Neuropathological changes in mouse models of cardiovascular diseases
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General discussion
CHAPTER 7
General discussion

Cardiovascular diseases (CVDs) such as atherosclerosis, heart failure, hypertension and small vessel disease are associated with brain pathology and an increased risk of dementia. Neuropathological observations associated with cognitive impairment and dementia are white matter lesions (WML), decreased blood-brain barrier (BBB) integrity, inflammation, synaptic loss, neuronal degeneration and amyloid beta (Aβ) accumulation.1-4 The main hypothesis presented in this thesis is that different CVDs contribute to the development of neuropathological changes related to cognitive impairment and dementia. Our specific hypotheses were 1) intracranial atherosclerosis is not present in mice and thus does not contribute to the neuropathological changes; 2) although the absence of the ApoE protein plays an important role in the development of neuropathology in atherosclerotic mouse models, severe extracranial atherosclerosis also contributes to the development of these changes and 3) cerebral hypoperfusion is the common denominator in the different cardiovascular diseases and this is the key initiator of neuropathological changes and cognitive decline. To test these hypotheses animal models with comparable etiology and neuropathology compared to humans are needed in order to perform mechanistic studies and study cause-consequence relationships.

Mouse models with neuropathological changes associated with cognitive impairment

In this thesis we present studies in which neuropathological changes in different cardiovascular mouse models were investigated. In chapter 2 we present the results of a literature review on cardiovascular mouse models for which cerebral changes have been reported, such as models for atherosclerosis, heart failure, hypoperfusion, hypertension and the small vessel disease CADASIL. Since the amount of literature is limited for most models and no standard experimental design is used, we were not able to unambiguously select the mouse models with the best comparable etiology and neuropathology compared to humans. The bilateral common carotid artery stenosis (BCAS) model seemed the most promising model for investigating the link between cerebral hypoperfusion and cognitive impairment as it induces cerebral hypoperfusion and cerebral changes like decreased BBB integrity, cerebral inflammation, WML, decreased metabolism and cognitive deficits. The BCAS model is the only CVD mouse model in which WML have been observed, which is a common feature in patients with vascular cognitive impairment and vascular dementia. A disadvantage of the BCAS model, as discussed in this chapter and reoccurring in chapter 6, is that it is very
sensitive to the degree of stenosis. Variations in the inner coil diameter by 0.02 mm make the difference between gray matter loss starting at 1 month or 8 months after surgery. Furthermore, it is known that in the rat version of this model, the visual performance is also affected.\(^5\) This needs to be tested in the mouse BCAS model to exclude a confounding effect of damage in the visual pathway on the performance of these mice in cognitive tests.

Two other surgically induced models with promising, although limited, evidence are the myocardial infarction (MI) and the transverse aortic constriction (TAC). In the MI model, a reduction in cerebral blood flow (CBF) has been reported 4-6 weeks after surgery, in addition to an increase in the inflammation cytokine TNF-\(\alpha\), though spatial memory performance was normal.\(^6\) Since CBF reduction is probably an early event in the disease progression, a lack of cognitive decline at the same time-point does not exclude that the MI model could be a useful animal model to study the link between CVD and cognitive impairment, but might indicate that longer follow-up times are needed. This has indeed been confirmed in later studies, in which a decline in spatial memory impairment was detected 3 months after surgery, while nonspatial memory was already impaired at 6-8 weeks after surgery.\(^7\)-\(^9\)

In the TAC model, a model for hypertension and heart failure, a reduction in CBF, an increase in BBB leakage and inflammation, and a decrease of cognitive function have been reported.\(^10\)-\(^12\) The effects of TAC on cerebral changes reported in literature are however based on conclusions drawn by one academic group only and reproducibility of these effects has to be established by other groups.

