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Biochemical changes induced by general anesthesia with romifidine as a premedication, midazolam and ketamine induction and maintenance by infusion in donkeys

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Abstract

The objective of this study was to determine the effects of the general anesthesia on same biochemical changes in donkeys. The anesthesia was induced by intravenous (IV) injection of romifidine 0.1 mg/kg as a premedication, after 5 minutes induction of general anesthesia by (IV) of mixture midazolam 0.1 mg/kg and ketamine hydrochloride 2.2 mg/kg in the same syringe. The maintenance of anesthesia was performed by (IV) infusion of a mixture of the midazolam 0.065 mg/kg/hr. and ketamine 6.6 mg/kg/hr. The biochemical parameters changes in serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphate (ALP) activity as liver enzymes and serum glucose were estimated in zero, 15, 30, 60, 120, 240 and 480 minutes. The results revealed significant differences ($P<0.05$) in the means of AST (U/L) between zero 199.6 with 30 min 192.5 and 60 min 191.5. No significant differences ($P>0.05$) in mean enzyme activity of the ALT and ALP. Serum glucose results were shown no significant differences ($P>0.05$) in the (control, 15, 30 minutes) and (60,120 and 240) respectively and significant differences in between and within 480 minutes. The general anesthesia in this protocol was good and had little effect on the liver function and showed increase in serum blood glucose in donkeys.

Keywords: general anesthesia, romifidine, midazolam, ketamine biochemical change.
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التغيرات الكيموحياتية المحدثة بالتخديرِ العام مِن قِبل الرومفدين كعلاج تمهيدي و الميدازولام كيتامين للاستحداث و الادامة في الحمير

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الخلاصة

هدفت الدراسة الحالية الى تحديد تأثير التخدير العام الوريدي على بعض التغيرات الكيمو حياتية في الحمير. تم احداث التخدير العام بحقن عقار رومفدين بجرعة ٠,١ ملغم /كغم كعلاج تمهيدي والتخدير العام بإعطاء مزيج الميدازولام بجرعة ٠,١ ملغم /كغم والكيتامين بجرعة ٢,٢ ملغم /كغم من وزن الجسم في نفس المحقنة وادامة التخدير العام بواسطة التسريب الوريدي لمزيج الميدازولام بجرعة ٠,٠٦٥ ملغم / كغم / ساعة والكيتامين بجرعة ٦,٦ ملغم / كغم/ ساعة. تم تسجيل التغيرات الكيموحياتية في المصل لمستويات AST وALT وALP كبعض معايير كفائة الكبد و سكر المصل في الاوقات صفر (سيطرة) و١٥ و ٣٠ و٦٠ و١٢٠ و٢٤٠ و ٤٨٠ دقيقة. اظهرت نتائج معدلات AST وجود فرق معنوي بين الاوقات صفر ١٩٩,٦ مع الوقت ١٥ دقيقة ١٩٢,٥ وحدة/لتر و الوقت ٦٠ دقيقة ١٩١,٥ وحدة/لتر في حين لم تظهر فحوصات ALP و ALTاي فروقات معنوية في ما بينها وكذلك أظهرت نتائج سكر مصل الدم فرق معنوي بين الاوقات(صفر و١٥ و٣٠ دقيقة) مع (٦٠ و١٢٠و ٢٤٠ دقيقة) وكذلك اشرت وجود فرق معنوي مع ٤٨٠ دقيقة. التخدير العام بواسطة هذا المزيج كان جيدا مع تأثير قليل على بعض فحوصات كفاءة الكبد واظهر تأثير على سكر المصل في الحمير.

