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Paradoxical exacerbation of neuronal injury in reperfused stroke despite improved blood flow and reduced inflammation in early growth response-1 gene-deleted mice

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Abstract

Objectives—Early growth response gene-1 (Egr-1) coordinates the rapid upregulation of diverse inflammatory and coagulation-related genes following ischemia/reperfusion. Genetic deletion of Egr-1 results in attenuated post-ischemic injury in diverse tissue systems. In the present study, we utilized a murine model of transient middle cerebral artery occlusion to probe the functional effects of Egr-1 deletion following cerebral ischemia/reperfusion.

Methods—The time course of Egr-1 expression was established by Northern/Western blot analysis, and immunocytochemistry localized Egr-1 to specific cell populations. Flow cytometry was then employed to characterize the ischemic cellular infiltrate of both wild-type (+/+) and Egr-1-null (–/–) mice. Next, the functional effect of Egr-1 deletion was investigated in Egr-1-deficient mice and their wild-type littermates subjected to middle cerebral artery occlusion. Infarct volumes, neurological scores, and reperfusion cerebral blood flow were compared between cohorts.

Results—Rapid upregulation of Egr-1 was observed in the ischemic hemisphere, and localized primarily to neurons and mononuclear cells. Egr-1 deletion led to a suppression of infiltrating neutrophils and activated microglia/macrophages ($P < 0.001$). Additionally, although Egr-1 deletion enhanced post-ischemic cerebral blood flow, Egr-1-deficient mice suffered larger infarcts ($P = 0.01$) and demonstrated a trend towards worse neurological scores ($P = 0.06$) than wild-type controls.

Discussion—Despite a reduction in the proportion of infiltrating inflammatory cells/activated microglia and improvement in post-ischemic reperfusion, Egr-1-deficient animals suffer larger infarcts in our model. Therefore, cerebral Egr-1 expression may function to protect neurons despite its adverse modulatory consequences for inflammation and thrombosis.

Keywords

Egr-1; Inflammation; Mouse; Neuroprotection; Stroke

Introduction

Early growth response gene-1 (Egr-1, also known as Krox-24, Zif268, and NGFI-A) is an immediate-early gene transcription factor that was first recognized for its role in the growth and differentiation of cells along a macrophage lineage *in vitro*.^{1,2} In an effort to probe the *in vivo* functional effects of Egr-1 signaling, Egr-1-null mice (Egr-1 $-/-$) were developed and found to be phenotypically normal, suggesting that the *in vivo* functions of Egr-1 manifest primarily under conditions of stress.³ Egr-1 gene-deficient mice have recently been employed to uncover a role for this transcription factor as a pivotal regulator of many pro-inflammatory and pro-coagulant genes involved in ischemic injury.⁴ For example, Egr-1 was demonstrated to rapidly upregulate pro-coagulant, pro-inflammatory, and vascular permeability-related genes, as well as chemokines.^{4,5} In keeping with this novel characterization as a 'master switch' for ischemic injury, targeted genetic deletion of Egr-1 was shown to ameliorate functional outcome following ischemia/reperfusion (IR) across several tissue systems.^{4,6-8}

Several studies have sought to evaluate the expression of Egr-1 in ischemic brain tissue.⁹⁻¹¹ However, this early work was purely descriptive and no attempt was made to probe the functional significance of cerebral Egr-1 expression. Given the established role of Egr-1 as a central mediator of multiple downstream pathogenic cascades following ischemia, coupled with more recent investigations demonstrating that Egr-1 regulates these same cascades following cerebral IR,¹² we hypothesized that Egr-1 would promote post-ischemic cerebral injury and would thus serve as an effective therapeutic target at the intersection of the inflammation and coagulation axes. In the present study, we thus evaluated the functional effects of Egr-1 gene deletion in a murine model of transient focal cerebral ischemia.

