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Differences in amyloid-β and tau/p-tau deposition in blood-injected mouse brains using micro-syringe to mimic traumatic brain microhemorrhages

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ABSTRACT

Background: Cerebral microbleeds (CMBs) due to traumatic brain injuries (TBI) have been shown to lead to cognitive decline and impairment. CMBs caused by TBI may be associated with pathophysiological mechanisms involving inflammation and the accumulation of amyloid-β (Aβ), tau, and phosphorylated tau (p-tau), contributing to cognitive abnormalities. However, their relationships remain unclear.
Objectives: To test our hypothesis that Aβ, tau, and p-tau are accumulated and regulated separately in mice with injuries imitating CMBs from TBI, we studied.
Methods: Seven-week-old C57BL/6 male mice were injected with 15 μL of heparinized autologous blood or saline by micro-syringe into the front lobe. Expression profiles and regulation of Aβ, tau, and p-tau were assessed immunohistochemically over time.
Results: On day 7 after blood injection, Iba-1$^+$ and S100B$^+$ cells in damaged cortex adjacent to the injection site were higher than saline injection group and non-injected sham. On days 3–14, Aβ deposition were gradually increased but normalized by day 28. In contrast, tau/p-tau deposition gradually increased during days 14–28 and dispersed along the corticomedullary junction adjacent to hem deposits, indicating different expression profiles from Aβ. Deposits of Aβ, but not tau/p-tau, were phagocytosed by CD163$^+$ macrophages increased by Gc-protein macrophage-activating factor during days 7–28, suggesting different mechanisms of deposition and regulation between Aβ and tau/p-tau.
Conclusion: Deposition and regulation differ between Aβ and tau/p-tau in mice with injuries mimicking CMBs from TBI. Further clarification of relationships between the pathologies of cognitive impairment and their neurodegenerative consequences is needed.

1. Introduction

Mild traumatic brain injury (TBI) has been shown to lead to the development of cerebral microbleeds (CMBs), which are thought to be associated with long-term cognitive decline and gait disturbance in patients (McInnes et al., 2017). Small, hypointense foci on T2*-weighted magnetic resonance imaging (MRI) are considered to represent a marker of CMBs in individuals with TBI (Haller et al., 2018) and CMB signals on MRI are attributed to iron-laden macrophages in the perivascular space along a network of injured vessels (Griffin et al., 2019).

TBI from concussion in athletes or falls in elderly individuals can result in chronic cognitive impairment (Witcher et al., 2021). Importantly, ageing is known to exacerbate microvascular fragility and promote the formation of CMBs (Toth et al., 2021). CMBs caused by TBI may be associated with axonal injury and/or a form of traumatic vascular injury and the prevalent form of dementia in the elderly has been projected to affect more than 40 million individuals worldwide (GBD, 2017 Disease and Injury Incidence and Prevalence Collaborators. Gobal., 2018). While an association between TBI-related CMBs and the pathology of cognitive impairment is suspected, details of the etiological and pathological mechanisms underlying these disorders remain unknown. The pathogenesis of CMBs caused by TBI therefore needs to be elucidated.

Amyloid-β (Aβ) is the major component of senile plaques, and Aβ

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protein dimers have been isolated directly from brains with impaired synaptic plasticity and memory and disturbed metabolism of Aβ peptides at the molecular level leads to the formation of toxic oligomers (Gallardo and Holtzman, 2019). Based on such findings, Aβ is considered a driving factor in cognitive impairment (Nakamura et al., 2018). Of the two major Aβ species in the human brain, $A\beta_{1\text{-}40}$ is soluble and several times more abundant than $A\beta_{42}$, which is mainly deposited in neurons and as the major component of amyloid plaques (Gu et al., 2016). Disturbed metabolism of Aβ peptides may be observed in patients who have experienced CMBs as a result of TBI. Other factors implicated in cognitive impairment include neuronal survival, synaptic function, glial cell activation, inflammatory responses, and impairment of the blood-brain barrier (De Strooper and Karran, 2016). Advances in imaging analyses, biomarkers and mouse models are now allowing the redefinition of this original hypothesis, as Aβ, tau and other pathophysiological mechanisms such as inflammation are likely to come together at a crossroads ultimately resulting in the development of cognitive dysfunction (Gallardo and Holtzman, 2019).

Tau is a microtubule-associated binding protein that provides cytoskeletal support allowing axonal transport. Under normal physiological conditions, this protein plays a role in stabilizing neuronal microtubules and promoting axonal outgrowth (Wang and Mandelkow, 2016). However, tau undergoes post-translational modifications and phosphorylated tau (p-tau)-containing neurofibrils represent a molecular hallmark of tauopathy (Acosta et al., 2022). Abnormal phosphorylation triggers the formation of microtubule-bound tau, the accumulation of neurofibrillary tangles as main tau tangles is known to correlate with cognition and clinical symptoms (DeVos et al., 2018, Gallardo and Holtzman, 2019). Tau aggregation and spreading can be induced by TBI (Edwards et al., 2020). However, non-local associations between increased Aβ accumulation rates and increased tau deposition are of great interest and support the idea that Aβ pathology might have remote effects in disease pathology, potentially spread via the intrinsic connectivity networks of the brain (Tosun et al., 2017).

Tau tangles, but not Aβ plaques, have recently been reported to correlate with cognition and clinical symptoms (DeVos et al., 2018). The prevailing view on the pathogenesis that initiates the deleterious cascade involving tau pathology and neurodegeneration has been thought to involve Aβ and tau acting independently and not actually interacting (Koss et al., 2016). New evidence, however, suggests synergistic effects of the two pathologies, based on negative results from anti-Aβ clinical trials. Studies of tau alone thus need to be reconsidered (Aisen et al., 2017). Pathological tau can appear in regions distant from the site of injury, but connected synaptically, implying the dissemination of tau aggregates. However, the association between tau deposition and antecedent Aβ accumulation remains unclear in early symptomatic individuals. Given this background, we hypothesized that Aβ, tau, and p-tau would accumulate separately over time and would be associated with CMB after TBI. We therefore injected mouse brains with blood by microsyringe to mimic CMBs from TBI, and examined the subsequent regulation of Aβ, tau, and p-tau.

