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The Study on Neural Remodeling of Medullary Visceral Zone in Early Sepsis and Interfered by Cholinergic Anti-Inflammatory Pathway

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Research

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Abstract

Background

Studies including our own have shown that the Medullary Visceral Zone (MVZ) can effectively regulate systemic inflammation and immunity through the Cholinergic Anti-inflammatory Pathway (CAP). Sepsis usually causes neuroinflammation in the Central Nervous System (CNS), which will inevitably affect the structure and function in related brain areas such as MVZ, whether the intervention of CAP can affect the structure and function of the MVZ in sepsis needs to be further verified.

Methods

64 adults, specific pathogens free Sprague-Dawley male rats were used in this study. The septic models were prepared by cecum ligation and puncture (CLP) method, GTS-21 (a selective α7 nicotinic acetylcholine receptor agonist which can mimic CAP's activation) and MLA (a powerful and selective nicotine acetylcholine receptor antagonist which can mimic CAP's blocking) were used to interfere CAP. The pathological changes, apoptosis, the expressions of Tyrosine Hydroxylase (TH) and Choline acetyltransferase (CHAT), the expression levels of GAP-43 mRNA, Olig-2 mRNA, VEGF mRNA, GFAP mRNA, MMP-9 mRNA in MVZ were analyzed among different groups.

Results

In this study, we found that sepsis induced apoptosis and functional suppression of catecholaminergic and cholinergic neurons and gliosis in MVZ, up-regulated key genes' expressions such as GAP-43 mRNA、GFAP mRNA、VEGF mRNA、MMP-9 mRNA, down-regulated the expression of Olig-2 mRNA. GTS-21, a selective α7 nicotinic acetylcholine receptor agonist, obviously mitigated the above changes; whereas, methyllycaconitine (MLA), a powerful and selective nicotine acetylcholine receptor antagonist, significantly aggravated these changes.

Conclusions

Our research shows that activating CAP can effectively mitigate the neural remodeling and neuronal suppression induced by early sepsis in MVZ, the mechanism may involve with its control of systemic and local inflammation. This study reveals that MVZ and CAP may be potential targets to curb the inflammatory storm in early sepsis.

Background

The uncontrolled systemic inflammation storm induced by early sepsis is closely related to high mortality [1]. Anti-inflammatory therapy can evidently improve the mortality and prognosis of septic patients [2]. Therefore, seeking the body's own inflammation and immune regulatory pathways to suppress the early uncontrolled inflammation storm of sepsis has significant theoretical innovation meaning.

The Medullary Visceral Zone (MVZ) is a relaying station to transmit peripheral inflammatory information to the high-level nervous center [3]. Previous studies have found that MVZ effectively regulates systemic inflammation and immune strength through Cholinergic Anti-inflammatory Pathways (CAP) [4]. GTS-21，a selective α7 nicotinic acetylcholine receptor agonist，can evidently down-regulate the serum levels of inflammatory mediators such as soluble CD14 (Presepsin), High Mobility Group Box-1 (HMGB-1), interleukin (IL)-10, Tumor Necrosis Factor (TNF)-α, IL-1α, IL-6 and CD4+CD25+Treg, CD4+IL-17+TH17 lymphocyte percentage; On the contrary, methyllycaconitine (MLA), a powerful and selective nicotine acetylcholine receptor antagonist, greatly aggravates systemic inflammation and immunity of sepsis. It is well known that both GTS-21 and MLA can pass through the blood-brain barrier and play multiple central roles [5-6]. Recent studies also suggested that intervention with the central CAP had a wide range of effects such as improving the impaired cognitive function [7] and regulating systemic inflammation [8]; then, a problem emerges, besides peripherally mimicking or antagonizing CAP to influence systemic inflammation, whether GTS-21 and MLA also affect the structure and function of septic MVZ through which further affect systemic inflammation and immunity is still unclear.

Studies have confirmed that while MVZ regulates systemic immunity and inflammation, systemic inflammation also induces MVZ's neuro-inflammation and even leads to its disorder of autonomic regulation [9-10-11]. In Clinic once patients in Intensive Care Unit were diagnosed with septic encephalopathy, the mortality rate is significantly higher than those without encephalopathy [12]. From these researches we reasonably believe that MVZ is definite to be attacked by systemic inflammation in the early stage of sepsis, which may underlie the disorder of CAP’s regulatory function and the inflammatory storm in the early stage of sepsis. Based on the theory that various constitutions of nerves have different functional states, which may be an important mechanism for individuals survival adaptation and even modifying the social role they played [13], it is necessary to explore the MVZ pathology in sepsis and the interfered effect by CAP activation or blocking to provide ideas of central intervention to combat septic inflammation storm.

Material And Methods

Rats management and septic models preparation

64 adults, specific pathogens free Sprague-Dawley male rats [8 weeks old, 250-280g, License Number: SYXK (Hubei)2018-0104] were purchased from Experimental Animal Center of Three Gorges University [Sale License Number: SCXK（Hubei）2017-0012]. Rats were housed in Guizhou Medical University Experimental Animal Center and maintained under temperature control (21 ± 0.5 °C) and a 12-h light-dark cycle (lights on during 05:00–17:00). Standard chow and tap water were provided ad libitum. Rats were acclimatized for 7 days before experimental manipulations. All experimental procedures were carried out in compliance with the guidelines of the institutional animal care and use committee (IACUC) of the First Hospital of Guiyang (IACUC number 20190107).

The septic models were prepared by cecum ligation and puncture (CLP) way[14], the main processes were included: Rats were deeply anesthetized with isoflurane inhalation[15-16], the right lower abdomen were shaved and disinfected prior to be cut open to abdominal cavity. The cecum were dissociated and ligated at the middle with a 5-0 suture, the ligated pare was pierced with a 21G needle twice and gently squeezed out a small amount of intestinal contents from the puncture holes. At last the cecum was returned into the abdominal cavity, the inner layer was sutured with 5-0 sutures and the outer layer with 3-0 sutures (Fig 1). After operation rats were accepted intraperitoneal injection of Piperacillin (50 mg/Kg, i.p. tid×3d).

After operation, rats were delayed to recover to their consciousness, their bodies were sluggish and often curled up, they were looked inactive, eat and drink less, they breathed quickly and difficultly. These appearances suggested that the model is successful. After 12 hours of operation, some rats began to die. As time went by, most rats showed low skin temperature, weakened muscle strength, bloody secretions in the eyes, stopped eating and drinking and began to diarrhea. Further development, they appeared shortness of breath, no resistance to passive supine. Some rats excreted large amount of mucus watery stool and eventually died.

