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Influence of Hypertension with Multiple Risk Factors on Brain Tissue Pathomorphology and Cognitive Impairment-Related Biomarkers

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Key words: Hypertension, Cognitive impairment, Vascular dementia, high - fat diet, high - glucose - high - fat diet, Salt - sensitive diet

1. Abstract

Background: Hypertension is the most common chronic disease in the elderly. In China, according to a survey conducted in 2012–2015, the prevalence rate of hypertension in people aged ≥60 years was 53.24%, and for people aged ≥80 was 60.27%. Not only is hypertension the primary cause of stroke in China, it is also a risk factor for cognitive impairment diseases such as vascular dementia and Alzheimer's disease (AD). Compared with healthy people, patients with hypertension have a 1.4-fold increased risk of developing dementia. Therefore, it is important to study the characteristics of hypertension models to reveal the potential and changeable etiologies and risk factors and to slow the progress of cognitive impairment through systemic intervention, especially for vascular cognitive impairment, and prevent AD. Hyperlipidemia, diabetes mellitus, and a high-salt diet are risk factors for hypertension and cognitive impairment. It is essential to explore how these diseases injure cognitive function by assessing the pathological changes and biomarkers.

Purpose: Using a spontaneously hypertensive rat (SHR) model of metabolic syndrome (hyperlipidemia, diabetes mellitus, salt-sensitive) by feeding SHRs complex model diets (high-fat diet, high-glucose diet, and salt-sensitive diet), we assessed changes in brain tissue pathomorphology and cognitive impairment-related biomarkers.

Materials and Methods: Thirty-two male SHR were randomly divided into four groups (n=8 rats per group): SHR group (fed routine diet), HFD–SHR group (fed high-fat diet), HSD–SHR group (fed high-salt diet), and the DM -SHR group (fed high-fat, high-glucose mixed diet and injected with streptozotocin, 25 mg/kg). Eight male Wistar–Kyoto (WKY) rats (that share the same genetic background with SHR) comprised the blank control group. After 24 weeks, blood plasma was collected by the abdominal aortic

method, and the plasma levels of interleukin (IL)-6, IL-1β, and hypersensitive C-reactive protein were assessed. Cortex and hippocampal homogenate was used to determine levels of inflammatory factors, acetyl cholinesterase, acetylcholine, and β-amyloid protein (β-AP). Meanwhile, pathological changes in the CA1 area of the hippocampus and the cortical histopathological structure were observed under light microscopy following hematoxylin and eosin staining, Nissl's staining, and Golgi-Cox staining.

Results: Compared with WKY controls, the level of inflammatory and cholinergic factors in the blood and brain tissue of the four model groups decreased significantly ($P<0.05$), whereas the amount of β-AP increased ($P<0.05$). Changes in the 3'H' model group were more obvious; however, there was no significant difference among the model groups. Compared with WKY group, other groups were obseved the decrease in the number of neurons, space broadening, a disorganized structure, and pyknosis and condensed nuclei by HE staining. Decreasing in the number of Nissl bodies in the CA1 area were observed by Nissl's staining.

Conclusion: Hypertension combined with lipid metabolism disorders, diabetes, or a high-salt diet can result in pathological changes to the brain tissue and influence the levels of cognitive impairment-related biomarkers. This study presents interactions between multiple risk factors and the increase in the degree of vascular dementia. And it is of great significance for identifying biomarkers to aid in the early intervention or delaying the progress of cognitive deficits caused by hypertension.

2. Introduction

Hypertension is characterized by raised arterial blood pressure of the systemic circulation (systolic pressure ≥140 mmHg, diastolic pressure ≥90 mmHg) with functional disturbances and physical lesions in the heart, brain, kidney, etc.[1,2].

In recent years, the number of patients with hypertension, hyperlipidemia (HLP), diabetes mellitus (DM) (glucose/lipid metabolic disease), and resultant cognitive impairment, either alone or simultaneously, is increasing with the ageing population. As a result, hypertension is becoming a major public health issue[3,4]. Current evidence suggests that controlling blood pressure can reduce the risk of stroke, cardiovascular diseases (CVD), and the possibility of patients with mild cognitive impairment developing dementia[5]. Vascular cognitive impairment (VCI), which involves damage to at least one cognitive area, is a clinical syndrome consisting of clinical stroke or subclinical injury of the cerebral vessels caused by CVDs and related risk factors[6,7]. Epidemiological studies suggest that risk factors of vascular dementia (VaD) and Alzheimer's disease (AD) include hypertension, DM, and HLP[8-14]. The probability of developing VaD in patients with diabetes with insulin resistance is 2-3-fold higher than in normal people[15-17]. Although there is no reliable clinical study showing that salt-sensitivity contributes to the development of VaD, Dahl salt sensitive (DSS) rats on a high-salt (HS) diet were accompanied by a risk factor for dementia due to damage of endothelial function[18,19]. In daily life, promoting a low-salt diet and more aerobic exercise is good for VaD prevention and treatment.