2. Materials and methods

2.1. Study approval

This study was approved by the ethics committee of Tokushima University Graduate School of Biomedical Sciences (approval no. T2019–17) and was performed in compliance with the animal care guidelines of Tokushima University, Tokushima, Japan. All animal experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use and the ARRIVE guidelines of Laboratory Animals (Kilkenny et al., 2010). Before all procedures, animals were anesthetized by inhalation of 2–4% isoflurane. Six-week-old male C57BL/6 J mice (Charles River Laboratories Japan Inc, Yokohama, Japan) were housed and acclimated in a temperature- and humidity-controlled room (approximately 23 °C and 50% relative humidity) under a 12-h light cycle (08:00–20:00) with ad libitum access to food and water. All mice were anesthetized at 7 weeks old and subjected to surgical procedures.

2.2. Murine model of CMBs to mimic TBI

Paveliev et al. proposed a model for use as an advanced tool to study the cellular mechanisms underlying the pathophysiological consequences of acute trauma in mammalian brains in vivo. Referring to that model, we injected blood by stereotaxic prick with a micro-syringe into brain parenchyma of anesthetized 7-week-old male C57BL/6 J mice by microsyringe to imitate CMBs caused by TBI (Paveliev et al., 2014). Under inhalation anesthesia with isoflurane, a stereotactic apparatus was placed in the right brain and a small hole was bored into the skull 2.0 mm lateral to the bregma using a dental drill. Heparinized autologous blood was collected just before injection, then 15 μL of blood was injected by microsyringe prick into the right cerebral hemisphere 2 mm below the brain surface, based on a volume of 30–50 ml/60 kg body weight as a general hemorrhage volume.

To examine the effect of CMBs from TBI, expressions of Aβ, tau and p-tau, along with and proinflammatory Iba-1 as a marker of microglia and S100B as a marker of astrocytes, after microinjection of blood were assessed immunohistochemically and compared with findings from control mice that received microinjection of an identical volume of saline and non-injected sham mice. Assessments were made on days 3, 7, 14, and 28 after injection (n = 6 in each group at each time point).

In addition, to confirm whether these expressions due to the brain damage are prevented, we used the Gc-protein macrophage activating factor **(**GcMAF) as an activator of M2-type macrophages (Kurashiki et al., 2022, Nabeshima et al., 2020). GcMAF protein (known as vitamin D3-binding protein) is the precursor for the principal macrophage-activating factor (MAF) and was obtained from Prof. Yoshihiro Uto, Faculty of Bioscience and Bioindustry, Tokushima University and intraperitoneally injected every day at a dose of 40 ng/kg/day daily on days 7–14 after blood microinjection into brain.

2.3. Immunohistochemistry

Anesthetized mice were perfused with 0.9% NaCl solution followed by 4% buffered paraformaldehyde, and their brains were immersed overnight in 4% buffered paraformaldehyde and successively dehydrated in 10%, 20%, and 30% sucrose gradient. Brain tissue was embedded in optimal cutting temperature compound for immunofluorescent studies. Using a brain matrix (Bioresearch Center, Nagoya, Japan), each brain was cut into equal 2-mm-spaced slices and 5 serial coronal sections.

Coronal sections (thickness, 10 μm) were sliced with a cryotome (CM 1850; Leica, Heidelberger, Germany). After 30-min serum-free protein blockade (Dako, Carpinteria, CA, USA), sections were incubated overnight at 4 °C with primary antibodies against **rabbit** $A\beta_{42}$ (ab2539; abcam, Cambridge, UK), tau (ab32057; abcam) or p-tau (#44–7680; Thermo Fisher Scientific, Waltham, MA, USA), S100B (ab41548; abcam), Iba-1 (#019–19741; Wako, Osaka, Japan), and $CD16^+$ (ab203883; abcam) and antibodies against **rabbit** or mouse $CD163^+$ cells (ab182422 or ab17051; abcam) and $CD36^+$ cells (SC-7641; Santa Cruz Biotechnology, Dallas, Texas, USA). These antibodies were diluted with Canget signal immunostain (1:100 dilutions with the exception of 1:1000 for Iba1; Toyobo, Osaka, Japan). Sections not treated with primary antibody were used as negative controls. For visualization, we used 3,3′-Diaminobenzidine (DAB) buffer with a few drops of hydrogen peroxide solution and counterstained with hematoxylin. For immunofluorescent staining, fluorescein-conjugated secondary antibodies against mouse or rabbit (Alexa Fluor 488 for CD163 and 594 for Aβ; Molecular Probes, Waltham, MA, USA) in Canget signal immunostain (1:1000 dilution; Toyobo) were incubated for 1 h at room temperature.

Slides were mounted with fluorescence mounting medium (Dako, Osaka, Japan) and examined under a fluorescence microscope (BZ-X710; KEYENCE, Osaka, Japan). The positive area per 40,000 μm^2 was analyzed using the image analysis software provided with the microscope.

2.4. Statistical analysis

All data are presented as the mean ± standard deviation. Differences between two groups were examined using Student's *t*-test, and differences among 3 groups using the Tukey-Kramer test. Differences of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using JMP version 13.2 (SAS Institute, Cary, North Carolina, USA).

3. Results

3.1. Cerebral inflammatory response after blood microinjection into mouse brains

To imitate CMBs due to TBI, we modified the model described by Paveliev et al. (Paveliev et al., 2014). Each mouse brain was subjected to injection of 15 μL of autologous blood by microsyringe prick (Fig. 1A). Neither the size of the injured brain area nor body weight changed during days **7–28** after blood microinjection (Fig. 1B), and both were comparable to those in mice injected with saline as a volume control (data not shown), reflecting the effects of the microsyringe. However, expressions of Iba1$^+$ microglia and S100B$^+$ astrocytes according to immunohistochemical assessments increased significantly in brain parenchyma adjacent to the injection site by day 7 after blood microinjection, compared to the non-injected sham and saline groups (Fig. 1C), indicating a higher inflammatory response due to CMBs.