Rats grouping and treatment

After 7 days of adaptive feeding, rats were divided into three groups according to the random number table: Control Group: Rats (n=8) were feed as usually without any treatment; Sham Group: Rats (n=8) were subjected to open and suture the abdominal cavity without CLP manipulation, afterwards they were accepted intraperitoneal injection of Piperacillin (50 mg/Kg, i.p. tid×3d); Sepsis Group: The sepsis rats (n=48) were prepared with CLP method as mentioned above. One hour after the septic rats return to their consciousness, they were randomly divided into 3 groups, 16 rats in each group. a: Model Group: accepting intraperitoneal injection of Piperacillin (50 mg/Kg, i.p. tid×3d) and saline (1 mL/100 g, i.p. tid×3d); b: GTS-21 Group, beside Piperacillin was used as Model Group, intraperitoneal injection of GTS-21 (a selective α7 nicotinic acetylcholine receptor agonist which can mimic CAP in acting on the α7 nicotinic acetylcholine receptors on monocytes and neutrophils to down-regulates NF-κB and inhibits the transcription and release of inflammatory factors, produced by MCE, Lot: 29834. Dosage and duration: 4 mg/Kg, i.p. tid×3d) was given to each rat [17]; c: Methyllycaconitine (MLA) Group: beside Piperacillin was used as Model Group, intraperitoneal injection of MLA (a powerful and selective nicotine acetylcholine receptor antagonist, since the terminals of CAP release acetylcholine, so it can simulate to block CAP's effect on immune cells and aggravate inflammation, produced by MCE, Lot: HY-N2332A/CS-0021211. Dosage and duration: 4.8 mg/Kg, i.p. tid×3d) was given to each rat [18]. After 3 days, the rats were sacrificed to collect the medullary tissue for analysis under anesthesia with isoflurane inhalation.

Paraffin section preparation and pathological observation

Three days after operation, all survival rats in each group were deeply anesthetized with2-4% isoflurane inhalation, brain tissues were quickly stripped and fixed in 4% paraformaldehyde buffer solution (PBS) for

12 ~ 24 hrs. 3 rats' Medulla Oblongata from each group were cut off and dealt with conventional dehydration, paraffin embedding to prepare sections (30 μm), which were used for haematoxylin and eosin (HE) staining, TdT mediated dUTP Nick End Labeling (TUNEL) and Immunofluorescence analysis. The rest Medulla Oblongata were preserved in the refrigerator at -80℃ for PCR detection.

TUNEL

5 paraffin sections from five groups were taken, they were dewaxed and immerged in Proteinase K solution.then the Terminal Deoxynucleotidyl Transferase (TdT) buffer was added to incubate. In the end, DAPI (Beyotime Biotechnology, lot number: C1002) and fluorescence quencher (southernbiotech, lot number: 0100-01) were added to stain the nuclei and the apoptotic cells. Sections were observed with a fluorescent microscope (Olympus BX53 biological microscope). The survival cell nuclei were stained blue, the apoptotic cell nuclei were stained red. Select 3 fields of view (the left side, the middle and the right side) to count labeling index (LI) under high magnification(400 times) on every sections, LI = the number of positive cells in each field/all cells in the field, and the apoptosis index (AI) of each group is the average value of LI for each section

Immunofluorescence double labeling for TH or CHAT combined with Caspase 3

After dehydration，sections were blocked with 10% normal goat serum for 1 hour and then incubated for 24 h in a cocktail of primary antibodies for the labeling of caspase3 (produced by Wuhan Sanyan Bio. Co., China. Lot: 66470-2-IG, dilution: 1:50), Tyrosine Hydroxylase (TH, produced by Wuhan Boster Co., China. Lot: BM4568, dilution: 1:50) or Choline acetyltransferase (CHAT, produced by Wuhan Bioss Co., China. Lot: bs-2423R, dilution: 1:50). Afterwards, sections were incubated with two fluorescent-labeled secondary antibodies，FITC labeled goat anti-rabbit IgG, used to mark TH or CHAT, produced by Wuhan Boster Co., China. Lot: BA1105, dilution: 1:100, Cy3 labeled goat anti-mice IgG, used to mark caspase3, produced by Wuhan Boster Co., China. Lot: BA1031, dilution: 1:100) for 4 hours. Finally, the sections were mounted on gelatin-coated slides and covered with mounting medium with DAPI, for nuclear staining of all cells present in the slice [19]. Images from the different experimental groups were captured with Olympus BX53 Biological Microscope. The normal nuclei were stained blue, Cholinergic neurons expressing CHAT and catecholaminergic neurons expressing TH were stained green, apoptotic neurons expressing caspase3 were stained red. Three images with 400 folds' enlargement from every group were analyzed with imagepro (ipp6.0) software [20] and the average densities were acquired.

Quantitative RT-PCR

All the rest rats' Medulla Oblongata specimens from each group were used for the target RNA detection. RNA extraction accorded to the instruction of Reagant Trizol kit (produced by Aidlab. Co. Lot: 252250AX).

In a gene bank database standard cDNA sequences of related gene were obtained, DNA starting primers were designed, synthesized and supplied by Qingke Co. Ltd. The sequences are as follows (Table1). Select Rat GADPH as inner reference to compute the expression levels of GAP-43 mRNA, Olig-2 mRNA, VEGF mRNA, GFAP mRNA, MMP-9 mRNA.

0.1g fresh sampling tissue of Medulla Oblongata from every group was taken and homogenized in 1mL Trizo reagent (Aidlab, Lot:252250AX) by bead mill (Retsch Tissue Lyzer II, Qiagen, Valencia CA, USA). RNA was isolated from medulla homogenates by addition of 10% BCP and standard phase separation, followed by overnight precipitation with isopropanol at -20°C. RNA was purified using the Qiagen RNeasy Mini kit (Qiagen), Start PCR amplification reaction in PCR instrument. The reaction conditions are as follows: Predenaturation: 50℃ for 2min.; denaturation: 95℃ for 10min; annealing: 95℃ for 30sec, extension 60℃ for 30sec, altogether for 40 cycles. Electrophoresis analysis: taking the product of 5ul PCR and 6 x DNA loading buffer 2ul to mix, then take the mixture into 2% agarose gel (containing EB) to electrophoresis at 150V voltage for 35min prior to being observed under ultraviolet lamp, after focus adjustment, the images were photographed with gel analysis system. With gray scale scanning software existing in the gel imaging system the electrophoresis target zone was analyzed, the intensity ratios of the target gene compared to the reference gene were acquired.

Tab. 1 Primer sequence list

Name	Primer	Sequence	Size
Rat β-actin	Forward	5‘-CACGATGGAGGGGCCGGACTCATC-3’	240bp
	Reverse	5‘-TAAAGACCTCTATGCCAACACAGT-3’	
Rat GAP-43	Forward	5‘-ATAACTCGCCGTCCTCCAAG-3’	237bp
	Reverse	5‘-GGTCTTCTTTACCCTCATCCTGT-3’	
Rat Olig-2	Forward	5‘-TCATCTTCCTCCAGCACCTCCTCGT-3’	311bp
	Reverse	5‘-TGACCCCCGTAAATCTCGCTCACCA-3’	
Rat VEGF	Forward	5‘-CGTCTACCAGCGCAGCTATTG-3’	145bp
	Reverse	5‘-CTCCAGGGCTTCATCATTGC-3’	
Rat GFAP	Forward	5‘-ACGAACGAGTCCTTGGAGAG-3’	181bp
	Reverse	5‘-CGATGTCCAGGGCTAGCTTA-3’	
Rat MMP-9	Forward	5‘-GCTGGGCTTAGATCATTCTTCAGTG-3’	109bp
	Reverse	5‘-CAGATGCTGGATGCCTTTTATGTCG-3’	

Statistics

Measurement data were expressed as mean ± standard deviation ($\bar{x}$±SD). Data were statistically processed with SPSS 19.0 software package. Intergroup differences were analyzed with analysis of variance (ANOVA). Leven homogeneity test is performed first. Comparing of homogeneous data is determined by Bonferroni Test, otherwise they were judged by Tamhane Test; $P < 0.05$ was considered statistically significant.

