Dexamethasone Prevents Cerebral Infarction Without Affecting Cerebral Blood Flow in Neonatal Rats

U.I. Tuor, PhD; C.S. Simone, BSc; J.D.E. Barks, MD; and M. Post, PhD

Background and Purpose: We recently demonstrated that pretreatment with the synthetic glucocorticoid dexamethasone prevents hypoxic-ischemic brain damage in neonatal rats. Presently, we examine whether this protective effect of dexamethasone is due to an improvement in local cerebral blood flow.

Methods: Neonatal rats were treated with either vehicle or 0.1 mg/kg i.p. dexamethasone 24 hours before hypoxia-ischemia (right carotid artery occlusion+3 hours of 8% O₂). Cerebral blood flow was measured with [¹⁴C]iodoantipyrine autoradiography after either 2 (n=17) or 3 (n=15) hours of hypoxia-ischemia. Additional animals (n=20) were perfusion-fixed 3 days after hypoxia-ischemia. The area of cerebral pathological changes was measured from hematoxylin and eosin–stained coronal sections taken at three different levels.

Results: Pathological outcome differed between groups. In vehicle-treated rats, sections from anterior, mid, and posterior portions of the cerebrum all had extensive infarction or cellular necrosis ipsilateral to the occlusion (mean areas of damage were 62.6±10%, 70.2±9%, and 54.2±8%, respectively). However, in dexamethasone-treated animals, brain damage in sections at corresponding levels was minimal (0%, 1.6±2%, and 1.5±1%, respectively; p<0.0002). In contrast to the pathological results, cerebral blood flow was equivalent in the dexamethasone- and vehicle-treated groups. After either 2 or 3 hours of hypoxia, cerebral blood flow was reduced 60–80% ipsilateral to the carotid artery occlusion in animals treated with either vehicle or dexamethasone.

Conclusions: Despite ischemic levels of cerebral blood flow, pretreatment with dexamethasone prevents cerebral damage in neonatal rats. Instead of improving local cerebral perfusion, dexamethasone presumably acts via peripheral or central glucocorticoid receptors to produce some alteration in the brain that decreases its susceptibility to hypoxia-ischemia. (Stroke 1993;24:452–457)

KEY WORDS • cerebral blood flow • dexamethasone • neuroprotection • rats

We demonstrated recently that glucocorticoid administration 24 hours but not immediately before an episode of cerebral hypoxia-ischemia has a surprising effect in that it prevents the pathological changes associated with a neonatal model of hypoxia-ischemia. The mechanism by which the synthetic glucocorticoid dexamethasone acts to protect the brain from hypoxic-ischemic damage remains to be identified. Since the pathogenesis of cerebral necrosis in the model of hypoxia-ischemia employed has previously been shown to be related to a reduction in local cerebral blood flow ipsilateral to the occlusion, an obvious mechanism of action for dexamethasone is to ameliorate the degree of ischemia ipsilateral to the occlusion (e.g., via reductions in cerebrovascular resistance or...
the tail vein and glucose oxidase reagent strips (Chemstrip bG, Boehringer Mannheim, Mannheim, FRG).

The rats were subjected to hypoxia-ischemia on the seventh day of life as described in detail previously. Briefly, they were prepared for surgery by infiltrating the incision site with 2% lidocaine and injecting 0.1 ml i.p. saline. The rats were anesthetized with halothane (3–4% for induction, ½–1% for maintenance), and the right carotid artery was isolated and ligated with 5-0 silk suture. A 3-hour recovery period with the dam was followed by 3 hours in a hypoxic chamber (8% O2/92% N2 at 37°C). This consistently produces neuronal damage with infarction of the striatum, thalamus, hippocampus, and overlying cortex ipsilateral to the ligation. The technique used to measure local cerebral blood flow in 7-day old rats is similar to that developed by Lyons et al12 and uses methods based on those established for adult rats. Briefly, 0.1 ml s.c. saline containing 7 μCi of the blood flow tracer 4-[N-methyl-14C]iodoantipyrine (New England Nuclear-Du Pont, Mississauga, Canada) was injected in the back. The tracer was gradually absorbed into the systemic circulation, and 2 minutes after injection the animal was decapitated and a blood sample was collected from the severed trunk vessels. The concentration of tracer in the blood sample was determined using liquid scintillation counting methods. The brain was removed, frozen, and processed for autoradiography as described elsewhere. Briefly, brain sections 20 μm thick were cut semiserially in a cryostat, mounted on coverslips, and dried on a hot plate. Autoradiograms were obtained by applying x-ray film (OMC1, Kodak, Rochester, N.Y.) to the sections and a series of 16 calibrated standards (0–1,260 nCi/g).