3.2. Deposition of Aβ associated with cerebral inflammatory response in the early phase after blood microinjection into brain parenchyma

Next, we examined the relationships between Aβ deposition and expressions of Iba-1 as a marker of microglia and S100B as a marker of astrocytes during days 3–28 after blood microinjection. Expressions of Iba-1 and S100B were observed in brain parenchyma adjacent to the injection site and gradually were elevated during days 3–14, then they decreased and normalized by day 28 (Fig. 2A, B). In parallel with Iba-1 and S100B deposition, Aβ observed in the same area was gradually elevated and peaked on day 14, then decreased and scarce on day 28 (Fig. 2C), suggesting that deposition of Aβ was associated with inflammatory response after blood microinjection.

3.3. Deposition of tau and p-tau in the late phase after blood microinjection into brain parenchyma

The deposition profile for tau after blood microinjection was similar to that for p-tau (Fig. 3A). During the 28-day observation period, expressions of tau and p-tau were gradually elevated on days 14–28 after blood microinjection (Fig. 3A), in association with the presence of hem at the corticomedullary junction. The timing and localization of tau and p-tau expressions seemed to differ from those of Aβ.

To further clarify whether expressions of tau and p-tau were associated with inflammation, expression profiles for CD16$^+$ pro-inflammatory M1-type and CD163$^+$ anti-inflammatory M2-type macrophages were assessed. After blood microinjection, expression of

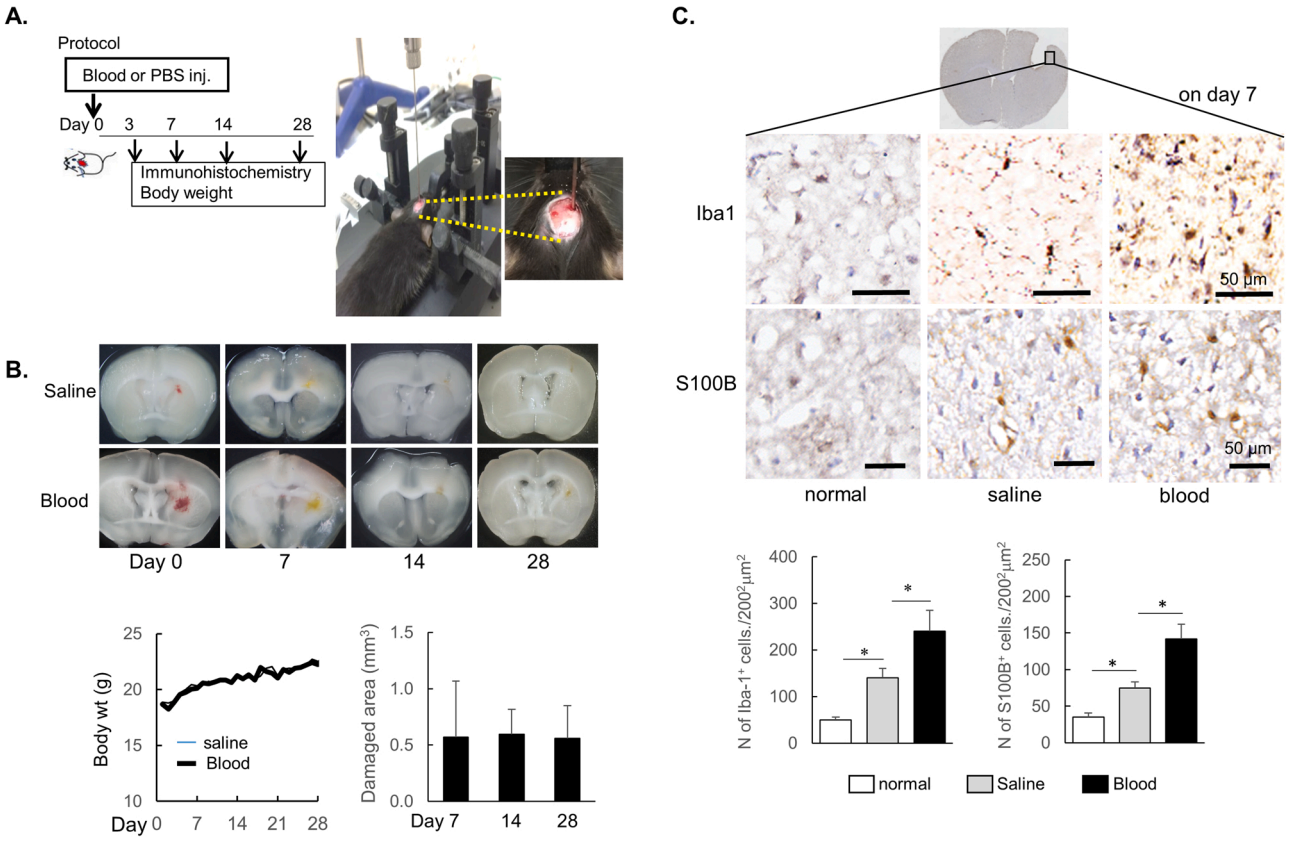

Fig. 1. Effects of blood microinjection into the brains of mice to mimic cerebral microhemorrhages. A)Effects of blood microinjection into the brains of mice to mimic cerebral microhemorrhages. A) Experimental protocol. B) Representative photographs before and on day 7–28 after saline or blood injection, changes of body weight and damaged area on day 7–28. C) Representative immunohistochemistry of Iba-1 and S100B on day 7 after blood microinjection were compared to saline and sham groups. Assessments were performed on day 7.Each column indicates the mean ± SD (each n = 6).*$p < 0.05$ by Student's *t*-test.

