Summary. Currently, it is accepted that brain injury promotes endogenous neurogenesis in mammals, primarily in the subventricular zone (SVZ), and newborn cells can migrate to the injured area. We examined the pattern of endogenous neurogenesis in adult rats after intracerebral hemorrhage (ICH) that was caused by intrastrial administration of collagenase type IV. Our results showed that ICH induced strong endogenous neurogenesis between 72 hours and 7 days after injury, but that the majority of newborn cells did not survive longer than 3 weeks due to apoptosis-mediated cell death. Furthermore, endogenous neurogenesis remained into a small extent at least 1 year after ICH. Because of the growing interest in new strategies for brain regeneration, these data suggest endogenous neurogenesis and inhibiting apoptosis of newborn neuroblasts as potential strategies to improve the consequences of hemorrhagic stroke in humans.

Key words: Intracerebral hemorrhage, Endogenous neurogenesis, Brain injury, Stroke

Introduction

In contrast to the belief that the cells of the adult mammalian brain cannot be regenerated after injury, recent findings have indicated not only the presence of stem cells in the brain throughout life but also that neurogenesis is an ongoing process into adulthood (Johansson et al., 1999; Song et al., 2002). Using several preclinical models of neuronal disease, studies have shown that brain injury could promote endogenous neurogenesis (Gould and Tanapat, 1997; Snyder et al., 1997) and that newborn cells can mature, express neuronal markers and migrate to the lesion zone (LZ) (Liu et al., 1998; Parent et al., 2002; Yamashita et al., 2006; Hou et al., 2008).

Previous studies have shown that there are two areas in the mammalian brain, the subventricular zone (SVZ) and the hippocampal dentate gyrus (DG), where neurogenesis occurs throughout adulthood. The newborn neurons in the DG become incorporated into the normal hippocampal circuitry (Cameron et al., 1993), whereas neuroblasts generated in the SVZ migrate toward the olfactory bulb (OB) where they differentiate into neurons (Luskin, 1993; Doetsch et al., 1999). Similar studies have established that neurogenesis also occurs in the DG and SVZ of adult primates, including humans (Eriksson et al., 1998; Gould et al., 1999). Furthermore, some studies suggest that new neurons are produced throughout adult life in the striatum or cortex of normal adult brains. These results raise the possibility of enhancing the recruitment of these newborn neurons to improve the outcome of brain injury that affects the striatum (Bedard et al., 2002, 2005; Kronenberg et al., 2005; Burns et al., 2007; Shimada et al., 2010).

In contrast, previous studies revealed that after brain injury, proliferating cells in the subgranular zone (SGZ) of the DG migrate to the granular cell layer (GCL) but do not migrate into the striatum or cortex (Jin et al., 2003). In this study, we examined neurogenesis in the SVZ because studies suggest that stroke caused the long-term formation of new striatal neurons from stem/progenitor cells in the SVZ (Altman and Das, 1995; Arvidsson et al., 2002). Stroke leads to increased proliferation of cells in the SVZ and migration of neuroblasts into the damaged striatum (Thored et al., 2007). However, only a small fraction of newly emerged neurons survive. Most of the neurons die before contributing to synaptic connections that are essential for functional recovery, suggesting the existence of an endogenous regulation process that prevents massive and
uncontrolled neuronal plasticity in the adult brain (Arvidsson et al., 2002; Zhang et al., 2006). Similar neuroanatomical remodeling is normal during early developmental stages, and the key molecular mechanisms regulating this process have been well characterized. During this period, active proliferation of embryonic stem cells is followed by programmed cell death, which occurs in a precisely controlled manner to form a well-organized, target-driven neural circuitry. Although the factors that regulate neuromorphogenesis in adulthood are not well characterized, it is likely that some regulatory mechanisms involved during early development also continue to play important roles in shaping the mature brain (Oppenheim et al., 1990).

Currently, the majority of studies investigating neurogenesis after stroke are based on an ischemic model. Our present study examined neurogenesis that was activated post-hemorrhagic injury. In previous studies, we demonstrated that cell therapy using bone marrow stromal cells (BMSCs) enhanced endogenous neurogenesis in the SVZ and compensated for the loss of neural tissue after intracerebral hemorrhage (ICH) (Otero et al., 2010, 2011). Therefore, we studied the temporal pattern of endogenous neurogenesis after ICH as a first step to find new therapeutic strategies that enhance this phenomenon. Furthermore, we studied apoptosis of the newly formed cells, which could restrict the possible functional recovery obtained by endogenous neurogenesis.

