Journal of the American Association for Laboratory Animal Science
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 46, No 3
May 2007
Pages 21–23

Effects of Ketamine and Thiopentone Anesthesia on Serum Lipid Parameters in Adult Bonnet Monkeys (*Macaca radiata*)

Nagarajan Perumal,[1,*] Venkatesan Ramasamy,[1] MJ Mahesh Kumar,[2] and Subeer S Majumdar[1]

We investigated the effect of anesthesia on serum lipid parameters in adult bonnet macaques (*Macaca radiata*). We treated 10 animals with ketamine hydrochloride (15 mg/kg intramuscularly) and, on the next day, thiopentone sodium (25 mg/kg intravenously). Blood samples were obtained before and after anesthetic treatment. Serum cholesterol, triglycerides, very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) were measured by autoanalyzer. Ketamine anesthesia significantly decreased serum cholesterol and HDL levels, whereas thiopentone significantly reduced triglycerides and VLDL and increased HDL values. Although the effects of ketamine hydrochloride and thiopentone sodium on serum biochemical values have been reported, no literature addressing the effect of anesthesia on lipid parameters in bonnet macaques is available. These findings will be useful in designing experiments assessing pathologic and toxicologic changes in serum lipid parameters and interpreting data obtained from adult bonnet monkeys.

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein

Nonhuman primates, both Old and New World species, are used widely for reproductive and toxicologic studies. When used under laboratory conditions, these animals present unique hazards to handlers, particularly the danger of bites and zoonotic infection.[24] Restraint or immobilization is very important to minimize the risk of harm to humans and animals when procedures like surgery, physical examination, treatment of wounds, tattooing, tuberculosis testing, and transportation are performed on animals. In nonhuman primates, the most common mode of immobilization is treatment with intramuscular ketamine hydrochloride (10 to 15 mg/kg body weight)[20] or thiopentone sodium (25 mg/kg body weight). The advantages of ketamine anesthesia are its high bioavailability (93%), broad therapeutic index, and short-acting anesthetic effect (approximately 20 to 30 min).[3,13] Thiopentone sodium is an ultra short-acting barbiturate typically used for brief surgical procedures or examination.

Standardized physiologic parameters are essential prerequisites for diverse scientific investigations. The effects of drugs vary in severity and can complicate interpretation of laboratory results and lead to erroneous diagnosis. Because anesthesia can modify biochemical parameters, precise knowledge about the effects of ketamine and thiopentone on the biochemical parameters of laboratory primates is extremely important.

Compared with rhesus macaques, bonnet macaques are underutilized for biomedical research because of their limited availability worldwide and the fact that available published reports on the hematologic and biochemical values of bonnet monkeys are limited.[11,15] Unlike rhesus macaques, bonnet monkeys reproduce throughout the year in the wild and therefore typically are used for reproductive studies.[14,17] These monkeys are abundant in the south Indian peninsula, smaller than rhesus monkeys, docile, easy to handle, and adaptable to captivity under laboratory conditions. Because, unlike rhesus macaques, bonnet monkeys are quite resistant to tuberculosis (unpublished observation),[12] they can be used conveniently for most biomedical research.

The effects of ketamine on hematology and biochemical values have been reported widely for many nonhuman primates, including the capuchin monkey (*Cebus apella*);[8] vervet monkey (*Cercopithecus aethiops*);[22] common marmoset (*Callithrix jacchus*);[19] and rhesus (*Macaca mulatta*),[1,9] cynomolgus (*Macaca fascicularis*),[7,23] and bonnet (*Macaca radiata*)[21] macaques. However, the effects of anesthesia on serum lipids in adult bonnet macaques has not been reported. To this end, we undertook the present study to determine the baseline values and effects of ketamine and thiopentone anesthesia on the serum lipid profile of adult bonnet monkeys.

Materials and Methods

Animals. This study used 10 adult female bonnet monkeys that were 12 to 14 y old and weighed 9 to 10 kg. All animals were bred and reared at the Primate Research Centre, National Institute of Immunology (New Delhi, India), and they were kept in accordance with guidelines for care and use of animals in scientific research (Indian National Science Academy, New Delhi, India)[18] in a facility registered with the Committee for the Purpose of Control and Supervision of Experimental Animals; this study was approved by the institutional animal ethics committee. The animals were maintained under standard environmental conditions (22 to 25 °C, 55% to 60% humidity, 12:12-h dark: light photoperiod) and housed individually in stainless-steel nonhuman primate cages (27 × 24 × 34 in.). Daily each animal was fed 100 g commercial pellet primate feed (Golden Feeds, New Delhi, India) and 50 g soaked chick peas (*Cicer arietinum*) in the morning, 2 slices of bread in the afternoon, and 350 g fruits or vegetables in the evening and had ad libitum access to water. Every 2 wk, they were given 5 ml oral B-complex vitamins (Visyneral syrup, USV, Mumbai, India) and calcium (Ostocalcium, Glaxo, Mumbai, India). All animals were tuberculosis-free, as determined by semiannual testing using bovine and mamma-