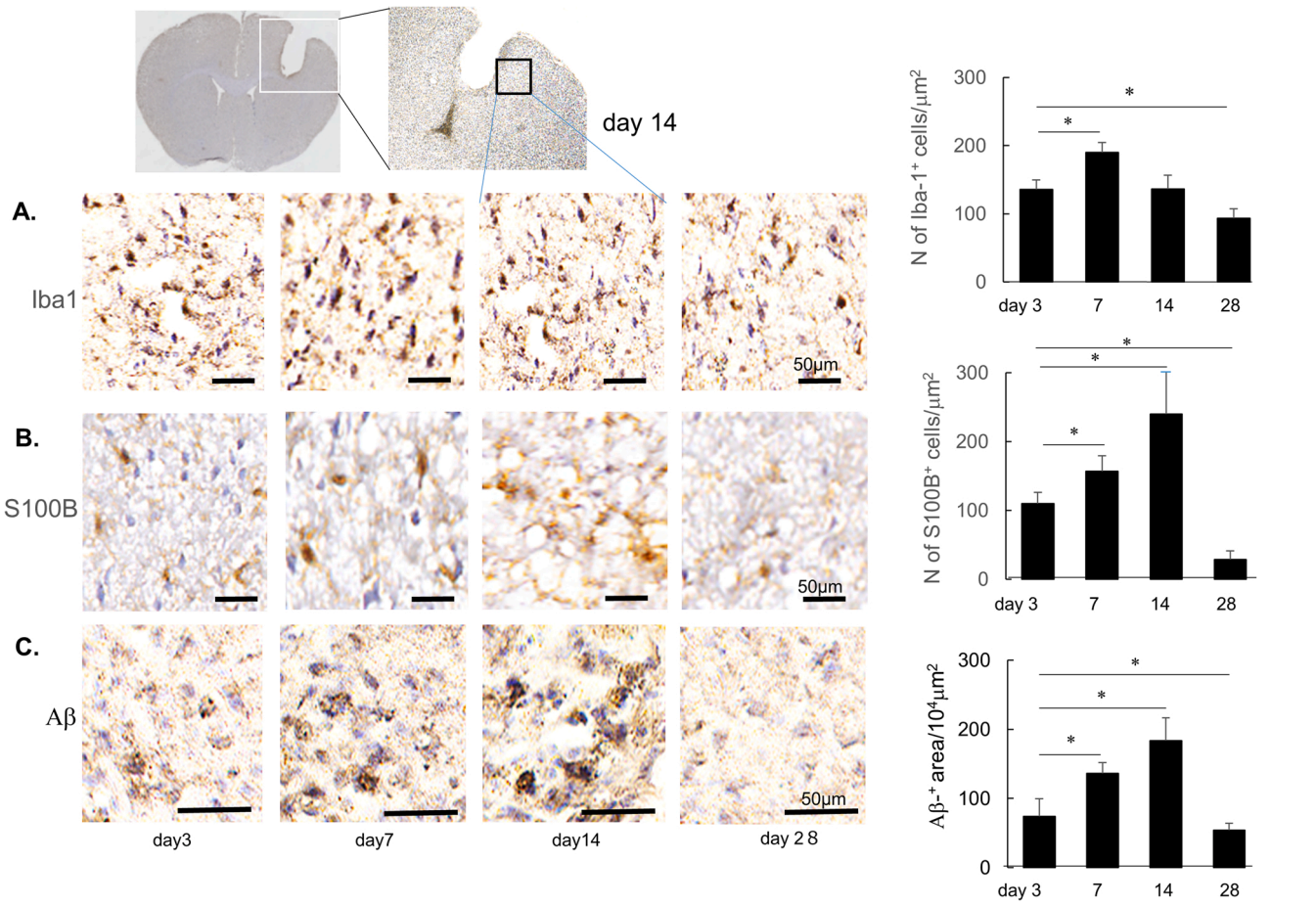

Fig. 2. Expression of Aβ, Iba-1 and S100B in mice brains subjected to blood microinjection. Time course for expressions of Iba-1 (A), S-100B (B) and Aβ (C) on days 3, 7, 14 and 28 after blood microinjection. Each column indicates mean ± SD (n = 6 each group). *p < 0.05, Student's *t*-test vs. each expression on day 3.

$CD16^+$ cells was elevated on day 3, peaked on day 7, and decreased on day 28, indicating higher expression in the acute phase (Fig. 3B), similar to Aβ deposition. In contrast, expression of $CD163^+$ macrophages gradually increased until day 14 and remained at a high level on day 28, indicating a similar expression profile to tau and p-tau in different deposition areas.

3.4. Different manners of deposition for Aβ, tau and p-tau after blood microinjection into brain tissue

We further assessed the co-localization between Aβ, tau, p-tau and $CD163^+$ macrophages, because the expression profiles for tau and p-tau, but not Aβ, were similar to that for $CD163^+$ macrophages (Figs. 2C, 3A, B). Interestingly, expression of Aβ colocalized with $CD163^+$ and $CD36^+$ phagocytic macrophages in the damaged area adjacent to the site of blood microinjection on day 14 (Fig. 4A), suggesting the phagocytosis of Aβ by $CD163^+$ macrophages. Despite tau and p-tau being associated with the presence of hem at the corticomedullary junction on days 14–28 after blood microinjection, neither tau nor p-tau deposition co-localized with $CD163^+$ macrophages (Fig. 4B). Taken together, these findings indicate different timings for deposition and separate expression profiles for Aβ and tau/p-tau.

3.5. Regulation of inflammatory changes by Gc-MAF

Finally, to clarify whether phagocytosis by $CD163^+$ macrophages was associated with reductions in Aβ, we used GcMAF as an activator of M2-type macrophages to indicate anti-inflammatory effects (Nabeshima et al., 2020) (Fig. 5A). Exposure to GcMAF within 7 days after blood microinjection did not exert anti-inflammatory effects. However, treatment with GcMAF during days 7–28 significantly increased $CD163^+$ M2-type macrophages (Fig. 5B), as seen in another study (Kurashiki et al., 2022), resulting in decreased deposition of Aβ (Fig. 5C) and reduced numbers of Iba-1^+ cells (Fig. 5D) without affecting expressions of tau or p-tau (data not shown). This suggests that the deposition of Aβ, unlike that of tau and p-tau, may be at least partly associated with inflammatory response after microbleeds.

