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Insulin-Like Growth Factors and Recurrent Hypoglycemia Associated with Renal Cell Carcinoma in a Horse

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The case was examined at the Easter Bush Veterinary Hospital, The University of Edinburgh. A 520 kg, 6-year-old Thoroughbred gelding was referred for evaluation of acute onset profound depression, head pressing, and lack of response to external stimuli. The horse had a history of moderate weight loss over several months despite adequate nutrition and a good appetite. Previous episodes of weakness had been observed following administration of anthelmintics, namely, alternate doses of pyrantel and ivermectin, administered every 8 weeks. In an attempt to eliminate a possible parasitic cause of weight loss, over the 7 days prior to presentation, the referring veterinary surgeon treated the horse with a 5-day course of fenbendazole[a] (7.5 mg/kg PO q24h) and moxidectin[b] (0.4 mg/kg PO), 2 days later. A 5-day course of prednisolone[c] (1 mg/kg PO q24h) was initiated on the same day as the fenbendazole. This dose was then tapered to 0.5 mg/kg PO q24h, and was still being administered at the time of presentation.

On physical examination, the horse was thin, profoundly depressed, unresponsive to external stimuli, and had marked weakness and ataxia. There was bilateral mydriasis and sluggish pupillary light responses. Clinical examination was otherwise unremarkable.

There was a peripheral blood neutrophilia (9.2×10^9/L; reference range = $2.7–6.8 \times 10^9$/L), hypoglycemia (24 mg/dL; reference range = 50–100 mg/dL) and hypoinsulinemia (<2 μU/mL; reference range = 5–36 μU/mL). Other blood analytes were within normal limits. Urinalysis revealed a specific gravity of 1.019 and pH of 8.0. The urine was negative for blood, glucose, protein, and bile pigments, contained no cells of renal origin, but contained numerous calcium carbonate and calcium oxalate crystals.

Two liters of 5% glucose[d] were administered intravenously. The horse responded rapidly and, within 10 minutes, was markedly brighter, less ataxic, and able to eat succulent feed and molasses. Concentrates were provided at intervals of 4 hours and hay was provided ad libitum. The dose of prednisolone was reduced by 0.05 mg/kg daily. The following morning, the horse was dull and ate feed slowly, despite being normoglycemic (58 mg/dL). The horse remained relatively stable, with the exception of one episode of mild colic, until day 6, when it exhibited head pressing, mild focal seizures (facial twitching), and profound ataxia. Venous blood analyses revealed hypoglycemia and hypoinsulinemia (16 mg/dL and <2 μU/mL, respectively). Intravenous administration of 2 L of 5% glucose resulted in a clinical improvement within 5 minutes. A further hypoglycemic episode occurred early in the morning of day 7. Although a blood sample was not taken on this occasion, the clinical signs resolved within 5 minutes after intravenous administration of 1 L of 5% glucose. No hypoglycemic episodes were observed between days 8 and 10, at that time, prednisolone treatment ceased.

On day 12, rectal examination identified a smooth mass occupying a large proportion of the right side of the abdomen, cranial to the cecum. It was not possible to determine the cranial extent of the mass. Transabdominal and transrectal ultrasonography confirmed the presence of a large mass in the region of the cranial pole of the right kidney, occupying most of the right side of the abdomen, and extending toward the liver. The mass extended to a depth of at least 25 cm and exhibited variability in echogenicity and structure. The caudal pole of the right kidney appeared normal. Five biopsies were taken from the pararenal mass using a 14-gauge, 20-cm disposable trucut biopsy instrument.[e] As histopathologic examination of the biopsy revealed monotonous fields of neoplastic cells, the horse was euthanized.