Received: 19 Sep 2006. Revision requested: 8 Dec 2006. Accepted: 19 Dec 2006.
[1]Primate Research Center, National Institute of Immunology, New Delhi, India; [2]Center for Cellular and Molecular Biology, Hyderabad, India.
[*]Corresponding author. Email: nagarajan@nii.res.in

Table 1. Effects of ketamine hydrochloride and thiopentone sodium on serum lipid parameters in adult female bonnet monkeys (n = 10)

		Ketamine hydrochloride		Thiopentone sodium	
	Preanesthesia sample	Postanesthesia sample	Absolute difference	Postanesthesia sample	Absolute difference
Cholesterol (mg/dl)	141.6 ± 22.9	121.7 ± 24.7	19.9[a]	128.9 ± 29.7	12.7
Triglycerides (mg/dl)	244.8 ± 7.6	215.6 ± 8.01	29.2	164.4 ± 74.3	80.4[a]
HDL (mg/dl)	108.4 ± 30.9	91.0 ± 32.9	17.4[b]	124.4 ± 39.2	–16[a]
VLDL (mg/dl)	48.3 ± 7.0	42.8 ± 13.5	5.5	32.8 ± 13.5	15.5[a]

Data are presented as mean ± 1 standard deviation.
[a]$P < 0.05$ compared with preanesthesia value.
[b]$P < 0.01$ compared with preanesthesia value.

lian purified protein derivative. The animals remained active, alert, and clinically healthy and were not pregnant, nursing, or carrying an infant during the study period.

Blood sampling. After overnight fasting, animals were physically restrained at the front of a squeeze cage. The right hindlimb was brought forward and held firmly and the injection site cleaned with alcohol. A 4-ml blood sample was drawn from the right saphenous vein using 23-gauge needle. The needle was removed from the syringe, and the blood sample was expressed slowly into a vial, allowed to clot for 1 h at room temperature, and refrigerated to retract the clot. The serum was collected within 2 h of bleeding (day 1) and then stored at –20 °C for biochemical analysis, which was done the next day. At 2 h after blood collection, the animals were anesthetized with ketamine hydrochloride (15 mg/kg; Ketmin, Themis, Mumbai, India) injected into the thigh by use of a 23-gauge needle. At 30 min after ketamine administration, 4 ml blood was withdrawn from the right femoral vein of each of the 10 animals and processed for biochemical examination as described.

On day 2 each animal was placed in an individual squeeze cage and anesthetized with thiopentone sodium (25 mg/kg; Thiosol, Neon, Mumbai, India) injected into the left saphenous vein. Because these animals are colony-born, they were acclimated to their surroundings. At 30 min after injection of thiopentone, 4 ml blood was withdrawn from the left femoral vein and processed for biochemical examination as done previously. Only 1 preanesthesia sample was taken from each animal, to minimize the stress to the animals.

Serum biochemistry. Serum cholesterol, triglycerides, very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) were estimated using a serum autoanalyzer (Screen Master 3000, Tulip, Goa, India).

Statistical analysis. Values for lipid parameters obtained from blood samples collected before and after anesthesia were compared by using paired *t* tests (Prism, version 4.02, GraphPad Software, San Diego, CA). A *P* value of 0.05 was used as the threshold for statistical significance.

Results

The effects of ketamine hydrochloride and thiopentone sodium on lipid parameters on adult female bonnet monkeys are shown in Table 1. Among the 10 animals, 7 showed significant decreases in cholesterol level ($P = 0.02$) after ketamine anesthesia, and 8 showed decreases in HDL ($P = 0.0055$). Thiopentone anesthesia decreased the serum triglycerides ($P = 0.0115$) and VLDL ($P = 0.0121$) in 9 animals, whereas HDL levels increased significantly ($P = 0.0128$) in 9 animals.

