Journal of the American Association for Laboratory Animal Science
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Vol 55, No 5
September 2016
Pages 534–540

Effects of Transportation on Antioxidant Status in Cynomolgus Macaques (*Macaca fascicularis*)

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To evaluate the effects of transportation on oxidative stress in cynomolgus monkeys, we measured serum levels of reduced glutathione (GSH), malondialdehyde, and protein carbonyl (PC) and the activities of total antioxidant capacity (TAOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase in cynomolgus macaques before transportation (day 0), on the day of arrival (day 1), and on days 7, 14, and 21 after transportation. Compared with that on day 0, TAOC and catalase activities on days 1, 7, and 14 after transportation were significantly decreased, reached their nadirs on day 7, and increased thereafter to reach their pretransportation levels by day 21 after transportation. Compared with day 0 levels, mean SOD activity and GSH concentration were decreased significantly on day 1; they thereafter increased to reach their pretransportation measures by day 7 after transportation. In contrast, PC and malondialdehyde concentrations in serum and the activity of GSH-Px were increased on day 1 compared with day 0 and thereafter decreased to reach their pretransportation levels by day 14 after transportation. In summary, GSH, TAOC, catalase, and SOD levels decreased and malondialdehyde, PC, and GSH-Px concentrations increased in cynomolgus macaques after transportation. These results suggest that transportation might imbalance oxidant and antioxidant levels to create excess oxidative stress in cynomolgus macaques. Therefore, cynomolgus macaques should have at least 21 d to recover after transportation and regain their healthy status.

Abbreviations: GSH, reduced glutathione; GSH-Px, glutathione peroxidase; PC, protein carbonyl; ROS, reactive oxygen species; SOD, superoxide dismutase; TAOC, total antioxidant capacity

Long- or short-distance transportation of NHP from a vendor to a research facility is inevitable, due to the uneven geographic distribution of NHP (for example, cynomolgus macaques [*Macaca fascicularis*]).[20] The stress associated with the transportation of NHP used in scientific research influences both their physical and mental health.[15] A previous study on transportation-related stress in cynomolgus macaques showed increases in cortisol levels and the neutrophil:lymphocyte ratio on the day of arrival.[27] Stressful conditions can lead to an imbalance between oxidants and antioxidants in favor of oxidants at the cellular or individual level.[26,38] Alteration of the oxidative balance produces oxidative stress that causes cellular damage[13] and makes the organism sensitive to disease.[31] These alterations are also explained as a resisting mechanism to the negative effects of reactive oxygen species (ROS)— including hydroxyl (HO^-), peroxyl (RO_2^-), alkoxyl (RO^-), and superoxide anion ($O_2^{-\bullet}$) radicals and nonradical species, such as hypochlorus acid (HOCl), ozone (O_3), and hydrogen peroxide (H_2O_2)—all of which cause the peroxidation of membrane lipids and protein oxidation.[41,9] To protect against damage by free radicals, cells have a variety of antioxidant systems in different cellular compartments, some of which are enzyme-based whereas others are not.[1,12] Main antioxidant enzymes in the body include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), and glutathione (GSH) reductase. These antioxidant enzymes react indirectly or directly with ROS.

ROS damage all types of biologic molecules, including protein, lipid and DNA. Protein carbonyl (PC) groups are produced on protein side chains when they are oxidized. As biomarkers of oxidative stress, the usage of PC groups has some advantages because of their relative early formation and the relative stability of carbonylated proteins.[9] Malondialdehyde, one of the end-products of lipid peroxidation, has been widely applied as the most common biomarker for the assessment of lipoperoxidation in biologic and medical sciences.[5,29,43] Total antioxidant capacity (TAOC) reflects overall response of the nonenzymatic antioxidants and enzymatic antioxidants. To date, few scientific studies have addressed the effects of transportation on oxidative stress in cynomolgus macaques used in the safety evaluation and research of new drugs, chemical agents, vaccines, biologic products, and other substances. Because cynomolgus macaques are widely used in biomedical research[6] and given that transportation from the breed supplier to a research facility is inevitable, it is worthwhile to evaluate the effects of transportation on oxidative stress in cynomolgus macaques. The aim of this study was to evaluate the effects of transportation on antioxidant status (SOD, catalase, GSH-Px, TAOC, and GSH), the production of lipid peroxidation (malondialdehyde), and protein modification (PC) in cynomolgus macaques to identify biomarkers to facilitate improving transportation conditions and to provide useful information for veterinarian and researchers for monitoring animal health and welfare.

Materials and Methods

Animals and transportation. Clinically healthy cynomolgus macaques (n = 16; 8 male and 8 female; age, approximately 4 y; weight, approximately 4 kg) were evaluated in this study. All monkeys were free of *Cercopithecine herpesvirus* type I (B virus), SIV, simian poxvirus, simian T lymphotropic virus type 1, simian retrovirus type D, *Mycobacterium tuberculosis*, *Shigella* spp., *Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter jejuni*, pathogenic dermal fungi, ectoparasites, and endoparasites.