Materials and Methods

Mice

All experiments were conducted under the approval of the Columbia University Institutional Animal Care and Use Committee. The mice were originally generated in 129S2/SvPas mice and were back-bred into a C57BL/6 background for six generations.^{3,4} All experiments comparing genotypes were carried out using adult male mice (aged 8–10 weeks, 23–26 g at surgery) and their littermate controls. Mice were housed in animal care barrier facilities with free access to food and water on a 12-hour light/ dark cycle. Due to the sterility of the homozygous-null ($-/-$) females, heterozygote ($+/-$) animals were bred in-house and genotype was confirmed by polymerase chain reaction as previously described.^{3,4} The operator was blinded to the genotype of animals at all times.

Transient cerebral ischemia model

Expression and functional studies utilized the intraluminal filament model of cerebral ischemia described previously, with minor modifications.¹³ Briefly, mice were anesthetized, intubated, and artificially ventilated with 1.5% isoflurane in 50% O₂/50% N₂, titrated to maintain a mean arterial pressure of 75 mmHg assessed by cannulation of the femoral artery. Middle cerebral artery occlusion was induced by advancing a heat-blunted, silicone-coated, 7-0 nylon monofilament (Ethicon, Somerville, NJ, USA) a distance of 8.0 mm from the carotid bifurcation to rest at the origin of the middle cerebral artery. Following 120 minutes of ischemia, the occluding filament was withdrawn to establish reperfusion. Normothermia (37.0±0.1°C) was maintained throughout the surgery and for 2 hours post-reperfusion using a rectal temperature probe and a heating lamp.

Transcranial measurements of cerebral blood flow (CBF) were performed using laser Doppler flowmetry (PeriFlux System 5000; Perimed, Stockholm, Sweden) through the intact skull as previously described.¹³ CBF was recorded continuously in all animals beginning prior to occlusion and continuing until 15 minutes post-reperfusion, and strict criteria were utilized to prospectively exclude animals with insufficient CBF drop-off (<30% baseline) as well as inadequate reperfusion (>90% baseline).

Northern blot analysis

Following homogenization of each hemisphere, total RNA (12 µg per lane) was subjected to electrophoresis in 0.8% agarose–formaldehyde gels and transferred to Duralon-UV membranes (Stratagene, La Jolla, CA, USA). Membranes were hybridized with [³²P]-labeled probes for mouse Egr-1 as well as [³²P]-labeled beta-actin as an internal control.¹⁴ Membranes were exposed to Kodak BioMax film (Eastman Kodak, Rochester, NY, USA) at –80°C. Blots are representative of two replicates per time point.

Western blot analysis

After homogenization of each hemisphere, protein (20 µg per lane) was loaded into a sodium dodecyl sulfate polyacrylamide gel. The gel was electrophoresed, and proteins were transferred to nitrocellulose membranes. Immunoblotting was performed using primary rabbit anti-mouse Egr-1 IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Secondary antibodies were horseradish peroxidase-conjugated goat anti-rabbit whole IgG (Sigma, St Louis, MO, USA). Blots are representative of two replicates per time point.

Immunocytochemistry

Sections of 40 µm were obtained from wild-type mice killed at 3 and 24 hours post-reperfusion. Sections were stained with commercially-available primaries, a rabbit anti-mouse Egr-1 IgG (1 : 200; Santa Cruz Biotechnology), anti-MAP2 (Sigma), anti-NeuN (Chemicon International, Temecula, CA, USA), anti-CD68 (AbD Serotec, Raleigh, NC, USA), anti-vWF (Novocastra Laboratories, Newcastle, UK), and anti-Nissl (Molecular Probes, Eugene, OR, USA). Primary antibody binding was visualized using biotinylated goat anti-rabbit IgG (1 : 100; Sigma), biotinylated mouse anti-hamster IgG (1 : 50; Pharmingen; BD Biosciences, San Jose, CA, USA), or biotinylated rabbit anti-rat IgG (1 : 100 dilution; Sigma). Blocking experiments were performed by pre-absorbing the antibody

with Egr-1 protein (Santa Cruz Biotechnology). Images were obtained using a Bio-Rad 2000 confocal laser-scanning device (Bio-Rad, Hercules, CA, USA) attached to a Nikon E800 microscope (Nikon, Tokyo, Japan).

