Modeling of Inflammation of Cerebral Vasculature Caused by MA & HIV

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Methamphetamine (MA) is medically used for the treatment of narcolepsy and mood disorders. Prominent responses induced by MA include arousal, euphoria, accelerated heart rate, elevated blood pressure, increased respiration rate, and increased body temperature. MA has addictive properties, and high doses can cause complications such as anxiety, hypertension, appetite suppression, aggressive thoughts, and throbbing headaches. MA can be inhaled, intravenously injected, or smoked. It is the second most popular drug world-wide.

Intravenously injected, or smoked. It is the second most popular such as anxiety associated with neurotoxic effect of methamphetamine exposure, especially at high doses, increases the risk of cardiovascular and cerebral vascular diseases such as ischemic stroke, subarachnoid hemorrhage, intracerebral hemorrhage, and vasculitis.1 There is also evidence supporting neuroinflammatory impairment and brain abnormalities, including inflammation associated with neurotoxic effect of methamphetamine consumption. A previous study suggested that MA may also contribute to accelerated atherosclerosis which predisposes users to ischemic stroke.2

Another possible mechanism of MA-related vascular pathology is that MA damages endothelial cells and disrupts the blood-brain barrier (BBB). Rats that received acute exposure to MA showed morphological abnormalities of cerebral endothelial cells. Bleb formation and perivascular edema was observed on capillary endothelium. The development of MA on the functions of the BBB was proved in vitro. The suggested mechanism involved induction of oxidative stress in brain endothelial cells.

In light of this, we first hypothesized that acute exposure to MA causes damage or activation of endothelial cells, which leads to adherence of leukocytes to the endothelium. Tat protein expressed in the brain as a transgene causes inflammation of the brain including edema.3 We further hypothesized that Tat protein damages endothelial cells and recruits neutrophils to the site of damaged endothelium. Psychological stress or adrenaline infusion causes a significant increase in peripheral circulating leukocytes and the number of activated circulating leukocytes.4 Since MA is sympathomimetic, acute exposure of MA induces a sympathetic-like response. We lastly hypothesized that acute exposure to MA will lead to an increase in numbers of leukocytes in cerebral vasculature.

Based on previous studies that took advantage of intravital microscopy3,6,7, we established a model using 2-photon imaging to observe and measure the flow of leukocytes and neutrophils in cerebral vasculature. To validate our model, we used systemic exposure to LPS to induce cerebral inflammation.8 We then examined leukocyte movement and recruitment in mice that either did or did not express HIV-1 Tat within the CNS, using either GFP-expressing CD18+ cells or Gr-1 labeled neutrophils. either did or did not express HIV-1 Tat within the CNS, using either GFP-expressing CD18+ cells or Gr-1 labeled neutrophils.

Materials and Methods

Animals and dosing

All animals involved were housed with a 12 hour light/dark cycle according to UCAR guidelines. Adult (8-10 week old) C57BL, or Tat-tg mice were used.

Mice were anesthetized with an intrapertioneal injection of urethane (1.25 g/kg) and xylazine (~2.4 mg/kg). Anesthesia was maintained by injection of a 5-10% of initial dose of urethane (~0.25g/kg) and xylazine (~0.5mg/kg) mixture every 45 min, if necessary. A thin skull cranial window was prepared. Texas Red dye (Dextran, Texas Red, 70,000mw, Invitrogen) diluted in 0.9% saline in the final volume of 200μL was injected intravenously into the femoral vein (17mg/kg). For C57BL mice, 17mg/kg of Texas Red was mixed with 1.7mL/kg of fluorescent-labeled anti-Gt-1 antibody (R302, Alexa Fluor 488) and diluted in 0.9% saline for a final volume of 200μL. Needle (30G 2) catheters connected to syringes with urethane/xylazine solution and MA solution were implanted intrapertioneally and stabilized. 2mg/kg of lipopolysaccharide (LPS) was intrapertioneally injected to C57BL mice 24 hours before imaging. 1.7mL/kg anti-Gt-1 antibody was used to label neutrophils.