Received: 07 Nov 2015. Revision requested: 21 Dec 2015. Accepted: 29 Feb 2016.
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The temperature and relative humidity in the animal holding room at our facility (National Shanghai Center for New Drug Safety Evaluation and Research, Shanghai, China) were kept at 18 to 24 °C and 40% to 70%, respectively; these parameters were recorded every hour by a built-in automatic system. Lights were on from 0700 to 1900 at 150 to 300 lx, with 15 to 20 fresh-air changes (100%) hourly. The environmental parameters at Hainan Jingang (Hainan Jingang laboratory animal Co. Ltd., Haikou, Hainan province, China) were kept naturally. Twice each day, macaques were fed a high-quality commercial pelleted feed (at the research facility: Beijing Ke'ao Xieli Feed, Beijing, China; at Hainan Jingang: Guangzhou Guolong Technology, Guangdong, China) that met or exceeded the nutrient recommendations of the National Research Council; fruits were supplemented once daily at both places. Environmental enrichment consisted of commercial toys. All macaques were housed individually in stainless steel wire cages (800 × 900 × 900 mm) that included a squeeze board and 2 side boards that could be removed to accommodate pair-housed animals. Macaques had free access to fresh deionized drinking water.

The monkeys experienced combined air and road transportation from Hainan to Shanghai. The total transportation time of 8 h was divided as following: 1 h loading, 0.5 h road transportation to the airport, 1 h waiting to board in Hainan, 3 h in airplane, 1 h waiting after landing in Shanghai, 1 h road transportation to the research facility, and 0.5 h unloading. The transportation cages (400 × 240 × 500 mm) were made of plywood, and the macaques could see and hear each other through the center grid wall of the transportation cage. Each set of transportation cages consisted of 5 individual cages. Air-conditioned vans were used in the transportation from the animal vendor facility to the airport at Hainan and from the airport to the final facility in Shanghai. To prevent from causing physical harm, unnecessary discomfort, overheating, excessive cooling, behavioral stress, or trauma, all animals were handled as carefully as possible during the transportation process. The daily minimal and maximal outdoor temperatures were 19 and 22 °C in Hainan and 3 and 13 °C in Shanghai, respectively.

The protocol was reviewed and approved by the IACUC of the National Shanghai Center for New Drug Safety Evaluation and Research, which is AAALAC-accredited.

Preparation of serum. Blood samples (2 mL) were collected from conscious macaques through venipuncture of cephalic vein by the same experienced technician specialist using disposable syringes before transportation (day 0), on the day of arrival (day 1), and at 1 wk (day 7), 2 wk (day 14), and 3 wk (day 21) after transportation. Blood samples were added to EDTA-treated serum separator tubes; serum was obtained after centrifugation at 1000 × *g*, 4 °C for 10 min and stored at –80 °C until analysis.

Assays of SOD, catalase, GSH-Px, and TAOC activities and GSH, PC, and malondialdehyde levels. Serum antioxidant enzymes activities (SOD, catalase, GSH-Px, TAOC) and the levels of GSH, PC, and malondialdehyde in serum were determined by using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

The SOD activity was measured based on the ability of xanthine oxidase and xanthine to generate superoxide radicals, which reacted with 2-(4-iodophenyl)-3-(4-nitrophend)-5-phenyltetrazolium chloride to form a red formazan dye. The colorimetric absorbance was determined at 550 nm. The SOD activity was calculated by the degree of inhibition of this reaction. One unit of SOD activity was defined as a 50% inhibition of reduction of 2-(4-iodophenyl)-3-(4-nitrophend)-5-phenyltetrazolium chloride under the condition of the assay.[21,47] The SOD activity was expressed in U/mL serum.

The malondialdehyde level in serum was measured based on the reaction of malondialdehyde and thiobarbituric acid to produce a pink-colored complex, which was detected at 532 nm by using colorimetric absorbance.[34,44] The malondialdehyde level was expressed in nmol/mL serum. The kit for PC used the reaction of 2,4-dinitrophenylhydrazine with the carbonyl groups in proteins to produce a compound that was quantified by colorimetry at 370 nm.[9,14] The PC level was expressed in nmol/mg protein. The GSH-Px activity assay is based on the fact that GSH-Px catalyzes peroxide and reduces glutathione to produce water and oxidized glutathione. GSH-Px activity can be represented by its enzymatic reaction rate and can be calculated by measuring GSH consumption rate in this enzymatic reaction. These 2 substrates (GSH and peroxide) can undergo redox reaction without enzyme (nonenzymatic reaction). Therefore, the GSH consumption caused by nonenzymatic reaction was deducted during the calculation of GSH-Px activity. GSH reacts with dithiodinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anions, which has a relatively stable yellow color that can be detected at 412 nm to estimate the change in the GSH level.[16,22,34] In a reaction at 37 °C for 5 min, a decrease of 1 µmol/L GSH concentration (less the effect of the nonenzymatic reaction) per 0.1 mL serum was considered as 1unit of GSH-Px activity.

GSH (deproteinization) is a tripeptide composed by glutamic acid, glycine, and cysteine and is the main nonprotein sulfhydryl compound. Dithiodinitrobenzoic acid reacts with sulfhydryl compounds to produce a yellow compound, which can be quantitatively estimated by colorimetry at 420 nm. The GSH concentration was expressed in mg/L. Catalase activity was determined based on the H_2O_2 substrate, and the enzymatic reaction was stopped by the addition of ammonium molybdate. The intensity of the yellow complex formed by molybdate and H_2O_2 was measured at 405 nm.[36,37] Catalase activity was defined such that 1 µmol H_2O_2 decomposition per mL serum per second was considered as 1 unit of activity.

