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ORIGINAL ARTICLE

Cleaved β-Actin May Contribute to DNA Fragmentation Following Very Brief Focal Cerebral Ischemia

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Abstract

Our previous study demonstrated caspase independent DNA fragmentation after very brief cerebral ischemia, the mechanism of which was unclear. In this study, we explore whether actin is cleaved following focal cerebral ischemia, and whether these structural changes of actin might modulate DNA fragmentation observed following focal ischemia. Results showed that a cleaved β-actin fragment was identified in brains of rats 24 hours following 10-minute and 2-hour focal ischemia. Though granzyme B and caspase-3 cleaved β-actin in vitro, the fragment size of β-actin cleaved by granzyme B was the same as those found after 10-minute and 2-hour focal ischemia. This was consistent with increases of granzyme B activity after 10-minute and 2-hour ischemia compared with controls. Cerebral extracts from 10-minute and 2-hour ischemic brains degraded DNA in vitro. Adding intact β-actin to these samples completely abolished DNA degradation from the 10-minute ischemia group but not from the 2-hour ischemia group. We concluded that β-actin is likely cleaved by granzyme B by 24 hours following 10-minute and 2-hour focal cerebral ischemia. Intact β-actin inhibits DNase, and cleavage of β-actin activates DNase, which leads to DNA fragmentation observed in the brain following very brief focal ischemia.

Key Words: Actin, Apoptosis, Brain ischemia, DNase, Granzyme B.

INTRODUCTION

Apoptosis is frequently observed in neurodegenerative diseases (1) such as Parkinsonism, Huntington disease, and Alzheimer disease, as well as following acute injury including cerebral ischemia (2). One of the most important intranuclear structure changes that occurs during apoptosis is degradation of nuclear DNA into nucleosomal units (3). DNA fragmentation is considered the check point that makes cell death irreversible (4). Deoxyribonuclease (DNase) is one of several enzymes implicated in DNA fragmentation and cell death. Molecular characterization of this process identified a specific DNase (i.e. caspase activated DNase [CAD]) that cleaves chromosomal DNA in a caspase-dependent manner. CAD remains inactive when complexed with the inhibitor of CAD (ICAD). Caspases, in particular caspase-3, cleave ICAD to dissociate the CAD: ICAD complex, allowing CAD to cleave chromosomal DNA.

In our previous study, we found that 10-minute transient focal cerebral ischemia was associated with apoptosis as early as 24 hours after reperfusion, while caspase-3 was not activated until 72 hours (5). This result implied that there must be a caspase-independent DNase that led to the DNA fragmentation following 10 minutes of focal cerebral ischemia.

DNase I is a secretory glycoprotein with an endonuclease ability that cleaves double stranded DNA to yield 5′-phosphorylated polynucleotides. DNase I has high affinity for actin ($K_d = 5 \times 10^8\ M^{-1}$) (6), which leads to its natural inhibition by actin (7). In the absence of salt, actin can exist as a globular actin, G-actin. In the presence of salt, actin changes into a fibrous protein, F-actin. In vitro DNase I interacts strongly with G-actin leading to inhibition of DNase I activity (8). These facts imply that structural changes of actin might modulate DNase I activity and promote apoptosis.

Previous studies have shown DNase I and actin to be involved in the pathogenesis of several neurological diseases such as Alzheimer disease (9), Down syndrome (9), and traumatic brain injury (10). In addition, we have shown following brief cerebral ischemia many changes of actin gene expression in peripheral blood including assembly of actin filaments and actin stress fibers, interaction of actin filaments, formation of an actin comet, and biogenesis of the actin cytoskeleton (11). Since β-actin is the dominant form of actin in the brain, we explored the structural/functional changes of β-actin in an animal model of very brief focal cerebral ischemia.

We found that β-actin is cleaved at 24 hours following 10-minute and 2-hour focal cerebral ischemia in rats, and this cleavage appears to be mediated by granzyme B. Extracts from 10-minute and 2-hour ischemic brains demonstrate an endonuclease/DNase activity which can be blocked by adding intact β-actin to the 10-minute but not the 2-hour extracts. These data suggest that very short durations of ischemia lead to cleavage of β-actin which releases inhibition of DNase I to produce caspase-independent DNA fragmentation.

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The authors have no duality or conflicts of interest to declare.

MATERIALS AND METHODS

Animals

Multiple brain samples from twelve adult male Sprague-Dawley rats weighing 280–350 g (Charles River Labs, Wilmington, MA) were studied. The University of California Animal Care Committee at Davis reviewed and approved the animal protocol in accordance with NIH guidelines.