Introduction

The α-2-adrenoceptor agonists drugs have been recognized as worldwide use in veterinary medicine for their sedative, analgesic and muscle relaxation properties in large and small animals (1). The commonly α_2-agonists which used in veterinary practice are xylazine, detomidine, medetomidine and romifidine. Romifidine is a recent α-2 adrenoceptor agonist marketed for use in horses (2). Romifidine has been available since 1985 (3). It has been used successfully for sedation, analgesia, and premedication in horses in several countries since 1988 (4) with effects similar to other α-2 adrenoreceptor agonists (5). Midazolam is a short-acting benzodiazepine with hypnotic, anticonvulsant, muscle-relaxant and anxiolytic properties. In clinical practice, it was using for the induction of anesthesia (6). The midazolam metabolites are conjugated and then excreted as glucuronides in the urine (7,8). Ketamine is a phencyclidine derivative that produces a dissociative state of anesthesia. Dissociative anaesthesia was characterized by dissociation between the thalamo-cortical and limbic system on the electroencephalogram (EEG) (9,10). Ketamine has been used as an anesthetic agent in equine medicine since the mid-70s (11). Initially, ketamine was applied just as an induction agent, producing amnesia, loss of consciousness, analgesia and immobility. In later years, based on these properties, the application of ketamine in equine anaesthesia was extended by using in different total intravenous anaesthesia protocols (12,13). Blood plasma contains three major protein fractions: albumin, globulin, and fibrinogen. In humans, sheep, goat, rabbits and rat there are mostly albumins, while in horse, pig and cattle the ratio of albumins and globulins is almost equal, or globulins prevail (14). Aspartate aminotransferase (AST) is a widely distribute enzyme, which is found in many tissues and organs, with high activity in the liver (15). The increase in the AST activity in the serum is a sensitive marker of liver damage (16). There are two main isoenzymes: mitochondrial and cytosolic, which prevail in the total concentration in the blood plasma because they have a longer half-life (17). Activity of AST in horses is much higher than in other animals (18). But alanine aminotransferase (ALT) activity in horses is not specific for the liver changes (17). Alkaline phosphatase ALP is relatively nonspecific enzymes hydrolyzes a variety of ester orthophosphates under alkaline conditions and exhibits optimal activity between pH 9 and 10. Several plasma isoenzymes of ALP are recognized, including hepatic, osseous, intestinal, and placental forms; the relative proportions of these isoenzymes in plasma vary with species (19). The plasma and urinary glucose act as broad indicators of the severity of any disturbance to carbohydrate metabolism, the homeostatic mechanisms for maintaining blood glucose are influenced by intestinal absorption and both hepatic and tissue metabolism. The balance is influence by several hormones in addition to insulin and glucagon, and these other hormones include corticosteroids, growth hormones, adrenocorticotrophic hormone, and biogenic amines (19). Plasma or serum should be rapidly separated after collection to avoid the effects of erythrocyte glycolysis.

Materials and methods

Ten clinically healthy female donkeys weighing between 70-100 kg aged 8-12 months have been used in this study. Romifidine was used as a premedication drug (Sedivet® 1.0% Boehringer Ingelheim Vetmedica, Inc., Spain). Midazolam (15 mg in 3 ml, Alsaad pharmaceuticals, Syria) and ketamine (kepro pharmaceuticals, 100 mg/ml Holland), was used for induction and maintain the general anesthesia. The regime of general anesthesia was made by administration of romifidine at a dose of 0.1 mg/kg B.W. injected intravenously in the jugular vein as a premedication, then after five minutes, midazolam at a dose of 0.1 mg/kg B.W. and Ketamine at a dose of 2.2 mg/kg B.W. mixed in the same syringe (20) have been injected intravenously.

Fifteen minutes later an infusion of midazolam 10 mg (2 ml) mixed with ketamine 1000 mg (10 ml) in 500 ml normal saline was administrated to maintain the anesthesia, the rate of dripping was 100-110 drops per minute (20 drops equal to 1ml). 5 ml of blood samples were collected via jugular vein puncturing with 23 G needle, the blood in the plain tubes was allowed to form serum at room temperature and centrifuged to harvest serum. Serum was stored at -20^0C until analysis by using diagnostic kits and spectrophotometer. The biochemical changes of this regime are evaluated by liver enzyme (AST, ALP and ALP) U/L and serum blood glucose (mg/dl). The (ALT) and (AST) enzyme kits were manufactured by RANDOX laboratories Antrim, United Kingdom. While the (ALP) kit manufactured by BIOLABO SA, Maizy, Frances. Serum blood glucose, kit manufactured by SPINREACT, S.A., Girona, Spain. The results were expressed as means (M) stander error (SE). Parametric data were analyzed by one ways analysis of variance (ANOVA) continued with Least Significant Difference (L.S.D.), and $P<0.05$ was considered to be significant. Statistical Package for Social Sciences (SPSS) was used (21).