TAOC activity was measured by its ferric reducing ability of the supernatant. The stable color of the Fe^{2+}-o-phenanthroline complex (produced by the reducing agents in serum reducing Fe^{3+} to Fe^{2+}, which reacted with the substrate o-phenanthroline) was detected at 520 nm. TAOC was expressed in U/mL serum where 1 U was defined as an increase in absorbance (520 nm) of 0.01/min/mL serum at 37 °C.[47]

Statistical analysis. Data were analyzed using SPSS Statistics 21.0 (IBM, Armonk, NY). All parameters including TAOC, catalase, SOD, GSH, PC, GSH-Px, and malondialdehyde were analyzed for statistical significances between before and after transportation by homogeneity of variance according to the Levene test. When group variances were homogeneous, one-way ANOVA was used to determine whether any statistically significant differences existed between the pre- and posttransportation groups. If the analysis of variance gave a significant result, the Dunnett test was performed to detect any significant differences between the pre- and posttransportation groups. For all tests, a *P* value of 0.05 (2 tailed) was used as the criterion for significance. Percentage change and principal components analysis were applied to analyze the relationship of TAOC, catalase, SOD, GSH, PC, GSH-Px, and malondialdehyde in cynomolgus macaques after transportation.

Results

The TAOC, catalase, SOD, malondialdehyde, GSH, GSH-Px, and PC activities or levels at pretransportation and different

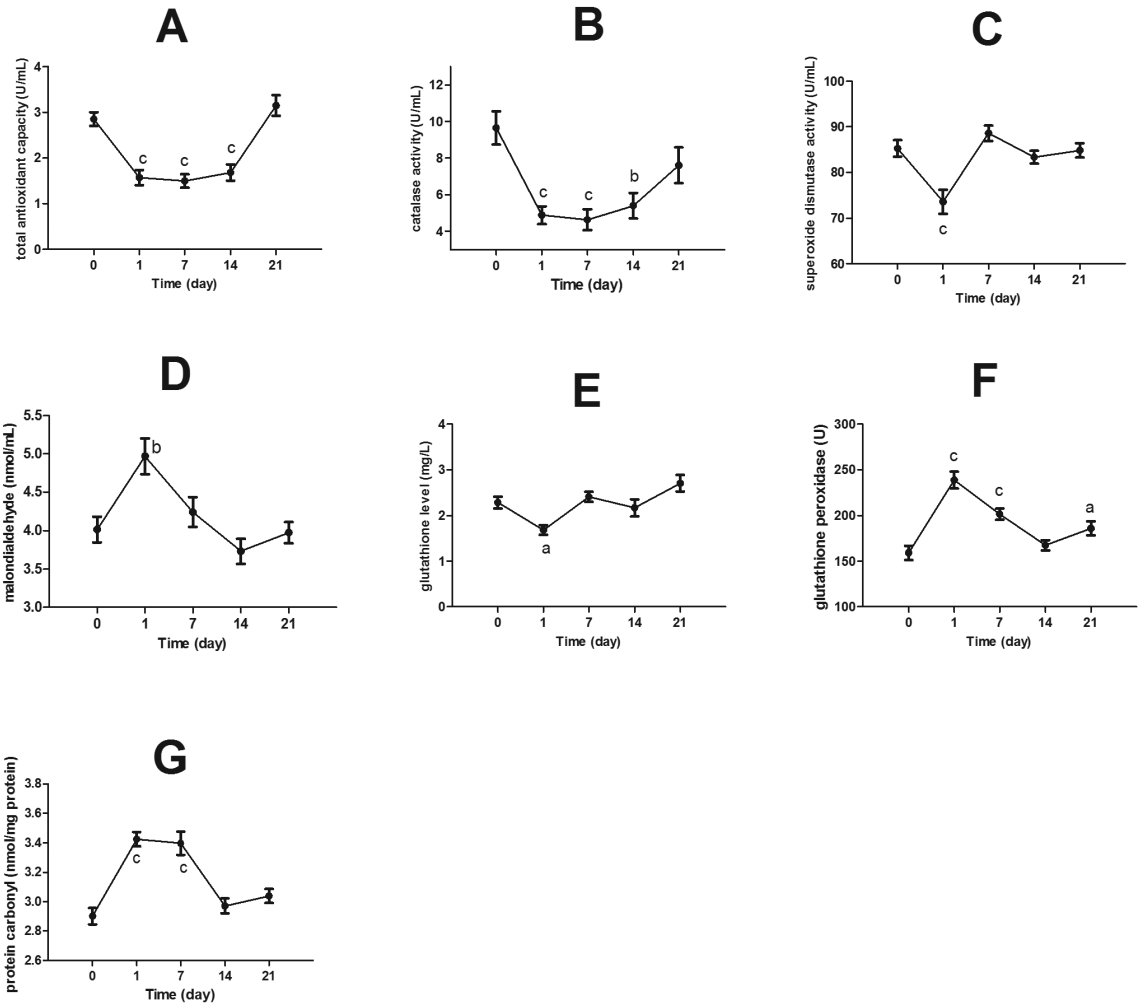

Figure 1. Measures (mean ± 1 SD; $n = 16$) of (A) total antioxidant capacity, (B) catalase, (C) superoxide dismutase, (D) malondialdehyde, (E) glutathione, (F) glutathione peroxidase, and (G) protein carbonyl before transportation (day 0), on the day of arrival (day 1), and on days 7, 14, and 21 after transportation. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$.