Discussion

Anesthetic agents can affect both the structure and function of organs and biological systems; this effect has been studied in many species of macaques. However, the effects of commonly used anesthetics on biochemical parameters in bonnet monkeys are poorly understood. The effects of a drug on the outcome of laboratory tests may be altered due to a biological effect, in which the drug affects an organ or interferes with the serum resulting in a change in the levels of some constituent of the blood, thereby altering the value obtained. Alternatively, an analytic effect, in which the drug interferes with the method of analysis, can lead to inaccurate results.[2] Our results revealed alteration in lipid parameters after administration of either ketamine hydrochloride or thiopentone sodium.

In rabbits, ketamine and thiopentone cause significant increases in cholesterol and triglycerides.[5] In our study, ketamine anesthesia of adult bonnet monkeys caused significant decreases in serum cholesterol and HDL, whereas thiopentone caused significant decreases in triglycerides and VLDL but increases in HDL values. In contrast, our earlier studies[21] on ketamine anesthesia in aged bonnet monkeys caused no significant alteration in serum cholesterol level; this apparently contradictory result might reflect the age of the animals studied.

Plasma cholesterol is under the direct influence of the liver and the stress response.[4] Lipolysis increases under the influence of catecholamines and corticosteroids and contributes to fat metabolism. A stress response associated with obtaining the preanesthesia samples in restrained animals could affect biochemical markers in these samples. To prevent this effect, we provided an interval of 120 min between the pre- and postanesthesia samples. Stress-related changes in heart rate and body temperature as well as hematologic and biochemical indicators are stabilized after 105 min after physical restraint.[10]

There are many causes of high blood triglycerides, including dietary indiscretion, metabolic diseases, drugs, and rare genetic mutation of an enzyme in the lipid metabolism pathway (type I hyperlipoprotinemia). The most frequent cause of an elevated serum triglyceride level is inadequate patient fasting,[16] because a recent meal will cause fat in the form of triglycerides to be transported from the gastrointestinal tract to the rest of the body. Typically when obese people lose weight, levels of both triglycerides and HDL (so-called 'good' cholesterol) improve.[16] We noticed increases in the triglycerides and HDL levels compared with published reports on bonnet macaques[6,21] that might be due to obesity and increased caloric intake. Other factors that can affect serum lipid levels include compounds such as diuretics, corticosteroids, male sex hormones (androgens), estrogen, birth control pills, antibiotics, and niacin (vitamin B3).[16]

In addition, physical stressors, such as infection, heart attack, surgery hypothyroidism, diabetes, and kidney or liver disease, can induce changes in the serum lipid profile. Pregnancy is known to cause an increase in the lipid profile during the third trimester. The ability of anesthesia to augment lipid levels in the blood may be kept in mind while using pregnant monkeys for the reproductive studies. The monkeys we studied were

free from infectious diseases, and no drugs or medicines were used before the study.

In summary, the present study shows that ketamine and thiopentone anesthesia can significantly alter serum lipid levels in adult female bonnet macaques. These potential changes should be considered when designing studies for or interpreting data from bonnet monkeys. In addition, this study provides essential baseline information on the normal lipid profile of bonnet monkeys and the effect of ketamine on these values, which data are not readily accessible from the existing body of scientific literature on nonhuman primates.

Acknowledgments

The authors thank the Director of the National Institute of Immunology for providing necessary facilities and financial support, which originated from the Department of Biotechnology, to conduct and report this study. We also wish to thank Rajesh Kumar Sharsar, Bhardwaj JP, Rajender K Thappa, and Kaniram Tanwar for assistance with blood collection and analysis.