Focal Cerebral Ischemia

Focal cerebral ischemia was produced by occluding the middle cerebral artery (MCA) using the intraluminal suture technique (5). Rats were anesthetized with isoflurane. Body temperature was monitored during the MCAO procedure. Occlusion lasted 5 minutes, 10 minutes, or 2 hours before removal of the suture and reperfusion (n = 3 each group). Rats were allowed to recover and then were decapitated 24 hours later under deep isoflurane anesthesia. Sham-operated control rats (n = 3) were subjected to the identical surgical protocol without insertion of the suture into the artery.

Western Blot Analysis

Brains were carefully removed and the cortex and basal ganglia in the MCA territory of the ischemic hemisphere were collected on dry ice and stored at −70°C. Brain was later homogenized in ice-cold RIPA buffer (50 mM Tris-HCl [pH 7.4] 150 mM sodium chloride, 1.0% NP-40, 0.1% SDS, 0.5% sodium deoxycholate) with complete protease inhibitor cocktail (Sigma, P8340, St. Louis, MO) at 4°C. The homogenate was centrifuged at 14 000 g for 30-minute at 4°C and the supernatant was collected. One hundred microgram of protein was used unless mentioned otherwise. Twelve percent SDS-polyacrylamide gels were used during electrophoresis. After blocking with 5% nonfat milk in PBS, the membrane was incubated overnight at 4°C with an antibody for antiβ-actin (1:1000 dilution, Sc-69879, Santa Cruz Biotechnology, Inc., Dallas, TX), antigranzyme B (1:500 dilution, 3073-100, BioVision, Milpitas, CA), antifractin (1:500 dilution, AB3150, Chemicon, Billerica, MA), or antic-Myc (1:1000 dilution, 9402, Cell Signaling Technology, Danvers, MA). Horseradish peroxidase-conjugated antimouse or antirabbit IgG (Bio-Rad, Hercules, CA) was used according to the primary antibody species. The signal was detected using the ECL chemiluminescent detection system (Pierce Inc., Rockford, IL). Relative intensity of each band was determined using Image J software.

In Vitro Cleavage of β-Actin by Granzyme B and Caspase-3

Recombinant β-actin (3 μg, 3777P, ProSci, Inc., Poway, CA), granzyme B (10U, BioVison, 1118-5), and caspase-3 (10U, 1083-100, BioVision) were used. Actin was incubated either alone, or with granzyme B, or with caspase-3 in 100 μl buffer (50 mM Tris [pH 8.0], 150 mM NaCl, 0.01% Triton 100) at 37°C for overnight. Fifty microliter of samples were loaded onto 12% SDS-polyacrylamide gels for electrophoresis followed by 2-hour staining with Simply Blue (LC6060, Invitrogen, Carlsbad, CA) at 24°C.

Enzyme Activity Analysis

Twelve samples total were used (n = 3 in each group). Granzyme B activity was analyzed using a granzyme B assay kit (BML-AK711, Enzo Life Sciences, Farmingdale, NY). The assays were performed according to the manufacturer's instructions. Twenty microgram of sample proteins were incubated with substrates and buffer for 30 minutes at 24°C and the absorption at 405 nm was read with a microplate-reading spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA).

In Vitro DNA Degradation by Ischemic Cerebral Extract

Sample homogenates containing 100 μg total proteins from 10-minute ischemia, 2-hour ischemia, and sham control were used for in vitro DNase activity analysis. Twenty microgram of calf DNA (D3664, Sigma) was used as substrate. The cerebral extract protein and calf DNA were incubated 30 minutes at 37°C in DNA digest buffer (final volume 100 μl, 1028475, Qiagen, Valencia, CA). The reaction was stopped by adding 25 μl of 50 mM EDTA. To test the inhibiting role of actin on DNase activity, 0.5 μg of globular actin (actin dissolved in globular actin buffer: 2 mM Tris-HCl [pH 8.0], 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM DTT) was pre-incubated with samples for 30 minutes at 24°C before performing the DNA degradation assay described above (12). After the reaction, 1 μl of each sample was analyzed using DNA 7500 chips (5067-1506, Agilent, Santa Clara, CA) in an Agilent 2100 Bioanalyzer.

Statistics

Data are given as mean ± SE. One-way ANOVA was performed with Fisher LSD Post Hoc test; p value of <0.05 was considered statistically significant.