Current studies have not determined the mechanism by which hypertension induces cognitive impairment and contributes to disease development. Thus, identification of the mechanism and related biomarkers deserves further study. In this study, we used spontaneous hypertensive rats (SHR) combined with multiple factors to induce cognitive impairment, including HFD–SHR (fed high-fat diet), HSD–SHR group (fed high-salt diet), and DM -SHR (fed high–fat, high–glucose mixed diet and injected with streptozotocin (STZ), 25 mg/kg). We also observed changes in the brain tissue pathomorphology and the expression of VaD-related biomarkers to explore the interaction between HLP, DM, salt-sensitivity, and cognitive impairment in SHR. This research is of great significance for identifying biomarkers to aid in the early intervention or delaying the progress of cognitive deficits caused by hypertension. This research provides scientific evidence for the prevention and cure of cognitive deficits associated with high blood pressure.

3. Materials and Methods

3.1 Experimental Animals

Laboratory rats were purchased from Weitong Lihua Laboratory Animal Technology Co., Ltd. (SCXK2016-0006, Beijing). Thirty-two male specific-pathogen free (SPF) level SHR aged in 5 weeks and weighing approximately 180 ~ 200 g and eight male SPF Wistar–Kyoto rats (WKY) controls aged in 5 weeks and weighing approximately 180 ~ 200 g were used in this study.

Rats were reared in the barrier system of Xiyuan Hospital, China Academy of Chinese Medical Sciences, and were fed compound feed (provided by Beijing Ke' ao Xieli Feed Co., LTD.) regularly (production certificate: (2019) 06054, Beijing). The room temperature was controlled at 22°C to 25°C, humidity was 50% to 70%, with illumination for 12 h and darkness for 12h. The rats were allowed to acclimatize

for 1 week before starting the study. Initially, rats were fed mixed feed, which was then replaced in some models with a HFD (high-fat diet), HCD (high-carbohydrate diet), or a HSD (high-salt diet). The HFD–HCD was comprised of 10% lard, 20% sucrose, 2.5% cholesterol, 0.5% cholate, and 67.0% normal feed; the HFD was comprised of 10% lard, 5% egg yolk, 1% cholesterol, 0.2% propylthiouracil, and 83.8% normal feed; and the HSD was comprised of normal feed with 4% salt content.

3.2 Laboratory medicines and reagent

Laboratory reagents included STZ (lot number: S0130, provided by Merck Millipore, Germany), an acetylcholine (Ach) kit (lot number: 20210512-N, provided by Nanjing Jiancheng Bioengineering Institute); an acetylcholinesterase (AchE) kit (lot number: 20210520-N, provided by Nanjing Jiancheng Bioengineering Institute); an amyloid-beta (β-AP) kit (lot number: 20210510-B, provided by Beijing Sino-uk institute of Biological Technology); and the rat inflammatory cytokines interleukin-1β (IL-1β) Enzyme Linked Immunosorbent Assay (ELISA) kit (lot number: 20210921), rat IL-6 ELSIA kit (lot number: 20210920), and rat hypersensitive C-reactive protein (hs-CRP) ELSIA kit (lot number: 20210915, all provided by Beijing Xinbosheng Biological Technology Co., Ltd.).

3.3 Laboratory Apparatus

Laboratory apparatus included the COBAS INTEGRA 800 automatic biochemical analyzer (Roche Pharmaceutical Ltd.); the DR-200BS automatic enzyme micro-plate reader (Wuxi Hua Wei Delang (China) Instrument Co., Ltd.); the T18 basic high-speed disperser (IKA, Germany); the 3-18K high-speed refrigerated air dryer (SIGMA, America); the ACCU-CHEK active glucose meter (Roche Diagnostics Co., Ltd., Germany); the 4K-CH Microscopic color image processing system (Beijing Sagely Aoheng Technology Co., Ltd., China); the Pannoramic MID digital slice scanning system (3D Histech, Hungary); and the BX53 optical microscope (Olympus Corporation, Japan).