time point after transportation are presented in Figure 1. TAOC activity on days 1, 7, and 14 after transportation showed a significant decrease ($P < 0.001$) compared with that on day 0, reached its lowest activity on day 7, and increased thereafter to reach its pretransportation activity by day 21 after transportation. Catalase activity on day 1 ($P < 0.001$), day 7 ($P < 0.001$), and day 14 ($P < 0.01$) after transportation showed a significant decrease compared with that on day 0, reached its lowest activity on day 7 posttransportation, and increased thereafter to reach its pretransportation activity by day 21. Significant decreases in the mean SOD activity ($P < 0.001$) and GSH level ($P < 0.05$) were noted on day 1 after transportation and returned to their pretransportation levels by day 7. The mean levels of PC ($P < 0.001$), GSH-Px ($P < 0.001$), and malondialdehyde ($P < 0.01$) showed a significant increase on day 1 as compared with those before transportation and thereafter decreased to their pretransportation levels by day 14. The PC level and GSH-Px activity on days 1 and 7 after transportation showed a significant increase as compared with those on day 0. By day 14, both PC and GSH-Px had returned to normal levels, but a higher GSH-Px activity was noted on day 21 ($P < 0.05$). There was no statistically significant difference between male and female animals.

The percentages of change in TAOC, catalase, SOD, malondialdehyde, GSH, GSH-Px, and PC are shown in Figure 2. Generally, elevated parameters were malondialdehyde, PC, and GSH-Px and decreased parameters were GSH, catalase, TAOC, and SOD. Results of principal components analysis are shown in Figure 3. The 7 parameters of antioxidative and oxidative statuses were classified into 3 groups by using the 2-factor loading plots: (1) SOD and GSH; (2) TAOC and catalase; and (3) PC, GSH-Px, and malondialdehyde. Parameters within the same group were correlated with each other.

Discussion

Reactive species are produced by body tissues and fluids. Imbalance between reactive species production and endogenous antioxidants can result in oxidative stress, which disrupts or damages essential cellular functions. The present study investigated the effects of transportation on oxidative stress damage. The transportation was able to change serum antioxidant enzyme activities and contents of production of oxidative stress. Here, increased levels of malondialdehyde, PC and activity of GSH-Px, and decreased activities of SOD,

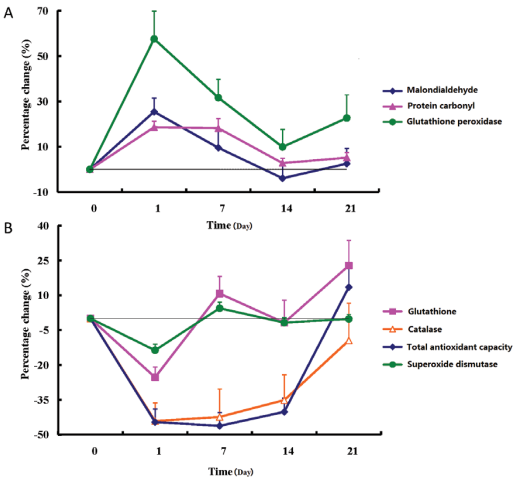

Figure 2. Percentage change of markers of oxidation status in cynomolgus macaques after transportation. (A) Increased level or activity of malondialdehyde, protein carbonyl, and glutathione peroxidase after transportation. (B) Decreased level or activity of glutathione, superoxide dismutase, total annntttioxidant capacity, and catalase after transportation.

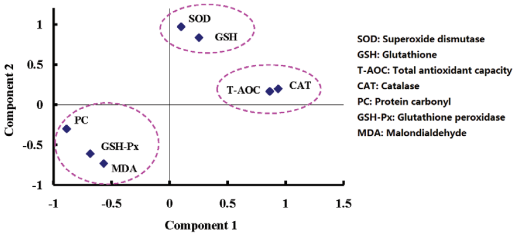

Figure 3. Principal components analysis. The 7 antioxidative and oxidative variables were classified into 3 groups by using the 2-factor loading plots. (1) Superoxide dismutase and glutathione. (2) Total antioxidant capacity and catalase. (3) Protein carbonyl, glutathione peroxidase, and malondialdehyde.

catalase, TAOC and level of GSH after transportation were observed, which suggested that there was an influence of transportation on the balance between oxidants and antioxidants in cynomolgus macaques.

Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids, leading to the production of a degraded product, malondialdehyde.[3,35] The increase in malondialdehyde level on day 1 was significant as compared with that on day 0 (pretransportation). The malondialdehyde level was increased significantly through day 3 after transportation in Boer goats,[39] and posttransportation malondialdehyde levels were increased significantly in camels and cattle as well.[24,33] Another study demonstrated that malondialdehyde concentrations in horses rose after transportation and reached the maximal level at slaughter.[45] The increased malondialdehyde level could be explained by higher levels of glucocorticoids and adrenaline-induced pathways of aerobic energy production associated with stress, which generated reactive oxygen metabolites and thus lipid peroxidation.[48] The serum cortisol level of cynomolgus macaques reportedly was markedly increased on the day of arrival.[27] Cynomolgus macaques also showed increased abnormal behaviors (for example, appetite, affiliative behavior, locomotion, exploratory behavior, aggression) and urinary concentrations of cortisol after transportation.[15] Glucocorticoids, the final effectors of the hypothalamic–pituitary–adrenal axis, are important in the control of whole-body homeostasis and the organism's response to stress.

