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Title

The Common Marmoset in Biomedical Research: Experimental Disease Models and Veterinary Management

Running head

MARMOSET DISEASE MODEL & VETERINARY CARE

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Abstract

The common marmoset, *Callithrix jacchus*, is increasingly being used as the preferred nonhuman primate (NHP) model in biomedical research. Marmosets share several physiological and biological similarities with humans, as a Simiiformes species, and their use in research programs advances knowledge of several fields. Their unique characteristics, such as small size, high fecundity, and rapid growth, offer additional advances in laboratory settings. This article reviews the developments in experimental disease models using marmosets based on our experience at the Central Institute for Experimental Animals (CIEA) in Japan. The development of genetically modified marmoset models using advanced genome editing technology attracts researchers, particularly in neuroscience-related fields. In parallel, various marmoset models of human diseases induced by surgery or drug administration have contributed to preclinical and translational studies. Among these are models for Parkinson's disease, induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; spinal cord injury models; a model for type 1 diabetes, induced by the combination of partial pancreatectomy and streptozotocin administration; and a hepatic fibrosis model induced by thioacetamides. The development of these models has been supported by refinements in veterinary care, such as the careful design of anesthetic protocols and better understanding of pathogenic microorganisms. In the second part of this review, we present a compilation of practices currently in use at CIEA that provide optimal animal care and enable safe experimentation.

41 Keywords: anesthesia protocols, disease model, marmoset, microbiology, translational research

42

43

Introduction

The common marmoset (*Callithrix jacchus*), a species of New World monkeys, shares many biological and physiological similarities with humans and is an increasingly valuable laboratory animal model. Several unique traits make marmosets an advantageous model, such as small size (average body weight: 350 g), easy handling, high fecundity with frequent twin delivery, relatively short lifecycle, and rapid sexual maturity (by 12–18 months of age) [1]. Marmoset models have been widely used in biomedical research particularly in neuroscience, infectious diseases, and preclinical studies for the development of novel drugs and therapies [1, 2]. Recent advances in genetic engineering based on stable assisted reproductive technology have further expanded the usefulness of marmoset models [3, 4]. Since the 1970s, the Central Institute for Experimental Animals (CIEA) in Japan has conducted research and development programs using marmosets as a nonhuman primate (NHP) model to bridge the critical gap between rodent models and humans. In particular, over the last decade, marmoset models of human disease for preclinical research developed at CIEA include genetically modified models and experimental models induced by drug administration or surgery. Development of these programs has been largely supported by refinements in veterinary care and animal management. In the first part of this article, we review the current status of experimental marmoset models of disease at CIEA; a discussion on current anesthetic protocols and microbiome surveys as part of veterinary management of the marmoset colony follows.

1. Experimental disease models for translational research using marmosets

1.1 Overview of marmoset research at CIEA

Historically, marmosets have been maintained as pets and zoo animals; their use as laboratory animals begun in earnest in the 1960s and 1970s [5]. During this period, breeding colonies of common marmosets for laboratory use were founded in the United Kingdom and European countries, and the United States. CIEA imported 12 species of small NHPs, including marmosets and tamarins, in the 1970s to develop NHP models for biomedical research. Since the introduction of common marmosets in 1976, CIEA has improved husbandry methods and established a breeding colony of this species from 12 marmoset pairs originally imported from the former Imperial Chemical Industries, UK in 1983 [6]. The breeding colony was transferred to a commercial breeder, CLEA Japan (formerly Japan EDM), in 1991. CLEA Japan has maintained the colony until now without crossbreeding with animals from other origins, while they have introduced animals a few times from other colonies of domestic facilities. Animals bred from this colony have been supplied to research institutes in Japan and worldwide, including in Korea and the United States.

Since the introduction of marmosets, CIEA has continued basic research projects for animal care and scientific use, such as husbandry, reproduction, experimental techniques, and veterinary care and published these outcomes as handbooks for researchers and animal technicians in Japan [7, 8]. Over the last two decades, alongside basic research programs, CIEA has conducted translational biomedical research projects using marmosets, particularly in the fields of