4. Discussion

This study provides the first description of the expression profiles for Aβ and tau/p-tau in the mouse brain after injection of autologous blood by micro-syringe prick to imitate CMBs caused by TBI. Importantly, deposition of Aβ was observed adjacent to the damaged area, while deposition of tau and p-tau was observed in the late phase in areas adjacent to hem deposits at the corticomedullary junction, distant from Aβ deposits. Interestingly, deposition of Aβ was associated with inflammatory changes in the early phase, while accumulation of tau and p-tau gradually increased without affecting inflammatory changes. In addition, deposition of Aβ, but not tau or p-tau, co-localized with $CD163^+$ macrophages and was abrogated under augmented expression of $CD163^+$ macrophages induced by GcMAF. These findings indicate that the timing and profiles of deposition differed between Aβ and tau/p-tau. Thus, the present mouse model may be useful for further studies to clarify the pathogenesis of Aβ and tau/p-tau depositions and their regulation.

The TBI model mice described by Paveliev et al. may be associated with axonal injury and/or a form of traumatic vascular injury (GBD,

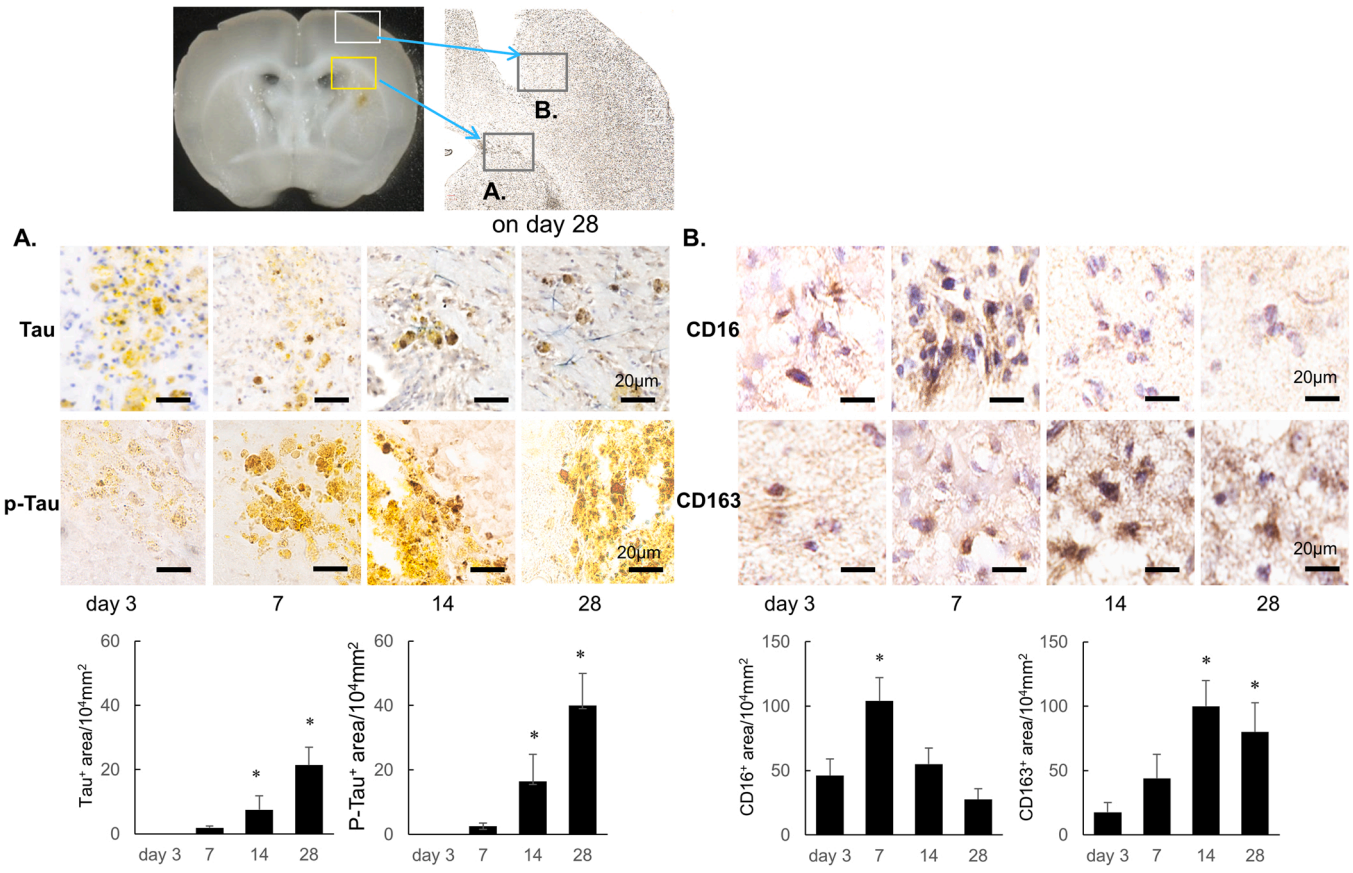

Fig. 3. Expressions of tau, phosphorylated tau (p-tau) and $CD16^+$ and $CD163^+$ macrophages on days 3, 7, 14 and 28 after blood microinjection. A) Time course of expressions of tau and p-tau observed at the corticomedullary junction adjacent to hem deposits. B) Time course of expressions of $CD16^+$ M1-type and $CD163^+$ M2-type macrophages after blood microinjection. Data indicate the mean ± SD (n = 6 each group). *$p < 0.05$ vs. each expression on day 3 (B) or day 7 (A) by Student's *t*-test.

2017, Paveliev et al., 2014). Microbleed signals on MRI are also attributable to iron-laden macrophages in the perivascular space along a network of injured vessels (Griffin et al., 2019). The model of Paveliev et al. represents a clinically relevant model close to TBI (Paveliev et al., 2014). Based on that study, we injected blood by microsyringe into mouse brains to imitate CMB caused by TBI. We identified greater inflammatory changes in treated mice, compared to sham-treated and saline control mice, suggesting that CMBs from TBI may have caused detrimental effects separate from those resulting from TBI alone. The present findings in our new mouse model are supported by findings from Irimia et al. that TBI frequently results in CMBs in clinical settings (Irimia et al., 2018).