Calculation of local cerebral blood flow according to the theory for the measurement of flow with a freely diffusible tracer requires knowledge of the final concentration of tracer in the tissue, the arterial input curve, and the partition coefficient. Local tissue concentrations of tracer were determined from the average of unilateral measurements from six sections. Gray levels were converted to tissue concentrations by using the gray levels of the standards and software provided with a microcomputer-based image analysis system (MCID, Imaging Research Inc., St. Catharines, Canada). The shape of the blood saturation curve, as tracer was absorbed systemically, was determined using 22 additional rats (either litter mates or pups of similar weights from other litters). These animals were treated with vehicle or dexamethasone and killed during hypoxia 0, 40, 60, 80, 100, 110, 120, or 130 seconds after subcutaneous injection of the tracer. A plot of kill time versus concentration of tracer in the blood provided an input curve that was similar to that of Lyons et al12 and was equivalent in vehicle- and dexamethasone-treated rats. For each blood flow experiment, the blood concentration at the kill time was used to estimate an input curve with a profile proportional to that determined from the additional experiments. Local cerebral blood flow was calculated using this input curve, the local tissue concentration of tracer measured from the autoradiograms, and the blood–brain partition coefficient for iodoantipyrine (1.0 in the rat pup12).

Cerebral pathological change was assessed in animals surviving 3 days after the hypoxia-ischemia. Under deep anesthesia produced with 50 mg/kg pentobarbital, the brains were perfusion-fixed with 10% buffered formalin, removed, and placed in formalin for several days. The presence or absence of gross cortical infarction was noted upon inspection of the brains at the time of removal. The cerebrum was sliced into three equal blocks, embedded in paraffin, and sectioned with a microtome, and the coronal sections were stained with hematoxylin and eosin. The extent of pathological damage was quantified by an investigator blinded to treatment. The total area of brain in the contralateral hemisphere and the ipsilateral area of intact brain at anterior, mid, and posterior levels of the cerebrum (approximately 2, –2, and –6 mm from the bregma) were measured using an image analysis system (MCID). The difference between the total contralateral and ipsilateral areas provided the area of infarction or necrosis at each level.

Statistical analyses were performed with the SAS statistical program for personal computers (SAS Institute Inc., Cary, N.C.). Means of variables measured in vehicle- and dexamethasone-treated rats were compared using Student's two-tailed t test. Differences were considered significant at p<0.05. Nonparametric values were compared with Fisher's exact test.

Results

Local cerebral blood flow was reduced ipsilateral to the carotid artery ligation in the distribution of the middle cerebral artery territory in both vehicle- and dexamethasone-treated rats (Figure 1). There was a distinct regional distribution of the ischemia ipsilateral to the occlusion that corresponded closely to the regional distribution of brain damage observed in perfusion-fixed brains. The greatest reductions in cerebral blood flow occurred in the cortex and lateral parts of the striatum and thalamus, with the least marked reductions in flow occurring in structures closer to the midline.

Quantitative analysis of the autoradiograms demonstrated that blood flow at either 2 or 3 hours of hypoxia was similar in rats pretreated with vehicle or dexamethasone (Figure 2). Since some of the interanimal variability in absolute blood flow values might have obscured subtle ipsilateral/contralateral differences in flow between treatment groups, we also examined the effect of treatment on left/right differences in flow (data not shown). Comparison of these left/right differences in flow demonstrated further convergence rather than a divergence in the similarity of the results between groups.

A comparison of blood flow at the two different times studied indicated that, although there was a trend for the level of flow to be lower at 2 hours than at 3 hours of hypoxia, the overall flow responses were similar at the two times (Figure 2). The majority of flows (26 of 28) on either side in dexamethasone- or vehicle-treated rats were similar at 2 and 3 hours (p>0.05), with the exceptions being the contralateral septal nucleus and corpus callosum of dexamethasone-treated animals (p=0.04 and p=0.03, respectively). In all regions, the percent left/right differences in flow at 2 hours were equivalent to those at 3 hours of hypoxia.

