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Clinical Evaluation in Geriatric Rhesus Monkeys (*Macaca mulatta*): Through Serological Studies and Physiological Constants in Captivity

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Abstract: Normal hematological and biochemical values are vital for sanitary assistance in a breeding population of rhesus monkeys. It is essential to count with a wide spectrum of biochemical and hematological references in geriatric rhesus populations, these values provide a refining management techniques used in these animals for biomedical research. The objective was to evaluate the health status of a rhesus monkey (*Macaca mulatta)* geriatric population in captivity for the purpose of biomedical research through the evaluation of physiological constants, body composition and serological studies hematological and biochemical tests. Twelve non human primates, 6 geriatric monkeys (20-28 years old) and 6 adult monkeys (10-15 years old) were used. The researcher was divided in two phases. According to the performed study, phase one consisted on the evaluation of physiological constants as well as the body composition and phase two consisted of the realization of serological studies. Statistically significant differences were observed in the physiological constant evaluation and in the blood chemistry for females and males as well as changes in body composition of males. No statistically significant differences were seen in the hematological tests for both groups. These results provide a framework for the proper management and refinement of applied techniques performed in geriatric rhesus monkeys.

Key words: Geriatric monkeys, physiological constant, blood chemistry, hematological test, body composition, Mexico

INTRODUCTION

The use of Non-Human Primates (NHP) as biological material in biomedical science and public health has been a fundamental element for research. This is because of the position that they stand in the evolutionary tree; they are the animal species most phylogenetically similar to humans (Cruzen and Colman, 2009; Fowler *et al*., 2010). Rhesus monkeys (*Macaca mulatta*), among other NHP are the most popular species used for biomedical research. The lifespan of these organisms is of approximately 30 years in captivity making it easier to be useful for longitudinal studies like research for biological processes such as development, maturation and aging (Cruzen and Colman, 2009; Hernandez-Godinez *et al*., 2011). Regarding the use of NHP in the study of aging and its effects, the researchers found multiple researches (Wu *et al*., 2005; Ebersole *et al*., 2008; Tang *et al*., 2008; Cruzen and Colman, 2009) but a big dilemma exists concerning aging as a biological and natural process or and aging and its related pathologies. Tigno *et al*. (2004), mention that with the decline of physiological functions as well as the increment of the prevalence of degenerative diseases, a big debate arises on weather aging and disease constitute a dichotomy or simply a continuum. Based on the above, a big urge arises in knowing the effects produced by age in a group of geriatric rhesus monkeys through clinical evaluations of physiological constants, body composition and serological studies, all these with the finality of improving and refining management techniques used in these animals for biomedical research.

MATERIALS AND METHODS

This research was undertaken in adherence to international guidelines of the World Organization for Animal Health (WOAH), Non-Human Primates Section; the International Guidelines for the Acquisition, Care and

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Breeding of Non-Human Primates of the International Primatological Society (IPS) and performed in accordance to the NOM-062-ZOO-1999, after approval of the local IACUC and the Ethics and Research Commission of the CAMINA Research Center, A.C.

Twelve Non Human Primates (NHP) were used and divided into to groups. The first group consisted of 6 geriatric animals: 3 females of 20 years of age average and a weight of 6 kg and 3 males of 28 years of age average and a weight of 7.5 kg. The second group used as a control consisted of 6 adult animals; 3 females of 10 years of age average and a weight of 6 kg and 3 males of 15 years of age and a weight of 11 kg.

The study subjects were NHP of the species *Macaca mulatta* which are housed in captivity at Centro de Investigacion Proyecto CAMINA para Curar la Paralisis A.C. at Mexico D.F. C.P. 1405. The NHP unit of Centro de Investigacion Proyecto CAMINA A.C., consists of 74 individuals living in group housing conditions where there are 44 females and 30 males of different age (Between 0 and 29 years of age). The installations of the centre are divided in 4 areas, each area forms an independent social group of animals with the objective of promoting the animals well being. The construction materials consist of concrete floors and all the surfaces are covered with ceramic tiles while the external walls are made of cyclone mesh firmly anchored to the walls and floors, all of these with the intention of exposing the animals to a natural photoperiod. The ceilings are waterproof and the doors are provided with locks to guarantee the safety of the animals and the keeper. Each area contains an automatic waterer. The food deposits are located outside the walls of each area to avoid contact of the food with the urine and feces. The monkeys were fed based on 4% of their body weight with Monkey chow Purina 5045® (Monkey Diet 5038, PMI Nutrition International, St Louis, MO) twice a day and *ad libitum* water. Medical veterinary care is provided regularly in adherence to the Mexican NOM-062-ZOO. Tuberculosis testing is performed twice annually as it is routine parasite monitoring with negative results.