Material and methods

In the present study, care of the animals complied with that stipulated by the guide for the care and use of laboratory animals issued by the American National Society for Medical Research, and all our experiments were approved by the animal care and experimentation committee of Puerta de Hierro Hospital.

Experimental model

The current study used 72 adult Wistar rats weighing 200 to 250 g that were subjected to ICH and 8 non-ICH control animals. The animals subjected to ICH were sacrificed (8 animals each) at 24 hours (24 HAH), 48 hours (48 HAH), 72 hours (72 HAH), 7 days (7 DAH), 14 days (14 DAH), 21 days (21 DAH), 28 days (28 DAH), 6 months (6 MAH) and 12 months (12 MAH) after the induction of ICH. To induce ICH, anesthesia was given using an anesthetic chamber with 8% sevoflurane in 5 l/min oxygen flow, and the animals were maintained using a face mask with 4% sevoflurane in 2 l/min oxygen flow. After a subcutaneous injection of meloxicam (2 mg/kg) and morphine (2.5 mg/kg), the animals were placed in a stereotactic frame. Craniotomy was performed on the right side adjacent to bregma, and intrastriatal hemorrhage was induced by administration of bacterial collagenase type IV (Sigma-Aldrich, Madrid, Spain). Using a microinjector pump (mod 310 Stoelting Co., Wood Dale, IL, USA), 2 µl of saline containing 0.5 U of collagenase was injected into the striatum over a period of 5 min. The stereotactic coordinates of the injection site, with respect to bregma, were as follows: 0.04 mm posterior, 3.5 mm lateral on the right side and 6 mm ventral. Rectal temperature was monitored and maintained between 36 and 37°C using an electric blanket. Subsequently, the scalp was closed.

Collagenase-induced intracerebral hemorrhage is a reproducible animal model for the study of the effects of hematoma and brain edema.

Histological studies

For sacrifice, the animals were deeply anesthetized with 8% sevoflurane with a continuous oxygen flow of 3 l/min and perfused transcardially with 20 ml heparinized saline followed by 60 ml of 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brains were then dissected and post-fixed in 4% formalin for 1-2 days at room temperature, and for each rat a block containing the zone of the ICH was processed for paraffin sectioning. A series of 3 µm thick sections were cut with a microtome through each block and mounted on glass slides for histological observations after hematoxylin-eosin staining. For immunohistochemical studies, adjacent slides were placed in a boiled citrate buffer (pH 6) in a microwave oven (650-720 w). After rinsing in PBS, the sections were exposed to 3% H₂O₂ for 15 minutes to quench endogenous peroxidase activity. Before incubation of primary antibodies non-specific binding was blocked for 2 hrs with 3% normal serum from the species in which the secondary antibody was raised. The primary antibodies used were Ki-67 monoclonal antibody (1:200 Master Diagnostica, Granada, Spain), nestin monoclonal antibody (1:100, Chemicon International Inc., Temecula, CA, USA), doublecortin (DCX) polyclonal antibody (1:200, Santa Cruz Biotechnology SL, CA USA) and glial fibrillary acidic protein (GFAP) monoclonal antibody (1 µl/ml, Lab Vision Corporation; CA, USA). 3',3'-diaminobenzidine (DAB) was used as a chromogen. Control slices, lacking primary or secondary antibodies, were analyzed with each series. The sections were studied using light microscopy.

To analyze a possible increase in the proliferating activity following ICH, 10 sections corresponding to the LZ and SVZ were selected at random from each animal, and the number of Ki-67-positive cells in a minimum of 10 different microscopical fields at 100x was recorded from each histological section.

