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Evaluation of creatine kinase (CK) and aspartate aminotransferase (AST) activities after laparoscopic or conventional ovariectomy in queens

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Summary

Creatine kinase (CK) and aspartate aminotransferase (AST) are mainly muscle-specific enzymes, which can be associated with muscle tissue damage. The aim of this study was to assess the activities of CK and AST during the postoperative period, after conventional (G1) and videolaparoscopic ovariectomy (G2), in queens. A further group (G3) was subjected to anaesthesia only. Results demonstrate that there were significant differences between groups. The highest levels of CK were recorded in G1, however at a confidence level of $p < 0.05$ there was no significant difference between groups during the first 6 hours after surgery. A significant ($p < 0.05$) increase of CK values was identified between 0h and 3h in both groups (G1 and G2). Regarding AST activity there was no significant variation between groups, but again there was a significant difference between values at 0h and 3h after surgery. In conclusion, ovariectomy performed by videolaparoscopy seems to cause less muscle damage when compared to the conventional method.

Keywords: videolaparoscopy, queen, muscle enzymes

Bestimmung der Kreatinkinase (CK) und Aspartat-Aminotransferase (AST) Aktivität nach laparoskopischer oder konventioneller Ovariohysterektomie bei der Kätzin

Kreatinkinase (CK) und Aspartat-Aminotransferase (AST) sind vor allem muskelspezifische Enzyme, die das Ausmass einer Muskelschädigung wiedergeben können. Ziel dieser Arbeit war es, die Aktivitäten von CK und AST nach konventioneller (G1) oder videolaparoskopischer (G2) Ovariohysterektomie bei der Kätzin zu bestimmen. Tiere der Kontrollgruppe (G3) wurden nur narkotisiert. Die Ergebnisse zeigen, dass zwischen den einzelnen Gruppen signifikante Unterschiede bestanden. Die höchsten CK Werte zeigte G1, doch waren die Enzymaktivitäten zwischen den einzelnen Gruppen während der ersten 6 Stunden nach der Operation nicht signifikant verschieden. Ein signifikanter ($p < 0.05$) Anstieg der CK war in beiden Gruppen (G1 und G2) während der ersten 3 Stunden nach Operationsbeginn zu beobachten. Bezüglich der AST Aktivität gab es zwischen beiden Gruppen keine signifikanten Unterschiede, doch waren auch hier die Werte zwischen 0 und 3 Stunden signifikant verschieden. Zusammenfassend lässt sich folgern, dass eine videolaparoskopisch durchgeführte Ovariohysterektomie die Muskulatur weniger schädigt als nach Anwendung der konventionellen Methode.

Schlüsselwörter: Videolaparoskopie, Katze, Muskelenzyme

Introduction

Population control of dogs and cats decreases the incidence of potential public health hazards, since these animals may transmit zoonoses. There are various methods of contraception, but surgical sterilization is most often used because it is an efficient and safe technique. However, ethical concern about animal suffering and welfare is unquestionable (Rollin, 2002) and therefore, procedures using videolaparoscopy have been developed. The

advantages of these techniques, compared to open surgery, is the small incision needed, minimal tissue damage, reduced postoperative discomfort and pain and a faster recovery (Brun et al., 1999; Malm, 2003). However, the application of this technique will be limited, because of the high cost of the equipment and the requirement to gain sufficient experience, before these benefits can be realised. In animals, creatine kinase (CK) is a cytosolic enzyme with highest activity in skeletal muscle, cardiac muscle and brain, with lesser amounts in the intestine, uterus, urinary bladder, kidney, and thyroid (Duncan et al., 1994). CK has mainly been used as a marker of skeletal muscle damage (Aktas et al., 1993). CK is important in energy production in muscular cells, as it catalyze the conversion of creatine phosphate and adenosine diphosphate (ADP) into creatine and adenosine triphosphate (ATP). CK enters the blood when the permeability of the cell membrane is altered or when necrosis of cells occurs (Cardinett III, 1997; Chaney et al., 2004). Aspartate aminotransferase (AST) catalyzes the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. AST occur in almost all cells, but is used as a diagnostic enzyme for liver and muscle disease because of its high activity in these tissues. The objective of the present study was to evaluate serum activities of CK and AST during the postoperative period in queens submitted to ovariectomy performed by videolaparoscopy or a open technique.

