Effect of hyperglycemia on progressive paraparesis in a rat metastatic spinal tumor model

Laboratory investigation

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Object. Hyperglycemia has been shown to potentiate ischemic injury of the spinal cord by quenching vasodilators and potentiating tissue acidosis and free radical production. Steroid-induced hyperglycemia is a common event in the surgical management of metastatic epidural spinal cord compression (MESCC). The goal in this study was to determine whether experimentally induced hyperglycemia accelerates neurological decline in an established animal model of MESCC.

Methods. Sixteen Fischer 344 rats underwent a transabdominal approach for implantation of a CRL-1666 breast adenocarcinoma cell line within the vertebral body of L-6. After 72 hours of recovery from tumor implantation, the animals received intraperitoneal injections every 12 hours of either 2 g/kg dextrose in 5 ml 0.09% saline (hyperglycemia, 8 rats) or 5 ml 0.09% saline alone (normoglycemia, 8 rats). Weights were taken daily, and the hindlimb function was tested daily after tumor implantation by using the Basso-Beattie-Bresnahan (BBB) scale (score range 1–21). Animals were killed at time of paralysis (BBB Score < 7), and the volume of epidural tumor growth within the spinal canal was measured. To determine the degree of hyperglycemia induced by this dextrose regimen, a surrogate group of 10 Fischer 344 rats underwent intraperitoneal injections of 2 g/kg dextrose (5 rats) or 0.09% saline (5 rats) every 12 hours, and serum glucose levels were assessed 1, 3, 6, 8, 10, and 12 hours after injections for 24 hours.

Results. Dextrose versus saline injections resulted in elevated mean serum glucose at 3 (259 vs 103 µg/dl), 6 (219 vs 102 µg/dl), 8 (169 vs 102 µg/dl), and 10 hours (118 vs 99 µg/dl) after injection, returning to normal levels by 12 hours (96 vs 103 µg/dl) just prior to subsequent injection. All rats had normal hindlimb function for the first 8 days after tumor implantation. Hyperglycemic versus normoglycemic rats demonstrated a worsened median BBB score by postimplantation Day 9 (Score 20 vs 21, p = 0.023) through Day 16 (Score 8 vs 12, p = 0.047). Epidural tumor volume demonstrated a near-linear growth rate across both groups; however, hyperglycemic rats developed paralysis earlier (median 15.5 vs 17.5 days, p = 0.0035), with significantly less epidural tumor volume (2.75 ± 0.38 cm³ vs 4 ± 0.41 cm³, p < 0.001) at time of paralysis.

Conclusions. In a rat model of metastatic epidural spinal cord compression, rats maintained in a hyperglycemic state experienced accelerated time to paralysis. Also, less epidural tumor volume was required to cause paralysis in hyperglycemic rats. These results suggest that hyperglycemic states may contribute to decreased spinal cord tolerance to compression resulting from MESCC. Clinical studies evaluating the effect of aggressive glucose control in patients with MESCC may be warranted. (DOI: 10.3171/2008.10.SPI08333)

Key Words • glucose • hyperglycemia • metastatic spinal tumor • rat

Approximately 1.4 million patients receive a cancer diagnosis annually in the US, and half a million of them die of metastatic disease. The spinal column is the most common site of skeletal metastasis, which occurs in up to 40% of patients with preexisting nonskeletal bone metastasis. Because survival times for patients with metastatic cancer are increasing, the incidence of symptomatic spinal metastatic disease can be expected to increase as well.

Metastatic epidural spinal cord compression is a debilitating complication of spinal metastasis occurring in up to 14% of cancer patients, resulting in progressive paraparesis in many of them. Although radiotherapy and resection remain the mainstay of therapy for MESCC, corticosteroids are widely used in the perioperative management of this disease. Frequent steroid-induced hyperglycemia as well as diabetic hyperglycemia aggravated by systemic oncological stressors have made...
perioperative occurrences of this entity a common event in the management of MESCC.22

Hyperglycemia may have detrimental effects on spinal cord tolerance to compression and ischemic injury from MESCC. Hyperglycemia has been shown to potentiate ischemic damage after CNS injury by quenching important vasodilators, potentiating lactate accumulation, worsening tissue acidosis, and increasing free radical–induced reperfusion injury.11,14,18,26,31 Furthermore, studies of ischemic SCI have suggested that induced hypoglycemia attenuates ischemic SCI.35,37 However, the effect of hyperglycemia on spinal cord compression remains unknown.