Several of the measured parameters differed in dexamethasone- and vehicle-treated rats. Glucocorticoid treatment reduced the rate of somatic growth since the
increase in body weight of animals treated with vehicle exceeded that of those treated with dexamethasone (Table 1). Blood glucose levels also differed. Before hypoxia, blood glucose concentrations were similar in vehicle- (n=13) and dexamethasone- (n=11) treated rats (4.5±0.4 and 4.0±0.4 mM, respectively), but after hypoxia the blood glucose concentration was reduced in vehicle-treated animals and increased in dexamethasone-treated animals (2.5±0.5 and 7.9±1.2 mM, respectively; p<0.002). Simple visual examination of the brains for gross pathological damage demonstrated that dexamethasone was effective in preventing infarction ipsilateral to the carotid artery ligation (Table 1).

Histological examination of the brains confirmed the marked neuroprotective effect of dexamethasone (e.g., Figure 1). The majority of vehicle-treated rats had areas of infarction or cellular necrosis ipsilateral to the occlusion. The cerebral cortex was the region most severely damaged, with the striatum, thalamus, and hippocampus also being affected in a large majority of vehicle-treated animals. Infarction consistently occurred in areas that had the lowest blood flow. In contrast to vehicle-treated rats, only a small area of pseudolaminar necrosis was observed in the cortex of one animal treated with dexamethasone. Neuronal necrosis or extensive cell loss were not evident in other regions such as the hippocampus. Quantitative analysis of the damage in sections from anterior, mid, and posterior portions of the cerebrum (Figure 3) demonstrated that in vehicle-treated rats a majority of the hemisphere was damaged ipsilateral to the occlusion (62.6±10.2%, 70.2±9.5%, and 54.2±8.5%, respectively) whereas in dexamethasone-treated animals damage was minimal (0%, 1.6±2%, and 1.5±1.0%, respectively; p<0.0002).

Figure 1. Photomicrographs of two representative histological sections stained with hematoxylin and eosin (H&E) and four cerebral blood flow (CBF) autoradiograms from six rats. CBF was measured after 2 hours of hypoxia, whereas H&E-stained sections were obtained from animals perfusion-fixed 3 days after hypoxia-ischemia. Neonatal rats were pretreated with either 0.1 ml vehicle (Veh) (top row) or 0.1 mg/kg i.p. dexamethasone (Dex) (bottom row) 24 hours before hypoxia. Rat treated with vehicle has extensive infarction or necrosis throughout cortex and striatum, whereas rat treated with dexamethasone has no evidence of cerebral damage (left column). CBF at level of striatum (center column) or anterior hippocampus (right column) is reduced markedly ipsilateral to carotid artery occlusion in both dexamethasone- and vehicle-treated animals.

Figure 2. Bar graphs demonstrating mean±SEM local cerebral blood flow in 7-day-old rats subjected to either 2 or 3 hours of hypoxia-ischemia (right carotid artery occlusion +8% O2). Cerebral blood flow was equivalent in vehicle- (filled bars) and dexamethasone- (shaded bars) treated groups whether duration of hypoxia was 2 hours (upper panel; for vehicle, n=9; for dexamethasone, n=8) or 3 hours (lower panel; for vehicle, n=7; for dexamethasone, n=8). White, corpus callosum; hippo, hippocampus; thal, thalamus; cortex, parietal cortex; caud, caudate; sept, septal nucleus; hyp, hypothalamus.
Table 1. Body Weight and Incidence of Pathological Change in Rats Treated With Vehicle or Dexamethasone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Day 6 (g)</th>
<th>Day 7 (hypoxia) (g)</th>
<th>Growth days 6–7 (g)</th>
<th>Pathology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF 2 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
<td>11.2±0.3</td>
<td>12.4±0.5</td>
<td>1.1±0.2</td>
<td>–</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>8</td>
<td>11.6±0.4</td>
<td>11.5±0.7</td>
<td>−0.1±0.4*</td>
<td>–</td>
</tr>
<tr>
<td>CBF 3 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>12.0±0.6</td>
<td>13.4±0.6</td>
<td>1.4±0.2</td>
<td>–</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>8</td>
<td>12.2±0.6</td>
<td>12.2±0.6</td>
<td>0.0±0.2*</td>
<td>–</td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>11</td>
<td>11.3±0.5</td>
<td>12.1±0.6</td>
<td>0.8±0.4</td>
<td>91</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>9</td>
<td>10.4±0.6</td>
<td>10.1±0.6†</td>
<td>−0.3±0.1†</td>
<td>0§</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n, number of rats. Pathology, cerebral cortical infarction ipsilateral to carotid artery occlusion; CBF, cerebral blood flow.