Flow cytometry

Flow cytometry was utilized to characterize the cellular infiltrate of the ischemic hemispheres of both wild-type (+/+) and Egr-1-null (-/-) mice subjected to reperfusion stroke and killed at 24 hours post-reperfusion. Gradient purification was performed by modification of a previously published protocol.¹⁵ Single mouse brains were harvested, minced (as two hemispheres) in medium (Roswell Park Memorial Institute: 10% fetal bovine serum), and carefully but vigorously drawn through an 18-gauge syringe (10–20 times). The mixture was then passed through a 70- μ m strainer (BD Falcon; BD Biosciences). An additional medium was added and the mixture was centrifuged (1500 rev/minute, 7 minutes). The supernatant was discarded, and the pellet was resuspended in 10 ml of 30% Percoll (Amersham Biosciences, Uppsala, Sweden) and spun at 27 000 *g* (12 000 rev/minute) for 30 minutes. The myelin layer was discarded, the pellet was carefully collected and washed with fluorescence-activated cell sorting (FACS) buffer, then resuspended in $\times 10$ diluted red blood cell buffer (PharM-Lyse no. 555899; BD Biosciences). This suspension was then incubated at room temperature for 15 minutes, and washed twice in FACS buffer. This protocol yielded a cell fraction enriched in leukocytes and microglia. Anti-CD11b fluorescein isothiocyanate and CD45 R-phycoerythrin-conjugated antibodies (BD Biosciences) were then used to identify three different cell populations: CD11b⁺/CD45^{high} (granulocytes and macrophage-like cells), CD11b⁺/CD45^{low} (resident/resting microglia), and CD11b⁻/CD45⁺ (lymphocytes). Flow cytometry was performed with a FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA), and data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

Hemoglobin assay

A spectrophotometric hemoglobin assay was employed to quantify intracerebral hemorrhage as previously described by our group.¹⁶ In brief, mouse brains were homogenized, sonicated, and centrifuged, and methemoglobin in the supernatants was converted (using Drabkin's reagent) to cyanomethemoglobin. Concentration was assessed by measuring optical density at 540 nm. The optical density reported is relative to that of a group of control uninfarcted brains.

Functional outcome

Neurological outcomes were quantified using a previously-described four-tiered grading system.¹³ In this system, a score of 1 reflects normal spontaneous movements; 2 indicates that the animal was circling clockwise; 3 indicates that the animal was observed to spin longitudinally around the axis of the tail; and 4 reflects that the animal was unresponsive to noxious stimuli. All assessments were conducted by an observer blinded to the identity of individual animals at all times.

Following neurological examination, mice were killed. The brains were sectioned (1 mm sections), and immersed in 2% triphenyltetrazolium chloride in 0.9% saline and incubated

for 25 minutes at 37°C. Infarcted brain was identified as an area of unstained tissue. Infarct volumes (% hemispheric infarction) were determined using the indirect method to correct for cerebral edema and reported as the average of values obtained by two blinded, independent observers from planimetric analysis of digitized serial images.¹⁷

Statistical analysis

All analyses were performed using a commercially-available software (JMP 8.0; SAS institute Inc., Cary, NC, USA). Between-group analyses of continuous variables were performed using one-way analysis of variance followed by *post hoc* Tukey–Kramer comparisons or Kruskal–Wallis test, as indicated. Categorical variables were analyzed by creating a 3×2 contingency table and reporting values of chi-square or Fischer's exact test, as appropriate. Values are expressed as mean±standard error of the mean, with statistical significance defined as $P<0.05$.

Results

Ischemic regulation of Egr-1 in stroke

The time course of Egr-1 RNA expression (Fig. 1A) revealed robust Egr-1 upregulation in the ipsilateral hemisphere of wild-type mice following stroke. This expression peaked immediately prior to reperfusion (0 hour post-reperfusion), and persisted for at least 24 hours. By 48 hours, this increase in gene expression had abated. Consistent with this time course, Western blot analysis revealed an ipsilateral peak of Egr-1 protein at 6 hours post-reperfusion, which persisted for at least 24 hours (Fig. 1B).