RESULTS

Cleaved Actin Fragment Was Detected Following Focal Ischemia

At 24 hours following 10-minute and 2-hour ischemia, a band ~27 kDa in addition to the expected 42-kDa band was detected by Western blot analysis using antibody against N-terminal β-actin (Fig. 1A). The 27-kDa cleaved actin band was found in 0/3 of control, 0/3 of 5-minute ischemia, 3/3 of 10-minute ischemia, and 3/3 of 2-hour ischemia groups. The 27-kDa actin band was significantly more intense following 2-hour ischemia compared with 10-minute ischemia (Fig. 1B). The most significant differences occurred between 2-hour ischemia compared with 5-minute ischemia ($p < 0.001$) or sham control ($p < 0.001$) though there was also a significant difference in band intensity between 2-hour ischemia and 10-minute ischemia ($p = 0.044$). The band in the 10-minute ischemia was significantly more intense compared with 5-minute ischemia

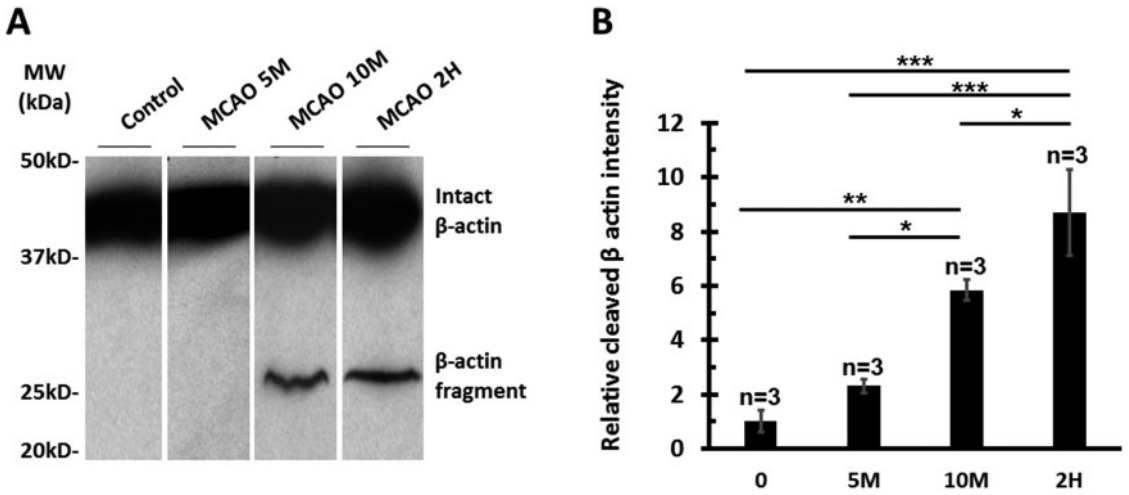

FIGURE 1. Western blot analysis of β-actin in brains of rats at 24 hours following brief focal cerebral ischemia. **(A)** Cleaved β-actin was detected at 27 kDa following 10-minute and 2-hour ischemia. The 27-kDa β-actin band was significantly more intense following 2-hour ischemia compared with 10-minute ischemia. **(B)** Relative intensity of 27 kDa β-actin (*Y* axis). One-way ANOVA showed a significant difference within the groups ($p = 0.018$), between 10-minute ischemia and sham control ($p = 0.004$), and between 2-hour ischemia and sham control ($p < 0.001$). In addition, there were also significant differences between 10-minute ischemia compared with 5-minute ischemia ($p = 0.018$), between 2-hour ischemia compared with 5-minute ischemia ($p < 0.001$), and between 2-hour ischemia compared with 10-minute ischemia ($p = 0.044$). $n = 3$ in each group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

($p = 0.018$) or sham control ($p = 0.004$). These results suggest that β-actin is partially cleaved during brief focal cerebral ischemia in rats. The cleavage is more severe following 2-hour focal ischemia which simulates human stroke compared with 10-minute focal ischemia which simulates human transient ischemia attacks. The antibody against fractin, a specific caspase-3 cleavage product, did not detect anything in the expected 32 kDa size (data not shown).

In Vitro Cleavage of β-Actin by Granzyme B and Caspase-3

Both granzyme B (13) and caspase-3 (14) can cleave β-actin in vitro. We performed in vitro actin cleavage assays and our findings agreed with the previous reports. Although both granzyme B and caspase-3 can cleave β-actin in vitro, the products are different molecular weights. The size of the major fragment of β-actin cleaved by granzyme B was ~27 kDa (Fig. 2, right lane), which was a smaller compared with the 32-kDa fragment of β-actin cleaved by caspase-3 (Fig. 2, middle lane).