To study the differentiation of the proliferating cells, we performed a double stain of Ki-67-positive cells with...
anti-doublecortin, anti-nestin and anti-GFAP antibodies. For the first reaction, the slides were placed in a boiled citrate buffer (pH 6) in a microwave oven (650-720 w). After rinsing in PBS, the sections were exposed to 3% H$_2$O$_2$ for 30 minutes to quench endogenous peroxidase activity. Before incubation of primary antibodies, non-specific binding was blocked for 1 hour with 3% normal goat serum. The primary antibody that we used was Ki-67 monoclonal antibody (1:200 Master Diagnostica). The sections were incubated with secondary antibody anti-rabbit conjugated with biotin, 1:200 (Vector). After rinsing in PBS, the sections were incubated with avidin-biotin-horseradish peroxidase complex (Vector). 3',3'-diaminobenzidine (DAB) was used as a chromogen. Control slices, lacking primary or secondary antibodies, were analyzed with each series. The sections were studied using light microscopy. After washing steps, slides were incubated with different primary antibodies: nestin monoclonal antibody (1:100, Chemicon International Inc., Temecula, CA, USA), GFAP monoclonal antibody (1 µl/ml, Lab Vision Corporation) or DCX polyclonal antibody (1:200, Santa Cruz Biotechnology). After the washing steps, sections were incubated for 1 hour with a secondary antibody to study the differentiation of proliferating cells using CyTM 2-conjugated anti-mouse antibody (1:200, Jackson ImmunoResearch Laboratories, Inc., Baltimore Pike, USA). After washing in PBS, the sections were incubated in 4’,6-diamidino-2-phenylindole (DAPI) and mounted in glycerol medium. Samples were studied using fluorescence microscopy.

Within each time point, the microscopical findings were compared with the controls for the frequency of Ki-67 positive and apoptotic cells. The recordings were made by image analysis morphometry (Optimas, 6.2 software package, Optimas Corporation, Bothell, WA, USA) using a macro application and conducted by two investigators who were trained in morphometric determinations and had no knowledge of the experimental group from which each sample had been obtained. The means recorded by these investigators were regarded as final values. For each experimental group, the total number of immunopositive cells was averaged, and the values were expressed as count means ± standard deviations. We used the unpaired Student’s t-test for a comparison of the number of immunopositive cells at each time point in the two experimental groups.

**Results**

In our experimental model, all animals suffered ICH after intracranial administration of collagenase. The morphological study identified the LZ in the striatum as a central cavity surrounded by a scar wall (Fig. 1).

We first assessed the distribution of proteins DCX, nestin and GFAP in ICH injured brains compared to the control brains. Second, we studied the proliferation activity enhanced by the ICH at different time points. In our study, we investigated the phenotypic identity of the proliferating cells using a double staining method. Finally, we assessed the distribution and the identity of the apoptotic cells.

48 hours after ICH, an increase in the number of cells in the SVZ was observed compared with the control animals. We observed the presence of a large number of immunostained cells showing nestin expression in the SVZ compared to the control rats. No evidence of differences in the number of DCX-positive cells between both experimental groups was observed. At 72 HAH, the injured animals presented more nestin and DCX-positive cells in the SVZ than control animals. Furthermore, the striatum of injured animals showed significantly more nestin and DCX-positive cells than the control samples. At 7 and 14 days, the presence of a large number of immunostained cells showing nestin and DCX expression was found in the SVZ and LZ. At 21 DAH, we found that regions showing nestin and DCX-labeled cells were localized primarily in the SVZ. Figure 2 shows the immunohistochemical expression of nestin and DCX in the SVZ and LZ at the different time points.
following ICH.

Quantification of the proliferating cells in the SVZ and LZ

To estimate the survival of newborn cells during a short-term and long-term period, the number of KI-67 positive cells was analyzed in the SVZ and in the LZ at different time points. When evaluated 48 hours after ICH, approximately 4 times more KI-67 labeled (85±9) cells were detected in the SVZ in injured animals compared to the controls (20±3 cells) (p≤0.05), indicating an enhancement of proliferating activity. At 72 HAH, KI-67 positive cells in the injured animals were located throughout the LZ, SVZ and parenchyma. In the SVZ, the brains of injured rats had significantly (10 times) more KI-67-positive cells compared to the control brains (212±22 cells) (p≤0.05). 1 week following ICH numerous KI-67-positive cells were found within the striatum (298±32 stained cells) and had migrated into the parenchyma from the SVZ to LZ (Fig. 3). These KI-67 cells were located more medially than laterally and displayed a rostrocaudal-decreasing gradient. At 14 days, the SVZ and LZ of the injured animals contained a total of 150±21 and 108±7 KI-67-positive cells, respectively. At 21 DAH, the brains with the ICH had fewer labeled cells (70 ± 13 in LZ and 110 ± 11 in SVZ). However, this proliferating activity was not present in the LZ after 28 days post-ICH. At 6 and 12 months after ICH, the brains showed 121±31 and 128±29 KI-67-positive cells, respectively. Figure 4 shows KI-67-positive cells in the SVZ, brain parenchyma surrounding the LZ and the LZ at the different time points following ICH. Furthermore, an increase in proliferating (KI-67-positive) and migrating (DCX-positive) cells was