Atherosclerosis models may also be used to study CVD effects on cerebral hypoperfusion, pathology and cognitive function. In humans, both cerebral and peripheral atherosclerosis have been associated with CBF reduction, brain pathology and cognitive decline.\(^13\)-\(^18\) Most studies with atherosclerotic mice have been performed in apolipoprotein E knockout (ApoE\(^{-/-}\)) mice or low density lipoprotein receptor knockout (LDLr\(^{-/-}\)) mice. ApoE\(^{-/-}\) mice show reduced CBF, increased BBB permeability, microvessel degeneration, increased inflammation, reduction of neurogenesis and synapses and cognitive impairment. Similar to ApoE\(^{-/-}\) mice, LDLr\(^{-/-}\) mice show microvessel abnormalities, increased inflammation, reduction of synapses and cognitive impairment. In contrast to ApoE\(^{-/-}\) mice, BBB permeability is not abnormal in young LDLr\(^{-/-}\) mice at 9 weeks of age on chow diet, while it is already increased at 2 weeks of age in ApoE\(^{-/-}\) mice on chow diet.\(^19\),\(^20\) The LDLr\(^{-/-}\) model is a less severe atherosclerotic mouse model compared to the ApoE\(^{-/-}\) model and does not develop atherosclerosis on chow diet.\(^21\) Therefore, older mice on a high-fat diet
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should be used to study if LDLr^/- mice will develop BBB permeability changes due to atherosclerotic lesions. However, it is unlikely that atherosclerotic lesions induced the CBF reduction and increased BBB permeability in ApoE^/- mice at the age of 2 weeks. In ApoE^/- mice on high-fat diet foam cell lesions start to appear at the age of 8 weeks. Therefore cerebral changes observed in ApoE^/- mice are probably not entirely due to the effects of atherosclerosis. This is further confirmed by the absence of increased BBB permeability at the age of 10.5 months in mice carrying a human mutated form of the ApoE gene, ApoE*3-Leiden (ApoE*3L), that develop atherosclerosis to a similar extent as ApoE^/- mice.

In order to investigate to which extent the presence of atherosclerotic plaques are responsible for cerebral changes in these mice, it is important to know where these lesions develop. Cerebral atherosclerosis could more directly damage the cerebral vessel walls and surrounding tissue, while peripheral atherosclerosis will affect brain tissue via reduced blood flow and possibly via increased plasma inflammatory factors or systemic endothelial dysfunction.

Intracranial atherosclerosis is absent in mice

Interestingly, in humans cerebral atherosclerosis develops later in life and shows a distinct phenotype compared to peripheral atherosclerosis. In mice the presence of intracranial atherosclerosis has barely been studied. A few studies report the absence of intracranial atherosclerosis in 6-18 months old ApoE^/- and 18 months old ApoE^/-LDLr^/- mice. One study shortly mentions the presence of macroscopic atherosclerotic lesions in the circle of Willis in chow fed LDLr^/-xhApoB mice at the age of 12 months. To determine the presence or absence of intracranial atherosclerosis in severe atherosclerotic mice, we studied the distribution of atherosclerotic plaques from the common carotid arteries to the bifurcations of the circle of Willis in ApoE^/- mice, ApoE^/-Fbn1C1039G^/-, and ApoB100/LDLr^/- mice in chapter 3. We did not find any intracranial lesions in these mouse models fed with a high-fat diet up to the age of 41 weeks. Interestingly, the lesions typically stopped in the internal carotid artery at the bifurcation with the pterygopalatine artery. At this point in the artery a decrease in vessel wall thickness, decrease in elastin layers, increase in smooth muscle actin, decrease in endothelial activation marker ICAM-1 and increase in antioxidant enzyme NQO1 was found in wild-type (C57Bl/6) mice without atherosclerosis. Although the lesions typically stopped at the bifurcation with the pterygopalatine artery, some of the ApoE^/-Fbn1C1039G^/- mice exhibited lesions beyond this point. However, even in
this severe model, these lesions did not occur within the cranial cavity. Differences in intrinsic vessel wall characteristics were also shown between these points. An increase in tight junction marker claudin-5 and increase in antioxidant enzyme heme oxygenase-1 (HO-1) were noted between the extracranial portion of the internal carotid artery and the intracranial portion. These intrinsic differences in vessel wall characteristics between different points in the vessel wall could lead to differences in susceptibility of the vessel to develop atherosclerotic lesions.