The research was divided in two phases; phase one consisted of the evaluations of physiological constants and changes in body composition, phase two consisted in obtaining blood samples for hematological test and blood chemistry.

Physiological frequencies monitoring: Heart Rate (HR), Respiratory Rate (RR), Temperature (T°), Oxygen Saturation (O_2S), Systolic Pressure (SP) and Diastolic Pressure (DP) were measured every 5 min during a 20 min period. This measurement was carried out using electrodes (Electrical conductors) applied on the surface of the skin from the hand palms and the left foot sole with an equipment for physiological constants of the brand Ohmeda Cardiocap/5® (Datex-Ohmeda Inc.1315 West Century Drive, Louisville, USA).

Body Mass Index (BMI): Body Mass Index (BMI) was obtained with a formula adapted for NHP that consists on dividing weight by the square of the Crown Rump Length (CRL) (Raman *et al*., 2005):

$$BMI = (Weight, kg)/(CRL, m)^2$$

Measurement of the weight was taken with a digital scale and CRL was measured with a non elastic measure tape from the Bregma craniometrical point all the way to the animal's rump (First caudal vertebrae).

Serological analysis: Blood samples were obtained from the puncture of the saphenous vein and collected in two different tubes BD Vacutainer® (BD Insyte™®, Becton, Dickinson and Company, New Jersey, USA) 23 gauges, one with EDTA for hematological test and the other one without anticoagulant for the blood chemistry. About 3 mL were collected on each tube and analyzed in a Cobas® autoanalizer (Cobas c111, Roche Centralized Diagnostics. Avda. Quilin 3750, Macul, Santiago) from the Dr. Manuel Gea Gonzalez General Hospital.

The analytes measured in the hematological test were: leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, erythrocytes, hemoglobin, hematocrit, VGM, MCHC, MCH, RDW, MPV and platelets. The blood chemistry measured the following parameters: glucose, ureic nitrogen, sodium, potassium, chlorides, total bilirubin, total protein, albumin, globulin, ALT, AST, lactate dehydrogenase, alkaline phosphatase, calcium and phosphorous.

The animals were handled by chemical containtment, accomplished by previously sedating the animals with tiletamine+zolazepam (Zoletil® Laboratorios Virbac Carros, France) on a doses of 4 mg kg^{-1} by intramuscular administration (Galvan-Montano *et al*., 2010; Hernandez-Godinez *et al*., 2011).

Statistical analysis: The program SPSS for Windows software 16 (SPSS, Chicago, IL) was used for the statistical analysis. The value $p<0.05$ was considered as statistically significant. The Student's t-test was used for independent samples on the physiological constants evaluation and serological studies (Hematological test and blood chemistry), comparing the means obtained between the two groups, geriatric monkeys and adult monkeys.

RESULTS AND DISCUSSION

According to the results obtained by the Student's t-test for independent samples, Table 1 shows the results of the physiological constants evaluation where significant differences are seen in the HR of females ($p = 0.004$) in T° ($p = 0.04$) and finally in the SP ($p = 0.04$). For the males, significant differences in the HR ($p = 0.004$) and in T° ($p = 0.001$).

In the serological studies observed in the hematological test, there were no statistically significant differences reported for males and females (Table 2). Otherwise, blood chemistry for the females showed differences in total proteins ($p = 0.02$) and globulin ($p = 0.002$) (Table 3). For the males, there was significant difference only in glucose levels ($p = 0.03$) (Table 3). Table 4 shows the evaluation of body composition from both groups. Statistically significant differences are shown only in weight and BMI from males.