Carbonyl (CO) groups (for example, in aldehydes and ketones) are produced on protein side chains (especially of Pro, Arg, Lys, and Thr) when they are oxidized.[25] CO groups, which are normally absent from cellular proteins, are thought to be formed after free-radical attack on amino acid residues. CO groups are only one byproduct that results from oxidative attack on proteins, but because the frequency at which the production of carbonyl residues on a typical protein alters its function is unknown, they may signal the occurrence of significant damage.[10,32] In the present study, the significantly increased level of PC in cynomolgus macaques on day 1 compared with that before transportation showed that the proteins in serum were attacked by free radicals and that antioxidant imbalance was present. The increase in both malondialdehyde and CO concentrations further suggested that the oxidative status was favored over the antioxidant status in cynomolgus macaques after transportation.

As the major defense against superoxide radicals, SOD plays an important role in the defense mechanism of biologic cells exposed to oxygen. SOD is the first line of defense against oxidative stress and represents a group of enzymes that catalyze the dismutation of superoxide anion radical ($O_2^{\cdot-}$) and the formation of hydrogen peroxide (H_2O_2).[30] Our results were consistent with the findings that the mean activity of SOD significantly decreased during and after transportation in goats and horses.[23,35] The decrease in SOD activity could be due to the excessive production of ROS during transportation-induced stress, which eventually exceeds the body's antioxidant capacity and therefore reduces SOD activity.

Catalase is one of the major antioxidant enzymes in cells, particularly cell structures that use oxygen-producing H_2O_2. Catalase catalyzes H_2O_2 into water and oxygen. Here, the decrease in catalase activity could be due to the excessive production of H_2O_2 during transportation, thus consuming catalase. Catalase activities were decreased in the liver and stomach of rats experiencing immobilization stress.[41]

GSH-Px exists in cell cytosol and mitochondria and has the ability to transform H_2O_2 into water. This reaction uses GSH and transforms it into oxidized glutathione. GSH-Px and catalase have the same action on H_2O_2, but GSH-Px has a higher affinity at high ROS concentration, and catalase has an important action with lower H_2O_2 concentration. To protect an organism against oxidative damage, GSH-Px catalyzes the reduction of H_2O_2 and lipid hydroperoxides to H_2O and their corresponding alcohols, respectively. A previous study showed that GSH-Px activity increased when an oxidative stress situation occurred, such as hyperglycemia or lipid oxidative damage.[17,28,40] Here, the increased GSH-Px activity of serum could be interpreted as a healthy defensive response against oxidative injury during oxidative stress in cynomolgus macaques.

Tripeptide GSH (γ-L-glutamyl-L-cysteinyl-glycine) is found in almost every cell at levels from 10 to 100- folds higher than its oxidized form under physiologic conditions. Due to its high concentration under physiologic conditions and its central role in maintaining the cell's redox state, GSH is the most important cellular antioxidant. Mixed-disulfide GSH is produced mainly by the catalysis of GSH-Px as well as through the direct interaction of GSH with electrophilic compounds, for example, radical species.[2] Here, cynomolgus macaques showed significantly decreased concentrations of serum GSH after transportation. This decrease indicated that our cynomolgus macaques experienced considerable oxidative stress during transportation. In addition, the decrease in GSH levels were in accordance with