Atherosclerosis lesion development can be stimulated by pro-atherosclerotic compounds like oxidized LDL (oxLDL). The response of endothelial cells of a certain vessel to oxLDL stimulation might indicate the susceptibility of atherosclerosis development in that vessel. Differences in extracranial and intracranial artery responses to oxLDL stimulation have been shown by others in quails. Quail brain microvascular endothelial cells have a lower death rate and a higher response of HO-1 mRNA and protein expression compared to quail carotid endothelial cells in response to oxLDL stimulation. In this thesis, we show that also human brain endothelial cells respond to oxLDL stimulation with an increase in HO-1 mRNA and protein expression. This increase coincided with an increased nuclear translocation of Nrf2, the transcription factor of HO-1, and a reduced production of reactive oxygen species compared to unstimulated cells. These results still have to be reproduced in a mouse brain endothelial cell line to confirm similar mechanisms in both species. In addition, to confirm the relationship between increased HO-1 expression and reduced atherosclerotic susceptibility, the HO-1 mRNA and protein expression has to be compared between the intracranial circle of Willis, which does not develop atherosclerosis in mice, and an extracranial vessel of the same size, which does develop atherosclerosis, e.g. a coronary artery.

To investigate the role of this intrinsic and oxLDL-induced increase of HO-1 in intracranial arteries in the decreased atherosclerotic susceptibility, further research is needed. It has already been shown that ApoE/- HO-1/- mice show accelerated and more advanced atherosclerotic lesion formation in the aortic arch compared to ApoE/- HO-1/-/- mice whereas knocking out Bach1, a suppressor of HO-1, leads to a reduction in total plaque area in the aorta of ApoE/- mice. For neither mouse model data is available on the intracranial atherosclerotic burden at this moment. If our hypothesis is true that HO-1 plays an important role in the resilience of intracranial arteries towards atherosclerotic plaque development, we expect that the ApoE/- HO-1/-/- double knockout mouse model would develop intracranial atherosclerosis.
Since intracranial atherosclerosis is known to develop about 20 years later in life compared to extracranial atherosclerosis in humans, we cannot exclude that longer follow-up times may be necessary to induce intracranial atherosclerosis in mouse models. We attempted to accelerate the plaque development by placing the animals on a high-fat diet, which generally leads to a faster progression of the atherosclerotic lesions compared to mice fed with chow. Unfortunately, we were not able to extend the duration of the experiments, due to sudden death and animal welfare-related issues. Seventy percent of the ApoE-/-Fbn1C1039G+/- mice died before the age of 41 weeks and 66% of the mice displayed neurological symptoms. The ApoB100/LDLr-/- mice exhibited large subcutaneous fat depositions in the paws and consequential muscle compression at 8.5 months of age. These subcutaneous xanthomas have also been described in 7 months old LDLr-/- mice on high-fat diet. In addition, mice of all groups, both on high-fat diet and chow, had cholesterol granulomas in their middle and inner ear affecting surrounding tissue and blocking the perception of auditory signals. In some animals the bone tissue surrounding the middle and inner ear was affected in such way that the granulomas extended into the cranium or into the esophagus. This indicates that harmful inner ear and subcutaneous fat depositions occur without or prior to fat depositions in intracranial vessels.