3.4 Rat model establishment and Grouping

Thirty-two SHRs were allocated into four groups (n=8 rats per group) using the random number table: 1) the SHR group (fed normal diet); 2) HFD–SHR group (fed high-fat diet); 3) HSD–SHR group (fed high-salt diet); 4) diabetic 3 'H'–SHR group (fed high-fat, high-glucose mixed diet and injected with STZ, 25 mg/kg; blood sugar increased stably over 72 h with the typical presentation of clinical polydipsia, polyuria, increased food intake, and weight loss. Rats with a blood sugar ≥11.1 mmol/L were incorporated into the group); and 5) eight male WKY rats, which share the same genetic background as SHRs, comprised the control group. Model rats were kept in fasting conditions the next day after continuous oral administration. Blood plasma was collected by abdominal aortic method following euthanasia with the injection of 1% sodium pentobarbital solution (50 mg/kg). The brain tissue was stripped and the cortical tissue and hippocampus were separated immediately after blood collection and centrifugation. Five samples were packaged in tinfoil according to random numbers and snap-frozen in liquid nitrogen before storing at −80°C until further analysis. The other three tissues were fixed (placed in a 4% neutral paraformaldehyde solution) for pathological analysis.

The care and use of animals in the experiment were in accordance with the requirements of the Beijing experimental animal management regulations and animal ethics regulations. The experiment was approved by Ethics Committee of Xiyuan Hospital, China Academy of Chinese Medical Sciences (No. 2021XLC0122).

3.5 Inflammatory factors testing

The frozen rat brain tissue was defrosted on ice, and 100 mg of cortex and hippocampus were weighed and homogenized separately in a pre-cooled saline solution in filled glass bottles on a high-speed dispersion machine. This process was repeated three times at settings of 1000 ~ 1500 r/min, 5–10 times/min. Centrifugation at 10000 × g was then performed for 10 min at 4°C and the supernatant was retained. The concentrations of IL-1β, IL-6, and hs-CRP were assessed in the cortex and hippocampus using ELISAs to quantify immunoreactivity in strict conformity with the instructions.

3.6 ACh, AChE, β - AP testing

ACh was measured using a colorimetry assay; Ach is broken down into choline and acetic acid after being catalyzed by serum cholinesterase. Acetylhydroxylamine is produced by the reaction between unhydrolyzed Ach and alkaline hydroxylamine, which reacts with ferric iron to form a brown colored compound. The optimal density (OD) was then measured. Colorimetry was used to detect the level of ACh and AChE; serum cholinesterase (ChE) hydrolyzes ACh to choline and acetic acid, dis-hydrolyzed ACh reacts with alkaline hydroxylamine to form N-acetylphenylhydrazine ($C_8H_{12}N_2O_2$), which then reacts with ferric iron to form brown complexes. The OD value was then measured at 540 nm. AChE hydrolyzes ACh to choline and acetic acid;

choline has color reaction with sulfhydryl groups to form trinitrobenzene (TNB), which is yellow, with the OD value measured at 412 nm. An ELISA was used to assess the concentration of β-AP per the manufacturer's instructions.

3.7 Processing and observing of brain tissues

Rat brain tissues were taken out of the pre-cooled 0.9% sodium chloride solution and fixed with 4% paraformaldehyde at 4°C. The tissues were then embedded in paraffin, dewaxed, and cut into 4–6 μm thick coronal slices. The slices were dehydrated to transparency, following by hematoxylin and eosin (HE) staining and Nissl's staining. Step I: the paraffin-embedded sections were placed in a 37°C thermotank with hematoxylin (5 min) and eosin (3 min) for HE staining, with 1% toluidine blue (40 min) for Nissl's staining. Step II: sections were washed with distilled water (2 min) and dehydrated with 95% ethanol. Step III: sections were visualized under the microscope for color separation. Step IV: sections were soaked into 100% ethanol I (2 min) and 100% ethanol II (1 min), in turn, to dehydrate. Step V: sections were soaked into dimethylbenzene I (5 min) and dimethylbenzene I (5 min), in turn, to vitrify. Step VI: permount TM mounting medium was used to seal the sheet glass. Finally, ultrastructural changes of brain tissues were observed after editing by the Pannoramic MID digital slices scanning system (HE staining) and 4K-CH color microscopic for image processing.

