNS serotonin regulates the feeding behaviour, body weight, sleep/wake cycle, aggression and impulsivity, anxiety and depression. Beside this, serotonin is involved in the pathogenesis of several diseases like migraine, obsessive-compulsive disorders, suicidal behaviour, bipolar disorders, HE and others (Lozeva-Thomas, 2004).

In HE, the tryptophan uptake into the brain is increased (Gerber and Schomerus, 2000). Several studies have shown an increase in the concentration of tryptophan in the blood of rats with a PSS and in humans with HE. The elevated tryptophan level increases the serotonin synthesis (Lozeva-Thomas, 2004), causing an increased serotonin level and consequently an increased level of its degradation product: 5-hydroxyindoleacetic acid (5-HIAA) (Gerber and Schomerus, 2000).

Other studies showed that there is a lack of significant changes in the 5-HT, but instead 5-HIAA – a serotonin metabolite - is significantly elevated. This result suggests that an

elevated serotonergic synthesis during HE is counterbalanced by the monoamine oxidases (MAO) activity. MAO increases the serotonergic metabolism and there is also a higher density of catalytic sites and expression of the brain MAO-A. These steps are resulting in a significantly decreased serotonin turnover rate and a synaptic deficiency of serotonin in the brain. It is suggested that due to the intracellular localisation of MAO, the significant amounts of 5-HIAA are produced in the nerve ending from the serotonin which was never released as a neurotransmitter (Lozeva-Thomas, 2004). It is assumed that the serotonin-receptors in the brain are decreased, but with an accompanying elevation of their affinity especially in the reticular formation which can explain the changes in the sleep-wake rhythm in humans with HE (Gerber and Schomerus, 2000).

Analysis of several brains by fluorescence spectroscopy showed the way how the neurotransmitters are distributed in the different parts of the brain of patients suffering from HE. The result of the analysis showed that there is a significant general elevation of brain tryptophan, dopamine was uniformly decreased and 5-HT was increased in the brainstem and striatum. Lastly, there was a quite uniform increase in the turnover of 5-HT, estimated by the level of 5-HIAA in the brain (Jellinger et al., 1978).

There is another finding of recent studies about the relationship between serotonergic dysfunction and the degree of PSS. The outcome of latter studies showed that the serotonergic turnover is exquisitely and selectively sensitive to the degree of portosystemic shunting and hyperammonemia (Lozeva-Thomas, 2004).

5. Therapy

5. Surgical therapy

The surgical treatment of a PSS is considered as the first choice treatment. The surgical procedure is the closure of the portosystemic shunting vessels. The surgical intervention is depending mainly on the type and on the size of the portosystemic shunt. Attenuation of an intrahepatic shunt is more difficult than extrahepatic PSSs due to its location. A complete ligation of the shunt is not always possible. It depends on the volume of the blood flowing through the shunting vessel, if the volume is too big, complete ligation can induce a portal hypertension of a hypoplastic hepatic portal circulation (Hunt and Hughes, 1999).

Possible complications that can lead to death are hypertension, haemorrhage, or intractable seizures, also called postligation seizure syndrome. Neurological alteration after the surgery can appear in 0-18% of the dogs. The alterations can differ from ataxia to generalized convulsive seizures which often can progress to status epilepticus (Fryer et al., 2011). The occlusion of a shunt is often associated with a good life quality. The long-term outcome of complete ligature and of partial ligature of a shunt were compared but the findings were controversial. In a study, it was not possible to find any differences but other results were associated with a more favourable outcome with the complete ligature. It was noticed that dogs with a partial ligation needed a shorter term for improvement but on the other hand 50% of the animals had recurrent signs 2 to 6 years postsurgical (Hunt and Hughes, 1999).

A clinical examination before the surgical intervention of dogs is of utmost importance: whether the dog is not cachectic, weak, unstable or encephalopathic. If this is the case a medical treatment should be performed in order for the dog to overcome stress during anaesthesia and surgery (Slatter, 2003). Postoperative seizures are reported to appear in 5-18% of the surgical treated dogs. To decrease the risk of postoperative seizures and status epilepticus, levetiracetam, an antiepileptic drug may be effective (Fryer et al., 2011).