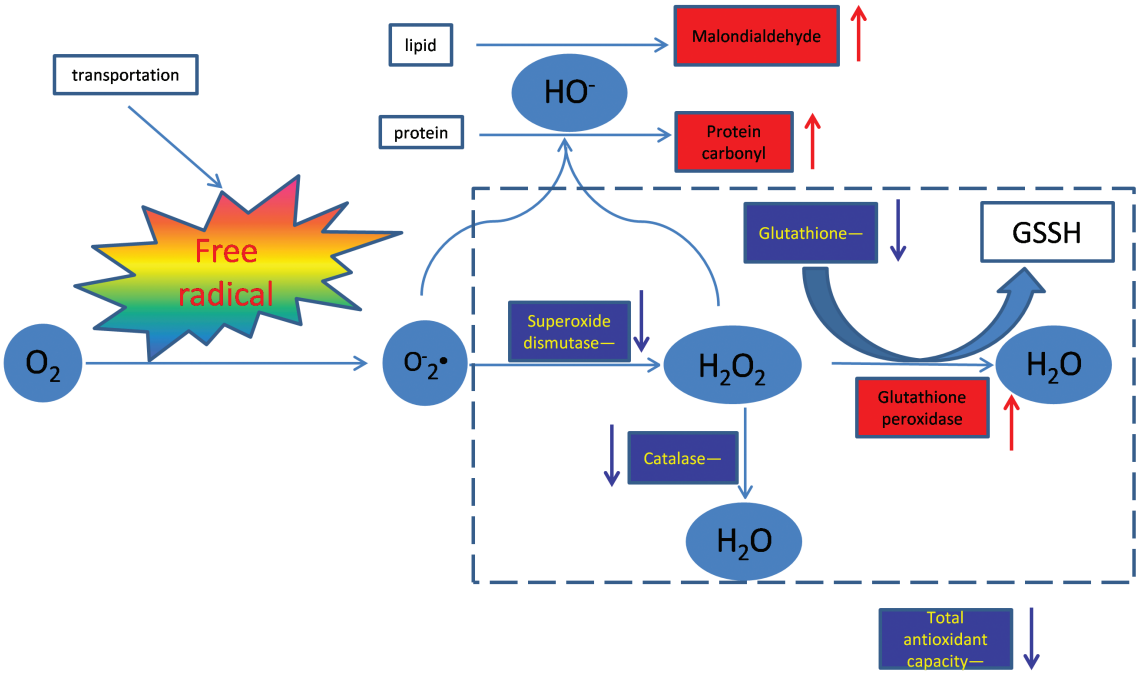

Figure 4. A diagram of transportation oxidative stress. The decreases in superoxidase, total antioxidant capacity, catalase, and glutathione and increases in protein carbonyl, malondialdehyde, and glutathione peroxidase indicate that cynomolgus monkeys experienced increased oxidative stress after transportation.

the increased activities of GSH-Px, given that GSH-Px can cause reduced GSH to revert to oxidative GSH.

TAOC has been described as a critical tool for assessing redox status. TAOC is a reliable and sensitive biomarker to monitor changes of in vivo oxidative stress, which might not be measurable through the detection of single, more specific, antioxidants.[7,18] TAOC depends mostly on the concentrations of albumin and uric acid,[2] whereas the remaining biomarkers depend on other molecules such as low-molecular-weight antioxidants, macromolecules, and different types of enzymes, such as catalase, SOD, GSH-Px and so on.[16] In the present study, the decrease in TAOC activity in cynomolgus macaques after transportation is consistent with previous reports that transportation stress significantly decreased the mean serum TAOC activity of beef cattle.[8] In addition, the decrease in TAOC activity is in accordance with the decreases in the main enzymatic compounds, such as catalase and SOD activities and GSH levels, noted in the current study.

All of these antioxidant enzymes not only synergistically prevent the damage caused by ROS, but they also protect against each other. The superoxide anion can inactivate catalase and GSH-Px, but SOD can catalyze the dismutation of superoxide anion into H_2O_2. H_2O_2 inactivates SOD and catalase, but GSH-Px catalyzes H_2O_2 into H_2O and O_2 (Figure 4). Therefore, once a member in this mutual protection system is abolished or decreased, the entire enzymatic protection system might collapse, leading to irreversible cell damage and, potentially, clinical symptoms including decreased appetite, activity, immunity, and so on.[15]

The antioxidative parameters of SOD and GSH, of TAOC and catalase, and of the oxidative parameters PC and malondialdehyde were positively correlated with each other in cynomolgus macaques after transportation (Figures 2 and 3). In addition, GSH-Px was positively correlated with PC and malondialdehyde. Perhaps GSH-Px had higher affinity with H_2O_2 at high ROS concentration, and catalase had an important action at lower H_2O_2 concentration. However, GSH-Px and catalase had the same action upon H_2O_2.[17]

However, a few limitations in the present study might have influenced the results. Blood collection might be a potential stressor in conscious animals. Therefore, to minimize this effect, the procedure was conducted by the same experienced technician specialist. Repeated blood collection might be another factor to cause stress in our animals. In this study, only a minimal amount of blood (2 mL) was collected each time point. A previous study indicated that removal of 7.5% of the total blood caused no disturbance to the normal physiology of cynomolgus macaques.[11] In addition, the removal of as much as 40% of a rat's total blood volume over 24 h and again 2 wk later caused no gross ill effects.[4,42] Withdrawing 2 mL blood (that is, 0.6% to 0.8% of the total blood volume) from each macaque weekly for 3 wk likely did not affect the transportation-related effects noted in the current study.

A small sample size poses challenges to any statistical analysis and leads to decreased predictive potential when compared with models developed with more events. As sample size increases, accuracy typically increases also, until the maximal potential accuracy is achieved, thereby reaching an asymptote.[37] Having an appropriate number of animals for each experiment is crucial: too many animals can lead to unnecessary use, and too few animals might require repetition of the entire experiment due to difficulties in analysis of the results, thus wasting animals.[19] A previous study on transportation-related stress used 6 animals per group, which met the requirement of biostatistics.[35] In the current study, we used 8 animals per sex per group to assess the effects on oxidative stress in cynomolgus macaques after transportation, and we noted statistically significant changes in all parameters examined. In addition, to minimize interanimal

variability and to reduce animal use, each macaque served as its own control.[46]

In conclusion, our results showed that increases in the activity or level of malondialdehyde, PC, and GSH-Px and decreases in GSH, SOD, catalase, and TAOC occurred after transportation of cynomolgus macaques (Figure 4). These parameters returned to basal levels after approximately 21 d of recovery. Our findings indicate that transportation caused excessive oxidative stress and resulted in the imbalance of the oxidant and antioxidant systems in cynomolgus macaques. Veterinarians and researchers should be aware that these transportation-induced changes might affect animal health and research results. Therefore, cynomolgus macaques should be allowed to recover for at least 21 d to regain their healthy status after transportation.