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**Fig. 1.** Macrophotographical views of the brains after ICH. These coronal sections show different cases of the animals in this study. Intracerebral hemorrhage and progressive reabsorption, leaving a residual cavity in the area of injury, can be seen.
**Fig. 2.** Immunohistochemical expression of nestin and doublecortin (DCX), at different time points after ICH. V: ventricle. LZ: lesion zone. SVZ: subventricular zone.

<table>
<thead>
<tr>
<th>48 HAH</th>
<th>72 HAH</th>
<th>7 DAH</th>
<th>14 DAH</th>
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<thead>
<tr>
<th>21 DAH</th>
<th>28 DAH</th>
<th>6 MAH</th>
<th>12 MAH</th>
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</table>

**NESTIN**

**DCX**

SVZ | SVZ | LZ | LZ

Subventricular zone.
observed in the contralateral SVZ (Fig. 5).

**Characterization of the proliferating cells in the SVZ and LZ**

Coupling KI-67 labeling with staining for DCX, nestin and GFAP is shown in Table 1 and Figure 6. In the first 3 days, KI-67 was primarily found in the SVZ. The identity of these proliferating cells was studied using a double staining with GFAP, nestin and DCX. During the first 3 days after ICH, the majority of KI-67-positive cells in the SVZ were GFAP-positive cells (Table 1). Colocalization of KI-67 with DCX increased from 7.40% in the first 24 hours after ICH to 37.03% in the third day post ICH, which was significantly different between both days. Throughout the first 72 hours, the quantity of cells coexpressing KI-67 and nestin decreased from 35.26% during the first 24 hours to 20.57% at 72 hours after ICH. However, these cells were more abundant in the LZ 72 hours after ICH.

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**Fig. 3. Immunohistochemical findings, 7 days after ICH.**

**A.** Doublecortin (DCX) positive cells migrating from SVZ to LZ, can be seen. **B.** KI-67 positive cells from SVZ migrating to LZ can be seen. LZ: lesion zone. V: ventricle.
Quantification of these results (Table 1 and Figure 6) is described in detail below. At 7, 14 and 28 days after ICH, KI-67-positive cells were found in the LZ. 7 days following ICH, KI-67-positive cells coexpressing DCX and nestin were found in the LZ, whereas GFAP was detected primarily in the lesion surroundings. At 7 DAH,

<table>
<thead>
<tr>
<th>Table 1. Number of KI-67-positive cells in the subventricular zone (SVZ), at 24, 48, and 72 hours after hemorrhage (HAH).</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVZ</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>24 HAH</td>
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<tr>
<td>48 HAH</td>
</tr>
<tr>
<td>72 HAH</td>
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</tbody>
</table>

This table shows the percentage of KI-67 positive cells coexpressing nestin (N), doublecortin (DCX) and Glial fibrillary Acidic Protein (GFAP). In brackets, the total number of double stained cells and standard deviation.

Fig. 4. Immunohistochemical expression of KI-67, at different time points after ICH. Images show KI-67 positive cells in SVZ (A, E, F, G, H), brain parenchyma surrounding LZ (B, C) and LZ (D). V: ventricle. LZ: Lesion zone. P: Brain parenchyma.
Endogenous neurogenesis after intracerebral hemorrhage

Fig. 5. A, B. Contralateral distribution of cells expressing KI-67 (A) or DCX (B) in SVZ, 7 days after hemorrhage. C, D. Distribution of cells expressing KI-67 (C) or DCX (D) in SVZ of a control animal (without hemorrhagic pathology). V: ventricle. Arrows show immunopositive cells.