Gross postmortem examination revealed normal peripheral lymph nodes. The abdomen contained a small amount of grossly normal pale yellow peritoneal fluid. An enormous encapsulated mass occupied the right abdomen and had replaced most of the right kidney. The adjacent duodenum and jejunum were loosely adhered to the mass, which was easily dissected away from the body wall but had infiltrated the liver. The mass was approximately 50 cm in diameter and weighed 35 kg. On cut section, a small area of renal tissue was present at the caudal pole but the majority of the mass comprised multiple cream lobules. There were two central areas of necrosis that contained brown fluid. The remaining organs, including the left kidney, were macroscopically unremarkable.

Histopathologic examination of representative tissue sections, following routine hematoxylin and eosin staining, confirmed that the significant findings were confined to the right renal mass. The neoplastic cells were dense and monotonous (Fig 1), with poorly defined and often vacuolated cytoplasms and basophilic nuclei containing finely granular nuclear chromatin. The mitotic index was variable but high (approximately 3–4 per high-power field). In some areas, the cells were more elongated and streaming (Fig 2). Im-

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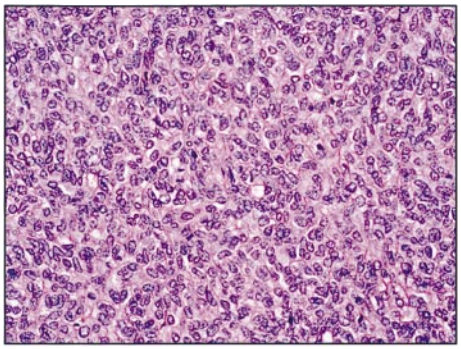

Fig 1. Histopathology of the mass reveals dense and monotonous areas of neoplastic cells (hematoxylin and eosin, 125×).

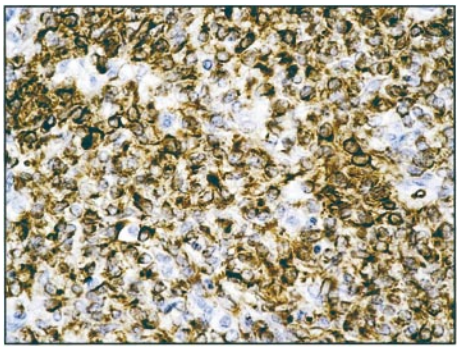

Fig 3. Immunohistochemistry revealed that the neoplastic cells were strongly positive to broad-spectrum cytokeratin staining, confirming them to be epithelial in origin (200×).

munohistochemical stains were used to identify the tumor type. Neoplastic cells were negative for vimentin antibody[f] and strongly positive for broad-spectrum cytokeratin[f] (Fig 3), confirming an epithelial origin. Additionally, positive cells were seen throughout the neoplastic areas following staining with a human renal cell carcinoma marker[1] (Clone 66.4.C2)[f] (Fig 4). These features were consistent with a diagnosis of renal clear-cell carcinoma.

Attempts were made to quantify IGF-1 and IGF-2 (insulin-like growth factors) in stored (−20°C) serum and compare results to an established normal range for age- and breed-matched horses. Serum was collected from 10 healthy thoroughbred horses (5 geldings, 4 mares, and 1 stallion) aged 4–9 years. IGF-1 and IGF-2 were measured using radioimmunoassay techniques,[g] employing specific, high-affinity polyclonal antibody against the human IGFs. No physical separation of IGFs from IGF-binding proteins was required. The cross-reactivity between IGF-1 and IGF-2 in this assay was <0.05%. The sensitivity of the assays for human IGF-1and IGF-2 were 0.02 ng/mL and 0.01 ng/mL, respectively, and interassay variation coefficients were 7.4% and 7.9%, respectively. Mean serum IGF-1 and IGF-2 levels from 10 normal horses were 194 ng/mL (range 124–327 ng/mL) and 26 ng/mL (range 12–44 ng/mL), respectively. Serum IGF-1 and IGF-2 from the present case were 16 ng/mL and 21 ng/mL, respectively.