For the anaesthesia, it is not advised to use drugs which are metabolised in the liver, highly protein bound, or hepatotoxic because of a low hepatic capacity and hypoalbuminemia. For example, isoflurane, ketamine with diazepine, opioids and propofol can be used. A

laparotomy must be performed to locate the extrahepatic PSS. Caution is needed when the incision is made to avoid any laceration of the shunting vessel which can be developed from a falciform ligament. The diameter of the extrahepatic PSS is mostly around 5-15 mm and has a turbulent blood flow. In dogs without a history of encephalopathy, the shunt can often be completely closed, but before the complete ligation, it is important to evaluate portal hypertension, increased intestinal peristalsis, cyanosis or pallor of the intestines, increased mesenteric vascular pulsations and cyanosis or oedema of the pancreas. This is performed by closing the shunt temporarily for a few minutes. Other methods for the closure can be ameroid constrictors or cellophane. Ameroid constrictors are implicated into the shunting vessel (Slatter, 2003).

The surgical procedure for an intrahepatic PSS is more complicated. Sometimes they are located between or within the liver lobes and usually it is surrounded by liver parenchyma. To realise the shunting vessel, it is possible to gently use a sterile ultrasonography transducer. The visual appearance of the vessel is dilatated with turbulent blood flow. If there is an external access to the vessel it is possible to use a ligature for occlusion, but alternative methods are also possible including inflow occlusion for transcaval approach or portal venotomy. Introducing coils to provoke an embolization of the shunt offers a less invasive method. The coils are installed through a stent. (Slatter, 2003).

Postoperatively, only a standard postsurgical care should be performed. And the medication should be continued for 2-4 weeks after surgery. The liver is a rapidly regenerating organ. For the postoperative care, an ammonia tolerance test or a scintigraphy can be performed (Slatter, 2003).

5.1. Medical therapy strategy

The treatment is made of two different phases which are the induction and the maintenance of remission. The maintenance is needed to prevent further HE episodes. In the initiation phase, non-absorbable disaccharides should be used as standard. If the patient is not responding to this treatment, non-absorbable antibiotics can be used (Butterworth, 2003).

One of the most important aim of the medical therapy of HE is based on the lowering of ammonia, which consists of several components. One part of the treatment acts on the intestines and the other on the liver.

1. **aim: Reduction of nitrogenous substances within the intestinal lumen**
   The first step for the intestinal part is the colonic cleaning. We want to achieve a decreased intestinal ammonia content and afterwards in the blood. The second step is to change the dietary protein. It was already demonstrated that a long-term reduction of dietary protein is potentially harmful for the animal, because a positive nitrogen balance is needed to encourage the regeneration of the liver and to elevate the elimination ammonia in form of glutamine in the skeletal muscles. The third step focuses on the decrease of ammonia production in the gut. This can be performed by the application of non-absorbable disaccharides and the application of different antibiotics. For the antibiotics, it is very important to evaluate the positive and side effects of the different active substances of the drug (Butterworth, 2003).

2. **aim: To stimulate the detoxicating ability of the liver to reduce the ammonia level in the blood**
   There are two possibilities that can be performed together. The first is to stimulate the urea cycle activity and the glutamine synthesis while the second is to support the liver function. For elevating the urea cycle activity there are the possibilities of the application of L-ornithine-L-aspartate (LOLA) and zinc (Williams, 2007; Summer et al., 1990) while to support the liver function it is possible to use a molecular adsorbent recycling system (Butterworth, 2003).

A detailed explanation about the different substances and possibilities is given below.

5.1.1. Non -Absorbable Disaccharides

Lactulose is a hyperosmotic laxative. The group of agents holds more water inside the intestines and achieves an osmotic effect, which leads to stimulate the peristalsis. The site of action is the *colon*. With *per os* application the onset of action starts after 12-72h and with rectal application after 0,25-1h (Berardi et al., 2006). In case of *per os* application, there is no breakdown of lactulose by the intestinal disaccharidase (Cordoba, 2014). In the *colon*, it is metabolised by bacterial fermentation to lactic, formic and acetic acids (Berardi et al., 2006). The production of lactic and acetic acids causes a decrease in the colonic pH (Cordoba, 2014), which leads to an elevation of the peristalsis (Berardi et al., 2006). The consequence is an increased elimination of nitrogenous substances with the faeces accompanied by a lowered nitrogen content in the portal blood circulation (Cordoba, 2014).