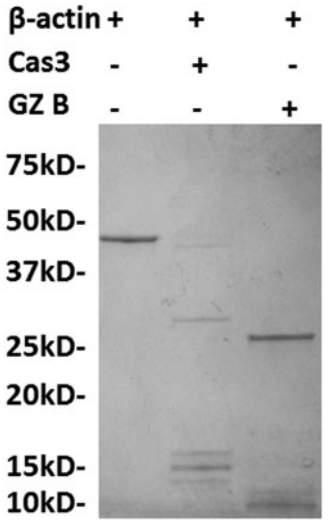

FIGURE 2. Cleavage of β-actin by caspase-3 or granzyme B. Cleaved β-actin was detected at 32 kDa or 27 kDa by caspase-3 or granzyme B, respectively. The intact β-actin was ~42 kDa in molecular weight (left lane); the major cleaved products produced by caspase-3 were ~32 kDa and ~15 kDa (middle lane); the major cleaved products produced by granzyme B were ~27 kDa and 10 kDa (right lane) B.

Expression and Activity of Granzyme B at 24 Hours Following Focal Ischemia

Since the in vivo cleaved actin fragment was ~27 kDa and this was the same size of cleaved actin product by granzyme B in vitro, we hypothesized that granzyme B may be expressed in rat brains following focal ischemia. We then measured the expression and activity of granzyme B in the rat brains in our study. A major band of granzyme B ~27 kDa was detected (Fig. 3A) by Western blot using antigranzyme B antibody. Statistical analysis in 12 animals ($n = 3$ in each group) showed that the expression of granzyme B was significantly increased following 10-minute ($p < 0.05$) and 2-hour ischemia ($p < 0.01$; Fig. 3B) compared with control. The enzyme activities of granzyme B were also significantly increased following 10-minute ($p < 0.01$) and 2-hour ischemia ($p < 0.05$) compared with control (Fig. 4).

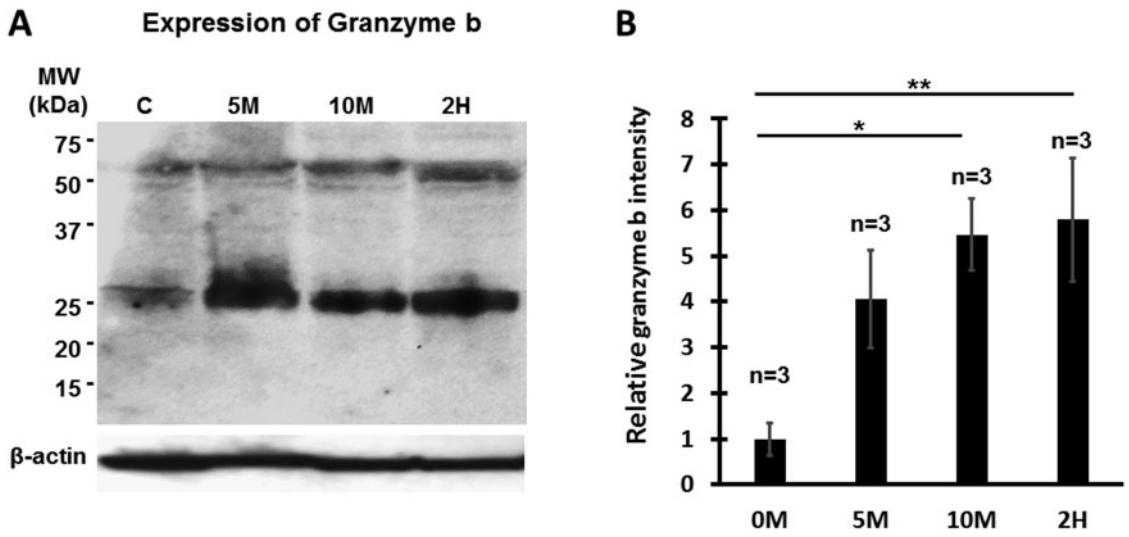

FIGURE 3. Western blot analysis of granzyme B in brains of rats at 24 hours following brief focal cerebral ischemia. **(A)** Western blot of samples from sham control, 5-minute ischemia, 10-minute ischemia, and 2-hour ischemia. A major band ~27 kDa was detected. **(B)** One-way ANOVA of the intensity of 27-kDa granzyme B band. Expression of granzyme B significantly increased in 10-minute ischemia ($p < 0.05$) and 2-hour ischemia ($p < 0.01$) compared with sham control. $n = 3$ in each group. *$p < 0.05$, **$p < 0.01$.