Animals, Material and Methods

Animals

Thirty healthy mixed breed queens, aged between 8 months and 3 years and weighing between 1.2 and 3.9 kg were used. The animals were randomly distributed to three groups with 10 animals in each. Group 1 (G1) was subjected to conventional ovariectomy, and Group 2 (G2) by videolaparoscopy. The control group (G3) was anaesthetized without surgery being performed.

Laparoscopic procedure

Pre-medication was performed using 0.05mg/kg of 0.2 % acepromazine maleate and 0.01 mg/kg buprenorphine hydrochloride[1] by intramuscular injection (IM). Tiletamine (5 mg/kg) and zolazepam[2] (5 mg/kg) (IM) were used for induction and maintenance. Conventional ovariectomy by a midline procedure was performed as described by Okkens et al. (1997).

For the laparoscopic procedure, the bladder of the queens was emptied, and the animal placed in the Trendelenburg position as necessary to visualize the uterine horns (approximately 15º). The first access to the abdomen was made using a Verres needle inserted through the midline 2 cm caudal to the umbilicus and a pneumoperitoneum was induced (8–10mmHg CO_2). After removal of the Verres needle, a 7mm trocar was inserted at the same position (camera portal). The second trocar (5mm) was placed 1cm caudal to the umbilicus (between umbilicus and the first puncture) and a third trocar (10mm) was placed 1cm cranial to the umbilicus. These trocars were used as an instrument portals. The ovaries were located and individually grasped with atraumatic forceps. They were adequately stabilised to place titanium clips over the ovarian pedicles and uterotubal junction. After clip placement, the fallopian tubes together with the cranial portion of the uterus and the ovarian pedicles were sectioned, and the ovaries were removed through the cannulas. The presence of any haemorrhage was assessed before instruments were removed, and then the pneumoperitoneum was deflated. The incisions were sutured conventionally in two layers (fascia and skin). G3 animals were premedicated and anaesthetized identically to the animals in groups 1 and 2, and were placed in the supine position for the same mean duration of the surgical procedures.

Enzyme analysis

Serum concentrations of creatine kinase (CK) and aspartate aminotransferase (AST) were determined in blood samples collected from the jugular vein, taken before pre-medicatication, and 3, 6, 12, 24 and 36 hours after surgery in G1 and G2 and after anaesthesia in G3 respectively. Samples were centrifuged at 800 G for 5 minutes and immediate analysis was performed with reagents for diagnostic[3] and spectrophotometric assay using specific reagents and kinetic methodology[4]. Reference values from Cardinett III (1997) for CK and AST measurements were adopted.

Statistics

Proc GLM of SAS[5] was used to compare treatment groups each comprising of 10 animals in a completely randomized experimental design. Multiple observations on the same animal across timepoints where controlled for by nesting observations within each animal. Tukeys post hoc test was used to identify treatment differences when the model was found to be significant.

[1] Temgesic ®, Schering-Plough S.A.
[2] Zoletil ®, Virbac S.A.
[3] Labtest – Labtest Diagnóstica S.A., Lagoa Santa, MG
[4] Labquest, CELM, model E-225-D
[5] Statistical Analysis System, Version 8

Table 1: Serum activities (m ± SD) of creatine kinase(CK) in animals submitted to conventional (G1) or laparoscopic (G2) ovariectomy at different times after surgery. Control group (G3) had anaesthesia only.

Time (Hours)	G1	G2	G3
0	0.42 ± 0.1^{Aa}	0.50 ± 0.11^{Aa}	0.69 ± 0.05^{Aa}
3	2.28 ± 0.54^{Ba}	3.06 ± 0.56^{Ba}	2.07 ± 0.15^{Ba}
6	3.37 ± 0.32^{Ba}	2.38 ± 0.39^{Ba}	3.28 ± 0.54^{Ba}
12	4.60 ± 0.56^{Ba}	1.61 ± 0.24^{Bb}	2.60 ± 0.61^{Bb}
24	3.35 ± 0.43^{Ba}	1.13 ± 0.56^{Ba}	2.96 ± 0.43^{Ba}
36	1.83 ± 0.23^{Ba}	0.72 ± 0.13^{Ba}	1.29 ± 0.35^{Aa}

Same letters in the columns and small letters in the rows are not different (Tukey's test $p < 0.05$).