Equine primary renal neoplasia is rare,[2] with the most common neoplasm being renal cell carcinoma.[3] Common clinical signs of equine renal neoplasia include weight loss,[4,5] colic,[4,6,7] and hematuria.[3,5–10] While weight loss and colic were observed prior to presentation and early in the hospitalization period, respectively, the presenting signs in this report of a horse with a renal carcinoma, namely encephalopathy associated with hypoglycemia, were atypical of renal neoplasia, thus making the diagnosis difficult. Hypoglycemia is rare in adult horses. As in most species, glucagon, somatostatin, growth hormone, epinephrine, and cortisol usually maintain blood glucose within normal levels and prevent fasting hypoglycemia, while insulin facilitates the entry of glucose into cells.[11] The differential diagnoses of hypoglycemia included hepatopathy,[12] hyperinsulinemia due to pancreatic neoplasia,[13] a paraneoplastic syndrome,[14,15] fraudulent insulin administration,[16] starvation, and extreme exertion.[17] The clinicopathologic findings were inconsistent with hepatic insufficiency, and the hypoinsulinemia eliminated the possibility of an insulin-secreting pancreatic islet B-cell tumor. The concurrent tapering of prednisolone was considered potentially significant in exacerbating hypoglycemia because glucocorticoids increase gluconeogenesis, antagonize insulin, and decrease peripheral utilization of glucose,[18] but was thought unlikely to have been the initiating factor.

Renal carcinomas arise from the tubular epithelium[19] and most are located in the pole of one kidney, are large and unilateral,[20] explaining why renal function is maintained.[21,22] Renal cell carcinomas have commonly been reported to metastasize to regional lymph nodes, liver, and lung[3,8] and

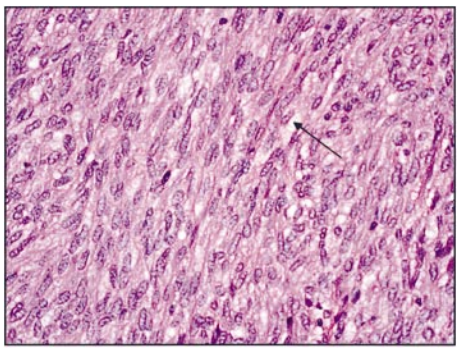

Fig 2. Histopathology revealed elongated cells in some areas. Note the pale cytoplasm and areas of vacuolation (arrow) (hematoxylin and eosin, 125×).

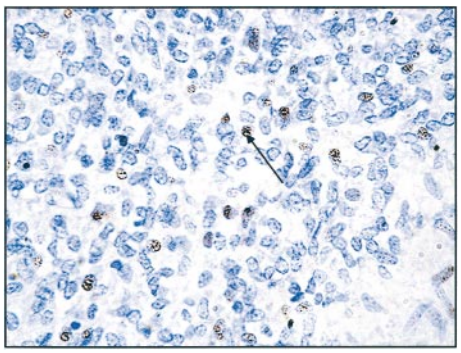

Fig 4. Positive cells were seen scattered throughout the neoplastic areas following staining with a human renal cell carcinoma marker (Clone 66.4.C2) (arrow) (200×).

more rarely to other organs.[10,23–25] Although locally invasive to the liver, no evidence of metastasis was seen in the present case or in the only other reported case of a renal carcinoma presenting with hypoglycemia.[15] The histopathologic features of renal carcinomas vary considerably,[8] making diagnosis challenging, and the use of immunohistochemistry was diagnostically beneficial in this case. In the domestic species, renal carcinomas are classified into three histological types: papillary, tubular, and solid,[26] the most common being tubular adenocarcinoma.[19] Clear-cell adenocarcinomas consist of large cells with pale or clear, vacuolated cytoplasm and, although rare in all domestic animals,[19] have been reported in horses.[23]