Transgenic mice expressing cyran fluorescent protein (CFP) under control of the CD18 promoter were used to visualize leukocytes. CD18-CFP mice were obtained from Dr. Minsoo Kim and Dr. Youngming Hyun. An acute dose of 5mg/kg MA was injected intrapertioneally during imaging.

For the short binge MA exposure, C57BL mice were intrapertioneally injected with four MA doses of 5mg/kg 1.5 hours apart, with the first dose at 24 hours before imaging. The fifth 5mg/kg dose was injected during imaging. 1.7mL/kg of anti-Gt-1 antibody was used to label neutrophils.

As a model of HIV-1-related brain inflammation, transgenic mice expressing human immunodeficiency virus (HIV) type 1 Tat protein under control of the GFAP promoter (Tat-tg) were used. A Tat-tg mouse was intraperitoneally injected with 1.7mL/kg of anti-Gt-1 antibody to label the neutrophils.

Mouse Thin Skull Cranial Window Surgery

To prepare a thin skull cranial window in a mouse skull, the far at the spot of intended cranial window was shaved. The scalp was cut, revealing a round-shaped cranial skin. 10% FeCl3 was applied to the skull to remove the tissue covering the skull more easily. A metal bar with a hole for the cranial window was glued to the head of the mouse. The mouse was attached to the holder and its legs were taped to stabilize the body. A lab electrical drill (Dremel JF5, 1900 rpm) was used to drill off the glue on the metal bar. A round cranial window of 1.5mm in diameter was thinned using both the drill and nontoxic bendable micro blade (900, Salvin Dental). The skull was made extremely thin using a drill and minute scratch so that the brain vessels could be observed clearly.

2-Photon Imaging

Laser imaging microscope (Olympus BX 51WI®) was used for imaging. The wavelength was 800nm for CFP/Texas Red or 930nm for fluorescein/Texas Red combination. The #660/680, #690, #600/54 and #645, 800/45 filters were used. 4 x and 10 x objectives were used subsequently to find and center field of view on the site of interest under light illumination using software Motic Images®. Then a 25 x objective was used for fluorescent imaging using Fluoview 10 software.

Line-Scan: The movable object stage was adjusted to find the vessels suitable for line-scan. After the desired vessels were located, the “line-scan” function in FV10® was performed. Line-scans were taken on venules and arterioles with diameters ~20μm because they provide clearer imaging. Line-scan is a function in FV10® in which a single line along the length axis of a blood vessel is scanned repeatedly at high speed. The plasma was fluorescently labeled with Texas Red while the red blood cells were not fluorescent. Thus one shot of a line-scan would show a fluorescent line with the red blood cells and red blood cells on it (Fig. 1). All the scans in a set were displayed from top to bottom with respect to the order of the scan taken. The dark dots would thus combine into stripes on a complete line-scan. Similarly, leukocytes in transgenic mice with CD18 pro motor driven CFP emitted blue fluorescence, displaying blue dots on each line-scan. A complete line-scan contained blue stripes, each reflecting a leukocyte. A line-scan was taken every minute for 30-40 minutes in each experiment. MA was injected 3 minutes after the start of a line scan. A typical result of a line-scan is shown in Fig. 1. The sample picture with stripes has a horizontal axis of position and a vertical axis of time. The flow of red blood cells corresponds to the black stripes in the line-scan. Similarly, leukocytes in CD18-CFP mice were fluorescently labeled and thus would display fluorescent stripes on a line-scan.

To determine the number of leukocytes passing through the vessel, the number of fluorescent stripes on each line-scan was manually counted. The counts were scored from 1-4, 1 being the vaguest and 4 being the most certain. A plot of leukocyte number was generated. A linear regression trend line was calculated to visualize the change in leukocyte number before and after injection of MA. Z-Stack: Z-stack is a function in FV10® which takes several scans of an adjustable range of depth for a particular region from top to bottom. The images from Z-stack can be combined together to form one picture.