Table 2: Serum activities (m ± SD) of aspartatoe aminotransferase (AST) in animals submitted to conventional (G1) or laparoscopic (G2) ovarectomy at different times after surgery. Control group (G3) had anaesthesia only.

Time (Hours)	G1	G2	G3
0	6.41 ± 0.85^{Aa}	8.12 ± 0.45^{Aa}	6.75 ± 0.21^{Aa}
3	7.50 ± 1.23^{Ba}	7.53 ± 0.67^{Ba}	7.48 ± 0.97^{Aa}
6	8.53 ± 0.91^{Ba}	8.94 ± 1.26^{Ba}	8.11 ± 1.01^{Aa}
12	9.14 ± 1.64^{Ba}	9.51 ± 1.04^{Ba}	8.06 ± 0.97^{Aa}
24	8.76 ± 0.93^{Ba}	11.10 ± 0.98^{Ba}	8.24 ± 0.74^{Aa}
36	8.22 ± 0.89^{Ba}	9.21 ± 0.20^{Ba}	7.37 ± 0.09^{Aa}

Same letters in the columns and small letters in the rows are not different (Tukey's test $p < 0.05$).

Results

Before the procedures there were no significant differences in CK levels between the groups. Mean serum CK activity was 2.64, 1.57 and 2.15 U/L for G1, G2 and G3, respectively. There was a significant difference ($p = 0.006$) between 0h and the other time periods in all groups, so that the levels increased significantly after all procedures (Tab. 1). Maximum CK activity in G1 was higher and occurred on average 12 hours after the procedure whilst in G2 it occurred 3 h after surgery. During the postoperative period, there were significant differences ($p = 0.0002$) between the levels of G1 and G2, and there was a greater increase in the activity of CK in G1 compared to G2. There was no significant difference in the activity of CK between G2 and G3 ($p = 0.08$).

The mean AST serum activity across all time points was 8.09, 9.06, and 7.66 U/L in G1, G2 and G3, respectively, and there were no significant differences between groups before and after the procedures (Tab. 2). There were differences between time points, with activity at 0h being significantly different from 3h ($p = 0.002$) in G1 and G2, but no differences between G3 and the others groups at any time point. Although serum AST activity in the laparoscopy group was apparently higher during the postoperative period, there was not a significant difference ($p = 0.09$), between groups (Tab. 2). In G3, the activities varied, but there were not significant differences between time points.

Discussion

Given the ethical concerns of animal welfare associated with surgery, videolaparoscopy may have advantages over open surgery because of reduced tissue damage, reduced pain and improved recovery. Surgical damage may also induce adverse neuroendocrine and metabolic activation (Bonica, 1992). Increase in serum CK activity is used in the diagnosis of neuromuscular diseases and to verify muscular damage (Fascetti et al., 1997; Auguste, 1992). In mammals, all creatine kinase is located in the cytosol of cells, mainly in the skeletal muscles, myocardium, and brain. Muscle injury causes release of intracellular enzymes and subsequent increases in serum activity CK.

In this study, during the postoperative period, there was a greater increase in the activity of CK in G1 compared to G2. This suggests that laparoscopy induces less tissue damage than the conventional technique. In general, the levels of CK followed the pattern described in the literature (Duncan et al., 1994). Elevated CK activity was observed after surgery in G1 and G2. The peak of CK activity in G2 occurred 3 h after surgery, whereas the peak in G1 was higher and occurred in average 12 hours after the procedure. According to Hjelms et al. (1987) the peak increase is observed within 6–12 h after surgery. In their study, CK increased to 20 times the base values after thoracotomy, and twice the base values after midline laparotomy. The values in the present study suggest that trocar placement for laparoscopy causes less muscular dam-

age than laparotomy incision. According to Harris et al. (1998), despite variations in the methods of anaesthesia and surgery, CK frequently increases in the hours following anaesthesia. AST levels in G2 were higher than in G1 and declined 24 hours after surgery, whereas the decrease in G1 occurred earlier (after 12h). These results are different from those decribed by Cardinett III (1997), who found that AST and CK levels showed similar behaviour. We hypothesize that an unknown process, perhaps related to carbon dioxide absorption in the liver, may have triggered the increase in G2, since the liver is responsible for AST production. Additionally, serum AST activity also increases with changes in muscular permeability (Duncan et al., 1994; Cardinet III, 1997). These factors should all be considered as explanations of the changes seen, but more studies are needed for confirmation.