The causes that lead to aging are multifactorial. It has been described that the mechanisms that lead to the manifestations of aging are resumed by the gradual decrease of cellular mass as well as the metabolic activity in every cell from the organism (Banks *et al*., 2007), mention that in higher organisms, a regulator process exists in order to ensure survival, lessening the consequences generated by aging.

On the physiological constants evaluation (Table 1) a decrease can be observed in the HR of the geriatric monkeys against the adult group, the significant difference seen is of $p = 0.004$, for both males and females. Also, females report a significant decrease in SP ($p = 0.04$). Yuan *et al*. (2009) and Salvi *et al*. (2010) mentions that in geriatric humans there are no significant modifications in HR however, there is a tendency to decrease with age because bradycardia is a common ailment in elderly people. Many theories exist that help understand the mechanisms that affect cardiac aging including the prolonged duration of the action potential, the deterioration in intracellular calcium homeostasis and the alterations in the membrane's structure and permeability (Wu *et al*., 2005; Li *et al*., 2007; Salvi *et al*., 2010).

According to the information previously described and the results obtained in this research, the HR decrease in both sexes of geriatric monkeys as well as the SP in females could be due to the decrease in calcium concentrations. Table 3 shows a non significant decrease in the calcium concentrations in geriatric monkeys, agreeing with what mentioned by Li *et al*. (2007) and Yuan *et al*. (2009).

On the other hand, Fischbach mentions that total protein concentrations tend to decrease with age. This event is generally associated with a low caloric and protein diet ingestion or to the presence of liver diseases, nephropathies, heart diseases or enteropathies. The present studies in Table 3 points out a decrease in total proteins from the geriatric group compared to the adult group for both sexes. However, for females the decrease is significant ($p = 0.02$), relating this to a decrease in serum globulin where a significant difference is observed

Table 1: Comparison of physiological constants obtained from an elderly population against an adult population of *Macaca mulatta* monkeys

			Physiological constants											
Groups	Gender	N	HR	p-value	RR	p-value	T°	p-value	O_2S	p-value	SP	p-value	DP	p-value
Adult	Female	3	137.90±16.33	0.004	22.66±6.20	0.06	37.76±0.38	0.040	97.20±3.83	0.39	116.60±15.95	0.04	63.73±17.36	0.96
Senile		3	120.46±18.27	-	20.60±4.64	-	37.38±0.91	-	96.36±3.71	-	106.53±21.26	-	63.50±26.38	-
Adult	Male	3	143.41±9.730	0.004	18.75±7.39	0.70	38.70±0.67	0.001	99.20±1.58	0.08	124.12±12.00	0.86	73.29±19.30	0.72
Senile		3	118.62±8.810	-	19.50±6.15	-	37.65±1.03	-	98.08±3.43	-	122.37±46.25	-	76.62±41.26	-

A statistically significant difference is observed between the adult group and the senile group in female and male; ±values shows the Mean±SD values

Table 2: Hematological parameters in male and female rhesus monkeys

			Hematological parameters											
Groups	Gender	N	Leukocytes	p-value	Neutrophils	p-value	Lymphocytes	p-value	Monocytes	p-value	Eosinophils	p-value	Basophils	p-value
Adult	Female	3	10.48±4.41	0.80	6.65±2.37	0.88	3.14±1.68	0.41	0.50±0.42	0.86	0.14±0.15	0.90	0.07±0.12	0.52
Senile		3	11.08±3.56	-	6.45±2.46	-	4.05±2.04	-	0.46±0.27	-	0.12±0.15	-	0.03±0.04	-
Adult	Male	3	8.77±5.13	0.45	4.51±1.53	0.28	3.50±3.35	0.62	0.67±0.70	0.82	0.08±0.07	0.38	0.02±0.02	0.57
Senile		3	6.97±2.49	-	3.55±1.43	-	2.72±1.69	-	0.58±0.65	-	0.13±0.10	-	0.03±0.04	-
Groups	Gender	N	Erythrocytes	p-value	Hemoglobin	p-value	Hematocrit	p-value	VGM	p-value	MCH	p-value	MCHC	p-value
Adult	Female	3	6.36±0.88	0.34	14.65±1.24	0.61	45.13±5.29	0.63	71.32±6.96	0.89	22.63±1.60	0.16	32.58±1.63	0.84
Senile		3	6.85±0.84	-	15.18±2.17	-	46.67±5.60	-	71.92±8.86	-	24.48±2.58	-	32.43±0.76	-
Adult	Male	3	7.06±1.11	0.62	16.40±2.04	0.38	51.15±6.44	0.50	72.70±3.37	0.91	23.33±0.81	0.49	32.08±0.79	0.35
Senile		3	6.78±0.76	-	15.53±1.11	-	49.05±3.74	-	72.50±3.14	-	22.98±0.89	-	31.68±0.65	-
Groups		Gender	N	RDW	p-value	Platelets	p-value	MPV	p-value					
Adult		Female	3	13.94±0.95	0.26	402.0±128.2	0.27	9.22±0.91	0.79					
Senile			3	14.49±0.60	-	332.7±74.29	-	9.11±0.37	-					
Adult		Male	3	13.77±0.58	0.49	296.33±81.7	0.83	9.57±1.21	0.18					
Senile			3	14.07±0.86	-	306.5±83.33	-	8.52±1.32	-					