TBI leads to the mechanical distortion of cerebral vessels, which may directly injure vascular walls and contribute to the formation of microhemorrhages around cerebral vessels, leading to oxidative stress and inflammation (Rubenstein et al., 2017). Those findings may resemble our observation that inflammatory changes resulted from the microinjection of blood into brain parenchyma. Although the results for cerebral microhemorrhages may differ slightly from those for traumatic brain damage, the deposition of Aβ, tau, and p-tau induced by microbleeds in our mice seems to at least resemble the deposition profiles for these proteins after CMBs following TBI (Witcher et al., 2021). Cerebral hemosiderin deposits induce progressive neurodegeneration, leading to the increase in neurofilament light protein, glial fibrillary acidic protein, total tau protein and p-tau, indicating that the neurodegeneration may be secondary to iron toxicity and oxidative stress, as well as Wojtunik-Kulesza K, et al. (Wojtunik-Kulesza et al., 2019).

Consistent with our findings, Cai et al. and Dourado et al. reported that a vicious cycle of inflammation forms between Aβ accumulation, activation of microglia, and microglial inflammatory mediators, enhancing Aβ deposition and neuroinflammation (Cai et al., 2014; Dourado et al., 2020). Cerebral amyloid angiopathy associated with inflammation is an important and increasingly recognized clinical condition that predominantly affects the older population and presents most commonly as cognitive decline, seizures, and headaches (Corovic et al., 2018). In our study, deposition of Aβ close to the damaged area was associated with augmented expression of $Iba1^+$ microglia and $S100B^+$ astrocytes, reflecting inflammatory responses in the acute to subacute phase after blood microinjection. Deposition of Aβ was associated with the expression profile for pro-inflammatory $CD16^+$ M1-type, but not anti-inflammatory $CD163^+$ M2-type, macrophages in our study. Famenini et al. reported that the expression profiles for microglia and astrocytes were associated with an increase in the numbers of M1-type pro-inflammatory macrophages, but not M2-type anti-inflammatory macrophages (Famenini et al., 2017). Since inflammatory changes were accentuated in mice subjected to blood microinjection compared to those that received saline injection as volume controls, this exacerbation seems likely to be attributable to some component of the blood.

On the other hand, expressions of tau and p-tau was gradually elevated in the chronic phase and appeared adjacent to hem at the corticomedullary junction. Expression patterns for tau and p-tau were similar to, but not co-localized with, those for M2-type macrophages. Importantly, areas of tau and p-tau expression were close to hem deposits and separate from the area injured directly by microinjection. Tau and p-tau deposition thus occurred separate from Aβ deposition, and the timings of deposition also differed, suggesting independent mechanisms from the deposition of Aβ.

Rubenstein et al. compared plasma levels of tau and p-tau in patients

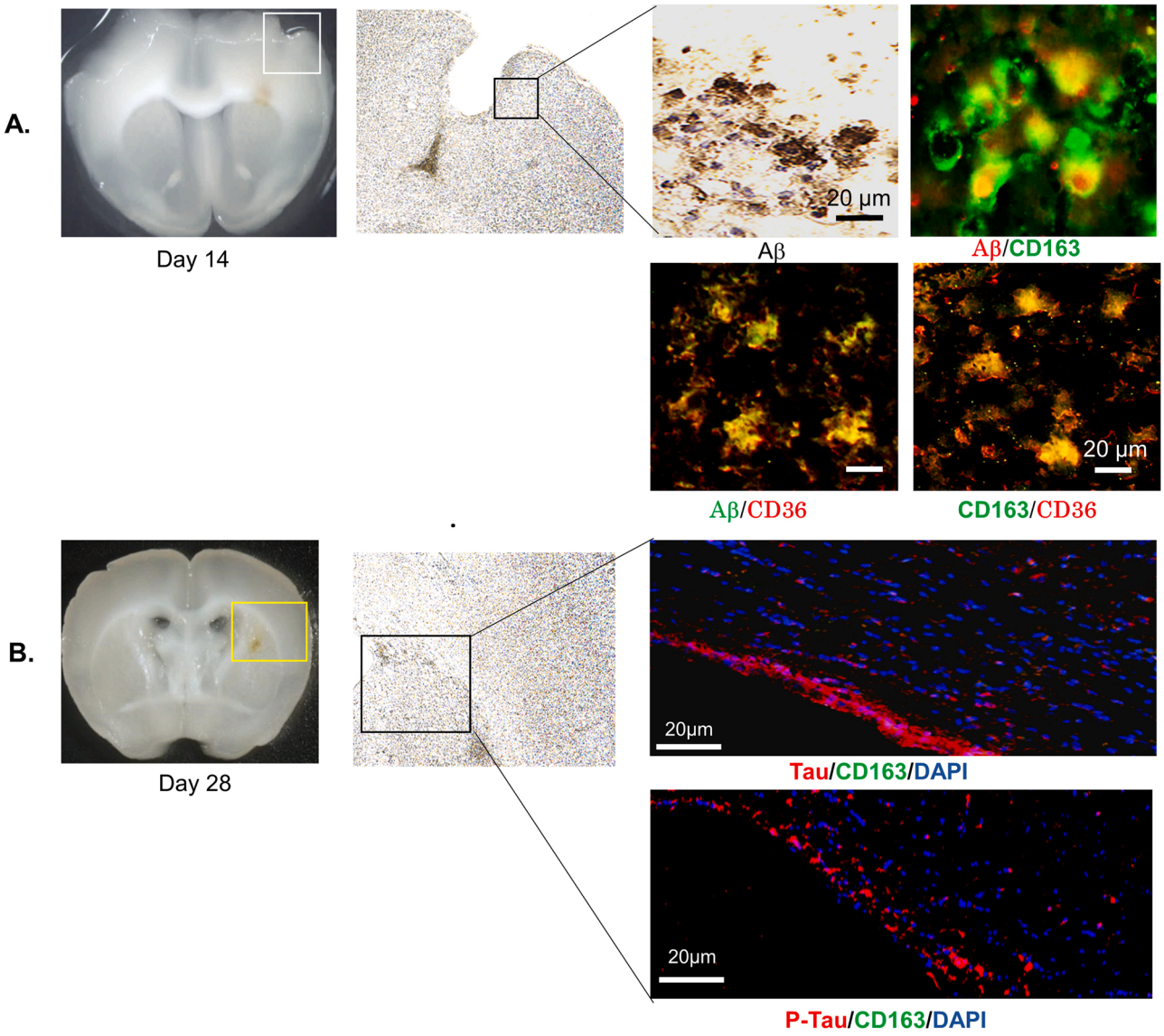

Fig. 4. Co-localization of CD163^{+} M2-type macrophage with Aβ, but not tau or p-tau, in mouse brains after blood microinjection. A) Aβ colocalizes with CD163^{+} M2-type macrophages in the damaged area on day 14 after blood microinjection. Both Aβ and CD163^{+} M2-type macrophages are CD36-positive. B) Tau and p-tau deposition adjacent to hem at the corticomedullary junction.