Acknowledgments

This work was supported by the Shanghai Municipal Science and Technology Commission (grant no. 12140903800). We thank Drs Jiaqin Yao and Xiaoping Zhao for their review of the manuscript.

References

1. **Andreyev AY, Kushnareva YE, Starkov AA.** 2005. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc) **70:**200–214.
2. **Aquilano K, Baldelli S, Ciriolo MR.** 2014. Glutathione: new roles in redox signaling for an old antioxidant. Front Pharmacol **5:**196.
3. **Belge F, Cmar A, Selcuk M.** 2003. Effects of stress produced by adrenocorticotropinon lipid peroxidation and some antioxidants in vitamin C treated and nontreated chickens. S Afr J Anim Sci **33:**201–205.
4. **Bernacky BJ**, **Gibson SV**, **Keeling ME**, **Abee CR**. 2002. Nonhuman primates, p 675–791. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, 2nd ed. New York (NY): Elsevier.
5. **Bird RP, Draper HH.** 1984. Comparative studies on different methods of malonaldehyde determination. Methods Enzymol **105:**299–305.
6. **Bourges-Abella N, Geffre A, Moureaux E, Vincenti M, Braun JP, Trumel C.** 2013. Hematologic reference intervals in cynomolgus (*Macaca fascicularis*) monkeys. J Med Primatol **43:**1–10.
7. **Cao G, Prior RL.** 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem **44 6 Pt 1:**1309–1315.
8. **Chirase NK, Greene LW, Purdy CW, Loan RW, Auvermann BW, Parker DB, Walborg EF Jr, Stevenson DE, XuY, Klaunig JE.** 2004. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. Am J Vet Res **65:**860–864.
9. **Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R.** 2003. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta **329:**23–38.
10. **Davies KJ, Delsignore ME, Lin SW.** 1987. Protein damage and degradation by oxygen radicals. II. Modification of amino acids. J Biol Chem **262:**9902–9907.
11. **Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van de Vorstenbosch C.** 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol **21:**15–23.
12. **Di Mascio P, Murphy ME, Sies H.** 1991. Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. Am J Clin Nutr **53 Suppl:**194S–200S.
13. **Fazio F, Casella S, Giannetto C, Giudice E, Piccione G.** 2015. Characterization of acute phase proteins and oxidative stress response to road transportation in the dog. Exp Anim **64:**19–24.
14. **Fernandes MS, Rissi TT, Zuravski L, Mezzomo J, Vargas CR, Folmer V, Soares FA, Manfredini V, Ahmed M, Puntel RL.** 2014. Oxidative stress and labile plasmatic iron in anemic patients following blood therapy. World J Exp Med **4:**38–45.
15. **Fernström AL, Sutian W, Royo F, Westlund K, Nilsson T, Carlsson HE, Paramastri Y, Pamungkas J, Sajuthi D, Schapiro SJ, Hau J.** 2009. Stress in cynomolgus monkeys (*Macaca fascicularis*) subjected to long-distance transport and simulated transport housing conditions. Stress **11:**467–476.
16. **Ferreira CS, Vasconcellos RS, Pedreira RS, Silva FL, Sá FC, Kroll FS, Maria AP, Venturini KS, Carciofi AC.** 2014. Alterations to oxidative stress markers in dogs after a short-term stress during transport. J Nutr Sci **3:**e27.
17. **Flores-Mateo G, Elosua R, Rodriguez-Blanco T, Basora-Gallisà J, Bulló M, Salas-Salvadó J, Martínez-González MÁ, Estruch R, Corella D, Fitó M, Fiol M, Arós F, Gómez-Gracia E, Subirana I, Lapetra J, Ruiz-Gutiérrez V, Sáez GT, Covas MI, PREDIMED Study Investigators.** 2014. Oxidative stress is associated with an increased antioxidant defense in elderly subjects: a multilevel approach. PLoS One **9:**e105881.
18. **Ghiselli A, Serafini M, Natella F, Scaccini C.** 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med **29:**1106–1114.
19. **Guhad F.** 2005. Introduction to the 3Rs (refinement, reduction and replacement). Contemp Top Lab Anim Sci **44:**58–59.
20. **Higashino A, Sakate R, Kameoka Y, Takahashi I, Hirata M, Tanuma R, Masui T, Yasutomi Y, Osada N**. 2012. Whole-genome sequencing and analysis of the Malaysian cynomolgus macaque (*Macaca fascicularis*) genome. Genome Biol **13:**R58.
21. **Horáková K, Sovcíková A, Seemannová Z, Syrová D, Busányová K, Drobná Z, Ferencík M.** 2001. Detection of drug-induced, superoxide-mediated cell damage and its prevention by antioxidants. Free Radic Biol Med **30:**650–664.
22. **Hu ML.** 1994. Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol **233:**380–385.
23. **Ishida N, Hobo S, Takahashi T, Nanbo Y, Sato F, Hasegawa T, Mukoyama H.** 1999. Chronological changes in superoxide-scavenging ability and lipid peroxide concentration of equine serum due to stress from exercise and transport. Equine Vet J Suppl (30):430–433.
24. **Kataria N, Kataria AK, Maan R, Gahlot AK.** 2010. Evaluation of oxidative stress in brucella infected cows. Journal of Stress Physiology & Biochemisty **6:**19–25.
25. **Kedzierska M, Olas B, Wachowicz B, Jeziorski A, Piekarski J.** 2012. Relationship between thiol, tyrosine nitration and carbonyl formation as biomarkers of oxidative stress and changes of hemostatic function of plasma from breast cancer patients before surgery. Clin Biochem **45:**231–236.
26. **Khadija, Ati AA, Mohammed S, Saad AM, Mohamed HE.** 2009. Response of broiler chicks to dietary monosodium glutamate. Pak Vet J **29:** 165–168.
27. **Kim CY, Han JS, Suzuki T, Han SS.** 2005. Indirect indicator of transport stress in hematological values in newly acquired cynomolgus monkeys. J Med Primatol **34:**188–192.
28. **Kohen R, Nyska A.** 2002. Oxidation of biologic systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol **30:**620–650.
29. **Lata H, Ahuja GK, Narang AP, Walia L.** 2004. Effect of immobilization stress on lipid peroxidation and lipid profile in rabbits. Indian J Clin Biochem **19:**1–4.
30. **McCord JM, Fridovich IW.** 1969. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem **244:**6049–6055.
31. **McCord JM.** 2000. The evolution of free radicals and oxidative stress. Am J Med **108:**652–659.
32. **Murphy ME, Kehrer JP.** 1989. Oxidation state of tissue thiol groups and content of protein carbonyl groups in chickens with inherited muscular dystrophy. Biochem J **260:**359–364.
33. **Nazifi S, Razavi SM, Hasanshahi F, Esmailnezhad Z.** 2009. Effect of the severity of *theileria annulata* infection on some haematological parameters and antioxidant enzymes in naturally infected cattle. Bulgarian journal of veterinary medicine **12:**63–71.
34. **Nazifi S, Saeb M, Baghshani H, Saeb S.** 2009. Influence of road transportation during hot summer conditions on oxidative status biomarkers in Iranian dromedary camels (*Camelus dromedarius*). African journal of biochemistry research **3:**282–287.