Cerebral xanthomas in atherosclerotic mice
Furthermore, xanthomas of different sizes were found in numerous ApoB100/LDLr-/-, ApoE-/-Fbn1C1039G+/- and ApoE-/- mice on high-fat diet. These xanthomas were mainly present in and around the choroid plexus and neocortex as described in chapter 4. The xanthomas were more often present in ApoE-/-Fbn1C1039G+/- compared to ApoE-/- mice. Interestingly, the location of the xanthomas coincides with the location of the most pronounced fibrillin-1 expression. Mutations in the fibrillin-1 gene lead to a defective formation of microfibrils, elastic fiber fragmentation, increased apoptosis, increased inflammation and subsequently a loosened basement membrane. These changes probably also contributed to the occasional development of atherosclerotic plaques beyond the pterygopalatine artery bifurcation in ApoE-/-Fbn1C1039G+/- mice as shown in chapter 3, while this was not present in the other atherosclerotic models. Due to the vascular effects of the fibrillin-1 mutation, we would expect that combining the fibrillin-1 mutation with other atherosclerotic mouse models, for example LDLr-/-Fbn1C1039G+/- mice on high-fat diet, will also have a more pronounced cerebral phenotype compared to LDLr-/- mice on a high-fat diet.
Although there is an important role for fibrillin-1 in the increased BBB/blood–cerebrospinal fluid barrier (BCSFB) damage, increased inflammation and xanthomas formation, these findings are also observed in ApoE−/− mice, however to a lesser extent. In chapter 4 we describe the presence of xanthomas in 6 months old ApoE−/− mice on high-fat diet. These findings have also been described in 6-19 month old ApoE−/− mice on high-fat diet, although only small lesions were seen in a few of the investigated ApoE−/− mice on chow diet at the age of 15-23 months. This indicates that high cholesterol levels also contribute to the progression of xanthomas formation. However, wild-type or ApoE*3L mice fed a high-fat diet did not develop any xanthomas, although the ApoE*3L mice had higher serum cholesterol levels compared to the ApoE−/− mice. This suggests that the deficiency of the ApoE protein will also play a major role in the increase of BBB permeability and the development of xanthomas.

**Importance of the ApoE gene in mice**

The difference in BBB permeability between ApoE−/− and ApoE*3L mice has also been reported by Mulder et al. Although ApoE−/− mice on high-fat diet showed a strong immunoreactivity of IgG in the brain at the age of 4 and 11 months, this was not present in the ApoE*3L mice at 11 months of age, despite the higher cholesterol levels in the latter. A subtype-specific effect of the ApoE gene on BBB function has been shown in *in vitro* and *in vivo* mouse models. Whereas the human ApoE2 and ApoE3 isoforms preserve an intact BBB in mice, the human ApoE4 isoform or the deletion of ApoE gene leads to a leaky BBB. This effect is thought to be mediated by the CypA – NFκB – MMP9 pathway. The difference in isoform functioning in the brain may also explain why ApoE4 carriers have an increased risk of developing Alzheimer’s disease. The important role of the ApoE gene in cerebrovascular functioning is probably also the reason that one year old ApoE−/− mice on chow did show neuropathological changes compared to C57Bl/6 mice (chapter 5), while one year old ApoE*3L.CETP mice on high-cholesterol diet did not show comparable changes compared to chow-fed ApoE*3L.CETP mice. As discussed in chapter 5, the lack of comparable neuropathological changes in ApoE*3L.CETP could not be attributed to low cholesterol levels, small atherosclerotic lesions or lack of systemic inflammation. As we used mouse tissue left over from other experimental studies, we were not able to match the study designs and therefore could not directly compare the ApoE−/− and ApoE*3L.CETP mice. Yet, the ApoE*3L.CETP mice on the high-cholesterol diet had similar cholesterol levels compared to chow-fed ApoE−/− mice reported in literature.
In addition, while chow-fed ApoE*3L.CETP mice barely developed atherosclerotic lesions, and if so lesions with a mild phenotype, the high-cholesterol-fed mice developed severe lesions in the aortic root. ApoE⁻/⁻ mice on chow are also known to develop severe lesions in the aortic root at the age of 13 months. Although the high-cholesterol-fed ApoE*3L.CETP mice did show an increase in plasma inflammation markers and an increase in endothelial activation marker ICAM-1 in and surrounding the hippocampus, this did not lead to an decrease in BBB integrity as shown with similar IgG staining and tight junction marker claudin-5 compared to ApoE*3L.CETP mice on chow diet. The presence of BBB leakage in the ApoE⁻/⁻ mice on the other hand, confirms the earlier suggestion that the absence or isoform of the ApoE gene might be more important for the induction of cerebrovascular changes than the presence of high cholesterol levels or atherosclerosis. Since ApoE⁻/⁻ mice at this age and diet did not develop intracranial atherosclerosis as shown in chapter 3, local plaques cannot be an additional cause for the cerebrovascular dysfunction.