3.8 Data Processing

All data were processed using SPSS 13.0 statistical software and are presented as the mean ± standard deviation (SD). Multiple groups were compared using the one-way analysis of variance (ANOVA), and assuming homogeneity of variances, differences among groups were compared by the least significant difference test. If the variance was not uniform, Welch's test was used to correct the F value, and Dunnett's T3 method was used for multiple comparisons. $P<0.05$ was defined as a significant difference.

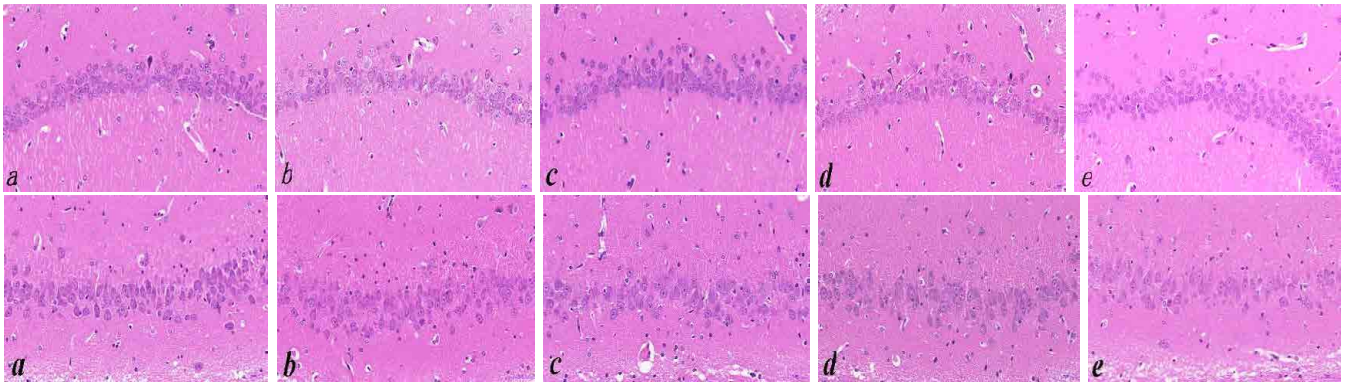
4. Result

4.1 Brain pathological changes (HE stain) of SHR with multiple risk factors

Pathological changes in brain tissues were observed by HE staining. The glial cells and neurons of the WKY control group in the CA1 and CA3 regions were clearly observed, regularly arranged, uniform, circular or oval shaped, with regular hyperchromatic nuclei. However, in the four model groups, neurons were irregular, triangular or spindle shaped, with a dilated intercellular space, and pyknosis and hyperchromatic nuclei. This was especially true in DM -SHR group, where the number of glial cells and neurons had decreased significantly and the nuclei were more hyperchromatic and pyknotic (Figure 1).

4.2 Brain pathological figure (Nissl's stain) of SHR with multiple risk factors

Neurons and glial cells in the CA1 and CA3 regions of WKY controls were observed with stained nuclei and abundant blue staining of large granular Nissl bodies; the Nissl bodies and nuclei were clearly separated and organized closely in the cytoplasm. Furthermore, the axon and dendrites were easily recognizable. The amount of Nissl bodies in the neurons and glial cells of SHRs and the three model groups decreased to



1) CA1 region of the brain tissue. a. WKY group, b. SHR group, c. HFD–SHR group, d. HSD–SHR group, e. DM -SHR group.

2) CA3 region of the brain tissue. a. WKY group, b. SHR group, c. HFD–SHR group, d. HSD–SHR group, e. DM -SHR group.

Figure 1. Brain pathological features (HE stain, × 200) of SHRs with multiple risk factors.