The simultaneous analysis of CK and AST in this study shows that CK half-life of CK is shorter than the half-life of AST, since CK activity increased and then decreased faster than AST activity. This result is in agreement with the study of Lindsay et al. (1989) and suggests that CK has higher specificity for indicating muscle damage and subsequent resolution, compared with AST. The latter may indicate other pathology and is less specific. Aktas et al. (1993) report that muscle diseases are the main source of plasma CK elevations. They mention inherited myopathies, malignant hyperthermia, hypothyroidism, vitamin E-selenium deficiency, prolonged decubitus, intramuscular injections, surgery, and experimental myocardial infarction. These authors also report that AST indicates muscle damage, with values increasing between 12 and 24 hours after muscle damage and remaining raised for 1 or 2 weeks.

The control group (G3) was included to evaluate possible alterations due to muscle injury caused by injections and manipulations during the anaesthetic procedure. In this group, CK activities varied at different blood sampling time points but without a sustained increase. According to Aktas et al. (1993), intramuscular injections of drugs can be responsible for significant increases of CK; elevation peaks at around four hours and elevations persists for about 24 h, before returning to base values on the third day. This indicates that needle insertion in tissues during sampling and IM injections causes measurable trauma, since they increase CK levels. Nevertheless, the increase in CK was not significant when compared to the tissue damage caused by surgery, since in groups G1 and G2 the values consistently increased and then decreased, without oscillation around the base line value. In conclusion, videolaparoscopy induced less muscular damage than conventional surgery. However, AST activity increased after videolaparoscopy compared to conventional surgery. The cause of this is unknown but may represent processes other than muscular trauma. Intramuscular injections during anaesthetic procedures also cause muscle injury, as demonstrated by rises in AST and CK, but this is less marked than that resulting from surgery.

Mesure de la créatine kinase (CK) et de l'aspartate amino-transférase (AST) après ovariohystérectomie par laparoscopie ou par méthode conventionnelle chez la chatte

La créatine kinase (CK) et de l'aspartate amino-transférase (AST) sont principalement des enzymes spécifiques aux muscles, qui reflètent l'importance des dégâts musculaires. Le but du présent travail était de déterminer l'activité de la CK et de l'AST après ovariohystérectomie conventionnelle (G1) ou par laparoscopie (G2) chez la chatte. Les animaux du groupe de contrôle (G3) n'ont été qu'anesthésiés. Les résultats montrent des différences significatives entre les divers groupes. Les valeurs de CK les plus élevées se trouvaient dans le G1, toutefois l'activité enzymatique ne différait pas significativement entre les groupes durant les 6 heures après l'opération. Une élévation significative de la CK ($p < 0.05$) pouvait être constatée dans les deux groupes G1 et G2 durant les trois heures après le début de l'opération. Pour ce qui est de l'AST, il n'y avait pas de différence significative entre les deux groupes, toutefois les valeurs étaient également modifiées significativement dans les 3 premières heures. En résumé on peut conclure, qu'une ovariohystérectomie effectuée par laparoscopie endommage moins la musculature que l'utilisation de la méthode traditionnelle.