Because the deletion of the ApoE gene has direct effects on cerebrovascular function and the presence of extracranial atherosclerosis does not seem to have an additional effect on neuropathological changes, mouse models lacking this gene cannot be used to study the cause-consequence relationships between atherosclerosis, brain pathology and cognitive impairment. On the other hand, at the age of 1 year the ApoE*3L.CETP mouse model on high-cholesterol diet is not severe enough to induce neuropathological changes comparable to patients with cognitive impairment. Thus we need to focus on other atherosclerotic models, for example the LDLr⁻/⁻ model. As mentioned above and in chapter 2, microvessel abnormalities, increased inflammation, a reduction of synapses and cognitive impairment are observed in LDLr⁻/⁻ mice. However, no WML or BBB deficits have been reported so far and the effects on CBF are still unknown.

**Myocardial infarction, transverse aortic constriction and bilateral common carotid artery stenosis**

To aggravate the neuropathological changes we combined the LDLr⁻/⁻ model with the TAC model in chapter 6. In addition, we examined the effects of the MI model on a C57Bl/6 background and a mouse model for amyloidosis (APP/PS1). Furthermore, we examined the BCAS model with a tapered cast of 0.20 mm on a C57Bl/6 background and with a LDLr⁻/⁻ genotype. For all studies, we used one experimental design, which allowed us to compare the outcomes for all models. CBF measurements...
were performed 6 and 12 weeks after surgery and neuropathological changes were examined 12 weeks after surgery. Although a CBF reduction at 4-6 weeks after MI surgery in C57Bl/6 mice is reported by Yang et al., we were not able to reproduce this finding at both the 6 week and 12 week time-point. The reason for this discrepancy may be the relatively small myocardial infarctions created in our surviving mice. We did not observe significantly lower cardiac output values in our MI mice compared to our sham mice, while Yang et al. did observe a significant difference in cardiac output between the two groups. Although the mean infarct size of our mice in the 6 week CBF scan was similar to the mice of Yang et al. at 6 weeks (i.e. ~20%), these infarct sizes may be too small to induce reproducible effects.

As our hypothesis is that CBF is an early event in the disease progression and a key initiator to neuropathological changes, it is not surprising that with a lack of CBF reduction, we also observed no neuropathological changes in the MI mice. This was found in both C57Bl/6 and APP/PS1 mice.

In the TAC model on LDLr−/− background and high-fat diet we did find a CBF reduction 12 weeks after surgery. This decrease was not yet present at 6 weeks after surgery and may therefore be of too short duration to induce neuropathological changes. Aβ plaques have been reported in TAC C57Bl/6 mice by Carnevale et al. 4-8 weeks after surgery, suggesting a link between hypertension or heart failure with Alzheimer’s pathology. Our group and Li et al. were not able to reproduce these effects in both C57Bl/6 or LDLr−/− mice. However, TAC surgery did increase the number of Aβ plaques after 3 months in an Alzheimer’s disease model, the transgenic APP DSL mice, suggesting TAC might accelerate the onset of Aβ pathology. The differences in findings between our group compared to Carnevale et al. could be due to the selection of responders to the TAC surgery based on a systolic trans-stenotic pressure gradient, performed in at least one of the papers by Carnevale et al., but absent in our study. However, all TAC mice finishing the 12 week scans had a lower ejection fraction and higher heart weight to body weight ratio compared to all sham mice, indicating all TAC mice showed at least some extent of hypertrophy and cardiac dysfunction.