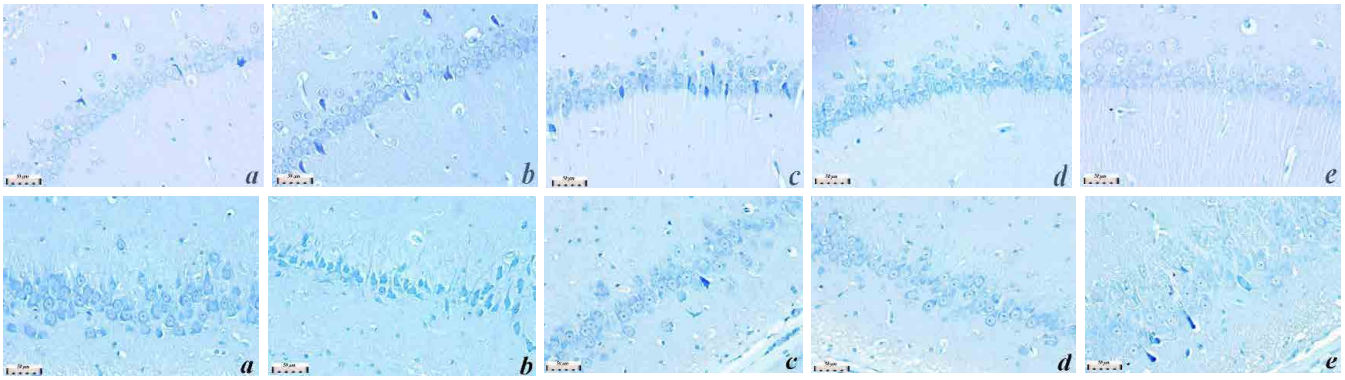
different degrees. In the CA1 region and CA3 region of the HFD–SHR group, we observed a dilated intercellular space, vacuole-like denaturation, and a decrease in Nissl bodies. The number of Nissl bodies decreased significantly, showing slight staining and dissolving to different degrees, especially in the CA3 region of the DM -SHR group (Figure 2).

4.3 Inflammatory cytokine levels in serums of SHR with multiple risk factors

The serum concentration of IL-1β, IL-6, and hs-CRP in the SHR group, HFD–SHR group, HSD–SHR group, and DM -SHR group increased significantly compared with the WKY control group, with a statistical difference *(P<0.05, P<0.01)*. The concentrations in the HSD–SHR and DM -SHR groups were notably higher than other groups, although no statistically significant difference was found among these groups *(P>0.05)* (Table 1).

4.4 Cholinergic and inflammatory levels in cerebral cortex of SHR with multiple risk factors

The concentration of IL-1β, IL-6, and β-AP in the cortex of the SHR group, HFD–SHR group, HSD–SHR group, DM -SHR group increased significantly compared with WKY control group, with a statistical difference (*P<0.05, P<0.01*). The levels of ACh and AChE in the cortex of the SHR group, HFD–SHR group, HSD–SHR group, and DM -SHR group were significantly decreased *(P<0.05, P<0.01)*. The ACh level in the cerebral cortex of the HFD–SHR group was notably decreased; however, there was no significant difference among these four groups *(P>0.05)* (Table 2).



1) CA1 region of the brain tissue. a. WKY group, b. SHR group, c. HFD–SHR group, d. HSD–SHR group, e. DM -SHR group.

2) CA3 region of the brain tissue. a. WKY group, b. SHR group, c. HFD–SHR group, d. HSD–SHR group, e. DM -SHR group.

Figure 2. Brain pathological features (Nissl's stain, × 200) of SHRs with multiple risk factors.

Table 1. Inflammatory cytokine levels in SHR with multiple risk factors (n = 10, $\bar{x} \pm s$).

Group	IL-1β (pg/ml)	IL-6 (pg/ml)	hs-CRP (mg/ml)
WKY	21.90 ± 2.58	132.59 ± 14.36	3.43 ± 0.68
SHR	33.57 ± 2.03**	160.04 ± 17.22**	6.68± 1.07**
HFD - SHR	32.37 ± 2.38**	166.62 ± 14.23**	7.07 ± 0.89**
HSD - SHR	34.53 ± 1.94**	169.03 ± 19.03**	7.56 ± 0.77**
DM -SHR	36.89 ± 1.83**	170.48 ± 12.31**	7.21 ± 0.84**

Table 2. IL-1β, IL-6, β-AP, ACh, and AChE levels in cortex of SHRs with multiple risk factors (n = 5, $\bar{x} \pm s$)

Group	IL-1β (pg/ml)	IL-6 (pg/ml)	ACh (ug/ml)	AChE (U/ml)	β-AP (mg/L)
WKY	1.86 ± 0.05	10.60 ± 0.56	75.71 ± 3.12	0.71 ± 0.06	0.10 ± 0.01
SHR	2.53 ± 0.25*	16.86 ± 0.65**	66.24 ± 5.32**	0.40 ± 0.02**	0.26 ± 0.03**
HFD - SHR	2.85 ± 0.53**	16.33 ± 0.98**	67.41 ± 4.41**	0.48 ± 0.09**	0.25 ± 0.02**
HSD - SHR	2.31 ± 0.31**	16.72 ± 1.31**	62.52 ± 3.67**	0.42 ± 0.02**	0.24 ± 0.04**
DM -SHR	2.32 ± 0.38**	16.61 ± 1.20**	67.60 ± 2.99**	0.43 ± 0.04**	0.24 ± 0.05**

Note. Compared with WKY control group, **P <0.01.