Immunohistochemistry demonstrated increased Egr-1 antigen in the peri-infarct regions of mice killed at both 3 and 24 hours (Fig. 2A–D). Control experiments employing antigen pre-absorption confirmed the specificity of our Egr-1 staining (Fig. 2E). Confocal microscopy demonstrated localization of Egr-1 antigen primarily to neuronal nuclei, with a triple stain (Nissl, Egr-1, and NeuN) unambiguously establishing the neuronal nuclear expression of Egr-1 (Fig. 3A–E). Additional immunostaining demonstrated Egr-1 antigen in the nuclei of both diapedesing macrophages and reactive microglia in the ischemic parenchyma (Fig. 3F–H).

Role of Egr-1 in leukocyte trafficking to ischemic brain

Flow cytometry was employed to distinguish three separate populations of cells. The proportions of cells in each of these populations are provided (Table 1). Egr-1 deletion resulted in a decreased proportion of activated microglia/infiltrating macrophages (CD11b⁺/CD45^{high}) in the ischemic hemisphere (+/+; 17.8±1.8%, $n=4$; -/-: 8.8±2.0%, $n=3$; $P<0.001$) when assessed at 24 hours post-ischemia (Fig. 4). Therefore, genetic deletion of Egr-1 attenuates inflammatory cell infiltration following reperfusion stroke.

Functional effect of Egr-1 gene deletion in stroke

A total of 39 mice were included in this arm of the study (-/-: $n=10$; +/-: $n=16$; +/+ : $n=13$). Of these mice, there were no significant differences between cohorts in the number of animals that were prospectively excluded for failure to meet the strict ischemic or

reperfusion threshold of CBF for our model ($-/-$: $n=3$ excluded; $+/-$: $n=4$ excluded; $+/+$: $n=3$ excluded; $P=0.93$). Of the remaining animals, mortality rates were higher in gene-deleted mice ($-/-$: 43%; $+/-$: 25%; $+/+$: 20%), although this difference did not reach statistical significance. Egr-1-deficient ($-/-$: $37\pm3\%$, $n=4$; $+/-$: $38\pm2\%$, $n=9$) mice suffered larger infarcts than controls ($+/+$: $29\pm2\%$, $n=8$; $P<0.05$; Fig. 5A). Neurological score evaluation also demonstrated worsening trends in the Egr-1-deficient mice ($-/-$: 2.5 ± 0.5 , $n=4$; $z/2$: 2.9 ± 0.3 , $n=9$; $+/+$: 1.9 ± 0.4 , $n=8$; $P=0.06$; Fig. 5B). There were no significant differences in the extent of intracerebral hemorrhage detected across cohorts ($-/-$: 1.2 ± 0.11 , $n=4$; $+/-$: 0.97 ± 0.12 , $n=9$; $+/+$: 0.96 ± 0.32 , $n=8$; Fig. 5C). However, while there were no differences between cohorts in the post-ischemic or pre-reperfusion cerebral blood flow, Egr-1 $^{-/-}$ animals demonstrated significantly improved post-ischemic CBF relative to wild-type mice ($-/-$: 1.45 ± 0.16 , $n=4$; $+/-$: 1.30 ± 0.08 , $n=9$; $+/+$: 1.07 ± 0.07 , $n=8$; $P=0.05$; Fig. 5D).

Discussion

Our data regarding the ischemic induction of cerebral Egr-1 expression are in accordance with those of prior studies.^{9–12} We demonstrate that post-ischemic Egr-1 expression begins early, with a peak of RNA expression occurring immediately prior to reperfusion. Protein production peaks 6 hours later, reflecting the time required for synthesis of the antigen. Immunohistochemistry identifies prominent Egr-1 antigen in the peri-infarct region accompanied by significantly reduced expression in the infarct core as early as 3 hours post-ischemia. Confocal microscopy unambiguously localized this Egr-1 antigen to neurons, as well as macrophages diapedesing through the vasculature walls and activated microglia in the ischemic region. In contrast to previous work in the pulmonary vasculature, cerebral endothelial cells revealed only minimal Egr-1 immunopositivity. The preponderance of neuronal Egr-1 suggests that neuronal expression may predominate over the vascular/monocytic expression in the brain following cerebral IR.