The high mortality rate in both the MI groups and TAC LDLr−/− group could have contributed to the lack of observed neuropathological changes, due to a selection bias of the animals with the smallest myocardial infarction or smallest response of the aortic constriction. However, in the APP/PS1 MI group there was no difference in mean infarct size between the animals that died 6 or 12 weeks after surgery. Similarly, in the TAC group the mice dying at 6 weeks after surgery did not have a lower cardiac
output at the 6 week time-point compared to the TAC mice that survived until 12 weeks after surgery. However, we cannot exclude that the biggest responders already died before the 6 week scans.

In the BCAS C57Bl/6 mice with 0.20 mm tapered casts we did find a CBF reduction 6 weeks after surgery, which returned to normal at the 12 week time-point. This temporary CBF decrease is in accordance with literature.\(^5,52\) In BCAS animals with a coil of 0.18 mm, the time it takes for CBF levels to return to those similar to sham animals differs per study from 4 weeks to 3 months. This large difference demonstrated in the same lab within the same mouse genotype shows that this model is highly susceptible to small changes.\(^5,52\) This may explain why the CBF of the LDLr\(^{-/-}\) mice is not significantly reduced when using a cast with a slightly larger inner diameter. Although both the C57Bl/6 mice and LDLr\(^{-/-}\) mice showed a subtle yet significant increase in Iba-1 immunoreactivity in the hippocampus, there was no overall increase in glial activation as has been shown with 0.18 mm and 0.20 mm coils by Shibata et al.\(^5\) The activated microglia and astroglia in the 0.20 mm coil group were present in the corpus callosum, caudatoputamen, internal capsule and optic tract. However, in our study only three BCAS C57Bl/6 mice (43%) with 0.20 mm tapered casts showed unilateral microgliosis and astrogliosis of the optical nerve 12 weeks after surgery. This could indicate initial damage to the visual pathway, like shown more extremely in the rat version of this model.\(^5,55\) However, WML were not present in the optical nerve, or other white matter regions, in our mice, suggesting a preserved function. WML were observed in the 0.20 mm coil group of Shibata et al.\(^5\) Differences in material between the piano wire coils and our polyethylene tapered cast might be responsible for differences in the observed neuropathological changes, due to differences in e.g. material stiffness. An increase of 0.02 mm in the coil diameter with the same material can lead to the absence of WML 4 weeks after surgery.\(^5\) These results suggests that this model is very susceptible to small changes in the extent of stenosis induced, the way the stenosis is induced and probably also for differences in age and genetic background as these will determine the carotid diameter of the animal before stenosis.

The different materials, i.e. piano wire and polyethylene, with similar inner diameter should be tested in one lab with one experimental design, on the same genetic background, gender and age, to establish its influence on the induced CBF reduction and neuropathological changes.

In the BCAS model, the placement of the coils causes an acute reduction in CBF, which is followed by a gradual recovery of the CBF. The cerebral hypoperfusion is
therefore not chronic. In patients with for example atherosclerosis we would however expect that stenosis will be induced in a more gradual manner and that the stenosis will lead to chronic cerebral hypoperfusion. Gradual increasing stenosis should be induced to make the model more easily translatable to the clinical practice. Recently, a new model for hypoperfusion is developed using ameroid constrictor devices instead of piano wire coils. These constrictors have an initial inner diameter of 0.5 mm, which decreases in time, due to water absorption and swelling of the hygroscopic casein material on the inside of the device. After 28 days, the device causes total occlusion of the common carotid arteries. The acute CBF drop after surgery is absent in this model, and instead a gradual and continuous reduction of the CBF is observed at least up to 28 days after surgery. One of the downsides of this model is that bilateral application of these devises leads to a 60% mortality rate within 2 weeks after surgery in mice of 10-12 weeks of age. In 1 year old mice, the mortality rate is even higher (80%). In addition, 75% of the mice develop multiple cerebral infarctions. This ameroid constrictor model may therefore be a good model for multi-infarct dementia. However, for other types of dementia which show a more gradual development of the disease without cerebral infarctions, other models should be developed.