Clinically apparent hypoglycemia is rare in adult horses and has only been reported twice in association with neoplasia,[14,15] despite hypoglycemia being a well-recognized paraneoplastic syndrome in noninsulin secreting tumors in dogs[27–30] and humans.[31–33] Mechanisms of nonislet-cell neoplasia-induced hypoglycemia are poorly understood but likely include secretion of insulin-like substances from extrapancreatic tumors, excessive glucose requirements by the tumor, and failure of compensatory mechanisms of glycogenolysis and gluconeogenesis associated with liver metastases.[34] In dogs and humans, the hypoglycemia is thought to result from the production of an abnormal form of IGF-2,[30,32,35–37] termed big IGF-2,[38] which has insulin-like activity. While this mechanism has been proposed as a cause of nonislet-cell neoplasia-induced hypoglycemia in horses,[15] this is the first report to investigate the role of IGFs in nonislet-cell neoplasia-induced hypoglycemia in the horse. Both forms of IGF-2 can be detected by radioimmunoassay, but as big IGF-2 circulates in a form more readily available to peripheral tissues than the normal IGF-2 complex, it leads to hypoglycemia. In humans and dogs with high circulating big IGF-2, increased insulin-like activity associated with uptake of glucose into tissues, primarily muscle, results in hypoglycemia[36] and secondary hypoinsulinemia. Serum levels of glucose and insulin closely parallel each other as a result of the precise feedback control of blood glucose on insulin secretion,[11] which may account for the hypoinsulinemia observed in this case. IGF-2 also suppresses the hypothalamo-hypophyseal axis, growth hormone secretion, and consequently IGF-1 production, and an IGF-2 : IGF-1 ratio of $>10:1$ is pathognomic for nonislet-cell neoplasia-induced hypoglycemia in humans.[37]

Hypoglycemia in dogs has been associated with several noninsulin-secreting tumors, including hepatocellular carcinoma, hemangiosarcoma, leiomyosarcoma, melanoma, and salivary adenocarcinoma.[29] Hypoglycemia associated with nonislet-cell neoplasia has only been reported twice in the horse. Roby et al[14] described a yearling filly with persistent hypoglycemia associated with a hepatocellular carcinoma and Baker et al[15] described a horse with intermittent hypoglycemia due to an anaplastic renal carcinoma. The hypoglycemia in the present case probably resulted from the production of an insulin-like factor or, less likely, from excessive glucose uptake by the tumor. There was no evidence of liver metastasis or failure. Attempts to measure serum levels of IGF-2 and IGF-1 were made and, although the markedly reduced IGF-1 was consistent with nonislet-induced hypoglycemia in humans[37] and dogs,[35] the IGF-2 data were inconclusive. The majority of IGF-2 levels in the 11 horses were below the assay's lowest standard of 40 ng/mL, probably reflecting a lack of detection of equine IGF-2 by the human-specific IGF-2 radioimmunoassay. The IGF-1 radioimmunoassay used in this study has been validated and used to successfully measure IGF-1 in a number of animal species other than the horse (Evans, personal communication). Additionally, other authors have successfully measured IGF-1 in equine plasma[39,40] and report similar reference ranges to those recorded in this study. Because different assays are available, comparison of published studies is impossible. Age-matched horses were selected for this study because young horses have significantly higher IGF-1 levels.[39]

Interestingly, clinically insignificant hypoglycemia is mentioned in many reports of equine renal carcinomas,[4,7,10] indicating that this paraneoplastic syndrome is possibly more common than is presently recognized. Further attempts to investigate the role of IGFs in such cases would be valuable.

Footnotes

[a] Panacur Equine Guard, Intervet UK Limited, Milton Keynes, Buckinghamshire, UK
[b] Equest, Fort Dodge Animal Health, Southampton, UK
[c] Prednisolone Tablets B.P. 5mg, Millpledge Pharmaceuticals, Nottinghamshire, UK
[d] Ivex Pharmaceuticals, Larne, Northern Ireland, UK
[e] Quick core biopsy needle, Cook UK Limited, Letchworth, Hertfordshire, UK
[f] Novocastra Laboratories Limited, Newcastle, UK
[g] Mediagnost, Reutlingen, Germany; assays performed by Cambridge Specialist Laboratories, UK

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