The present study also supports a critical role for Egr-1 in leukocyte trafficking to ischemic brain. We utilized flow cytometry to demonstrate a reduction in infiltrating neutrophils and activated microglia (CD11b⁺/CD45^{high}) in the infarcted hemisphere with Egr-1 deletion. These data are in concordance with previous findings of the suppression of chemokine induction (IP-10 and RANTES) in Egr-1-null mice, and the recently described role of Egr-1 in the upregulation of CCR5 expression in monocytes.^{4,18} We also observed a dose-dependent increase in post-ischemic reperfusion with gene deletion. This improved CBF likely derives from decreased fibrin deposition, as well as platelet and leukocyte accumulation in the microvasculature of the gene-deleted animals.^{4,12} Additionally, despite extinguishing pro-coagulant cascades in Egr-1-deficient mice, we did not observe a significant increase in intracerebral hemorrhage using our hemoglobin assay. Although these data support both a pro-inflammatory and pro-coagulant function for Egr-1 expression, we demonstrate a significant increase in infarct volume with Egr-1 deletion in our model.

This paradoxical exacerbation of infarction severity suggests alternative functions for Egr-1 expression in ischemic brain tissue. Although several *in vitro* experiments propose that neuronal Egr-1 expression is directly associated with delayed cell death following various

insults,^{19–21} it remains unclear whether Egr-1 serves a protective or deleterious role in ischemic neurons. In fact, a growing literature describes the role of Egr-1 in the survival and repair of various tissues, including the brain.^{22–26} These effects likely stem from upregulation of growth factor gene targets, including PDGF-A, PDGF-B, bFGF, and TGF-beta which have been shown to serve neuroprotective functions both *in vivo* and *in vitro*.^{27–29} In support of this concept, *in vivo* experiments have demonstrated that Egr-1 directly upregulates the neuroprotective epidermal growth factor receptor³⁰ and inhibits apoptosis following ultraviolet irradiation.³¹ A protective role for *in vivo* post-ischemic neuronal Egr-1 expression has also been supported by experiments utilizing hypoxic preconditioning which suggest that Egr-1 mediates neuroprotection through the induction of various growth factors.^{32,33} Another recent study has proposed a role for Egr-1 in functional recovery post-stroke. In this study, expression of Egr-1 was increased in rats exposed to environmental enrichment following stroke, and this expression correlated with improved functional recovery.³⁴ Given the profound neuronal expression of Egr-1 that we observe, as well as the robust suppression of acute inflammatory cell infiltrate with Egr-1 deletion, the overall lack of neuroprotection observed with genetic deletion suggests that Egr-1 directly protects neurons in our model. Further work to elucidate the specific function of neuronal Egr-1 expression in stroke must thus be undertaken in a system that functionally isolates the neuronal expression of Egr-1. These experiments could either make use of primary neuronal culture derived from gene-deleted animals or wild-type neurons in which Egr-1 is inhibited.⁶ Alternatively, mice possessing cell-specific Egr-1 deletions could be developed.

Simultaneous with the preparation of our manuscript, a competing group published a study evaluating the functional effect of Egr-1 deletion following stroke.³⁵ In this study, Tureyen *et al.* determined infarct volume using cresyl violet-stained frozen sections obtained from animals killed at 72 hours post-stroke. The authors found that Egr-1-null mice suffered smaller infarcts and exhibited improved neurological function relative to wild-type controls. We believe that experimental differences may primarily account for the discordant outcomes observed between the study by Tureyen *et al.*³⁵ and our own, and that a close analysis of these differences is critically important to understanding the true function of cerebral Egr-1 expression. For example, although both studies utilized a 120-minute occlusion period, our group observed a significant increase in post-ischemic reperfusion in the gene-deleted mice, in contrast to their report of comparable CBF across time points. This difference, necessary to interpret both the severity of the ischemic model as well as the extent of reperfusion, may relate to differences in the occluding filament, as the authors of the prior study utilized a 6-0, heat-blunted but uncoated filament. In our hands, larger, uncoated occluding filaments lead to increased endothelial disruption and decreased reperfusion.³⁶ The difference in extent of reperfusion may reflect an unanticipated difference in ischemic severity despite identical periods of occlusion. Additionally, our observed peak of RNA expression at 0 h, relative to their peak at 24 hours, as well as our demonstration of the overwhelming preponderance of neuronal Egr-1 expression, suggests model-related differences.