±values shows the Mean±SD values

Table 3: Biochemical parameters of male and female rhesus monkeys

			Blood chemistry											
Group	Gender	N	Glucose	p-value	Ureic nitrogen	p-value	Sodium	p-value	Potassium	p-value	Chlorides	p-value	Total bilirubin	p-value
Adult	Female	3	72.17±9.700	0.51	25.52±6.47	0.69	146.17±2.93	0.12	3.55±0.27	0.14	106.67±3.27	0.83	0.22±0.05	0.32
Senile		3	76.00±10.08	-	23.98±6.65	-	143.00±3.63	-	3.91±0.48	-	107.17±4.58	-	0.27±0.27	-
Adult	Male	3	72.2±11.160	0.03	28.75±6.66	0.95	145.17±3.43	0.43	3.78±0.58	0.58	107.33±4.27	0.34	0.31±0.14	0.43
Senile		3	88.83±7.030	-	28.94±3.37	-	147.00±4.34	-	3.62±0.44	-	105.17±3.25	-	0.26±0.09	-
Group	Gender	N	Total proteins	p-value	Albumin	p-value	Globulin	p-value	COC A/G	p-value	ALT	p-value	AST	p-value
Adult	Female	3	7.13±0.81	0.02	3.25±0.67	0.74	4.12±0.19	0.002	0.92±0.29	0.76	40.08±5.37	0.96	52.50±12.18	0.07
Senile		3	6.18±0.39	-	3.14±0.42	-	3.08±0.43	-	0.88±0.12	-	39.92±7.70	-	39.71±10.49	-
Adult	Male	3	7.03±0.76	0.53	3.65±0.67	0.57	3.67±0.51	0.280	0.85±0.27	0.96	38.92±6.99	0.63	46.33±9.830	0.51
Senile		3	6.75±0.75	-	3.42±0.71	-	3.38±0.35	-	0.85±0.08	-	40.83±6.37	-	49.67±7.000	-
Group	Gender	N	LDH	p-value	ALP	p-value	CK	p-value	Phosphorous	p-value	Calcium	p-value	Creatinine	p-value
Adult	Female	3	376.7±170.2	0.62	266.5±217.5	0.64	187.0±61.99	0.08	5.48±1.74	0.620	10.33±0.85	0.32	0.97±0.39	0.21
Senile		3	428.1±180.8	-	217.6±128.9	-	131.3±24.58	-	5.05±1.16	-	9.87±0.67	-	0.74±0.12	-
Adult	Male	3	551.6±488.9	0.36	244.1±125.6	0.19	244.72±59.6	0.32	4.99±0.81	0.052	10.07±0.67	0.33	1.14±0.36	0.88
Senile		3	341.0±210.5	-	160.2±77.92	-	204.67±73.8	-	5.87±0.56	-	9.67±0.69	-	1.11±0.37	-

LDH = Lactic Dehydrogenase; ALP = Alkaline Phosphatase; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ±values shows the Mean±SD values

(p = 0.002). For males, this decrease in total proteins does not show statistically significant differences but it is the same phenomenon seen in the females. Shum *et al.* (2005) and Kuzuya *et al.* (2007) mention that the decline in globulin is produced by nephropathies and cardiopathies, mainly heart failure or also by hemolytic anemia. Based on these arguments and to the above described the decrease in HR and SP in geriatric monkeys could be mainly caused by the sum of all these metabolic changes, related to the obtained results from the blood chemistry (Table 3).