Non-absorbable disaccharides facilitate the growth of the bacterium *Lactobacillus bifidus*. This bacterium has urease activity, which leads to a decrease in the production of ammonia in the *colon*. The complete mechanism of action is not fully understood, but it interacts with the enteric flora and causes a decrease in the generation of nitrogenous compounds (Cordoba, 2014).

Some studies in humans have shown a marked decrease in the recurrence of HE after one year of treatment with non-absorbable disaccharides and several studies have shown that the treatment of minimal HE with lactulose caused an *"improvement of neurophysiological test and quality of life index"* (Cordoba, 2014). This has led to lactulose being used as a standard care in case of HE.

During the therapy with non-absorbable disaccharides (lactulose or lactitol) gastrointestinal side effects like bloating, flatulence, and severe diarrhoea possibly leading to dehydration, can appear (Bass, 2010).

5.1.2. Antibiotics

Antibiotics have an important role in the treatment of HE. The antibiotics inhibit the activation of intestinal glutaminase, which leads to a decrease in ammonia level in the portal blood. Beside this, they lower the bacterial translocation.

The first antibiotic tested for the treatment of HE is an aminoglycoside, namely neomycin that is poorly absorbed by the intestines and which showed a similar effectiveness like lactulose (Cordoba, 2014). However, due to the ototoxic and nephrotoxic side effects reported, it is not an attractive treatment choice. Other antibiotics including metronidazole and vancomycin have been experimented as well, but these were banned due to their potential toxic effect in particularly on long term usage (Butterworth, 2003).

The antibiotic that is nowadays used as a treatment of HE is rifamixin. It is a broad-spectrum antibiotic, which is poorly absorbed by the intestines and it has a minimal toxic effect. This makes it possible to use this antibiotic over a longer term. Several different studies were made about the effectiveness of rifamixin compared to placebo, lactitol or lactulose (Cordoba, 2014; Leise et al., 2014). When compared to non-absorbable-disaccharides, rifamixin did not seem to be more effective (Leise et al., 2014) or just slightly improved but no significant results were obtained (Cordoba, 2014).

In another study, rifamixin combined with lactulose was administered and compared to rifamixin alone. This study showed a higher proportion of complete subsiding of HE and a shorter hospitalisation of human patients who got rifamixin together with lactulose (Leise et al., 2014).

An argument for the usage of rifamixin is that the safety profile is better due to a lower frequency of diarrhoea compared to lactulose (Cordoba, 2014). Rifamixin modifies the intestinal bacterial flora by decreasing the amount of ammonia producing bacteria. Another favourable effect is that rifamixin decreases the endotoxin (LPS) load derived from the intestines and lowers the level of proinflammatory cytokines (Bajaj, 2015). As a treatment strategy, it is often recommended to use lactulose for the first episode of HE. During a second

episode of HE it is advised to use rifamixin combined with lactulose due to the higher efficacy (Cordoba, 2014).

The risk of antibiotic-resistance is nowadays a very important point in the application of antibiotic treatments. A lower risk of inducing a resistance is expected with non-absorbable antibiotics like rifamixin compared to systemic antibiotics (Bass, 2010).

Another antibiotic is minocycline which is a semi-synthetic tetracycline, however this has also anti-inflammatory properties. Due to this effect, it can inhibit the microglial activation (Butterworth, 2015).

5.1.3. Zinc

Zinc is a cofactor in the urea cycle, the enzymes of this cycle are dependent on zinc, thus the rate of the cycle is reduced in case of a zinc deficiency (Gerber and Schomerus, 2000). In cirrhotic patients with a portosystemic shunt and portal hypertension occurs a lower zinc level in the body may due to the lower hepatic extraction of zinc. However, a higher volume of portal blood flowing into the systemic blood circulation, due to a big PSS or several PSS, is put in connection with an increased urinary loss of zinc. (Sengupta, 2015).

A deficiency causes an impaired immune function as well as decreases the resistance to infection (Sengupta, 2015) and it might take indirect part in HE through the effect on the ammonia metabolism (Williams, 2007). Despite of this, beneficial effect of a zinc supplementation (600 mg per day) in a human with an encephalopathy was not shown (Dragonjic et al., 2013).

The results of some studies about the application of zinc in patients with HE were not fully clear, but others showed an alleviation of HE at zinc supplementation. Zinc application is at least recommended when there is a proven deficit of zinc (Gerber and Schomerus, 2000).