with acute or chronic brain injury and healthy controls, revealing higher levels in patients with acute TBI compared to healthy subjects and markedly higher levels in patients with chronic TBI (Rubenstein et al., 2017). Those findings support our results that after blood microinjection, tau and p-tau were dispersed, then became elevated and deposited in the late phase and accumulated at the corticomedullary junction close to hem deposits separated from Aβ deposition. Edwards et al. reported that compared with sham mice, brain-injured tau transgenic mice display an accelerated tau pathology in different brain regions (Edwards et al., 2020). Further, the appearance of pathological tau occurs in regions distant from the site of injury but connected synaptically, suggesting dissemination of tau aggregates. These findings are similar to our results and supportive of the present findings.

In addition, Tosun et al. revealed non-local associations linking increased Aβ accumulation rates and increased tau deposition (Tosun et al., 2017). Busche et al. reported that Aβ-tau interactions might have remote effects in disease pathology via the intrinsic connectivity networks of the brain (Busche and Hyman, 2020). The prevailing view on the pathogenesis that initiates a deleterious cascade involving tau pathology and neurodegeneration has been that tau tangles correlate with cognition and clinical symptoms (DeVos et al., 2018). Although Aβ stabilizes tau aggregates, lowering total tau levels may be an effective strategy for treating tauopathies and neuronal loss even in the presence of Aβ deposition. To clarify these interactions, further studies of the mechanisms regulating tau and p-tau deposition are needed.

Macrophages are associated with brain injury, hemorrhage, and several aspects of vascular development and remodeling (Liu et al., 2016). Adenosine triphosphate released from injured parenchyma mediates a rapid response in microglia and thus plays a central role in inflammation and phagocytosis in association with brain injury, hemorrhage, and several aspects of vascular development and remodeling. In the present study, the injection of GcMAF in the subacute phase resulted in the activation of M2-type macrophages and reduced inflammatory responses, decreasing Aβ deposition, suggesting the phagocytotic effects of activated M2-type macrophages (Olivera-Perez et al., 2017). However, treatment with GcMAF within 7 days after microbleed displayed no clear benefits (data not shown). Another recent study supports our results that the timing of M2-type macrophage activation is crucial to elicit beneficial effects from GcMAF (Kurashiki et al., 2022).