![Image](https://via.placeholder.com/150)

Fig. 1. The green “box” shows the first three linescans out of 900 lines of a complete linescan. A line-scan is an x vs t scan in which a single line along the length axis of a blood vessel is scanned repeatedly (here n=900) at high-speed (>1 μm/sec). The plasma fluoresces red while red blood cells do not. As shown in Fig. 1, red blood cells are seen as dark dots on each line-scan and thus form dark stripes once all the line-scans are displayed from top to bottom. The horizontal axis indicates position whereas the vertical axis indicates time. The blood cell velocity is calculated using the formulas v(t)=<v> (t), where <v> is the average velocity of the cell, which is visually measured.
Acute MA exposure did not cause recruitment of neutrophils into cerebral vasculature

To find out if acute MA exposure causes cerebral vascular damage, a C57BL mouse was intraperitoneally injected with four doses of 5mg/kg MA 1.5 hour apart; the first dose was administered 24 hours before imaging. Stationary neutrophils were observed in the parenchyma, but no stationary neutrophils were observed in the blood vessels (Fig. 3). Another boost dose of 5mg/kg was injected during imaging in an attempt to visualize the development of an acute response. The boost dose of MA did not cause a change in neutrophil number. Stationary neutrophils were still only observed in parenchyma but not in blood vessels after the boost dose. Therefore, we conclude that neither short binge, nor acute MA exposure causes neutrophil adherence to cerebral blood vessels.

The number of leukocytes in cerebral blood vessels did not change after acute exposure to MA

Acute exposure to MA causes a sympathetic response in nervous system. The sympathetic response is known to cause an increase in the number of leukocytes in blood.10-11 The hypothesis of this experiment was that MA exposure might lead to an increase in leukocyte numbers in blood. To examine this, the number of blue stripes (each representing one CD18+ leukocyte on a line-scan) was manually counted. A scatter plot was generated (Fig. 5). No increase in leukocyte numbers within cerebral blood vessels after acute exposure to MA was evident. We conclude that acute MA exposure does not cause an increase in the number of leukocytes in cerebral blood vessels. This suggests that MA does not lead to an increased number of leukocytes in the blood stream, although additional experiments will be necessary to definitively show this (such as direct counting of leukocytes in peripheral blood samples).

Neutrophils were observed in parenchyma and possibly in blood vessels in Tat-tg transgenic mouse.

To study the mutual influence of MA and HIV-1 Tat protein in cerebral vasculature, a Tat-tg transgenic mouse was imaged (Fig. 6). No stationary neutrophils were observed in blood vessels. Many neutrophils were visible in parenchyma (Fig. 6). However, some neutrophils seemed to be in the process of extravasation as they were surrounding the blood vessels (Fig. 6A arrows). To better observe the neutrophils in parenchyma, we took a Z-stack (Fig. 6B). Many green fluorescent cells were found in view of several stationary neutrophils is shown both inside the blood vessels and in parenchyma (Fig. 2A, B).

This result convinced us that we established a suitable model to visualize recruitment of leukocytes in cerebral vasculature. Note that there were dark, circular shapes which were probably cells (Fig. 2A blue arrow) that were not fluorescent. They were probably leukocytes that did not express Ly-6g protein and thus were not bound by anti-Gr-1 antibody. The stationary or slowly migrating neutrophils in parenchyma were probably cells that extravasated from the blood vessels into the parenchyma due to the inflammatory change caused by LPS exposure. Alternatively, LPS-induced leakage of the BBB may have allowed anti-Gr-1 antibody to exit from blood vessels and label cells in parenchyma.

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We think that chronic exposure to MA can be detrimental to cerebral vasculature, especially in combination with HIV virotoxins, including circulating leukocyte activation. Stress, 2003. 6(1): p. 41-7.

REFERENCES