Results

Aspartate aminotransferase (U/L) activity showed decreased in level of enzyme assay through induction and maintenance of anesthesia and increased after recovery but still below the base line of the study (Table). The statistical

analysis revealed significant differences at the level of (P<0.05) between zero time 199.6±1.783 with 30 min and 60 min (192.5±1.579; 191.5±1.771) respectively. Results of ALT showed no significant difference P<0.05 between donkeys, the enzyme level within normal range in all-time of experiment. ALP values of this study showed no significant difference in the M ± SE values of the ALP between donkeys at level of P<0.05 the enzyme level within normal range in all-time of experiment. Serum glucose mg/dl was slowly increased in the 10 minutes (97.2±0.513 -100.77±0.855), and increased to (101.4±0.805) at the 15 minutes the serum blood glucose still increase to the end of experiment. The statistical analysis revealed no significant differences at the level of (P>0.05) in the (control, 15, 30 min) and (60,120 and 240 min) respectively and significant differences (P<0.05) between them with 480 minutes.

Table: Effect of general anesthetic regime on some liver enzyme and serum blood glucose in (10) donkeys.

Parameter	Time minutes						
	Zero	15	30	60	120	240	480
AST enzyme U/L	199.6±1.783 A	194.8±1.982 AB	192.5±1.579 B	191.5±1.771 B	195.1±2.024 AB	195.1±1.048 AB	197.3±1.453 AB
ALT enzyme U/L	25.55±0.450	25.75±0.512	24.73±0.265	25.23±0.668	25.21±0.747	25.79±0.580	25.28±0.612
ALP enzyme U/L	519.2±4.378	514.9±5.728	511.7±6.119	508.6±5.406	513±5.278	514.4±5.200	513.9±4.789
Serum blood glucoses Mg/dl	96.1±0.737 AB	95.0±1.229 AB	94.6±1.212 B	93.7±1.075 B	93.9±1.075 B	96.3±1.155 AB	96.0±0.714 AB

Value is expressed as M ± SE, Different in the capital latters refer significant differences (P<0.05) between time.

Discussion

The increase in the serum glucose during the anesthesia time agreed with khan *et al*., (23) and Almarsoumy, (24) whom used α-2 adrenocepter similar to romifidine in there effect, cause hyperglycemic effect by inhibition of insulin released by the stimulation of the pancreatic β cells and to an increased glucose production in the liver. Thakur *et al.* (25) exposed that ketamine hydrochloride generally increases norepinephrine blood levels and turnover, since norepinephrine affects gluconeogenesis and glycogenolysis and also decreases insulin production, enhanced hyperglycemic effects after ketamine administration are obvious. The continuously increase of the serum glucose after the end of anesthesia may be feeding and watering of the animals after fasting and continues effects of romifidine and ketamine. In the enzyme assay there are two main isoenzymes: mitochondrial and cytosolic, AST prevails in the total concentration in the blood plasma because it has a longer half-life (17). Activity of AST in horses is much higher than in other animals (18).In addition to species, breed and age, AST activity was influence by muscle activity (26). The AST enzyme is present in most tissue and increases with muscle injury especially cardiac muscle, as well as hepatocellular injury, also present in kidney, pancreas and erythrocytes. Thus AST assays should be run in conjunction with other enzymes assays, especially ALT when evaluating liver function. Increase ALT with normal to mildly elevated AST may indicate reversible liver damage. Marked elevation in ALT and AST indicate hepatocellular necrosis. Increased AST with normal ALT may indicate that the source of AST is not the liver (27,28). Thus AST has also been used as a cardiac marker (29). Result of the AST assay agreed with Hall *et al.*, (30) revealed that midazolam was substrate competition which causes inhibitors to the enzyme this factor effects lead to decrease the AST level in the time of anesthesia. Lemma and Moges, (31) shown that working donkeys have higher activity of the AST enzyme 288 U/L than donkeys which are at rest for several days 223 U/L. The AST enzyme assay in study was within the normal which ranged on 223.30±32.78 U/L (22). The ALT enzyme assay is using to detect liver injuries and long-term liver disease. Highly elevated levels may indicate active hepatitis from any cause, including virus, drug or toxin. Some prescription and over-the-counter medications can cause an increase in ALT levels, can be dramatically affected by shock, low blood pressure or any other condition that deprives the liver of blood and oxygen. The result of this study agrees with the other studies that the ALT enzyme is not present in enough amounts in liver cells of horse, ruminants and pig (27). ALT level of this study were in accordance with Gul *et al.*, (22) who reported a normal value of ALT 27.25±01.91U /L activity in healthy donkeys.