*p<0.01, †p<0.05 different from vehicle treatment by Student's t test.

Posthypoxia growth was similar between groups, with body weight at 10 days increasing to 20.5±1.6 g and 17.0±2 g in vehicle- and dexamethasone-treated groups, respectively.

§p<0.0001 different from vehicle treatment by Fisher’s exact test.

Discussion

In this study we have confirmed what was originally a surprising result by demonstrating that pretreatment of neonatal rats with dexamethasone prevents infarction. The present demonstration that local cerebral blood flow is similar in dexamethasone- and vehicle-treated animals is a crucial first step in better understanding the mechanism involved in dexamethasone's protection against brain damage due to hypoxia-ischemia. Rather than increasing cerebral perfusion via an effect on the systemic or cerebral circulations, dexamethasone treatment appears to act via another glucocorticoid-mediated effect that in some way alters susceptibility of the brain to hypoxic-ischemic damage.

Assessment of brain damage by visual inspection demonstrated that there was a high (91%) incidence of cerebral damage in vehicle-treated, control rats. This incidence of damage is similar to that observed previously by ourselves and others with this neonatal model of hypoxia-ischemia. In contrast to the vehicle-treated animals, rats treated with dexamethasone had no visual evidence of infarction, which is similar to our previous finding that dexamethasone administered 24 hours before hypoxia-ischemia prevents gross pathological damage in the cerebral cortex. The present study also included a morphometric analysis of hematoxylin and eosin-stained sections clearly demonstrating that dexamethasone treatment results in a striking reduction in damage throughout the cerebrum.

The relation between ischemia and cerebral pathology has been examined previously by others in this neonatal model of cerebral hypoxia. Originally, qualitative studies of cerebral blood flow demonstrated that the distribution of cerebral damage corresponded closely to a reduction in cerebral perfusion, suggesting that the injury observed is largely related to ischemia. Subsequently, using quantitative autoradiographic methods, Vannucci et al demonstrated a clear correlation between regions with the most severe blood flow reductions and those with the greatest sensitivity to pathological damage. In the present study, the distribution of damage also correlated well with the pattern of ischemia.

The results of several studies suggest that the level of ischemia is rather stable in this model of hypoxia-ischemia. Silverstein et al found that there was a relatively constant 60% left/right difference in brain [14C]iodoantipyrine concentrations between 30 minutes and 2 hours of exposure to hypoxia. Vannucci et al reported no difference in blood flow between 1 and 2 hours of hypoxia in most regions examined, except perhaps for a modest reduction in cortical blood flow. In addition, equivalent levels of low flow ipsilateral to the occlusion have been observed by Ringel et al at 1, 2, and 3 hours of hypoxia. In the present study, cerebral blood flows at 2 and 3 hours of hypoxia were similar, again supporting a rather uniform severity of ischemia with time in this model.

The major objective of this study was to determine whether in our experiments dexamethasone has an effect on cerebral blood flow. The results of previous studies investigating glucocorticoid influences on cerebral or spinal cord blood flow under normal and patho-

Figure 3. Bar graph showing cross-sectional area of damaged tissue ipsilateral to right carotid artery occlusion (infarcted) and total area of tissue measured contralaterally (total). Area of infarcted tissue ipsilateral to occlusion at anterior (ant), mid, and posterior (post) levels of cerebrum is markedly reduced in rats pretreated with 0.1 mg/kg dexamethasone (shaded bars, n=9) compared with those pretreated with vehicle (filled bars, n=11). All data presented as mean±SEM. *p<0.0002 different from vehicle.
logical conditions have been contradictory. Increases in spinal cord blood flow and improvements in cord perfusion have been observed after experimental spinal cord trauma and high-dose glucocorticoid therapy.18,19-21 Similarly, in patients with cerebral tumors increases in cerebral blood flow within both hemispheres have been observed following dexamethasone treatment at rather high doses of 1–0.5 mg/kg/day.18 Some of these blood flow increases have been suggested to be due to direct decreases in cerebrovascular resistance by dexamethasone. In contrast, other investigators have reported no change in cerebral blood flow following intracarotid administration of dexamethasone19 or a decrease in blood flow following dexamethasone treatment in tumor patients.20 In addition, Wagnerle et al21 recently examined the effect of dexamethasone on pial vessel reactivity in newborn piglets and found no change in diameter but a reduction in dilatatory reactivity to hypercapnia. The present results demonstrate that cerebral blood flow is similar in vehicle- and dexamethasone-treated rats during either hypoxia (contralateral hemisphere) or hypoxia+ischemia (ipsilateral hemisphere). A single injection of dexamethasone appears to have no major effect on cerebral blood flow 24 hours later.