Results

Specimens observation

Observed from the specimens, there was no significant difference in the appearance of the brain tissues among five groups of rats. Viewed from the ventral aspect, the anterior midlines of the brainstem of rats in Model Group and MLA Group were slightly shallower than the other groups, suggesting edema in the brainstem, see Figure 2.

Pathological observation

In the Control Group and Sham Group, The structure of the medulla oblongata is clear. The cells in the Nucleus Tractus Solitary (NTS), Dorsal Vagus Motor Nucleus (DVMN) and Ventrolateral Reticular Nucleus (VLRN) are neatly arranged. There are more neurons and fewer glial cells under high magnification. Whereas in the Model Group, GTS-21 Group and MLA Group, Decrease of neurons and increase of glial cells were obvious, cells were disorderly arranged, See Figure 3.

TUNEL and Dual Fluorescence Immunoassay（CHAT, TH / Caspase 3）

As depicted by our precedent study[4], compared with the Control Group and the GTS-21 Group, much more cells in MVZ of the Model Group and the MLA Group went through apoptosis; From the results of fluorescent double-labeled stains, we can see that sepsis induced decreased expression of CHAT and TH and increased expression of Caspase 3, suggesting the suppression of cholinergic neurons and catecholaminergic neurons and obvious apoptosis occurred, especially for the catecholaminergic neurons. The intervention of CAP can significantly affect the activity and apoptosis of neurons in MVZ, α7nAChR agonists can improve the activity or apoptosis of two kinds of neurons in MVZ, while α7nAChR antagonists are the opposite, see the Figure 4-6.

RT-PCR

RT-PCR showed that sepsis resulted in increased expressions of GAP-43 mRNA, GFAP mRNA, VEGF mRNA, MMP-9 mRNA in MVZ, and down-regulation of Olig-2 mRNA expression; GTS-21, an agonist of α7nAChR, could significantly reduce the expressions of GAP-43 mRNA, GFAP mRNA, VEGF mRNA, MMP-9

mRNA and had a tendency to increase the expression of Olig-2 mRNA. MLA, the antagonist of α7nAChR, is the opposite (Figure 7).

Discussion

In the Central Nervous System (CNS), LPS can activate astrocytes through Toll-like receptor4 (TLR4) to produce pro-inflammatory cytokines and arouse neuro-inflammation [21-22]. Moreover, cytokines elevated in the peripheral blood circulation can also be transported into the CNS to directly inspire neuro-inflammation [23-24].Neuro-inflammation boost nerve cells apoptosis, neurodegeneration and metabolic disorders through NF-kB/STAT3/ERK pathway and mitochondria-mediated apoptosis [25-262]. Based on these testimonies, we reasonably concluded that inflammation occurs in MVZ in early sepsis, what's more, the configuration and function of MVZ is bond to change under inflammation and they should be affected by the intervention of CAP. In this study we found that although the appearance of the whole brain specimens from five group didn't show significant difference in the early stage of sepsis, HE staining signified that MVZ neurons in three Sepsis Groups significantly lost and glial cells significantly proliferated. It is no doubt that neuro-inflammation should be responsible for these changes. The pathologic changes suggested that neuro-remodeling occurred in MVZ in early sepsis stage. Neuro-remodeling involves changes in the activity and number of functional neurons and regulations in the expression of key genes. What will happen to these functional neurons and key genes in sepsis? When CAP is interfered what will happened to them next? Solving these problems may help to explain the suppression of CAP and uncontrolled systemic inflammation in sepsis.

Analysis of TUNEL fluorescence images confirmed that sepsis induced apoptosis of MVZ neurons and central intervention of CAP had positive effect on apoptosis, GTS-21 could significantly reduce apoptosis of MVZ cells, while MLA could further aggravate it, indicating that central CAP activation played an anti-apoptotic role. Studies have confirmed that acetylcholine secreted from CAP terminal can activated microglial cells, the binding of acetylcholine to α7nAChR on the surface of microglia cell on one hand activate Janus kinase/signal transducers (JAK/STAT) signaling pathway, on the other hand inhibit nuclear factor kappa B signal transduction pathway, all contribute to reduce the production of inflammatory mediators such as TNF-α, HMGB-1, IL-1, and IL-6, and increases the levels of anti-inflammatory factors such as IL-10 [28]. What's more, acetylcholine also reduce the number of activated microglia cells [29] and restrain the growth of them [30] which underlie the suppression of MVZ neuro-inflammation and apoptosis.

There are multiple types of neurons in MVZ. Cholinergic neurons are directly involved in the regulation of systemic inflammation [31]. Catecholaminergic neurons project into DVMN and the network structure of the medulla oblongata which also participate in the regulation of inflammation, immunity [32-33] and stress in MVZ [34]. In order to explore the activity and apoptosis of these two kinds of neurons, we conducted CHAT/Caspase 3 and TH/Caspase 3 immunofluorescence double labeling detection. The results tell us that sepsis leads to obvious apoptosis of these two kinds of neurons. Along with apoptosis

of catecholaminergic neurons, the biosynthesis of TH declined obviously, GTS - 21 protected catecholaminergic neurons from apoptosis and promoted the biosynthesis of TH in sepsis, MLA significantly worsen the apoptosis and further reduced the production of TH in sepsis; For the cholinergic neurons, GTS-21 has the tendency to facilitate the production of CHAT, while MLA significantly reduce the output of CHAT in sepsis. Our results are consistent with other studies that CAP activation in CNS has multiple functions such as anti-apoptosis, which depend on its reduction of central inflammation, preventing the brain from injury and improving the activity of functional neurons [35-36]. Here, we found an interesting phenomenon that TH expression decreased simultaneously with the increasing apoptosis of catecholaminergic neurons, while CHAT expression had no significant reduction when there was evident apoptosis of cholinergic neurons, suggesting that sepsis promotes the functional activation of the cholinergic system of MVZ to increase the vagal output, which may be an important mechanism for its systemic anti-inflammatory effect [37-38]. This study indirectly indicates that cholinergic neurons may be the major adjusting mediators of systemic inflammation in MVZ.