Fig. 6. Phenotypic identification of the proliferating cells. KI-67-positive cells were co-stained for doublecortin (DCX), nestin (N) and GFAP, at 24, 48 and 72 hours after hemorrhage (HAH), in SVZ.
40% of KI-67-positive cells in the LZ were nestin positive cells. At 14 days, the LZ contained significantly fewer nestin-labeled cells (5%), but this area had 56% of DCX-positive cells. After 21 days post-ICH, the majority of KI-67-positive cells showed evidence of having acquired a neuroblast phenotype as measured by co-expression of DCX. Significant differences were observed in the number of DCX-positive cells at 14

Table 2. Number of KI-67-positive cells in lesion zone, at 7, 14 and 21 days after hemorrhage (DAH).

<table>
<thead>
<tr>
<th>Lesion zone</th>
<th>KI-67+ cells</th>
<th>KI67+ and DCX++</th>
<th>KI67+ and GFAP+</th>
<th>KI67+ and N+</th>
<th>Other KI+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 DAH</td>
<td>298±32</td>
<td>10% (29.6±7)</td>
<td>5% (14.9±3)</td>
<td>40% (119.2±11)</td>
<td>45% (134.2±12)</td>
</tr>
<tr>
<td>14 DAH</td>
<td>108±7</td>
<td>55% (59.4±3)</td>
<td>13% (14.04±2)</td>
<td>5% (5.4±2)</td>
<td>25% (27.3±4)</td>
</tr>
<tr>
<td>21 DAH</td>
<td>70±13</td>
<td>58% (40.6±5)</td>
<td>17% (11.9±3)</td>
<td>0.3% (0.21±0.1)</td>
<td>24% (16.8±2)</td>
</tr>
</tbody>
</table>

The table shows the percentage of KI-positive cells, coexpressing nestin (N), doublecortin (DCX) and Gliofibrillary Acidic Protein (GFAP). In brackets, the total number of double stained cells and standard deviation.

Table 3. Number of apoptotic cells in lesion zone.

<table>
<thead>
<tr>
<th>Lesion zone</th>
<th>F7-26+ cells</th>
<th>F7-26+ and DCX+</th>
<th>F7-26+ and GFAP+</th>
<th>F7-26+ and N+</th>
<th>Other apoptotic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 DAH</td>
<td>215±27</td>
<td>11% (23.65±3)</td>
<td>22% (47.3±6)</td>
<td>24% (51.6±7)</td>
<td>41% (88.15±7)</td>
</tr>
<tr>
<td>21 DAH</td>
<td>136±22</td>
<td>25% (34.0±4)</td>
<td>17% (23.12±3)</td>
<td>26% (35.36±4)</td>
<td>30% (40.8±6)</td>
</tr>
</tbody>
</table>

This table shows the percentage of apoptotic cells coexpressing nestin (N), doublecortin (DCX) and Gliofibrillary Acidic Protein (GFAP). In brackets, the total number double stained cells and standard deviation.

**Fig. 7.** Phenotypic identification of the proliferating cells (KI-67 positive cells) in LZ. Proliferating cells were co-stained for DCX, nestin (N) and GFAP, at 7, 14 and 21 days after hemorrhage (DAH).
DAH and 28 DAH in the LZ. The distribution of the proliferating and GFAP-positive cells was homogeneous in the LZ throughout the last 3 weeks. The majority of these cells remained in the lesion surroundings. Quantification and images of these results (Table 2; Figs. 7, 8) are described below in detail.

**Characterization of the apoptotic cells in the LZ**

To examine newborn cell survival, we studied and characterized apoptotic cells. At 24, 48, 72 hours and 7 days after ICH, the brains of the injured rats had very few apoptotic cells (data not shown). As expected, more apoptotic cells were present at 14 DAH. At 14 days, the injured animals had 10 times more apoptotic cells than at 7 days after ICH. Double immunofluorescence studies revealed that approximately 11% of the apoptotic cells expressed DCX, a marker for migrating neuroblasts, while 22% expressed GFAP, and 24% expressed nestin. At 21 DAH, the number of apoptotic cells was lower than at 14 DAH (136±22 and 215±27 cells, respectively). Overall, 25% of the apoptotic cells showed were immunopositive for DCX, 26% for nestin and 17% for GFAP. Interestingly, 1 month after ICH, there were 4 times fewer apoptotic cells than at 21 days (data not shown). At 6 and 12 months after ICH, the difference between the apoptotic cells in the LZ of the injured and control animals was similar in the two studies (25±12 and 32±11 cells, respectively) (Table 3; Fig. 9).