The present results also support the likelihood that blood pressure is similar in dexamethasone- and vehicle-treated animals. Clinical studies had indicated that hypertension may be an important side effect of dexamethasone treatment, particularly at the higher doses used to treat bronchopulmonary dysplasia (0.5 mg/kg/day).4,5 However, the fact that heart rate is similar in vehicle- and dexamethasone-treated rats during hypoxia suggests that blood pressure may also be comparable in the two groups.1 This is now further supported by the present demonstration of equivalent cerebral perfusion in the two treatment groups.

To conclude, an improvement in cerebral blood flow following dexamethasone administration does not account for the marked improvement in pathological outcome with dexamethasone treatment. Instead, dexamethasone must have another glucocorticoid-mediated effect that alters one or more of the biochemical processes in the brain involved in hypoxic-ischemic cell death. Since glucocorticoids have a large number of physiological and biochemical effects, there are many different potential mechanisms, several of which may ameliorate hypoxic-ischemic brain damage in the neonate. For example, hyperglycemia, which occurred in the dexamethasone-treated rats during hypoxia,1 tends to exacerbate ischemic damage in adults but may be beneficial in young animals.22-25 As observed in the present study, dexamethasone also inhibits somatic growth, and studies by others suggest that growth retardation may either reduce brain damage caused by hypoxia-ischemia in neonatal rats26 or increase damage in growth-retarded neonborn piglets.27 Irrespective of its overall physiological effect, dexamethasone likely binds to glucocorticoid receptors within the cell, and the hormone receptor complex then interacts with specific regulating DNA elements. This results in either positive or negative control of gene expression. Indeed, the fact that dexamethasone requires at least 3 hours to induce its effect1 suggests that a minimum period of time is required, perhaps for the synthesis of some protein involved in the neuroprotective effect. Indeed, other studies have demonstrated that the beneficial effect of dexamethasone on cerebral edema is prevented by a blockade of de novo protein synthesis.28,29 Clearly, there are several possible ways in which dexamethasone may produce its novel neuroprotective effects, and further experiments are required to identify the mechanism involved.

Acknowledgments

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Glucocorticoids can reduce edema associated with tumors and improve outcome in spinal cord injury. Although several animal studies of cerebral ischemia have found that brain edema is reduced by corticosteroids, in most studies they were ineffective in reducing or even potentiated the resulting brain damage. Thus, the recent report by Barks et al., which demonstrated that pretreatment with low doses of dexamethasone afforded striking protection in the neonatal rat hypoxia-ischemia model, was somewhat surprising. The study reported by Tuor et al. (the same group) replicated these results using quantitative histological analysis of the brain lesion. Additionally, they clearly demonstrated that the beneficial effect of the corticosteroid was not due to a smaller reduction in cerebral blood flow during the period of hypoxia-ischemia. Since postischemic hypoperfusion is not seen in this model, alteration of cerebral blood flow by dexamethasone is an unlikely explanation for its neuroprotective action.

Thus, a multitude of possible mechanisms remain. The effect of dexamethasone on somatic growth and blood glucose levels suggests the possibility that systemic changes could somehow provide protection in this model, which is known to be fairly sensitive to the conditions required to produce the insult. The blood-brain barrier of the neonatal rat has not reached adult function in regard to its passive permeability and transport properties. It is well documented that dexamethasone reduces blood-brain barrier permeability in normal and ischemic brain, and it may be that this action has greater impact on the outcome following hypoxia-ischemia when the barrier is less mature. Future studies aimed at understanding the developmental factors that influence the responses to ischemia may provide new insights into the pathophysiological process itself.

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Reference
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