As mentioned above, pathological studies suggest that sepsis in early stage leads to apoptosis of MVZ neurons and glial cell proliferation [39], manifesting neuro-remodeling occurs. In order to clarify the tendency and regulating mechanism of neuro-remodeling in MVZ in sepsis, we sequentially detected the expression levels of GAP-43 mRNA, Olig-2 mRNA, VEGF mRNA, GFAP mRNA, and MMP-9 mRNA in each group. Results show that compared with the control group, the expressions of GAP- 43 mRNA, VEGF mRNA, GFAP mRNA and MMP-9 mRNA were significantly up-regulated, while the expression of Olig-2 mRNA was significantly down-regulated in the Sepsis Group; GTS-21 significantly reduced the expressions of GAP-43 mRNA, VEGF mRNA, GFAP mRNA and MMP-9 mRNA, increased the expression of Olig-2 mRNA in sepsis; MLA was completely the opposite.

Gap-43 is involved in the regeneration of neurons, extension of axons, and regulation of synaptic development and reconstruction to restore impaired nerve function [40-413], it dynamically adjusts its expression level according to the severity degree of nerve damage [43-44]. In this study, it can be seen that sepsis leads to significant up-regulation of Gap-43 mRNA expression, which is down-regulated by GTS-21 through inhibiting inflammation, and further promoted by MLA through exacerbating inflammation. MMP-9 is a kind of protease capable of refactoring extracellular matrix [45-46]. The content, activity and gene expression of MMP-9 arouse after a variety of physiological stimulation and pathological damage in the nervous tissue. By shaping the dendritic spines and altering the function of the excitatory synapses, MMP-9 participates in the dynamic adjustment of synaptic plasticity [47-48]. Our study confirmed that through regulating central inflammation levels by CAP interfering, the gene expression level of MMP - 9 changed significantly, suggesting the need for neuro-remodeling. GFAP can be seen as a biomarker of neuro-inflammation [49-50], its increasing expression represents astrocyte activation [51]. The hyperplasia of glial cells in MVZ induced by sepsis is apparently related to the up-regulation of GFAP mRNA expression. The down-regulation of GFAP mRNA expression by GTS-21 shows that the activating of CAP suppress neuro-inflammation and gliosis, on the contrary, the up-regulation of GFAP mRNA expression by MLA shows that the blocking of CAP intensify neuro-inflammation and gliosis in MVZ. Vascular

endothelial growth factor (VEGF) promotes angiogenesis by stimulating endothelial cell migration, proliferation and formation, which is related to nerve recovery and neuroprotection [52-534]. VEGF mRNA up-regulation manifests that neuro-inflammation induced by sepsis results in damage to blood vessels and nerves in MVZ and the urgent need to restore. The down-regulation of its expression by GTS-21 should contribute to its central anti-inflammation, while MLA is the opposite. Olig-2 is a specific marker of oligodendrocytes. The decreased expression of Olig-2 mRNA indicates destruction of myelin [55], when the myelin is compensatory to regenerate, the Olig-2 expression level increases [56]. This study signified that neuro-inflammation caused by sepsis led to destruction of myelin, GTS-21 prompted myelination through anti-inflammatory, whereas MLA further worsened the damage to myelin through aggravating the inflammation. It can be seen that the decrease of CAP output in sepsis from our past research may be related to the destruction of myelin sheath in MVZ.

The expression levels of GAP-43 mRNA, VEGF mRNA, GFAP mRNA and MMP-9 mRNA are consistently up-regulated in sepsis in MVZ, they also have almost the same reaction to the intervention of CAP, indicating that MVZ has an integral internal regulatory mechanism on inflammation, injury and restoration, which jointly promotes the recovery of neurovascular unit and its functions. These key genes' expression levels can reflect the severity of MVZ inflammation, the degree of damage and the tendency of repairing and remodeling. At the same time, the consistency of key gene expression also reflects the complex communication connection of CNS at the cellular and even molecular level. Even if the nerve tissue is damaged, there is still communication help to promote the recovery of damaged nerve function [57-58]. Here, an issue emerged, that is if these key genes is subjected to the inflammatory level, or directly controlled by CAP through a specific mechanism needed to be answered by further research.

Conclusion

This study preliminarily confirmed that sepsis causes functional neurons' apoptosis and inactivity in MVZ, key genes' expressions related to glial activation, nerve repairing and remodeling significantly increased, indicating the tendency of MVZ restoring and remodeling in sepsis; CAP can significantly affect these process. Agonists of α7nAChR can improve the remodeling of MVZ and facilitate MVZ to "normalize", which may be one of mechanism that GTS-21 can effectively curb the septic inflammatory storm, while antagonists of α7nAChR do the opposite. Therefore, anti-inflammation in MVZ may be a prospective way to treat the early stage of sepsis. Of course, this study still has some limitations, for example, whether the effect of α7nAChR intervention on Neuro-remodeling of MVZ is through some specific pathways, or is it secondary to the adjustment of the level of systemic or central inflammation, or include both. These questions need to be studied in the future research.

List Of Abbreviations

AI: the apoptosis index

ANOVA: Analysis of Variance

CAP: Cholinergic Anti-inflammatory Pathway

CHAT: Choline Acetyltransferase

CLP: Cecum Ligation and Puncture

CNS: Central Nervous System

DAPI: 4',6-diamidino-2-phenylindole

DVMN: Dorsal Vagus Motor Nucleus

GAP-43: Growth Associated Protein-43

GFAP: Glial Fibrillary Acidic Protein

HE: haematoxylin and eosin

MLA: methyllycaconitine

MMP-9: Matrix Metalloprotein 9

MVZ: Medullary Visceral Zone

NTS: the Nucleus Tractus Solitary ()

Olig-2: Oligodendrocyte Transcription Factor 2

TH: Tyrosine Hydroxylase

TLR4: Toll-like Receptor 4

TUNEL: TdT mediated dUTP Nick End Labeling

VEGF: Vascular Endothelial Growth Factor

VLRN: Ventrolateral Reticular Nucleus

Declarations

Ethics approval

All experimental procedures were carried out in compliance with the guidelines of the institutional animal care and use committee (IACUC) of the First Hospital of Guiyang (IACUC number 20190107). and performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85–23; revised 1985).

Consent for publication

Not applicable.

Availability of data and materials

Because this study is supported by the Guizhou Provincial Science and Technology Foundation, the data will not make known to public until the research project is checked and accepted. Therefore, we declare that all the datasets used and/or analysed during the current study are available from the author on reasonable request. The link email is mrbright789@sina.com.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

H. B. Li, ZH. G. Shu and Q. F. Pi contributed equally to this research. H. B. Li and ZH. G. Shu performed the experiments, analyzed the data and were responsible for the conception and design of the study and manuscript writing. H.B. Li, ZH. G. Shu and Q. F. Pi performed animal experiments. L.L. Guo and Q. F. Pi processed the experimental data. All authors read, revised, and approved the final manuscript.