The result of the ALP enzyme assay agreed with Thakur *et al.* (25) that revealed ALP value in horses is wide that a change in its value is of no clinical relevance. Serum ALP activities usually increase in animals with biliary stasis,

steroid hepatopathy and occasionally bone lesions. In addition to that ALP is found in much tissue, including liver, bone, intestine, placenta and kidneys (32). Hepatic and bony metastasis can also cause elevated levels of alkaline phosphatase. Results of enzyme assay were in accordance with Gul *et al.* (22) who reported a normal value of ALP 485.46±98.74 U/L activity in healthy donkeys.

According to these results, the general anesthesia by this protocol has minimal effect on the liver and the value of enzyme still within normal value while the serum glucose was affected by this protocol and causes elevation in the value.

References

1. Luna SP, Nogueira CS, Cruz ML, et al. Romifidine or xylazine combined with ketamine in dogs premeditated with methotrimeprazine. Braz J. Vet Res Anim Sci.2000, 37(2): 3031-3040.
2. Muir WW, Julie S, and Wolfrom GW. Sedative and analgesic effects of romifidine in horses. Intern J. Appl Res Vet Med. 2005,3(3):249-258.
3. Gasthuys F, Martens L, Goosens L, and De Moor A. A quantitative and qualitative study of the diuretic effects of romifidine in the horse. J. Assoc Vet Anaes. 1996, 23: 6-10.
4. Martnell S and Nyrnan G. Effects of additional pre-medication on romifidine and ketamine anaesthesia in horses. Acta Vet Scand. 1996, 37: 315-325.
5. England GC and Clarke KW. Alpha 2 adrenoceptor agonists in the horse – a review. Brit Vet J. 1996,152 (6):641-657.
6. Nordt SP and Clark RF. Midazolam: a review of therapeutic uses and toxicity. J. Emerg Med. 1997, 15: 357–365.
7. Kronbach T, Mathys D, Umeno M, et al. Oxidation of midazolam and triazolam by human liver cytochrome P450 IIIA4. Mol Pharmacol. 1989, 36: 89–96.
8. Bauer TM, Ritz R, Haberthur C, et al. Prolonged sedation due to accumulation of conjugated metabolites of midazolam. The Lancet. 1995, 346:145-147.
9. Rieblod TW, Geiser DR and Goble DO. Local and regional anesthesia. In: T.W. Rieblod, D. R. Geiser and D. O. Goble, eds. Large Animal Anesthesia: Principles and Techniques. 2nd ed. Iowa State University Press Ames Iowa. 1995, Pp: 205-230.
10. Stoelting RK. No barbiturate induction drugs. In: R. K. Stoelting, ed. Pharmacology and Physiology in Anesthetic Practice. 3rded: Lippincott-Ravin Publishers, Philadelphia. 1999, Pp: 148-157.
11. Muir WW, Skarda RT and Milne DW. Evaluation of xylazine and ketamine hydrochloride for anesthesia in horses. Am J. Vet Res. 1977, 38: 195-201.
12. Taylor PM and Luna SP. Total intravenous anaesthesia in ponies using detomidine, ketamine and guaiphenesin: pharmacokinetics, cardiopulmonary and endocrine effects. Res Vet Sci. 1995, 59: 17-23.
13. Mama KR, Wagner AE, Steffey EP, et al. Evaluation of xylazine and ketamine for total intravenous anesthesia in horses. Am J. Vet Res. 2005, 66: 1002-1007.
14. Swenson MJ. Dukes' Physiology of Domestic Animals, 11thed. Cornell. University Press Itaca and London. 1993, Pp: 41-43.