Furthermore, their observation of neuroprotection with Egr-1 deletion at 72 hours relative to our 24 hours outcome may also reflect variation in the functional role for Egr-1 expression across post-ischemic time points. Similar differences in activation of various post-ischemic

factors with variation of time course and concentration have been observed in studies of vascular endothelial growth factor. For example, early expression of vascular endothelial growth factor appears to worsen stroke outcome as well as edema whereas later expression may actually improve outcomes.^{37,38} Although evaluating Egr-1 expression and its functional effects beyond 24 hours would have been extremely informative in our study, the severity of our middle cerebral artery occlusion model yields prohibitively-high baseline mortality beyond 24 hours and precludes meaningful 72 hour survival. Finally, there remains the possibility that anesthetic differences contribute to the variations observed between our experiments (halothane versus isoflurane), as isoflurane preconditioning has been shown to upregulate Egr-1 expression in an *in vitro* model of oxygen-glucose deprivation.³⁹

In summary, the present study suggests that Egr-1 serves an overall neuroprotective role in our model despite its adverse modulatory effect on post-ischemic inflammation and coagulation. These seemingly contradictory findings are similar to the divergent functional effects of other immune-related molecules such as TNF-alpha, which have been shown to function in both progressive post-ischemic injury as well as protection/repair, depending on experimental conditions.⁴⁰ The recognition of the pleiotropic functions of the Egr-1 transcription factor in ischemic brain is thus essential to understanding its role in the pathogenesis of cerebral ischemic injury as well as the ultimate translational utility of Egr-1 modulation. Furthermore, given conflicting evidence across experimental models, additional work is necessary to elucidate the complex functional effects of post-ischemic cerebral Egr-1 signaling prior to further translational efforts.

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Figure 1.

(A) Northern blot of total Egr-1 RNA at each time point post-reperfusion demonstrating the peak of total RNA expression at 0 hour. (B) Western blot of Egr-1 protein production at each time point post-reperfusion demonstrating peak protein production at 6 hours post-reperfusion.

Figure 2.

Light microscopy demonstrating Egr-1 immunoreactivity at 3 hours post-reperfusion. (A) Coronal image through infarcted brain. Colored squares depict areas of increased magnification. (B) Magnified image depicting contralateral cortex. Note the low level of Egr-1 immunoreactivity. (C) Section through ipsilateral cortex. (D) Section depicting ischemic penumbra near the core. Note that ipsilateral immunoreactivity decreases with proximity to the infarct core. (E) Negative control staining following Egr-1 antigen preabsorption.

Figure 3.