4.5 Cholinergic and inflammatory levels in cerebral hippocampus of SHR with multiple risk factors

The concentration of IL-1β, IL-6, β-AP in the hippocampus of the SHR group, HFD–SHR group, HSD–SHR group, DM -SHR group increased significantly compared with WKY control group, with a statistical difference *(P<0.05, P<0.01)*. The levels of Ach and AChE in hippocampus of SHR group, HFD–SHR group, HSD–SHR group, DM -SHR group decreased significantly, with a statistical difference *(P<0.05, P<0.01)*. In the SHR group, IL-1β, IL-6, and β-AP levels in the hippocampus were notably increased, and ACh and AChE levels were obviously decreased; however, there was no significant difference among these four groups *(P>0.05)* (Table 3).

5. Discussion

VaD is widely considered as the second most common dementia disease. Hypertension, in addition to obesity, dyslipidemia, and insulin resistance, form metabolic syndrome (MetS). Clinical observations suggest that MetS, a high-salt diet, alcohol drinking, and so on, are risk factors for cognitive changes in the elderly population[20-25]; however, the mechanism remains unclear. Hypertension is one of the most common risk factors that comprises the pathological basis of a series of cerebrovascular diseases[20,21,24-31]. Furthermore, hypertension is considered to be an independent risk factor for cognitive impairment[32,33]. A constant elevation of blood pressure can cause smooth muscle fibrosis, thickening and narrowing of small arteries, and increase in vascular resistance increasing, and a diminished flow rate. Therefore, hypertension leads to cognitive dysfunction due to neurodegenerative diseases with vascular injury[34]. In addition, a decrease in ACh caused by hypertension can lead to vascular endothelial injury[35].

Lipids are among the factors that cause VaD, and a connection between cholesterol and VaD has been established[36-38]. High cholesterol can promote the processing of amyloid protein precursor (APP), which can cause AD. The increase in serum cholesterol can injure cerebral arteries and vascular endothelial cell function in small vessels, reduce blood flow, and accelerate atherosclerosis, which increases the risk of cognitive impairment and dementia. A HFD can increase the dephosphorylation of the β-site amyloid precursor protein cleaving enzyme (BACE1) to improve the catalytic activity and thermal stability of the enzyme. This can accelerate the deposition of β-AP plaques, neurodegeneration, and leads to learning and memory impairment[39].

Type 2 diabetes mellitus (T2DM), which is characterized by chronic hyperglycemia and insulin resistance, is another risk factor of AD. T2DM can increase the risk of developing cognitive impairment and dementia by speeding up the process between mild cognitive impairment (MCI) and dementia. Hyperglycemia can activate a change from cell apoptosis to necroptosis [48]. In the process of necroptosis, cells generate excessive ROS and produce β-AP. Inflammation can also promote the production of β-AP as a key consequence of necroptosis. Necroptosis is vicious circle due to multiple risk factors, which can be a key therapeutic target of AD.

Furthermore, a HSD correlates with an increased risk of dementia [49]. DSS rats exhibited hypertension, leakage from microvessels in the hippocampus, and degenerative cognitive function, which were associated with increased brain angiotensin II levels, as well as decreased mRNA levels of tight junctions and collagen-IV in the hippocampus [50].

When observing the pathological results of the SHR group and three model groups, HE staining revealed a decrease in the number of hippocampal and cortical neurons, space broadening, a disorganized structure, and pyknosis and condensed nuclei. Nissl's staining revealed that the number of Nissl bodies was decreased in the CA1 area of the hippocampus. These findings were especially present in the DM -SHR group; the reduction in neurons, change in structure, and reduction of Nissl bodies were even more obvious and many cells had vesicular degeneration.