In the present study, we use an established animal model of metastatic spinal tumors to determine whether experimentally induced hyperglycemia accelerates neurological decline resulting from MESCC.3,4,15,28

Methods

Animals and Study Design

Twenty-six 10-week-old female Fischer 344 rats (Charles River Laboratories) weighing between 200 and 250 g were used for this experiment. Animals were maintained in standard facilities, 4 rats per cage, and given free access to Baltimore city water and rodent chow. All experimental protocols were approved by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine.

Experiment 1

To determine the effect hyperglycemia itself may have on neurological hindlimb function, 10 animals underwent serum glucose testing and hindlimb neurological assessment while being maintained in either a hyper- or normoglycemic state (5 rats each). Animals were given intraperitoneal injections of either 2 g/kg dextrose in 5 ml of 0.09% saline or 5 ml of 0.09% saline alone every 12 hours. To assess the degree of hyperglycemia induced by this cyclical injection regimen, serum glucose was measured from tail-vein blood samples obtained 1, 3, 6, 8, 9, 10, and 12 hours after each injection for the first 24 hours. Hindlimb function was assessed daily for 14 consecutive days during continued intraperitoneal dextrose or saline injections via the BBB locomotor rating scale.7 Serum glucose and BBB scores were compared between saline and dextrose cohorts. To determine whether dextrose injection–induced serum glucose levels would be affected by the presence of VB tumor, this experiment was then repeated in 10 rats (2 g/kg dextrose every 12 hours, saline every 12 hours, 5 rats each) receiving implantation of tumor into the L-6 VB.

Experiment 2

Sixteen Fischer 344 rats underwent implantation of CRL-1666 mammary adenocarcinoma into the L-6 VB. After 72 hours of recovery from tumor implantation, animals received intraperitoneal injections of either 2 g/kg dextrose in 5 ml 0.09% saline or 5 ml 0.09% saline alone (8 rats each) every 12 hours, as performed in the surrogate groups in Experiment 1. In pilot studies, intraperito-

M. J. McGirt et al.
Hyperglycemia and neurological decline in experimental spine metastasis

was continued dorsally (deep) until underlying bone was visualized. The Dumont #7 forceps were used to mobilize the tissue underneath the aorta and vena cava, exposing the intervertebral discs above and below the L-6 VB. A high-speed surgical drill (Dental Drill, Aseptico) was used in concert with a 2-mm bur to drill a cavity 1 mm deep into the VB for tumor implantation. A 1-mm³ tumor section was implanted entirely inside the hole in the VB and sealed into the bone with polymethyl methacrylate. Once the glue hardened, the retractor was removed, the abdominal muscles were sutured with 3-0 Vicryl (Ethicon), and the skin was closed with surgical autoclips.

Hindlimb Neurological Assessment

All animals underwent daily testing of hindlimb function following tumor implantation. Hindlimb function was graded by a single observer blinded to experimental group, and grading was performed according to the BBB locomotor rating scale.7 Briefly, the BBB scale includes 21 different levels of movement of the hindlimbs, and total scores range from 0 to 21 (0, no spontaneous movement; 21, normal locomotion). The animals were monitored for signs of poor grooming and the average weight of each group of animals was recorded twice weekly, and the individual rats were monitored for signs of weight loss. Animals were killed once neurological deterioration resulted in loss of ability to ambulate (BBB Score ≤ 7). Surgical inspection of the spinal cord and vertebral column was performed in all animals after they were killed, to confirm epidural tumor progression and spinal cord compression.

Epidural Tumor Volume at Time of Paralysis

Once neurological deterioration resulted in loss of ability to ambulate, surgical inspection of the spinal cord and vertebral column was performed to quantify volume of intracanalicular epidural tumor volume resulting in paralysis. A midline skin incision was made over the L5–S1 spinal segments, and the paravertebral muscles were dissected from the underlying laminae. The L5–S1 laminec- tomies were then performed and the compressed spinal cord was removed. The L5–S1 VBs were then observed and epidural tumor within the spinal canal was easily rec- ognized by its darkened color and the protuberance at the L-6 VB. The L5–6 and L6–S1 intervertebral disc spaces were observed, and with the aid of an operating microscope the intracanalicular epidural tumor was freed with a microcurette and collected with a Hamilton syringe.