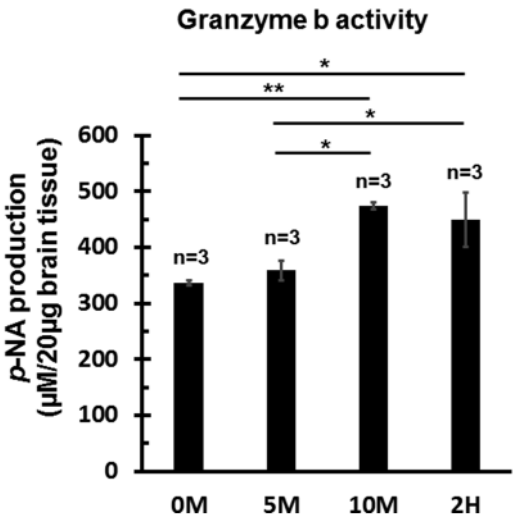

FIGURE 4. Enzyme activity analysis of granzyme B. Activity of granzyme B in brain tissues was assessed by the production of p-NA, a granzyme B cleavage product. The enzyme activity of granzyme B was significantly higher in 10-minute ischemia ($p < 0.01$) or 2-hour ischemia ($p < 0.05$) compared with sham control. In addition, the enzyme activity of granzyme B was significantly higher in 10-minute ischemia ($p < 0.05$) or 2-hour ischemia ($p < 0.05$) compared with 5-minute ischemia. $n = 3$ in each group. *$p < 0.05$, **$p < 0.01$.

Granzyme B Expression Was Positively Correlated to c-Myc Expression

c-Myc regulates the expression of granzyme B in hematopoietic tissues (15). We explored whether the expression of granzyme B associates with the expression of c-Myc in brains. We detected a band of c-Myc ~25 kDa (Fig. 5A) by Western blot using antic-Myc antibody. Statistical analysis in 12 animals ($n = 3$ in each group) showed that the expression of c-Myc was significantly increased following 5-minute ($p < 0.05$), 10-minute ($p < 0.001$), and 2-hour ischemia ($p < 0.001$) compared with control (Fig. 5B). There was a strong positive Pearson correlation between the expression of granzyme B and the expression of c-Myc ($r = 0.836$, $p = 0.0007$; Fig. 6).

DNase I-like Activity at 24 Hours Following Focal Ischemia

Intact β-actin is a natural inhibitor of DNase I (7). Since β-actin was cleaved after 10-minute and 2-hour ischemia, we hypothesized the inhibitory effect of intact β-actin on DNase I would be decreased and DNase I activity would increase. In vitro DNA degradation was measured when incubated with brain homogenates from 10-minute and 2-hour ischemia (Fig. 7, lane 10M and lane 2H). The DNA degradation was completely abolished by first adding intact β-actin to 10-minute ischemia sample (Fig. 7, lane 10M + β). However, DNA degradation was not abolished when pre-incubated with β-actin in the 2-hour ischemia sample (Fig. 7, lane 2H + β). The control sample did not degrade DNA in vitro (Fig. 7, lane Control). These results suggest that cerebral extracts following 10-minute and 2-hour ischemia (simulating TIA and stroke, respectively) contain DNase I-like activity. Intact β-actin can abolish or limit this activity and minimize DNA damage.

DISCUSSION

We demonstrated that β-actin was cleaved at 24 hours following 10-minute and 2-hour focal cerebral ischemia, and the ~27-kDa β-actin fragment was the same size as that produced by granzyme B but not by caspase 3. Cerebral extracts from 10-minute and 2-hour ischemia had endonuclease