**Discussion**

Although recently the majority of studies investigating neurogenesis after stroke are based on an ischemic model, the aim of this study was to ascertain the endogenous neurogenesis after ICH. Hemorrhagic stroke often requires emergency surgery, and the possibility of implementing new techniques, such as the application of cell therapy, when surgical intervention is performed (Otero et al., 2010) suggests the need to know the normal regenerative response of the brain after this
In our present study, we investigated endogenous neurogenesis after ICH in the SVZ, because previous studies have shown that the adult SGZ is a less likely source than the SVZ of neuronal precursors that migrate into injured brain areas after focal cerebral brain injury (Jin et al., 2003). Using the Ki-67 antigen, we quantified the total number of proliferating cells in the SVZ and in the lesioned striatum at different time points after ICH. The identity of these proliferating cells was determined using GFAP, nestin, and DCX for immunocytochemistry. The results indicate that ICH induced a significant proliferation of cells within the SVZ and the LZ. Our results were consistent with previous studies, showing that focal cerebral ischemia or traumatic brain injury induced proliferation and differentiation of precursor cells in the SVZ (Zhang et al., 2001; Chirumamilla et al., 2002). The induced stem cell division was most prominent between 72 hours to 7 days following ICH, but it decreased by 80% at 28 days. However, a small level of cell proliferation continues up until 12 months after ICH. These newborn cells can differentiate, as shown by their expression of precursor markers of immature neurons. In our experimental animal model, the ICH significantly increased the number of newly produced cells in the vicinity of the lateral ventricle, and neurogenesis was present in the SVZ. Proliferation was increased 48 hours after ICH in the SVZ. First, the proliferating stem cells in the SVZ co-expressed GFAP 72 hours after ICH and the proliferating cells increased their expression of nestin and DCX in the SVZ. There are many molecular candidates for stimulating this process, including basic fibroblast growth factor, epidermal growth factor, insulin like growth factor-1, sonic hedgehog and brain-derived neurotrophic factor (BDNF). An increased production of new brain cells after stroke can also be attributed to a reduction in the G1 phase of the cell cycle. The observation of a stroke- or cerebral injury-mediated increase in the production of brain cells is also consistent with the concept that the injured brain mimics an earlier stage of development (Chopp et al., 2007). Secondly, 7 days after ICH, the majority of the proliferating cells were found near the LZ. The Ki-67 positive cells from the areas surrounding the lesion co-expressed GFAP. They were present both inside and surrounding the lesion. However, there were no GFAP cells within the lesion. Nevertheless, in the LZ, proliferating cells co-expressed nestin and DCX. With regard to the possible origin of these cells, some studies have reported that DCX-expressing cells from the SVZ migrated to the lesioned striatum via the rostral migratory stream (RMS) and along the embryonic lateral cortical stream. These neurons appear to arise primarily in the SVZ, a site that normally provides neurons to the OB of the adult brain (Jin et al., 2003).