Statistical Analysis

Nonparametric data (BBB score) were expressed as a median and compared via the Mann-Whitney U-test. Time to loss of ambulation (BBB Score ≤ 7) was ex- pressed using the Kaplan-Meier method19 and compared between treatment cohorts via log-rank analysis (Stat- view, SAS Institute). Volume of intraspinal canal tumor (parametric) was expressed as the mean ± SD and com- pared via the Student t-test.
Results

Experiment 1

In the nontumor group of rats, the mean serum glucose level was significantly elevated in animals receiving injections of 2 g/kg dextrose versus 0.09% saline 3–10 hours after injection (Fig. 1). The mean serum glucose values returned to normoglycemic levels by 12 hours after dextrose injection, but were again elevated 1–9 hours after the second injection. In rats with tumor implants, the mean serum glucose level was also significantly elevated in those receiving injections of 2 g/kg dextrose versus 0.09% saline 3–10 hours after injection. The mean serum glucose values returned to normoglycemic levels by 12 hours after dextrose injection, but were again elevated 1–9 hours after the second injection. Peak serum glucose was similar between tumor (243 ± 30 µg/dl) and nontumor (259 ± 20 µg/dl) groups (p = 0.765).

This cyclical and peaking hyperglycemic pattern mimics the steroid-induced hyperglycemia typically observed in the clinical setting. All normo- and hyperglycemic animals maintained a hindlimb BBB score of 21 throughout 14 days of injections. Hence, hyperglycemia itself did not affect hindlimb function.

Experiment 2

All rats in the normoglycemic group maintained their weight after tumor implantation (Day 0: 224 ± 10 g; Day 6: 226 ± 10 g; Day 9: 228 ± 11 g; Day 12: 230 ± 11 g; Day 15: 232 ± 12 g). No rats in the hyperglycemic group demonstrated signs of systemic distress or weight loss after tumor implantation and glucose injections (Day 0: 243 ± 30 µg/dl and normoglycemic (259 ± 20 µg/dl) groups (p = 0.765).

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Discussion

Symptomatic metastatic spine disease affects up to 5% of patients in whom cancer has been diagnosed.42 Given the presence of an ever-aging population and improvements in systemic treatment for primary tumors, this incidence is likely to increase in the future, contributing significant pain, neurological dysfunction, and immobility in individuals afflicted with this disease. Although palliation is the goal in patients with MESCC, dramatic improvement in quality of life can be obtained when multimodality treatments such as surgical decompression, spinal stabilization, radiation treatment, and chemotherapy are appropriately applied.12,21,22,29,41

Recently, Patchell et al.32 demonstrated the efficacy of surgery for tumor resection in a prospective, randomized clinical trial in patients with MESCC. However, outcomes continue to vary widely among patients with MESCC, a phenomenon that probably results from various underlying physiological variables.10,40,48 Other than extent and duration of both preoperative neurological deficit and spinal cord compression, reversible physiological factors contributing to progressive neurological deterioration remain poorly understood.

In our study, we demonstrate that rats maintained in hyperglycemic rats demonstrated a decreased median BBB score compared with the normoglycemic animals (p = 0.023, Fig. 2). A decreased median BBB score in the hyperglycemic versus normoglycemic group persisted through postimplantation Day 16 (8 vs 12, p = 0.047; Fig. 2). The median time to paralysis was significantly reduced in the hyperglycemic group (15.5 vs 17.5 days, p = 0.0035; Fig. 3). There was no evidence of systemic or surgical site infection in any of the normo- or hyperglycemic rats in either experiment.

At the time of paralysis and planned death, surgical assessment of the L-6 spinal cord confirmed encasement and compression of the spinal cord in all 16 animals. Tumor volume as a function of time after tumor implantation suggested a near-linear tumor growth pattern within the epidural space; however, hyperglycemic rats developed paralysis with significantly less epidural tumor volume (2.75 ± 0.38 cm³ vs 4.0 ± 0.41 cm³, p < 0.001; Fig. 4).
Hyperglycemia and neurological decline in experimental spine metastasis

![Fig. 4. Scatterplot demonstrating epidural tumor volume at time of paralysis as a function of days after implantation in normo- and hyperglycemic rats. Tumor volume as a function of time after implantation demonstrated a near-linear tumor growth pattern within the epidural space. Hyperglycemic rats developed paralysis at a lower epidural tumor volume (mean tumor volume 2.75 ± 0.38 cm³ vs 4 ± 0.41 cm³, p < 0.001).](image)

A waxing and waning hyperglycemic state, similar to that frequently observed in the clinical setting, demonstrated accelerated hindlimb neurological decline and earlier onset of paralysis from MESCC. Furthermore, the epidural tumor volume at the time of paralysis was significantly less in hyperglycemic rats; because of this, accelerated tumor growth was probably not the cause of accelerated hindlimb paresis. In fact, a near-linear growth pattern was observed across both groups (Fig. 4). Furthermore, hyperglycemic rats demonstrated no signs of systemic distress or decline. Hyperglycemic rats maintained their grooming habits, dietary intake, and daily weight, suggesting that systemic sequelae of hyperglycemia did not directly influence activity and BBB score. These results suggest that hyperglycemic states may contribute to decreased spinal cord tolerance to compression resulting from MESCC, in turn resulting in accelerated functional decline.