Determinazione nei gatti dell'attività della creatinchinasi (CK) e dell'aspartato trasaminasi (AST) dopo laparoscopica o metodo convenzionale

La creatinchinasi (CK) e l'aspartato transaminasi (AST) sono sue enzimi specifici ai muscoli che possono rimediare al grado di una lesione muscolare. Scopo di questo studio è di determinare le attività della CK e della AST dopo un'isterectomia ovarica convenzionale (G1) o videolaparoscopia (G2) nelle gatte. Gli animali del gruppo di controllo (G3) hanno ricevuto solo una narcosi. I risultati mostrano che esistono delle importanti differenze tra i vari gruppi. Il valore più alto di CK si è riscontrato nel gruppo G1 ma durante le prime 6 ore dopo l'operazione le attività enzimatiche tra i diversi gruppi non si sono rilevate particolarmente differenti. Un aumento importante ($p < 0.05$) del CK si è osservato in entrambi i gruppi (G1 e G2) durante le prime 3 ore dopo l'inizio dell'operazione. Per quel che riguarda l'attività dell'AST non si è riscontrata nessuna differenza significativa ma anche in questo caso, nelle ore tra 0 e 3, i valori misurati erano ben differenti. In conclusione si può dire che un'isterectomia ovarica per videolaparoscopia danneggia meno la muscolatura che l'impiego del metodo convenzionale.

References

Auguste D., Aktas M., Fayolle P., Lefebvre H.P., Toutain and Braun J.P.: Etude preliminare de la cinetique plasmatique de la creatine kinase après administration intraveineuse ou intramusculaire chez le chien. Prat. Med. et Chirur. de l'anim. de Comp. 1992, 27:399–403.

Aktas M., Auguste D., Lefebvre H.P., Toutain P.L, Braun J.P.: Creatine Kinase in the dog: a review. Vet. Res. Comm. 1993, 17: 353–369.

Bonica, J.J.: Pain research and therapy history, current status and future goals. In: Animal Pain. Eds. C.E. Short, & A.V. Posnack, 1992, 1–29.

Brun, M.V.: Ovário-histerectomia em caninos por cirurgia laparoscópica. Master Thesis, Universidade Federal do Rio Grande do Sul, 1999.

Cardinet III, G. H.: Skeletal muscle function. In: Clinical biochemistry of domestic animals. Eds.J.J. Kaneco, J.W. Harvey, M.L. Bruss, 1997, 407–440.

Chaney, K., MacLeay J.M., Enns R.M., Al-Sobayil F., Kawcak C.E., Frisbie D.D.: Effects of induced lameness via carpal osteochondral fragmentation on plasma creatine kinase activity in horses. J. Equine Vet. Sci. 2004, 12: 531–534.

Duncan J. R., Prasse K.W.: Veterinary Laboratory Medicine: Clinical Pathology, Eds. Ames, IA: Iowa State University Press, 1994; 130–151.

Fascetti A.J., Mauldin G.E., Mauldin G.N.: Correlation between serum creatine kinase activities and anorexia in cats. J.Vet . Int. Med. 1997, 1: 9–13.

Harris, P.A.; Marlin, D.J.; Gray, J.: Plasma aspartate aminotransferase and creatine kinase activities in Thoroughbred racehorses in relation to age, sex, exercise and training. Vet. J. 1998, 3: 295–304.

Heljms E., Hansen B.F., Waldoff S., Steiness E.: Evaluation increased serum creatine kinase as an indicator of irreversible myocardial damage in dogs. Scan. J. Card. Surg. 1987, 21:165–168.

Lassen, E. D. Laboratory detection of muscle injury. In: Veterinary Hematology and Clinical Chemistry. Eds. Lippincott Williams e Wilkis, 2004, p. 417–550.

Lindsay, W. A., Robinson G.M., Brunson D.B., Majors L.J.: Induction of equine postanesthetic myositis after halothane-induced hypotension. Am. J. Vet. Res. 1989, 3: 404–409.

Malm, C.: Ovário-histerectomia – Estudo experimental comparativo entre as abordagens laparoscópicas e aberta na espécie canina. PhD thesis Universidade Federal de Minas Gerais, 2003.

Okkens, A.C., Kooistra, H.S., Neickel, R.F.: Comparison of long-term effects of ovariectomy versus ovariohisterectomy in bitches. J. Reprod. Fertil., 1997, 31: p.227–231.
Rollin, B.E.: A ética e o controle da dor em animais de companhia. In: Dor em animais-uma abordagem com orientação prática para o controle eficaz da dor em animais. Eds. L.J. Hellebreckers., 2002, p.17–35.

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