An ameroid constrictor with a larger initial inner diameter (0.75 mm) has been developed in order to overcome the high mortality rates. The 32 day survival rate in 10-12 week old male C57Bl/6 mice is 91%. These mice show a gradual decrease in CBF and an increase in WML, GFAP-positive astrocytes and Iba-1-positive microglia 28 days after surgery, while only a few infarctions were observed. Based on relatively mild WML in the optical nerves, the authors conclude that the visual system is preserved in this model and for that reason cognition measures can be obtained reliably. Mice with bilateral 0.75 mm ameroid constrictors show a decrease in motor function and spatial working memory, while the reference learning and memory are unaffected 28 days after surgery. These results show that the use of 0.75 mm ameroid constrictors may have a higher translational value for studying the effects of hypoperfusion on the brain compared to the BCAS model. A disadvantage of both sizes of ameroid constrictors is the big outer diameters of the devices (3.25 mm). Although side effects due to the implementation of these large devices have not been described, we cannot exclude that the tissue next to the devices is compromised. New models and devices should continue to be developed with the aim of generating a model which has a good translational value, a relatively low
mortality rate and is minimally invasive, to reduce undesirable secondary effects due to the implementation of the devices.

Since dementia is an age-related disease, aging models can also be used to study the underlying mechanisms of dementia development. An example of a spontaneous animal model for accelerated aging is the senescence-accelerated mouse prone 8 (SAMP8) model. Cognitive performance is impaired in the SAMP8 mice, and this is correlated with CBF reduction and increased hippocampal BBB permeability in 4 and 12 months old mice. In addition, Alzheimer’s disease pathology, hippocampal vascular endothelial senescence and neuronal loss are observed in these mice. Besides cerebral changes, systemic changes are also found. SAMP8 mice show increased cardiac fibrosis, diastolic dysfunction and dysfunctional aortic endothelium at the age of 6 months. Although the multifactorial nature of the disorders in these mice represent the human situation more realistically, it may be difficult to study the cause-consequence relationships of individual causative factors.

**Conclusion**

The goal of this thesis was to study the neuropathological changes in different cardiovascular mouse models to obtain more insights in which animal models are suited for future mechanistic studies to investigate the relationship between CVD and cerebral hypoperfusion, brain pathology and cognitive decline. To achieve this goal we examined neuropathological changes associated with CVD and cognitive decline in the different mouse models using one experimental design. Our hypothesis was that different CVDs in mice contribute to the development of neuropathological changes related to cognitive impairment and dementia. Cerebral hypoperfusion is thought to be the key initiator of neuropathological changes and cognitive decline. Although we did not perform any cognition tests in this thesis, we expect that cognitive changes will only occur in models with pronounced neuropathological changes. Therefore, we first focused on establishing a model with extensive neuropathological changes. We showed that in contrast to humans, cerebral atherosclerosis is not present in mice and will therefore not contribute to the development of CBF reduction and neuropathological changes. Deletion of the ApoE gene has a direct effect on BBB integrity and ApoE deficiency models are therefore not appropriate to study the effects of atherosclerosis on CBF reduction and neuropathological changes. The ApoE*3L.CETP model on
the other hand does not induce sufficient neuropathological changes at the age of 12 months. The lack of CBF reduction in the MI model and late CBF reduction in the TAC model are probably the reasons for the lack of or limited extent of neuropathological changes in these models. On the other hand, CBF reduction in the 0.20 mm BCAS model did lead to a few neuropathological changes, supporting our hypothesis. The neuropathological changes were however not as extensive as reported in literature, suggesting that a more pronounced CBF reduction with a smaller coil or cast is needed for reproducible effects. There appears to be a very delicate balance between 1) inducing a cardiovascular intervention in mice without any cerebral effect, 2) an intervention which reduces CBF and induces reproducible structural cerebral changes and 3) an intervention which induces a high mortality rate. There is still a high need to further investigate the development and validation of cardiovascular and chronic hypoperfusion mouse models with brain pathology and cognitive impairment.
References