15. Zimmerman HJ, Dujovne CA, and Levy R. The correlation of serum levels of two transaminases with tissue levels in six vertebrate species. Comp Biochemical Physiol. 1968, 25: 1081-1089.
16. Meyer DJ and Harvey J W. Veterinary Laboratory Medicine. Interpretation and Diagnosis. 2nded. W. B. Saunders Company Philadelphia, London, Toronto, Montreal, Sydney, Tokyo.1998, Pp: 157-187.
17. Kramer JW and Hoffman WE. Clinical Enzymology. In: Clinical Biochemistry of Domestic Animals. (Kaneko, J. J., J. W. Harvey, M. L. Bruss, Eds.). Academic Press. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto. 1997, Pp: 303-325.
18. Cornelius CE, Bishop J, Switzer J and Rhode EA.Serum and tissue transaminase activities in domestic animals. Cornell Vet.1958, 19: 116-126.
19. Evans GO. Animal Clinical Chemistry :In A practical Guide for Toxicologist and Biomedical Researcher.2nd ed. Taylor and Francis Group, Boca Raton, London, New York.2009, p:50.
20. Kilic N. Cardiopulmonary, biochemical, and hematological changes after detomidine-midazolam-ketamine anesthesia in calves. Bull Vet Inst Pulawy.2008, 52:453-456.
21. SAS, Statistical Analysis System for Windows. 2001, V.6.13.
22. Gul ST, Ahmad M, Khan A and Hussain I. Haemato-biochemical observation in apparently healthy equine species. Pakistan Vet J. 2007, 27(4): 155-158.
23. Khan MA, Ashraf M, Pervez K, et al. Effects of Detomidine with Chloral Hydrate Anaesthesia in Horses. Inter J. of Agri & Bio. 2003, 46:316–321.
24. Almarsoumy IN. Comparative study between two regimes for induction of general anesthesia in donkeys. (MSc. Thesis) Baghdad: Baghdad University, 2010. P: 44.
25. Thakur BP, Sharma SK, Sharma A and Kumar A. Clinical Evaluation of Xylazine-Butorphanol-Guaifenesin-Ketamine as Short-Term TIVA in equines. Vet Med Inter. 2011, 10: Online journal http:// www.hindawi. com/journals/vmi/2011/506831.
26. Weigert PK, Schec kB, Lemmer W. Labordiagnostische Haflinger Pferden und Maultieren (Tragtiere der Bundeswehr). Enzymaktivitäten im Serum. Tierärztl. Praxis.1980, 8:P387.
27. Hamel. Clinical Chemistry. In: Tighe, M. M. and Brown, M. (eds.). Mosby's Comprehensive Review for Veterinary Technicians. 2nd ed., Mosby. 2003, Pp: 98-99.
28. Weiss D J. Tests for evaluation of liver disease, Section VI, Liver and Muscle. In: (Ed.) Cowell, R. L., Clinical pathology secrets, Elsevier Mosby. 2004, Pp:168-172.
29. Nymblom H, Björnsson E, Simren M, et al. The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. Liver Int. 2006, 26(7): 840-845.
30. Hall LW, Clarke KW and Trim CM. General pharmacology of the injectable agents used in anaesthesia, In: Veterinary Anaesthesia, Hall L.W., Clarke K.W. and Trim C.M (Eds.), 10th ed., W.B. Saunders, Harcourt Publishers Limited London.2001, Pp:113-131.
31. Lemma A and Moges M. Clinical, hematological and serum biochemical reference values of working donkeys (Equus asinus) owned by transport operators in Addis Ababa, Ethiopia. Livestock Research for rural development.2009, 21 (8): 211-227.
32. Tilley L P and Smith FW.The 5 minute veterinary consult canine and feline. Williams and Wilkins, Philadelphia.1997, P: 182.