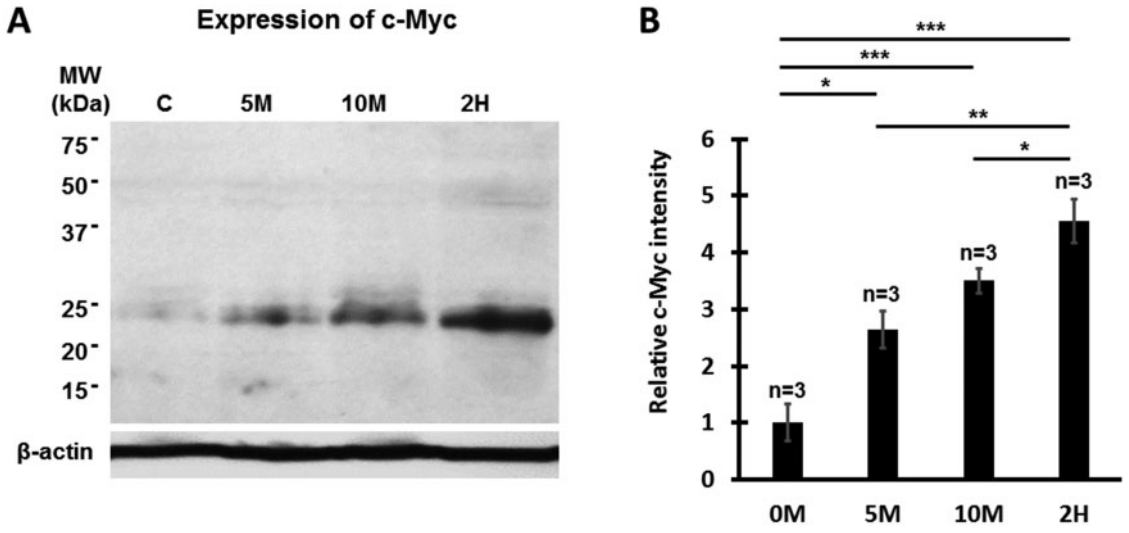

FIGURE 5. Western blot analysis of c-Myc in brains of rats at 24 hours following very brief focal cerebral ischemia. **(A)** c-Myc was detected as a band of ~25 kDa following brief focal ischemia. **(B)** Relative c-Myc intensity was significantly increased following 5-minute ischemia ($p < 0.05$), 10-minute ischemia ($p < 0.001$), or 2-hour ischemia ($p < 0.001$) compared with sham control. Significant differences also existed between 2-hour ischemia and 5-minute ischemia ($p < 0.01$) as well as between the 2-hour ischemia and 10-minute ischemia groups ($p < 0.05$). n = 3 in each group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

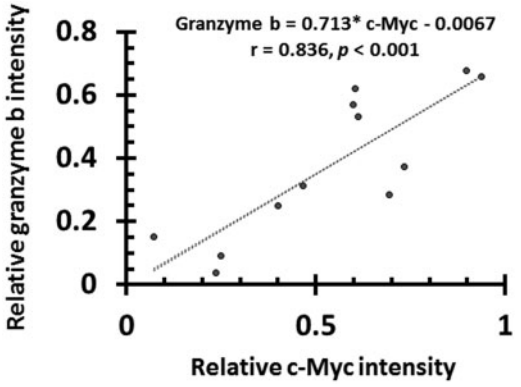

FIGURE 6. Pearson correlation analysis of granzyme B and c-Myc expression. A strong positive correlation was observed between expression of granzyme B and c-Myc relative to β-actin ($r = 0.836$, $p = 0.0007$). $N = 12$ in total.

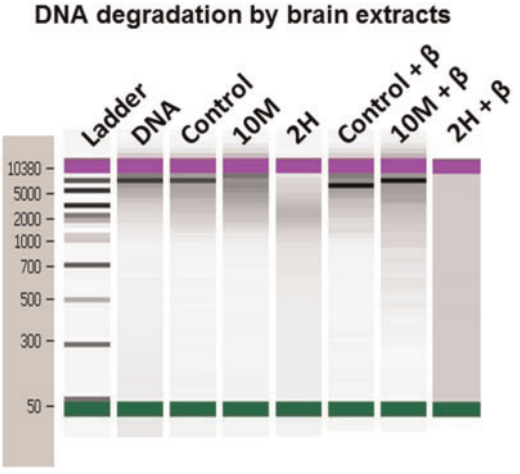

FIGURE 7. In vitro DNA digestion by cerebral extract of brains of rats at 24 hours following brief focal cerebral ischemia. DNase I-like activity was detected at 24 hour following brief focal ischemia. DNA was degraded by cerebral extracts from 10-minute (10M) and 2-hour (2H) ischemia but not from sham control (Control). DNA degradation was completely abolished by pre-incubation of β-actin with 10-minute ischemia sample (10M + β), but not with 2-hour ischemia sample (2H + β).

activity indicated by DNA cleavage activity which was blocked by adding β-actin to the 10-minute ischemic cerebral extracts but not the 2-hour ischemic cerebral extracts. We interpret these data to mean that very short durations of ischemia trigger granzyme B cleavage of β-actin which decreases inhibition of DNase I and leads to caspase-independent DNA fragmentation. DNA fragmentation following longer periods of ischemia appears to involve both β-actin dependent and β-actin independent DNA degradation mechanisms, and likely includes DNase I, caspases, and other enzymes.