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Figures

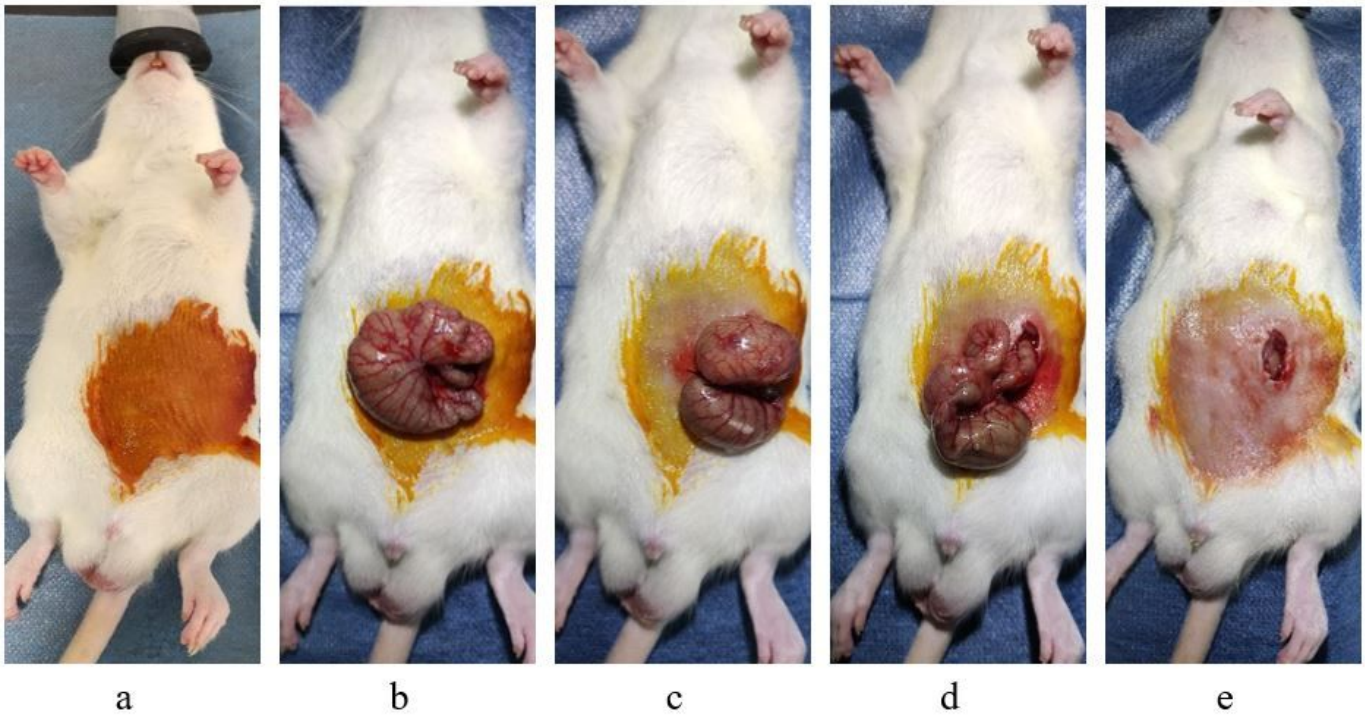

Figure 1

Fig 1 the main procedures of Cecum Ligation and Puncture for the sepsis models preparation Picture a: Anesthesia by inhalation and disinfection; Picture b: Paunch and isolated the cecum; Picture c:Ligation and puncture the cecum; Picture d: Squeeze the cecum; Picture e:Return the cecum into abdominal cavity