Confocal microscopy demonstrating cell-specific localization of Egr-1 antigen. (A) Note the low level of neuronal expression of Egr-1 in contralateral cortex at 3 hours [(A)–(C): MAP2 (neuronal marker, green), Nissl (Nuclear Stain, blue), Egr-1 (red)]. (B) High Egr-1 immunoreactivity in most neurons in the ipsilateral cortex at 3 hours, even those distant to the ischemic region. (C) Less staining in the region destined for the infarct core. (D) At 24 hours, the spatial pattern of Egr-1 deposition persists, but with relatively decreased expression in the peri-infarct cortex and minimal staining in the infarct core (note the lack of MAP2 immunopositivity in the infarct core). (E) Triple stain (orange) utilizing NeuN (antigen marker of mature neuronal nucleus) unambiguously demonstrates neuronal Egr-1 immunopositivity [Egr-1 (red), NeuN (green), Nissl (blue)] in periinfarction cortex. (F) Confocal images demonstrating Egr-1 expression by activated microglia in the peri-infarction cortex. Note the arborized morphology of the CD68⁺ cells as well as the prominent adjacent nuclear Egr-1 staining likely representing neuronal nuclei. Arrowhead=Egr-1 (green), short arrow=CD68 (microglia/macrophage marker, red), Nissl (blue). (G) Egr-1 immunoreactivity in the nuclei of macrophages in the blood vessel wall. Arrowhead=Egr-1 (green), short arrow=CD68 (red), long arrow=vWF (endothelial marker, blue). (H) Same as (G). Scale bar=30 μ m for all panels.

Figure 4.

(A) Representative dot-plot analysis of leukocytes isolated from non-ischemic (contralateral) and ischemic (ipsilateral) hemispheres of Egr-1-null and wild-type mice at 24 hours post-ischemia. Gating with anti-CD11b (granulocytes/ macrophages and microglia) and anti-CD45 (expressed in all leukocytes and at lower levels in resting microglia), allowed the identification of three different populations: CD45^{high}/CD11b⁺ [granulocytes/ macrophage-like cells (pink)]; CD45^{low}/CD11b⁺ [resident microglia (green)], and CD45⁺/CD11b⁻ [lymphocytes (blue)]. Depicts less leukocyte infiltrate in Egr-1 null ischemic cortex. (B) Ischemic hemispheres of both wild-type ($P < 0.001$) and Egr-1-null ($P < 0.05$) mice demonstrated a significantly increased proportion of granulocytes and macrophage-like cells (CD45^{high}/CD11b⁺) relative to the contralateral hemispheres. Egr-1 deletion resulted in a decreased proportion of CD45^{high}/CD11b⁺ cells in the ischemic hemisphere ($P < 0.001$) relative to wild-type animals.

Figure 5.

(A) Egr-1-deleted animals demonstrated significantly larger infarcts than wild-type mice, with *post hoc* analysis indicating that (+/+) animals exhibited significantly smaller infarcts than (+/-) animals ($P < 0.05$). (B) Neurological deficit scores of the animals assessed at 24 hours (1=no deficit, 2=circling, 3=longitudinal spinning, 4=unresponsive). Trend goes towards worse deficits in the gene-deficient animals ($P = 0.06$). (C) Effect of Egr-1 deletion on intracerebral hemorrhage assessed by a spectrophotometric hemoglobin assay. There were no differences in hemorrhage between the groups ($P = \text{not significant}$). (D) Relative cerebral blood flow was recorded in all cohorts. This demonstrates no differences between the cohorts until the post-reperfusion time point, where higher CBF is observed with Egr-1 gene deletion ($P = 0.05$).

Table 1

Percentage of cells in each region of interest in the contralateral or ipsilateral hemisphere of wild-type (+/+) or Egr-1-null (-/-) mice

	Percentage of total			n
	CD45 ^{low+} /CD11b ^{low+}	CD45 ^{high+} /CD11b ^{high+}	CD45 ⁺ /CD11b ⁻	
Contralateral (wild type)	16.4 ± 0.6%	3.9 ± 0.3%	1.9 ± 0.1%	5
Ipsilateral (wild type)	41.7 ± 2.3% ***	17.8 ± 1.8% ***	4.1 ± 0.3% ***	4
Contralateral (Egr-1 null)	34.9 ± 7.2% **	3.0 ± 0.6% §§§	2.0 ± 0.3% §§§	3
Ipsilateral (Egr-1 null)	20.6 ± 0.9% §§§, ‡	8.8 ± 2.0% *, §§§	2.7 ± 0.3% *, §§§	3

Note:

* P 0.05

** P 0.01

*** P 0.001 versus contralateral (wild type).

§§ P 0.01

§§§ P 0.001 versus ipsilateral (wild type).

‡ P 0.05 versus contralateral (Egr-1 null).