It is assumed that a zinc deficiency leads to an increase in the TNF production, however the supplementation of zinc may decrease the circulating TNF in dogs (Odeh, 2007). TNF is a serum cytokine which shows a high positive correlation to the HE severity (Goral et al., 2010).

5.1.4. L-Ornithine L-Aspartate (LOLA)

There are several therapy opportunities for humans who are not responding to disaccharides or non-systemic antibiotics. Recent studies have shown a significance in the treatment of HE with L-ornithine-L-aspartate (LOLA). It is a compound salt that stimulates the ornithine transcarbamolyase and carbamoyl phosphate synthetase. Furthermore, it is a substrate for the formation of urea (Leise et al., 2014). The carbamoyl phosphate synthetase is an ATP-dependent enzyme which synthetises carbomoyl phosphate out of glutamine or ammonia and bicarbonate. It is found in the mitochondria and is a part of the urea cycle in the liver (Summer et al., 1990). The other mentioned enzyme, the ornithine transcarbmolyase catalyses the reaction from carbamoyl phosphate and ornithine to citrulline and phosphate. It is located in the mitochondria and belongs to the urea cycle as well (Langley et al., 2000).

Beside these effects, LOLA stimulate the glutamine synthesis in the skeletal muscles thus lowers the ammonia level in the body. In recent studies, LOLA has been used as an oral and intravenous application form to patients with chronic HE. In both cases, oral and intravenous applications of LOLA the ammonia level and HE parameters are improving (Leise et al., 2014). Another study evaluated the LOLA as an adjunctive therapy compared to a placebo in patients who received a standard medical treatment. The result showed that patients with a Grade II or higher graded HE improved significantly better than the group of the placebo combined with the standard medical treatment (Leise et al., 2014). In pigs, LOLA had also a beneficial effect, because it caused a reduction of the circulating ammonia and a reduction in brain edema in acute HE (Butterworth, 2015).

The combination of L-ornithine with phenylacetate is a newer ammonia-lowering agent (Butterworth, 2015). The strategy is to stimulate the activity of glutamine-synthetase by ornithtine. The newly formed glutamine will be combined with phenylacetate and can be excreted with the urine. The advantage of the possibility is that a degradation of glutamine to ammonia in the intestines is prevented due to the fact that phenylacetylglutamine is eliminated via urine (Cordoba, 2014).

5.1.5. Branch Chain Amino acids

The branched chain amino acids belong to the essential, aliphatic, hydrophobic, proteinogenic amino acids. There are three amino acids belonging to this group which are L-Leucine, L-Isoleucine and L-Valine. They are named branched chain amino acids because they have a branched carbon chain (Rassow et al., 2016).

There is a high amount of branched chain amino acids in the muscles. They are one of the most important energy sources for the myocytes and they are important for the protein synthesis as well. The branched chain amino acids are competing with L-Phenylalanine, L-Tyrosine and L-Tryptophan at the BBB. A very high supplementation of the branched chain amino acids can decrease the concentration of L-Phenylalanine, L-Tyrosine and L-Tryptophan and thus the production of neurotransmitter can be decreased including serotonin whose low level is associated with the overdose of branched chain amino acid mentioned above (Holecek, 2010). The lower concentration of aromatic amino acids causes a decrease of the GABA level in the brain. GABA is an inhibitory neurotransmitter. However, longer time ago was advised to prescribe a restriction of the whole dietary protein during the treatment of HE, but the restriction is not advised due to lacks in scientific background (Afridi et al., 2014) and other studies showed no improvement in the outcome of HE. In addition, a normal protein intake is associated with a better metabolism. Nevertheless, ingestion of too large quantities of proteins could have a negative effect as well through the production of toxins (Butterworth, 2015).

Owing to the fact that branched chain amino acids stimulate the protein synthesis in the liver, they can improve the nutritional status of an individual. This is resulting in an better quality of life. It is also suggested that an appropriate protein intake together with a branched chain amino acids diet can be helpful for the prevention of the CNS signs of a HE. The beneficial effect of branched chain amino acids, especially in the course of HE, is the detoxification of ammonia, correction of the plasma amino acid imbalance and reduction of the influx of aromatic amino acids into the brain. Their beneficial effects have been proven in certain studies. A recent study compared the effectiveness of conventional treatment (antibiotics, lactulose) to the combination of the conventional treatment and a supplementation of branched chain amino acids. The clinical improvement of the group with the conventional

therapy plus the aminoleban infusion was significantly higher (76.7%) compared to the other group (23.3%). This shows that the conventional treatment together with branched chain amino acids is much more effective than the conventional therapy alone (Afridi et al., 2014). However, the metaanalysis of clinical trials judging the efficacy of branched chain amino acids in the therapy of humans did not show a favourable effect (Lidbury et al., 2016).