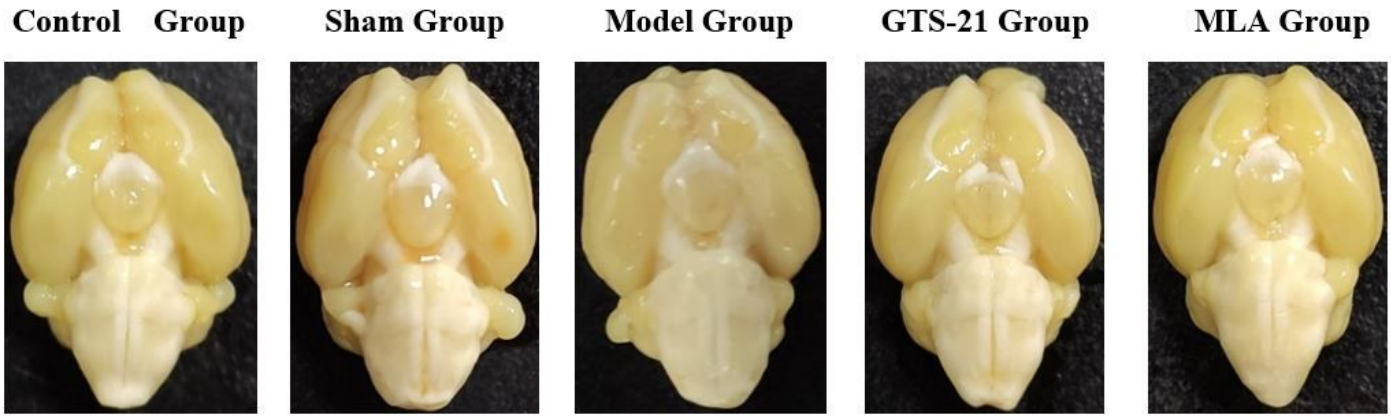

Figure 2

Brain specimens of five groups viewed from the ventral aspect

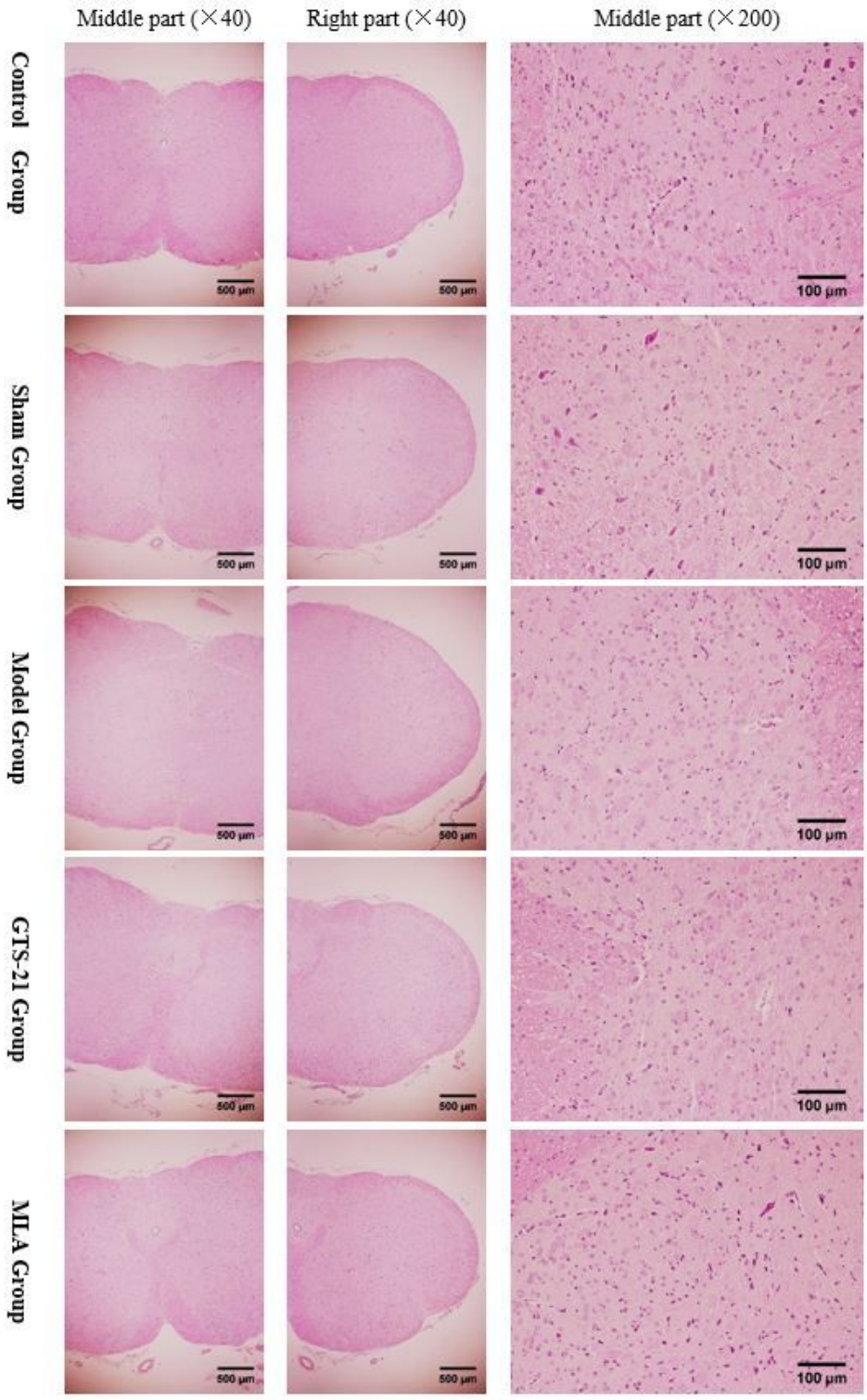

Figure 3

Medulla oblongata sections from five groups by H. E. staining The Nucleus Tractus Solitary (NTS), Dorsal Vagus Motor Nucleus (DVMN) can be seen from the middle part images, the Ventrolateral Reticular Nucleus (VLRN) can be seen from the right part images. All these parts show there were fewer neurons and more glial cells in Sepsis Groups when compared to the Control Group or Sham Group.

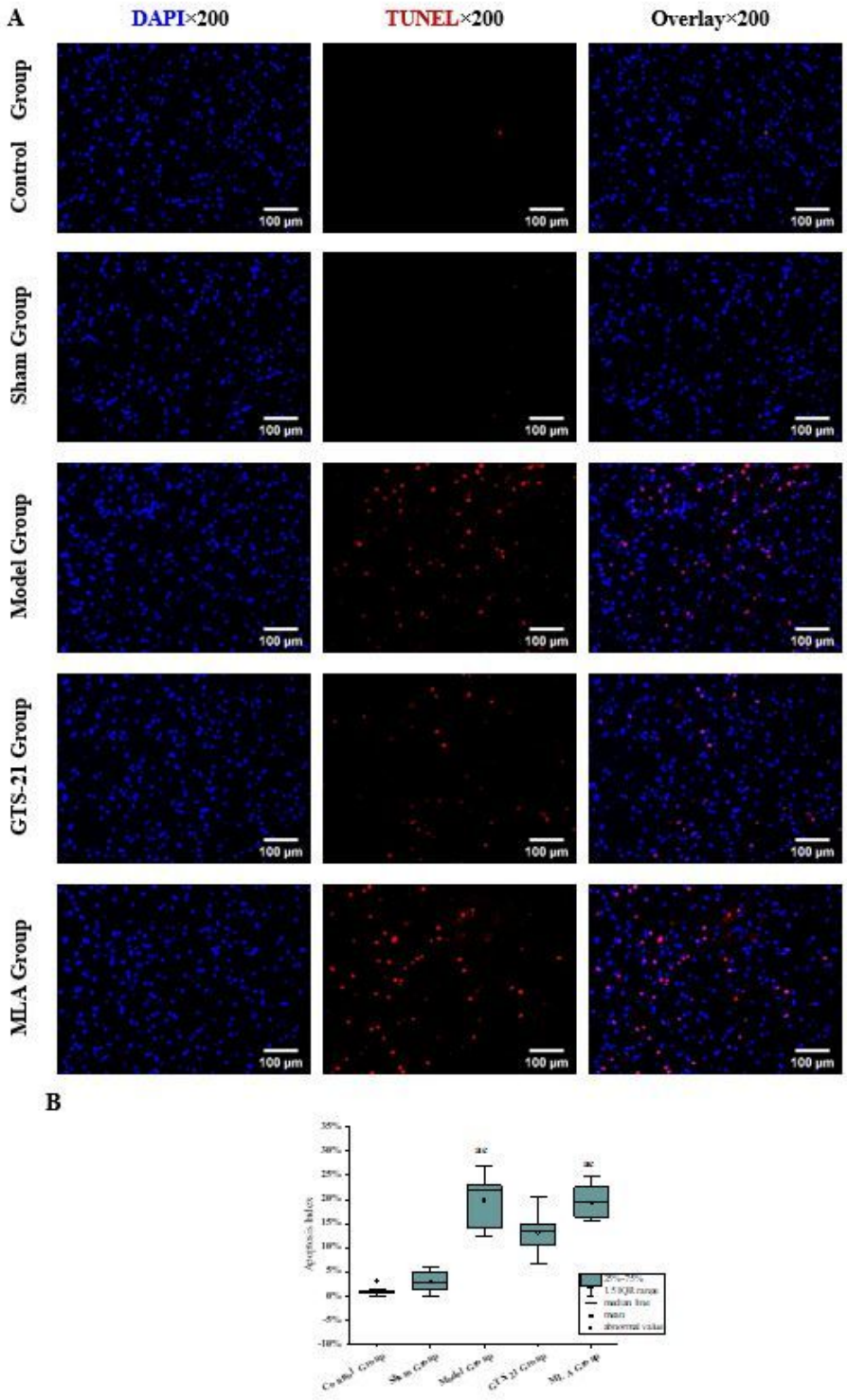

Figure 4

Cells apoptosis in MVZ among different groups Part A shows the representative TUNEL images of five groups. Part B, the histogram shows the comparation of Apoptosis Index among five groups. Results of TUNEL shows that much higher Apoptotic Index (AI) in Sepsis Group (including Model Group, GTS-21 Group and MLA Group) than that in Control Group, GTS-21 decreased AI in sepsis; MLA significantly

increased it, when compared to the GTS-21 Group, it also reached significant difference. The scale bars of bottom right in the images represent 100μm. a: P0.05 vs Control Group; c: P0.05 vs GTS-21 Group.