References

1. **Bennett JS, Gossett KA, McCarthy MP, Simpson ED.** 1992. Effect of Ketamine hydrochloride on serum biochemical and hematologic variables in rhesus monkeys. Vet Clin Pathol **21:**15–18.
2. **Bush BM.** 1991 Interpretation of laboratory results for small animal clinicians. Oxford: Blackwell Science.
3. **Clements JA, Nimmo WS, Grant IS.** 1982. Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. J Pharma Sci **71:**539–542.
4. **Gehlot A, Godhwani JL, Godhwani S, Aseri ML, Jain P, Vyas MCR.** 1997. Sound stress–induced changes and their modification by drugs in albino rats: an experimental study. Indian J Pharmacol **29:**187–189.
5. **Gil AG, Silvan G, Illera M, Illera JC.** 2004**.** The effect of anesthesia on the clinical chemistry of New Zealand White rabbits. **43:**25–29.
6. **Kaufman D, Smith EL, Gohil BC, Banerji M, Coplan JD, Kral JG, Rosenblum LA.** 2005. Early appearance of the metabolic syndrome in socially reared bonnet macaques. J Clin Endocrinol Metab **90:**404–408.
7. **Kim CY, Lee HS, Han SC, Heo JD, Kwon MS, Ha CS, Han SS.** 2005. Hematological and serum biochemical values in cynomolgus monkeys anesthetized with ketamine hydrochloride. J Med Primatol **34:**96–100.
8. **Larsson MM, Birgel EH, Benesi FJ, Birgel EH Jr, Lazaretti P, Fedullo JL, Larsson CE Jr, Molina SR, Guerra PC, Prada CS** [Internet]**.** Hematological values of *Cebus apella* anesthetized with ketamine [cited 26 Mar 2007]. Available at http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-95961999000300005&lng=en&nrm=iso.
9. **Loomis MR, Henrickson RV, Anderson JH.** 1980 Effects of ketamine hydrochloride on the hemogram of rhesus monkeys (*Macaca mulatta*). Lab Anim Sci **30:**851–853.
10. **Montane J, Marco I, Lopez-Olvera J, Perpinan D, Manteca X, Lavin S.** 2003. Effects of acepromazine on capture stress in roe deer (*Capreolus capriolus*). J Wildl Dis **39:**375–386.
11. **Mythili MD, Vyas R, Patra SS, Nair SC, Akila G, Sujatha R, Gunasekara S.** 2005. Normal hematological indices, blood chemistry, histology, and ultrastructure of pancreatic islets in the wild Indian bonnet monkeys (*Macaca radiata radiata*). J Med Primatol **34:**35–40.
12. **Nagarajan P, Venkatesan R, Majumdar R.** 2004. Unpublished observation.
13. **Ochsner AJ.** 1977. Cardiovascular and respiratory responses to ketamine hydrochloride in the rhesus monkey (*Macaca mulatta).* Lab Anim Sci **27:**69–71.
14. **O'rand MG, Widgren EE, Sivashanmugam P, Richardson RT, Hall SH, French FS, VadeVoort CA, Ramachandra SG, Ramesh V, Jagannadha Rao A.** 2004. Reversible immunocontraception in male monkeys immunized with eppin. Science **306:**1189–1190.
15. **Ramachandra SG, Ramesh V, Krishnamurthy HN, Ravindranath N, Shetty KT.** 1998. Normal hematological and plasma biochemical parameters of the captive bonnet monkey (*Macaca radiata*). Primates **39:**127–137.
16. **Reduced triglycerides.com** [Internet]. Elevated triglycerides: causes and associated condition [cited 9 Oct 2006]. Available at http://www.reducetriglycerides.com/causes_high_triglycerides_template.htm.
17. **Rosario GX, Sachdeva G, Manjramker DD, Puri CP.** 2005. Enhanced expressions of endometrial tumor necrosis factor alpha and its receptors during early pregnancy in bonnet monkeys. Cytokine **31**:459-464.
18. **Sahni SK.** 2000. Guidelines for care and use of animals in scientific research. New Delhi (India)**:** Indian National Science Academy. p 1–26.
19. **Taglioni A, Casetti AR, Bernardini A, Perretta G.** 1996. Effects of ketamine hydrochloride on haematological parameters in *Callithrix jacchus*. Folia Primatol **67:**65–68.
20. **Thurmon JC, Tranquilli WJ, Benson GJ, Lumb WV, editors.** 1996. Anesthesia of wild, exotic, and laboratory animals. In: Lumb and Jones' veterinary anesthesia. Baltimore: Lippincott Williams and Wilkins. p 727.
21. **Venkatesan R, Nagarajan P, Ravishankar R, Majundar SS.** 2006 Hematologic and serum biochemical values in aged bonnet macaques anesthetized with ketamine hydrochloride. J Amer Assn Lab Anim Sci **45:**45–48.
22. **Wall HS, Worthamn C, Else JG.** 1985. Effects of ketamine anesthesia stress and repeated bleeding on the hematology of vervet monkeys. Lab Anim **19:**138–144.
23. **Yoshida T, Suzuki K, Shimizu T, Cho F, Honjo S.** 1986. The effects of ketamine anesthesia hematological and serum biochemical values in female cynomolgus monkeys (*Macaca fascicularis*). Jikken Dobutsu **35:**455–461.
24. **Young SS, Schilling AM, Skens S, Ritacco G.** 1999. Short duration anesthesia with medetomidine and ketamine in cynomolgus monkeys. Lab Anim **33:**162–168.