Notably, in contrast to proliferation, migration does not appear to be symmetrical around the lesion. GFAP-positive cells localized to the surroundings of the lesion, and the neuroblasts localized in the lesion core. In the SVZ and RMS of the normal adult brain, neuroblasts showed chain migration where they migrated in association with each other inside glial tunnels, which enabled their efficient migration into the mature brain tissue. In the developing cerebral cortex, new neurons use the fibers of radial glial cells as a scaffold for their migration (Krupinski et al., 1994; Zhang et al., 2004; Robin et al., 2006). Because the adult striatum does not contain radial fibers or glial tubes the newly generated neuroblasts need an alternate physical scaffold. Consistent with this migratory role, it has been previously suggested that GFAP-positive cells may have similar properties to radial glia, which facilitate migration (Leavitt et al., 1999) and serve as a pathway for newly recruited cells, guiding them to the lesion or its proximity. Perhaps these distinct regions of astrocytes serve as a source of growth factors or a conduit for migrating cells from distant sites of RMS. Whereas neuroblasts migrate through the brain parenchyma using chain migration, a mechanism that allows migration of neuroblasts without the use of a glial scaffold (Garcia-Verdugo et al., 1998), migration of neuroblasts through the brain parenchyma might require radial glial-like support cells. Thus, it may be that the proximal astrocytes serve as both a source for migratory...
and differentiation signals (Menet et al., 2001) and a conduit or substrate for guided migration to the lesion site. However, it is not known why new neurons are not born directly in the place they need to reside. Progenitor cell migration may provide an additional level of control for cell positioning. The maintenance of stem cell niches also represents a potential source of cells for brain repair, but it may not be beneficial for the organism. The stem cell niche also requires specific features that restrict the structures where they can persist, which implies that cells need to be able to migrate from these discrete niches to their final destination (Cayre et al., 2009).

Some studies have suggested that cells are newly generated in the striatum of normal adult animals and that a distinct subset of these cells develop a mature neuronal phenotype. This finding suggests that DG and SVZ are not the only forebrain areas that generate new neurons throughout adult life. It is also possible that newly generated striatal cells result from the division of in situ progenitor cells in the striatal parenchyma because multipotent progenitor cells have been shown to reside in the striatum of adult rodents (Bedard et al., 2002, 2005; Kronenberg et al., 2005; Burns et al., 2007; Shimada et al., 2010).

After ICH it is likely that a synergistic function occurs. First, it promotes neurogenesis in the SVZ and the migration of the neuroblast to the damaged striatum. Second, new neurons could be produced in the striatum to replace the dead neurons in the injury.

In contrast, in our present study, SVZ activation has been observed not only in SVZ of ipsilateral hemisphere but also in contralateral SVZ and was characterized by an increase in cell proliferation and migrating neuroblasts (high number of KI-67- and DCX-positive cells). Although the exact mechanism underlying this reaction has not been clarified, 3 possibilities can be considered: a) factor(s) from the lesion site might disperse and affect many brain areas; b) commissural fibers might participate in this reaction; and c) hormonal factor(s) from other tissues or cytokines from the immune system might be involved (Masuda et al., 2007). Compared with the controls, ICH resulted in a large increase in the rate of production of new cells as indicated by KI-67 labeling. Unfortunately, we found a decrease over time, which was consistent with the post-injury period. Thus, from 21 days after ICH, non-proliferating cells remained in the LZ. Furthermore, at the same time, we found an important loss due to cell death.

In addition, we found that the ICH induced activation of apoptosis in the striatal region from 14 days to 28 days after injury. Active apoptosis in the LZ was co-localized with nestin, suggesting that apoptosis could mediate the loss of differentiation of neurogenic cells from 14 DAH, which was consistent with previous results where the majority of newborn cells die while they migrate toward the injured parenchyma via a caspase-dependent apoptotic process (Arvidsson et al., 2002; Zhang et al., 2006).

Despite the fact that newborn cells from several brain regions are able to form both neuronal and astroglial lineages (Jori et al., 2003), the majority of the surviving cells in our study had differentiated toward a neuronal phenotype in the LZ at 14 DAH, as demonstrated by DCX/KI-67 dual-positive cells in the injured animals. A large number of newly generated neurons in the adult are believed to undergo programmed cell death rather than achieving maturity (Morshead and van der Kooy, 1992). However, few of the DCX-immunopositive cells that we observed in the injured striatum underwent apoptosis. Therefore, the DCX-immunopositive cells that arose from hemorrhage-induced SVZ neurogenesis survived to reach injured regions.

As noted above, ICH induced endogenous neurogenesis, but the majority of the newborn cells did not survive longer than 3 weeks due to apoptosis. Enhancing endogenous neurogenesis and inhibiting apoptosis in the context of ICH-induced neurogenesis might provide an opportunity for functional repair and a therapeutic outcome in the wake of ICH.

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Doetsch F., Caille I., Lim D.A., Garcia-Verdugo J.M., and Alvarez-Buylla


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