Table 3. Content of IL-1β, IL-6, ACh, AChE and β-AP in the hippocampus of SHRs with multiple risk factors (n=5, $\bar{x} \pm s$)

Group	IL-1β (pg/ml)	IL-6 (pg/ml)	ACh (ug/ml)	AChE (U/ml)	β-AP (mg/L)
WKY	1.70 ± 0.09	9.15 ± 1.06	67.78 ± 1.89	0.62 ± 0.04	0.15 ± 0.01
SHR	1.96 ± 0.10**	12.81 ± 1.09**	62.76 ± 4.44***	0.43 ± 0.01**	0.20 ± 0.01**
HFD - SHR	1.91 ± 0.15**	10.93 ± 1.55**	61.14 ± 4.82**	0.51 ± 0.04**	0.20 ± 0.01**
HSD - SHR	2.14 ± 0.53**	12.95 ± 1.16**	64.47 ± 3.83**	0.49 ± 0.02**	0.21 ± 0.01**
DM -SHR	2.19 ± 0.27**	13.23 ± 2.11**	59.74 ± 2.31*	0.47 ± 0.09**	0.26 ± 0.05**

Note. Compared with WKY control group, ***P* <0.01, ***P* <0.01.

Real world studies suggest that patients with hypertension often have risk factors such as HLP, DM, and HSD. These risk factors interact with each other and lead to or accelerate the progression of AD. There are increased amounts of activated glial cells and inflammatory cells in pathological tissue from VaD and AD patients, and some known inflammatory factors take part in pathological process of inflammatory lesions[51]. A previous experimental animal study reported that after feeding a HFD to pR5 transgenic mice (with expression of P301L mutating into tau), glucose intolerance and insulin resistance appear, which develops into T2DM and aggravates the hyper-phosphorylation of tau [52]. At the same time, early inflammatory disorders result in the expression of apolipoprotein 4 (APOE4), leading to central nervous system damage caused by several injuries, and increasing risk of developing AD [53]. Insulin may affect metabolism of β-AP and tau protein; chronic hyperinsulinemia can increase the release of inflammatory markers and suppress oxidative stress [54]. The level of CRP, IL-6, TNF-α, and lipid peroxide increased, and the level of endothelial nitric oxide synthase (eNO) decreased, in the plasma of patients with AD [55,56]. The other studies reported that the level of hippocampal nuclear factor kappa-B (NF-κB) was raised significantly in 32-week-old SHRs [55,56], which suggests that the increase in inflammatory factors may be linked to cognitive impairment as a result of hypertension. Activation of the NF-κB pathway by extracellular factors is related to memory and synaptic plasticity. NF-κB is a type of cytokine produced by activated macrophages, in addition to TNF-α, IL-1β, IL-2, IL-6, iNOS, COX2, etc. Furthermore, NF-κB can adjust and control inflammatory and apoptosis-related molecules, including heme oxygenase 1 (HO-1) and TNF receptor-associated factors 1/2. IL-1β can stimulate the proliferation and differentiation of immunoreactive cells. IL-6 can adjust growth and differentiation of several kinds of cells; take part in the immune response, acute phase response, and hematopoietic function; and plays a role in fighting infections. TNF-α is a multiplex proinflammatory cytokine produced by fibroblasts, monocytes, macrophages, T lymphocytes, etc. TNF-α plays a key role in inducing inflammation and DM.

This study revealed that 24-week-old SHRs, HFD–SHRs, HSD–SHRs, and DM -SHRs exhibit increased serum and brain tissue levels of IL-1β, IL-6, and hs-CRP. The results were comparable with those reported previously that inflammatory markers (hs-CRP and IL-6) are related to primary hypertension, with inflammatory mechanisms resulting changes to vessels and causing blood-pressure fluctuations, thus promoting brain injury and the development of VaD [58]. In DM -SHRs, the level of IL-1β, IL-6, and hs-CRP in the serum and hippocampus were increased more significantly.

Cholinergic system dysfunction can damage brain flow and affect the function of the neuro-vascular unit (NVU) [59]. ACh is one of the most important neurotransmitters for retaining consciousness and has a physiological effect on learning and memory. AChE is the key enzyme in nerve conduction; it can guarantee that nerves send signals through hydrolysis of ACh to break up excitation of the neurotransmitter on the postsynaptic membrane. Mild depression on AChE has been proven to be related with curing AD; AChE inhibitors (for example donepezil hydrochloride, huperzine A, and rivastigmine) are commonly used in the clinic to improve activation of ACh. However, AChE can take part in growth and development of cells to promote neuron development and regeneration. Thus, excessive depression of AChE may lead to a cholinergic crisis. There is also no clinical study to prove that AChE inhibitors have an ideal therapeutic effect.