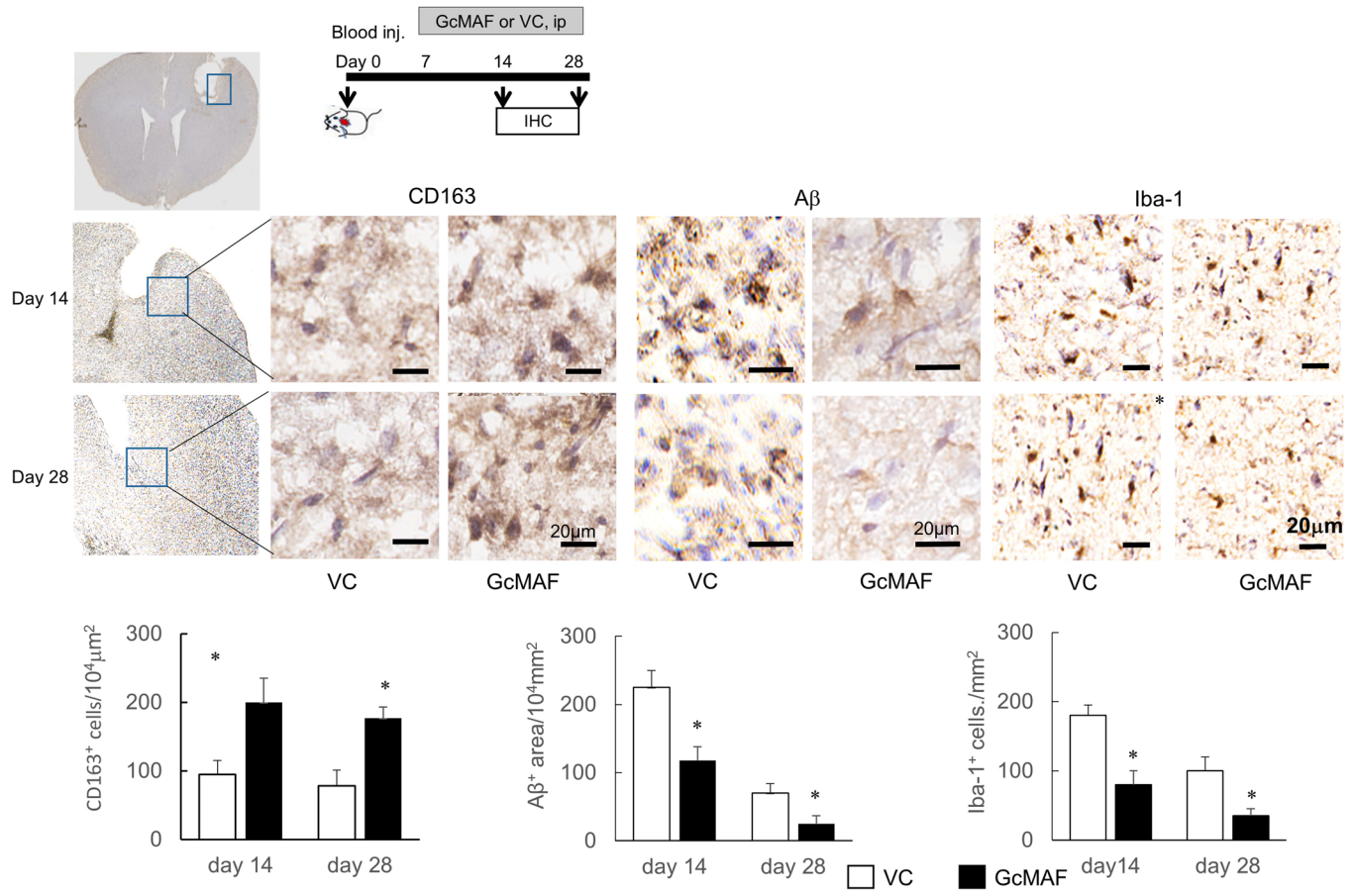

Fig. 5. Effects of GcMAF on deposition of Aβ and expressions of CD163$^+$ macrophages and Iba-1. Experimental protocol, increased expression of CD163$^+$ macrophages, and decreased expressions of Aβ and Iba-1 with GcMAF treatment during days 7–28. Data are given as mean ± SD (n = 6 each group). *p < 0.05 vs. saline (VC), Student's *t*-test.

5. Study limitations

To imitate CMBs that occur in humans with TBI, we injected blood by microsyringe into mouse brains. Although our study represents a new experimental method using mice to mimic CMBs from TBI, some study limitations must be considered. First, to clarify the localization of deposited Aβ and tau/p-tau in mouse brains mimicking microbleeds after TBI in adult male mice, we immunohistochemically assessed expression profiles as in another study by Ramos-Cejudo et al. (Ramos-Cejudo et al., 2018). They reported that TBI-induced neurovascular injuries accelerate Aβ production and accumulation of p-tau and amyloid precursor protein (APP) as observed at 6, 24, and 72 h post-TBI, indicating that TBI can initiate cerebrovascular pathology and is causally involved in the development of neurodegeneration. The expression patterns for these dementia-related proteins in aged humans may therefore differ from those seen in our animal study. Second, we assessed the expressions of dementia-related proteins Aβ and tau/p-tau, while the relationships between these proteins, neurological deficits and cognitive disorders remain unclear. On the other hand, Shishido et al. reported that significant Aβ plaques and APP accumulation were observed in mouse hippocampus 7 days after induction of TBI, whereas Aβ deposition was no longer apparent 28 days after TBI (Shishido et al., 2019). Such findings support the present results and suggest that the process of Aβ accumulation in TBI may different from that caused by aging. Regarding the relationship between Aβ and tau pathologies after trauma in TBI models, Tran et al. also reported that systemic treatment with compound E to inhibit γ-secretase activity, a proteolytic process required for Aβ production, successfully blocked posttraumatic Aβ accumulation in injured mice, but tau pathology was unaffected (Tran et al., 2011). Those findings support differences in regulation between Aβ and tau/p-tau in the present study. Habiba et al. reported that in aged dogs with cognitive dysfunction, p-tau deposits displayed a widespread pattern that closely resembled the typical human neuropathology but this pattern did not co-localize with Aβ plaques (Habiba et al., 2021). These findings are also consistent with our results. Although slow accumulation of Aβ and tau/p-tau may be associated with aging, the deposition pattern might differ from those in the case of CMBs after TBI (Jagust and Landau, 2021). TBI-related CMBs may be associated with axonal injury and/or a form of traumatic vascular injury (GBD, 2017 Disease and Injury Incidence and Prevalence Collaborators. Gobal., 2018) and age-related increase in oxidative stress is a likely factor enhancing the formation of CMBs (Toth et al., 2021) CMB signals on MRI are attributed to iron-laden macrophages in the perivascular space along a network of injured vessels as reported by Griffin et al. (Griffin et al., 2019). However, the direct relationships between amyloid deposition and tau accumulation and the different phenomena including bleeding, inflammation, oxidative stress, necrosis, apoptosis remain unclear.

Taken together, our findings showed expression of Aβ around the damaged area in the early phase and deposition of tau and p-tau in a more widespread pattern in the late phase. CMBs by TBI may independently affect Aβ and tau abnormalities.

Elucidation of these processes will expand our understanding of the pathogenesis leading to early impairments in memory and cognition. We will then need to devise therapeutic strategies to ameliorate the cognitive disorders accompanying CMBs caused by TBI. In this setting, we need to clarify whether deposition of Aβ and/or tau/p-tau is associated with cognitive deficiencies, and what therapeutic strategies are

beneficial to address these depositions. Since several pathological conditions are considered to be involved in the accumulation of dementia-related proteins, we are now assessing cognitive disorders using the Novel Object Recognition Test with or without pharmaceutical therapies in the present mouse model (Lueptow, 2017). Further investigations are required to clarify the essential elements related to cognitive dysfunction after CMBs by TBI and neurological disorders.

6. Conclusion

We offer a first demonstration of brain damage in a newly established model mice mimicking CMBs caused by TBI. In model mice, Aβ, tau, and p-tau accumulated in different brain areas at different time points. Only deposition of Aβ was associated with early inflammatory changes close to the damaged area. Deposition of Aβ, but not tau/p-tau, was abrogated by treatment with GcMAF, a specific activator of M2-type macrophages. Further studies are needed to elucidate the mechanisms regulating depositions of tau and p-tau as well as Aβ that may play roles in the development of human cognitive impairment.

Ethics approval and consent to participate

All animal procedures in this study were approved by the Animal Experimental Committee of Tokushima University, and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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CRediT authorship contribution statement

Hiroshi Kagusa: Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Izumi Yamaguchi:** Formal analysis. **Kenji Shono:** Methodology, Investigation. **Yoshifumi Mizobuchi:** Conceptualization, Writing – review & editing. **Eiji Shikata:** Formal analysis. **Taku Matsuda:** Formal analysis. **Takeshi Miyamoto:** Writing – review & editing. **Keiko T. Kitazato:** Conceptualization, Methodology, Data curation, Writing – original draft, Visualization, Supervision. **Yoshihiro Uto:** resources. **Yasuhisa Kanematsu:** Supervision, Project administration. **Yasushi Takagi:** Supervision, Project administration.

Data availability

Data will be made available on request.

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Disclosures

None.

Ethics statement

All animal procedures in this study were approved by the Animal Experimental Committee of Shihezi University, and proceeded in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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