35. **Nwunuji TP, Mayowa OO, Yusoff SM, Bejo SK, Salisi S, Mohd EA.** 2014. The ameliorative effect of ascorbic acid on the oxidative status, live weight and recovery rate in road transport stressed goats in a hot humid tropical environment. Anim Sci J **85:**611–616.
36. **Ozmen B, Ozmen D, Erkin E, Güner I, Habif S, Bayindir O.** 2002. Lens superoxide dismutase and catalase activities in diabetic cataract. Clin Biochem **35:**69–72.
37. **Hernandez PA, Graham CH, Master LL, Albert DL.** 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography **29:**773–785.
38. **Paltrinieri S.** 2013. Oxidative stress and canine leishmaniasis: more than a simple consequence of host-parasite interaction. Vet J **198:**547–548.
39. **Polycarp TN, Obukowho EB, Yusoff SM.** 2015. Changes in haematological parameters and oxidative stress response of goats subjected to road transport stress in a hot humid tropical environment. Comp Clin Path **25:**285–293.
40. **Rodrigo R, Libuy M, Feliú F, Hasson D.** 2013. Oxidative stress-related biomarkers in essential hypertension and ischemia-reperfusion myocardial damage. Dis Markers **35:**773–790.
41. **Sahin E, Gümüşlü S.** 2007. Immobilization stress in rat tissues: alterations in protein oxidation, lipid peroxidation and antioxidant defense system. Comp Biochem Physiol C Toxicol Pharmacol **144:**342–347.
42. **Scipioni RL, Diters RW, Myers WR, Hart SM.** 1997. Clinical and clinicopathologic assessment of serial phlebotomy in the Sprague Dawley rat. Lab Anim Sci **47:**293–299.
43. **Suttnar J, Másová L, Dyr JE.** 2001. Influence of citrate and EDTA anticoagulants on plasma malondialdehyde concentrations estimated by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl **751:**193–197.
44. **Tilyek A, Chai C, Hou X, Zhou B, Zhang C, Cao Z, Yu B.** 2016. The protective effects of Ribes diacanthum Pall on cisplatin-induced nephrotoxicity in mice, J Ethnopharmacol **178:**297–306.
45. **Wessely-Szponder J, Bełkot Z, Bobowiec R, Kosior-Korzecka U, Wójcik M.** 2015. Transport induced inflammatory responses in horses. Pol J Vet Sci **18:**407–413.
46. **Zeng XC, Yang CM, Pan XY, Yao YS, Pan W, Zhou C, Jiang ZR, Chang Y, Ma J.** 2011. Effects of fasting on hematologic and clinical chemical values in cynomolgus monkeys (*Macaca fascicularis*). J Med Primatol **40:**21–26.
47. **Zhang W, Miao J, Wang S, Zhang Y.** 2013. The protective effects of β-casomorphin-7 against glucose -induced renal oxidative stress in vivo and vitro. PLoS One **8:**e63472.
48. **Zhou SS, Cao LL, Xu WD, Cao J, Zhao ZJ.** 2015. Effect of temperature on oxidative stress, antioxidant levels and uncoupling protein expression in striped hamsters. Comp Biochem Physiol A Mol Integr Physiol **189:**84–90.