Earlier studies observed an increase in the concentration of AChE in plasma and tissue of patients with T2DM and AD; AChE was considered to be a possible predictive biomarker of T2DM and AD [55]. Hypertension, as well as other related risk factors, can also alter the production and release of AChE [55]. ACh-mediates it effects through the release of NO in WKY and SHRs [61]. An elevation of blood lipids, blood pressure, and insulin resistance can cause an increase in the level of lipocalin-2, which restrains the bio-utilization of NO [62]. An early study reported that the downward trend in ACh is associated with increasing age; ACh in the brains tissue of C57BL/6 and BALB/c mice decreased by 41% and 44% at 10 months of age, and further decreased by 64% and 75% at 30 months of age. The results suggested that the brain tissue of elderly people was more easily damaged by MetS, and that hypoxic-ischemic brain damage in all age groups could depress the synthesis of neurotransmitterss [63]. A more recent study found that in pre-senile and early-stage dementia, the increased activity of AChE is related to neurodegeneration, neurofibrillar tangles, and further inflammation; however, there was no relationship between AChE and the level of Aβ-42 in cerebrospinal fluid[64]. Several experiment results suggested that Ach and AChE expression were increased in the brain tissue of AD model mice (APP/PS1) and VaD/cognitive impairment model mice (2 - VO, p MCAO) [65-67]. Therefore, dysfunction of the cholinergic system was considered a crucial pathological mechanism

of cognitive impairment or dementia. Another study also suggested that cerebral ischemic injury caused neuronal damage, resulting in fewer neurons and synaptic cells in hippocampal CA1 region, decreased production of ACh, and decreased activity of AChE [68], cerebral ischemia injury caused neurons damage with fewer neurons and synaptic cells in hippocampus CA1 region, decreased production of ACh and decreased activity of AchE.

β-AP has neurotoxic effects; its formation of oligomers, which form beta-amyloid plaques, can cause vessel damage, NVU functional impairment, and cognitive impairment [59]. Hypertension and risk factors can weaken the ability to degrade β-AP in the brain and promote cognitive impairment caused by amyloid deposition [60,69]. Scholars observed a 3.71-fold increase in BACE1 expression in the cerebral microvessels of patients with hypertension [70]. The increase in BACE1 and β-AP are associated with hyperglycemia and hyperlipidemia, which promote the deposition of β-AP in the cerebral vessels and increase the chances of cerebral small vessel disease and neurological dysfunction [71]. The results suggested that in the cortex and hippocampus of the SHR group, HFD–SHR group, HSD–SHR group, and DM -SHR group, the level of ACh and AChE were significantly decreased and the level of β-AP was significantly increased. Changes were much more obvious in the DM -SHR group. It was interesting that although there was no statistical significance among these four groups, the level of AChE in the DM -SHR group was elevated significantly compared with the SHR group, with the same observed in the HFD–SHR group and HSD–SHR group.

This experiment was derived from one research topic of the cardiovascular system and made an association with cognitive impairment; therefore, we did not assess any behavioral changes. We examined the mechanisms of ACh and AChE and their effects on cognitive disorder, and assessed the mutual relationship between hypertension and multiple factors. Nowadays, more effective ways of protecting the function of the brain and preventing cognitive impairment are adjustments to lifestyle, preventing and treating the risk factors of CVD (especially blood pressure control, blood glucose control, and blood lipid control), and eating foods rich in ACh [71].

6. Conclusions

Epidemiological data suggests that vascular risk factors are present in AD patients. Hypertension, DM, obesity, etc., can increase the risk of AD [72-75]. Thus, hypertension combined with multiple factors, including lipid metabolism disorders, DM, and HSD can cause damage to the brain tissue and changes in the levels of biomarkers of cognitive impairment. Interactions between multiple risk factors exist that can increase the likelihood of developing vascular dementia, as observed in AD.

7. Data Availability Statement

The data used to support the findings of this study are included within the article.

8. Conflict of Interest

The authors declare no conflict of financial interest or benefit, with other people or organizations in this work.

9. Author Contributions

Su-rui Chang, Zhen Zhang, Jian-Gang Liu carried out the experiments, analyzed the results, and wrote the manuscript. Hao Li contributed equally to this work.

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11. Ethics Statement

The animal study was reviewed and approved by ethics committee of Xiyuan Hospital, China Academy of Chinese Medical Sciences in 2021 (№.2021XLC0122). All the experiment methods are abide by Guidelines on the good treatment of laboratory animals of Ministry of 'Science and Technology' and 'the People' s Republic of China.

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