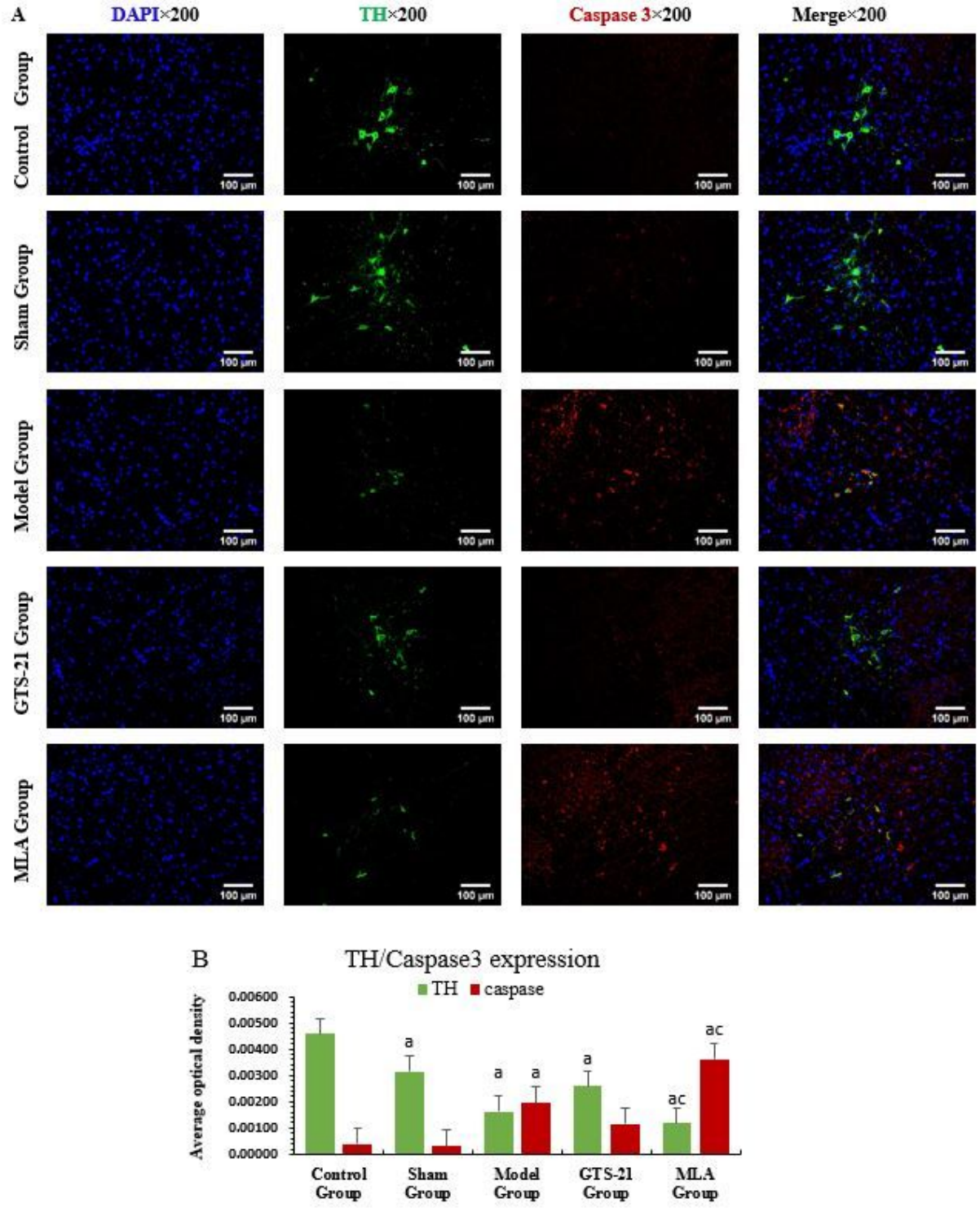

Figure 5

Catecholaminergic neurons apopsis in MVZ among different groups Part A The representative fluorescence double-label images and merging images of each group (200 times). DAPI: 4',6-diamidino-2-phenylindole, a fluorescent blue dye capable of binding strongly to DNA. TH: Tyrosine hydroxylase, used

for labeling catecholaminergic neurons (stained green with FITC); Caspase 3: indicator for apoptotic cell (stained red with Cy3). The scale bars of bottom right in the images represent 100μm. Part B The histogram shows the comparison of TH/Caspase 3 expression in each group. Compared with the Control Group, the TH expressions in the Sham Group and the Sepsis Group were significantly reduced; the expression of Caspase 3 in the Model Group was significantly increased, GTS-21 reversed this tendency, MLA aggravated the sepsis-induced apoptosis. It indicates that both stress and inflammation can reduce the activity of catecholaminergic neurons, and the intensity of inflammation is directly proportional to the apoptosis of catecholaminergic neurons. a: P□0.05 vs Control Group; c: P□0.05 vs GTS-21 Group.