Actin has 374 amino acids and is conserved in almost all species. In the absence or low concentrations of salt, β-actin can exist as a globular actin, G-actin. In the presence of high concentrations of salt, actin becomes the fibrous F-actin. β-actin interacts with DNase I and is a transcriptional mediator. DNase I has a high affinity for actin ($K_d = 5 \times 10^8\ M^{-1}$). In vitro DNase I is known to interact strongly with G-actin in a 1:1 complex leading to inhibition of DNase I activity and preventing actin polymerization (16, 7). The N-terminal peptide comprised of residues 1–207 of actin inhibits DNase I, while the tryptic fragments (cleavage sites between residues 62 and 63 or between 68 and 69) fail to bind to DNase I (17). These results indicate that cleavage of actin could cause the disinhibition of DNase I leading to DNA degradation.

Granzyme B (13) and caspase-3 (18) each cleave actin into different size fragments. Our results confirmed the major

cleaved sizes of β-actin were ~27 kDa by granzyme B and were ~32 kDa by caspase-3. Western blot analyses showed a 27-kDa band using antibody against N-terminal β-actin in the brains of 10-minute and 2-hour cerebral ischemia while no bands were detected for the caspase-3 product, fractin. As noted above, the size of β-actin fragment from ischemic brains was similar to those cleaved by granzyme B in vitro, suggesting that granzyme B or a similar enzyme cleaved intact actin following short and longer periods of cerebral ischemia. A previous study demonstrated that ischemia-induced cell death in adult brain is caspase independent and is likely granzyme B dependent (19). This conclusion is supported by our finding that granzyme B protein expression and enzyme activity increased significantly in the 10-minute and 2-hour ischemic samples compared with the sham control. These results also support the hypothesis that granzyme B was likely the upstream effector of actin cleavage. In addition to cleaving actin, granzyme B can also cleave poly (ADP-ribose) polymerase-1 (PARP1) and heat shock protein 70. Granzyme B can also facilitate nuclear translocation of AIF (20–22).

Granzyme B is a serine protease commonly found in the granules of cytotoxic T cells and natural killer cells. Along with the pore-forming protein perforin, granzyme B mediates apoptosis in the target cells. Elevated levels of granzyme B are implicated in amnestic mild cognitive impairment (23) and amyotrophic lateral sclerosis (24). A role for granzyme B in cerebral ischemia is supported by our finding that protein expression of c-Myc, a transcription factor and repressor of granzyme B (15), increased significantly at 24 hours following 5-minute, 10-minute, and 2-hour focal ischemia compared with sham control, and expression of c-Myc and granzyme B were positively correlated ($r = 0.839$, $p < 0.001$). Although we did not perform immunohistochemistry using c-Myc or granzyme B antibodies, another study demonstrated that granzyme B is released by T cells (25). Furthermore, granzyme B is secreted by $CD8^+$ T lymphocytes as cytotoxic protease in human stroke patients and in ischemic rat brains where CD8 colocalized with granzyme B (20, 21). The infiltration of $CD8^+$ T-lymphocytes has been associated with dying neurons (20, 21), though it is not known if granzyme B actually kills neurons or glial cells. The cellular source of granzyme B following cerebral ischemia in this study is likely NK and CD8+ T cells, but this will require future study.

In summary, we demonstrated that β-actin is cleaved following focal cerebral ischemia. The cleavage of β-actin is likely associated with increased expression of granzyme B, which is consistent with increases of granzyme B activity following focal ischemia. Intact β-actin inhibits DNase in vitro, and cleavage of β-actin activates DNase which leads to DNA fragmentation following very brief focal ischemia.