The effects of hyperglycemia on CNS injury, vascular injury, and clinical morbidity in the surgical critical care setting have been well studied. Acute hyperglycemia following ischemic and traumatic brain injury has been shown to be associated with larger infarct volume and worse neurological outcome. Elevated levels of advanced glycosylation end products, which quench vasodilators such as adenosine and nitric oxide, have been suggested to underlie this hyperglycemic effect. Nishikawa et al. identified 3 separate pathways of hyperglycemia-associated injury, all of which were blocked by limiting glucose-induced mitochondrial superoxide production. Finally, hyperglycemia potentiates lactate accumulation and tissue acidosis, further contributing to secondary CNS injury. Although these mechanistic effects of hyperglycemia have been demonstrated in brain injury and ischemic SCI, we did not perform histological analysis of the spinal cord at the time of paralysis. Hence it is unclear if hyperglycemia resulted in greater histological evidence of spinal neuronal necrosis and apoptosis.

Few experimental studies have investigated the effect of acute hyperglycemia on SCI and neurological outcome. Robertson and Grossman used a rabbit model of spinal cord ischemia to study the effect of induced hypoglycemia on functional recovery following spinal cord ischemia. They found that active reduction in blood glucose (mean blood glucose 97 µg/dl vs 172 µg/dl) attenuated the spinal cord lactic acid level and lessened neurological injury. The elevated peak lactate concentration observed during both ischemia and reperfusion correlated closely with the preischemia glucose concentration. These results suggest that not only avoiding hyperglycemia, but actively reducing blood sugar, may improve neurological outcome following ischemic SCI. Given these findings, it is not surprising that neurological dysfunction occurred earlier and with less spinal cord compression in rats with hyperglycemia in our study of MESCC. Furthermore, a plausible explanation may be a hyperglycemic effect on tumor growth. Because adenocarcinoma cells preferentially rely on anaerobic rather than aerobic metabolism, a hyperglycemic environment could theoretically influence tumor growth and time to spinal cord compression. Nevertheless, given our data, no firm conclusions can be made about the effect of hyperglycemia on oncological growth.

Our experimental model of MESCC, first reported by Mantha et al. in 2005, has been reproduced and validated in several experimental studies. Bagley et al. used this model to evaluate the effects of standard ionizing radiation treatment, reporting a significant delay in the onset of paresis when administering 10–30 Gy of radiation versus controls. This same model was effectively used to test the efficacy of a locally delivered paclitaxel-impregnated biodegradable polymer (ReGel). Most recently, this model was used to demonstrate delay of paresis after vertebrectomy in animals with MESCC. This model, which involves surgical placement of breast adenocarcinoma in the L-6 VB of a Fischer rat, reproducibly led to epidural tumor spread and paraparesis in all animals in the current study. Furthermore, on inspection of the spinal canal at time of paralysis, all animals demonstrated epidural tumor spread and significant spinal cord compression. Nevertheless, several limitations of this model of resection of MESCC should be recognized when interpreting these results.

Metastatic spinal disease in humans not only involves focal MESCC, but also has an element of biomechanical instability, is frequently associated with multifocal sites of spinal metastasis, may have more diffuse epidural seeding at the site of cord compression, and is often accompanied by physiological sequelae of systemic disease. This model of MESCC also does not account for physiological factors of systemic disease, nor does it address issues of global functionality or survival. Furthermore, steroid-induced hyperglycemia, as frequently observed in the clinical setting, may have a dissimilar effect on neurological decline. In our model, steroids were not given...
in rats with serum glucose elevation. It remains unknown whether concurrent steroid administration would provide a neuroprotective effect or rather augment this detrimental glucose effect. The interaction of hyperglycemia and steroid therapy on spinal cord function during MESC is a topic worthy of future investigation. Similar to all animal models, these experimental results cannot be directly applied to humans for the aforementioned reasons. Regardless, this model allows a platform for the specific study of novel factors contributing to neurological decline from MESC.

Conclusions

In a rat model of metastatic epidural spinal cord compression, the rats maintained in a hyperglycemic state experienced accelerated time to paralysis. The epidural tumor volume at the time of paralysis was significantly less in hyperglycemic rats. These results suggest that hyperglycemic states may contribute to decreased spinal cord tolerance to MESC, in turn resulting in accelerated functional decline. Clinical studies evaluating the effect of aggressive glucose control in patients with MESC may be warranted.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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