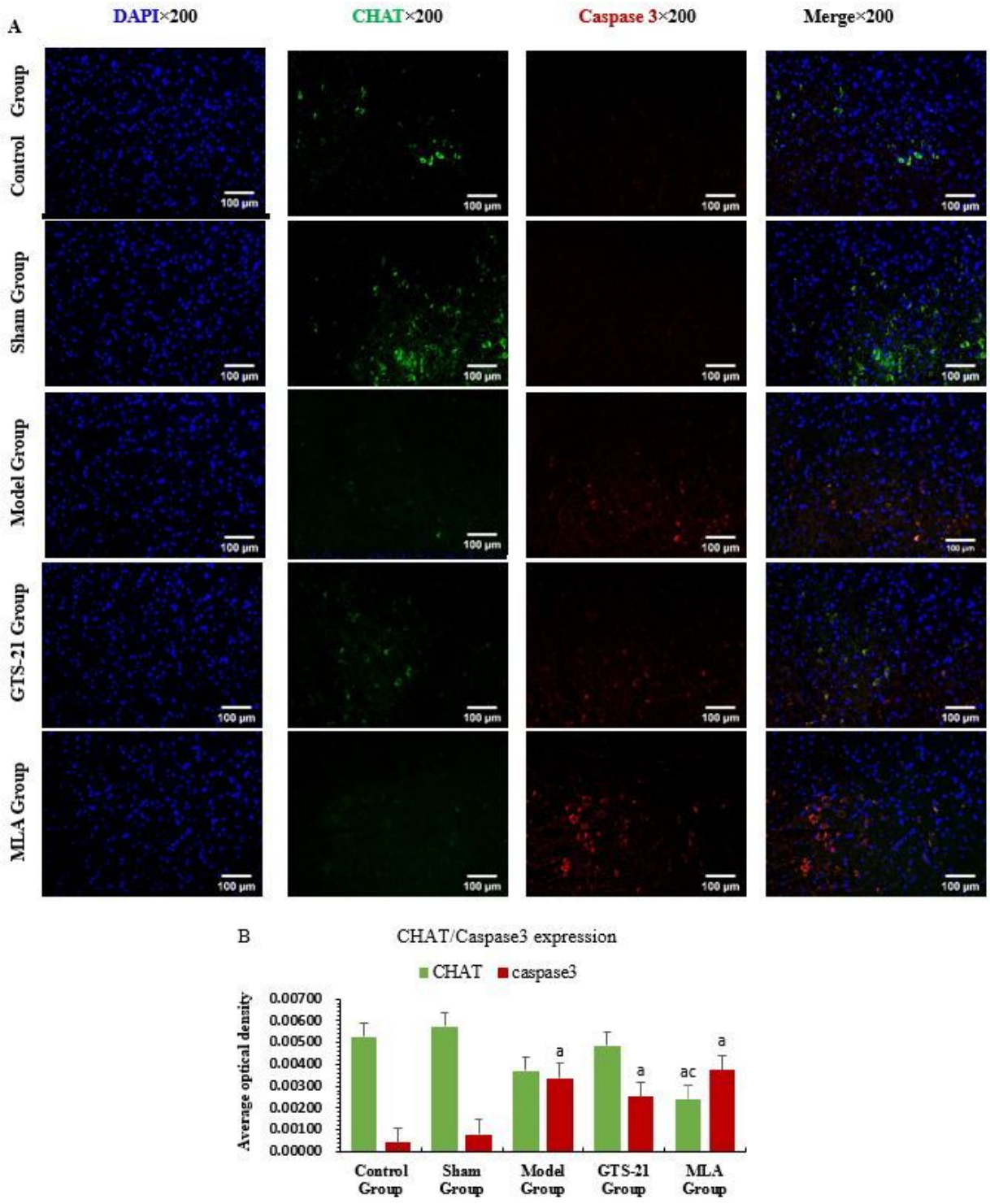

Figure 6

Cholinergic neurons apoptosis in MVZ among different groups Part A The representative fluorescence double-label images and merging images of each group (200 times). DAPI: 4',6-diamidino-2-phenylindole, a fluorescent blue dye capable of binding strongly to DNA. CHAT: choline acetyltransferase, used for labeling cholinergic neuron (stained green with FITC); Caspase 3: indicator for apoptotic cell (stained red with Cy3). The scale bars of bottom right in the images represent 100μm. Part B The histogram of Part B

shows the comparison of the expression of CHAT/Caspase 3 in each group. Compared with the Control Group, the expression of CHAT in the MLA Group was significantly reduced, GTS-21 had a tendency to increase it; the expression of Caspase 3 in the Sepsis Groups was significantly increased. It is suggested that inflammation can cause obvious apoptosis of cholinergic neurons, but has no obvious influence on the expression of CHAT, only worsening inflammation can lead to a significant decrease in the expression of CHAT. a: P<0.05 vs Control Group; c: P<0.05 vs GTS-21 Group.

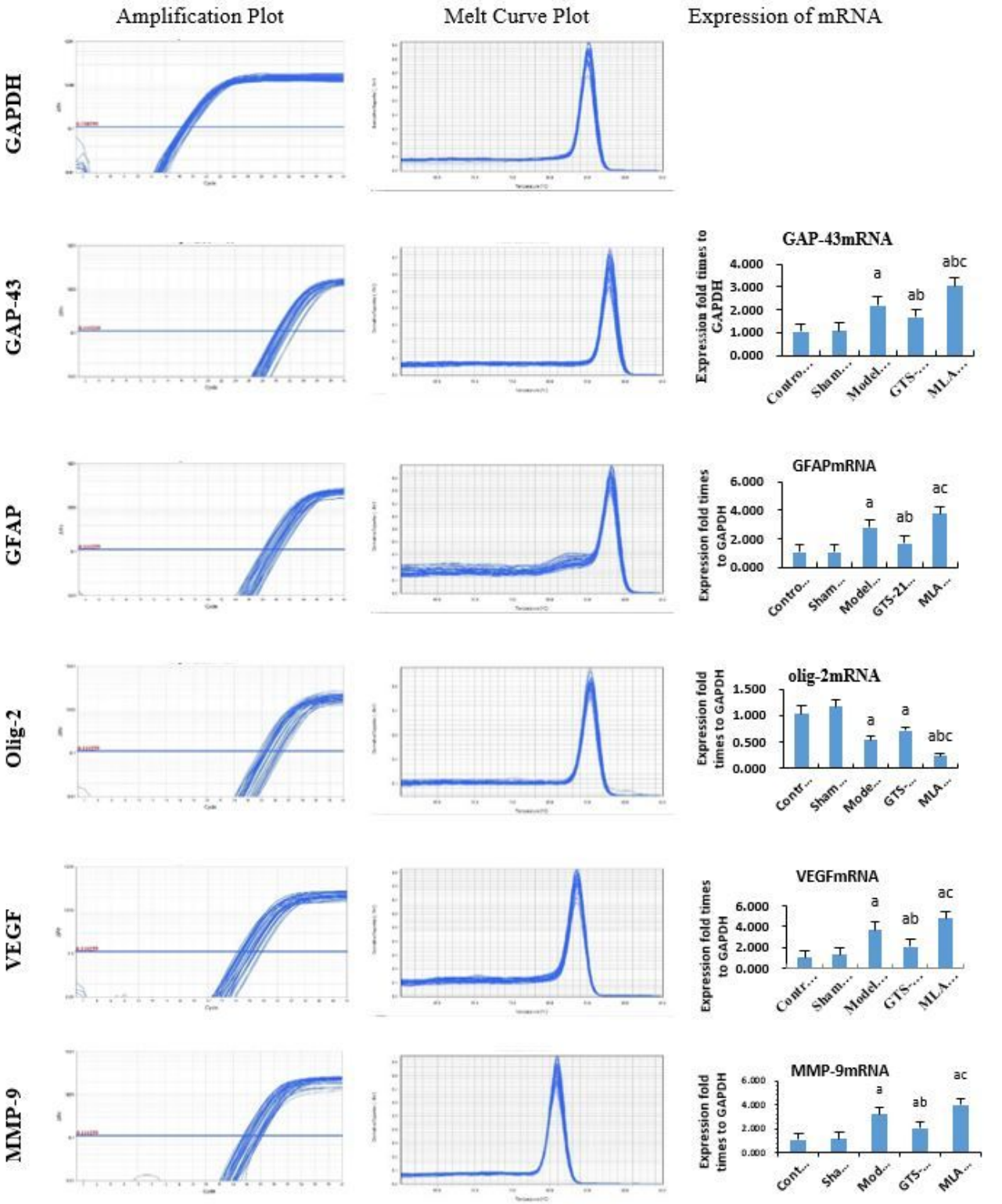

Figure 7

The expressions of key genes on Neuro-remodeling in MVZ in sepsis and interfered by CAP Using GAPDH as an internal reference, we can see that sepsis causes the simultaneous up-regulation of GAP-43 mRNA, GFAP mRNA, VEGF mRNA, and MMP-9 mRNA expression and down-regulation of Olig-2 mRNA. The expression of these genes are closely related to the intensity of systemic inflammation. a: P□0.05 vs Control Group; b: P□0.05 vs Model Group; c: P□0.05 vs GTS-21 Group.