5.1.6. Mild hypothermia

A reduction in 2–3°C have been shown to be effective in prolonging the survival and preventing brain oedema. A reduction in blood brain ammonia level and in brain lactate production is associated with hypothermia. The reduction of brain lactate production is leading to a normalisation of the brain energy. It is suggested that mild hypothermia has anti-inflammatory effect by reducing the microglial activation which happens parallel with a decrease of pro-inflammatory cytokines, which is leading to a reduction in the formation of brain edema (Butterworth, 2015).

5.1.7. SUR-1 regulation by NF-kB

In ammonia-treated astrocyte cultures a significant increase of nuclear translocation of nuclear factor kappa B (NF-kB) was observed and this conducts to the astrocyte swelling and consequently to the formation of lethal brain oedema (Rao et al., 2013), (Butterworth, 2015).

The cell culture was examined 24h later and there was a significant increase of the sulfonylurea receptor-1 (SUR-1) protein expression. SUR-1 protein expression is a marker of the non-selective cation channel (NCCa-ATP channel) activity (Jayakumar et al., 2014). Other cell cultures were pre-treated with an anti-inflammatory drug (an inhibitor of NF-kB). In the pre-treated cell cultures the SUR-1 protein expression was decreased. The result was a significant decreased in astrocyte swelling (Cordoba, 2014).

5.1.8. Repaglinide

Repaglinide is a drug that is usually used as an antidiabetic drug (Hasslacher, 2003). Studies have shown a significant reduction in swelling of cultured astrocytes which was caused ammonia. Repaglinide blocks the ATP-sensitive potassium channels (KATP-channel). It is now suggested that KATP-channel activation plays a role in the ammonia-induced astrocyte swelling (Alahmari et al., 2015).

5.1.9. Glibenclamide

Glibenclamide is a sulfonylurea compound like tolbutamide, which blocks the non-selective cation channel (NCCa-ATP channel) and reduced the astrocyte swelling. Studies have been conducted that suggest using glibenclamide as a treatment against the astrocyte swelling by inhibiting the ammonia-induced astrocyte swelling. Astrocyte cell cultures were treated with ammonia and showed after 24h the maximal increase in size due to swelling (40.2%). This was diminished by glibenclamide. Rats treated with 3 mg/kg of glibenclamide showed a significant reduction in brain oedema as well as improved clinical status (Jayakumar, 2014).

6. Conclusion

HE is demonstrated not only by the symptoms but also by the complexity of the pathogenesis. Three different organ systems are involved in the pathogenesis, which are the digestive system, nervous system and the endocrine system. An important factor for the breakthrough of pathogenesis is the modern diagnostic methods. The pathogenesis became much more well explained and most of the theories became proven by several studies.

Since this time, several different theories have been developed about the pathogenesis. The oldest theory of the pathogenesis in connection with hyperammonia has been experimentally verified, is the most feasible and widely accepted nowadays. Several studies indicate the pathogenesis in association with the different actors.

In my opinion, HE is a multifactorial disease and not a disease caused by different single actors.

Concerning the treatment in the last few years, several studies published longer time ago became evaluated again. An example is the theory about the restriction of the protein in the nutrition of the animals to lower the ammonia content in the body. These theories became refuted. Recent studies have shown that it is not the quantity, but the quality of the amino acids of the nutritional protein which have a great impact of the treatment.

I think this work offers itself as a reference work to get a good impression about this complexity of the pathogenesis and the recent treatment possibilities.

7. Acknowledgments

The deepest gratitude and immeasurable appreciation for the support and help are extended to the following persons who contributed in making this thesis possible.

Heike and Andreas Wildhage who helped me with advice and always supported me.

Department of Physiology and Biochemistry, which allowed me to write my thesis with.

Dr. Dávid Sándor Kiss who had the supervision and helped me with advices and assistance.

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