REFERENCES

1. Radi E, Formichi P, Battisti C, et al. Apoptosis and oxidative stress in neurodegenerative diseases. J Alzheimers Dis 2014;42(Suppl. 3): S125–52
2. Chen SD, Yang DI, Lin TK, et al. Roles of oxidative stress, apoptosis, PGC-1alpha and mitochondrial biogenesis in cerebral ischemia. Int J Mol Sci 2011;12:7199–215
3. Wyllie AH, Kerr JF, Currie AR. Cell death: The significance of apoptosis. Int Rev Cytol 1980;68:251–306
4. Nagata S. Apoptotic DNA fragmentation. Exp Cell Res 2000;256: 12–8
5. Zhan X, Kim C, Sharp FR. Very brief focal ischemia simulating transient ischemic attacks (TIAs) can injure brain and induce Hsp70 protein. Brain Res 2008;1234:183–97
6. Mannherz HG, Goody RS, Konrad M, et al. The interaction of bovine pancreatic deoxyribonuclease I and skeletal muscle actin. Eur J Biochem 1980;104:367–79
7. Lazarides E, Lindberg U. Actin is the naturally occurring inhibitor of deoxyribonuclease I. Proc Natl Acad Sci U S A 1974;71:4742–6
8. Chen T, Haigentz M Jr, Reisler E. Myosin subfragment 1 and structural elements of G-actin: Effects of S-1(A2) on sequences 39–52 and 61–69 in subdomain 2 of G-actin. Biochemistry 1992;31:2941–6
9. Schatzmann-Turhani D, Labudova O, Yeghiazaryan K, et al. Overexpression of DNAse I in brain of patients with Down syndrome. J Neural Transm Suppl 1999;57:353–62
10. Bareyre FM, Raghupathi R, Saatman KE, et al. DNase I disinhibition is predominantly associated with actin hyperpolymerization after traumatic brain injury. J Neurochem 2001;77:173–81
11. Zhan X, Ander BP, Jickling G, et al. Brief focal cerebral ischemia that simulates transient ischemic attacks in humans regulates gene expression in rat peripheral blood. J Cereb Blood Flow Metab 2010;30: 110–8
12. Nascimento AA, Cheney RE, Tauhata SB, et al. Enzymatic characterization and functional domain mapping of brain myosin-V. J Biol Chem 1996;271:17561–9
13. Hansen MH, Nielsen H, Ditzel HJ. The tumor-infiltrating B cell response in medullary breast cancer is oligoclonal and directed against the autoantigen actin exposed on the surface of apoptotic cancer cells. Proc Natl Acad Sci U S A 2001;98:12659–64
14. Du J, Wang X, Miereles C, et al. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. J Clin Invest 2004;113:115–23
15. Laurenti E, Varnum-Finney B, Wilson A, et al. Hematopoietic stem cell function and survival depend on c-Myc and N-Myc activity. Cell Stem Cell 2008;3:611–24
16. Kabsch W, Mannherz HG, Suck D, et al. Atomic structure of the actin: DNase I complex. Nature 1990;347:37–44
17. De Couet HG. Studies on the antigenic sites of actin: A comparative study of the immunogenic crossreactivity of invertebrate actins. J Muscle Res Cell Motil 1983;4:405–27
18. Mashima T, Naito M, Noguchi K, et al. Actin cleavage by CPP-32/apopain during the development of apoptosis. Oncogene 1997;14: 1007–12
19. Gill R, Soriano M, Blomgren K, et al. Role of caspase-3 activation in cerebral ischemia-induced neurodegeneration in adult and neonatal brain. J Cereb Blood Flow Metab 2002;22:420–30
20. Chaitanya GV, Eeka P, Munker R, et al. Role of cytotoxic protease granzyme-b in neuronal degeneration during human stroke. Brain Pathol 2011;21:16–30
21. Lee PR, Johnson TP, Gnanapavan S, et al. Protease-activated receptor-1 activation by granzyme B causes neurotoxicity that is augmented by interleukin-1beta. J Neuroinflammation 2017;14:131
22. Chaitanya GV, Schwaninger M, Alexander JS, et al. Granzyme-B is involved in mediating post-ischemic neuronal death during focal cerebral ischemia in rat model. Neuroscience 2010;165:1203–16
23. Le Page A, Bourgade K, Lamoureux J, et al. NK cells are activated in amnestic mild cognitive impairment but not in mild Alzheimer's disease patients. J Alzheimers Dis 2015;46:93–107
24. Ilzecka J. Granzymes A and B levels in serum of patients with amyotrophic lateral sclerosis. Clin Biochem 2011;44:650–3
25. Wang T, Lee MH, Johnson T, et al. Activated T-cells inhibit neurogenesis by releasing granzyme B: Rescue by Kv1.3 blockers. J Neurosci 2010;30:5020–7