As the same in humans, NHP present structural changes like reduction of height. Males showed a decreasing tendency in height even though there was no statistically significant difference. Body composition modifies because fat increases in the visceral area and the muscle mass decreases. Appetite reduces and weight loss may appear (Roth *et al.*, 2004). Weight loss in relation to height is observed in the BMI, shown in Table 4. The two groups decreased their BMI, showing a statistically significant difference in males from an average of 27.72±1.46 to a BMI of 20.86±0.75. In aging, a loss of muscular mass exists, so as a consequence, protein storage from skeletal muscle might be inadequate for protein synthesis (Mahan and Escott-Stump, 2004). All these summed up with a poor ingestion might cause a decrease in protein levels like mentioned before. Malnutrition risk is common during aging however, albumin values do not show significant difference between adult monkeys and geriatric monkeys although, a decreasing tendency is perceived associated to aging.

Finally, it is fully described that geriatric patients normally present an important reduction in thermoregulatory mechanisms, being common the event of hypothermia (Shum *et al.*, 2005). This argument supports the results found in this research where a statistically significant difference was observed in the body's temperature for males and females showing a decrease in the geriatric group compared to the control group (Table 1).

On the other hand, the blood chemistry for geriatric monkeys (Table 3) shows an increase in glucose compared to the control group in adults. For the males, this augment was statistically significant (p = 0.03), unlike females where there is an increase in glucose serum levels for geriatric females in relation to the adult group but no significant difference (Table 3). Research studies like the one from Tigno *et al.* (2004), Rezzi *et al.* (2009) and Wang *et al.* (2009) support this finding. They mention that serum glucose values in geriatric monkeys increase because glucose intolerance is common in aging. This occurs because of insulin resistance without reaching a diabetic state, equally seen in elderly patients (Banks *et al.*, 2007). For this reason, it is believed that one of the main mechanisms for insulin resistance is given by of an increase in free fatty acids derived from the adipose tissue, a common hyperlipidemia in aging. Free fatty acids are captured by peripheral tissues, especially muscle interfering with the signaling transmission produced by the insulin-receptor union.

At last, Table 2 shows the parameter's values derived from the hematological study, reporting a null presence of statistically significant differences in any parameter. Guillen and Ruiperez, explain that hematological parameters in geriatric human patients do not change with aging. They mention that an augment in fat is produced in the bone marrow and a decrease in hematopoietic tissue is seen, coupled with a decrease in the bone's marrow activity which in basal conditions do not show any clinic repercussion.

Table 4: Body composition from rhesus monkeys

Group	Gender	N	Age (years)	Weight (kg)	p-value	CRL (CM)	p-value	BMI	p-value
Adult	Female	3	11.00±2.64	6.23±0.81	0.480	53.00±3.50	0.23	22.24±2.86	0.240
Senile		3	20.33±4.04	6.26±1.57	-	55.16±3.21	-	20.37±3.18	-
Adult	Male	3	15.60±1.15	11.25±1.57	0.001	63.6±3.850	0.09	27.72±1.46	0.001
Senile		3	26.00±1.00	7.50±0.02	-	60.00±1.00	-	20.86±0.75	-

SD = Standard Deviation; BMI = Body Mass Index, CRL = Crown Rump Length; BMI = (Weight, kg)/(CRL, m)^2; Statistically significant difference ($p<0.05$); ±values show the Mean±SD values

CONCLUSION

Based on the conducted studies, degenerative and metabolic changes are seen in the geriatric animals group in relation with the adult group. These changes provide a framework for the proper management and refinement of applied techniques performed in laboratory animals. Remarkably, these degenerative processes observed in rhesus monkeys similarly occur in geriatric human population so, the use of these animals for biomedical research would generate an important source of knowledge in the evaluations of degenerative diseases and potential treatments in humans.

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