84 developmental biology, magnetic resonance imaging (MRI) applications, and preclinical
85 evaluation of novel therapies. In particular, the development of genetically modified marmosets
86 has been promoted with the advancement of developmental engineering technology [4]. Sasaki
87 and colleagues have established a protocol for stable assisted reproductive technology [9, 10],
88 developed a method for producing transgenic marmosets using the lentiviral vector, and were the
89 first to report the germline transmission of a transgene in primates [11]. Recently, they proposed
90 technologies for the knockout of target genes and point mutagenesis by genome editing tools and
91 produced novel disease models, including models for immunodeficiency and Alzheimer's disease
92 [12-14].
93
94 **1.2 Experimental disease models for transrational research using marmosets**
95 In addition to use in genetically modified (GM) disease models, marmosets have been used
96 in non-GM disease studies [1, 2, 15]. CIEA and collaborating institutes have developed various
97 non-GM disease models induced by surgery and/or drug administration (mentioned below) and
98 models for infectious [16, 17] and spontaneous diseases [18] for preclinical research, as outlined
99 in Table 1.
100 Marmosets and humans share the basic plan of nervous system organization, and marmoset
101 models of neurodegenerative disease are valuable for translational research [1]. A Parkinson's
102 disease (PD) model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
103 administration that causes degeneration of dopaminergic neurons in the substantia nigra is a

104 flagship marmoset model; the model has been extensively used in various applications, from basic
105 pathophysiological studies to the preclinical evaluation of novel drugs and therapies worldwide
106 [19, 20], whereas another PD model induced by 6-hydroxydopamine (6-OHDA) injection into
107 dopaminergic neural areas has been used in marmosets as in rodents [21]. Compared to other
108 NHPs, marmosets are particularly suited for behavioral measurements in parkinsonism and for
109 monitoring the safe management of the MPTP toxin because of their small body size and abundant
110 motor activity. Ando and colleagues [22, 23] established a simple dosing schedule of MPTP
111 administration to induce PD with subcutaneous injections of 2 or 1 mg/kg/day for three
112 consecutive days; the authors have also established care protocols for the acute toxic phase that
113 include oral administration of nutrient solution and subcutaneous infusion for hydration, as well
114 as protocols for behavioral measurements, such as automated counting of spontaneous motor
115 activity and dysfunction scoring systems. The MPTP-treated marmosets exhibited major signs of
116 PD, such as immobility (decrease of spontaneous motor activity), tremor, muscle rigidity, and
117 postural dysfunction in conjunction with dopaminergic degeneration of the substantia nigra [22,
118 24]. Furthermore, in MRI studies of MPTP-treated marmosets, voxel-based morphometry has
119 revealed decreased local tissue volume in the substantia nigra, and diffusion-tensor imaging
120 demonstrated fiber loss in the nigrostriatal pathway; these findings suggest a novel role for MRI
121 in the clinical diagnosis of PD [25, 26]. Furthermore, in the MPTP model, dyskinesia (involuntary
122 movements of the body), a side effect of long-term dopamine replacement therapy with L-DOPA,
123 was induced by repeated L-DOPA administration (10 mg/kg/day on 3 days/week for 6 weeks)

[27].

Marmoset models have further contributed to the preclinical evaluation of novel therapies, such as regenerative medicine research. For example, during the early stages of research and development projects, preparing large amounts of testing materials, such as induced cells, can prove technically and economically challenging. The small body weight of marmosets equivalent to that of rats (approximately one tenth of that of cynomolgus macaques) can facilitate experiments at a lower cost. In this vein, marmoset models of cervical spinal cord injury [28] have been used for the evaluation of regeneration-based therapies using hepatocyte growth factor (HGF) [29] and transplantation of iPS cell-derived neural stem/progenitor cells [30].

Several experimental disease models for translational research have been developed in marmosets; for example, a hypertrophic scar [31] model to test nucleic acid-targeting drugs, and models for myocardial infarction (Hattori et al., unpublished) and type 1 diabetes mellitus [32] for the preclinical assessment of cell transplantation therapies (Table 1). Preclinical models for liver regeneration therapies for cirrhosis would also be useful; however, marmoset models of experimental hepatic fibrosis were not available at the time. We attempted to induce liver fibrosis by administration of thioacetamide (TAA), a common hepatotoxin in rodents, and found that subcutaneous injection (SC) of TAA at doses of 2.5–40 mg/kg two or three times for more than 11 weeks caused hepatic fibrosis [33]. In a subsequent study, marked fibrotic lesions were induced by adjusting TAA doses at 30 mg/kg twice a week for an additional period of 12 months (Fig. 1a, b); TAA administration terminated when acute liver failure was suspected by weekly monitoring

144 of blood chemistry. Furthermore, non-invasive evaluation of the hepatic lesion by contrast-
145 enhanced MRI using gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-
146 DTPA), a hepatocyte-targeted contrast agent [34], was tested as an alternative to invasive hepatic
147 biopsy. MRI data were obtained on a 7.0T Biospec 70/16 scanner system (Bruker BioSpin GmbH;
148 Ettlingen, Germany) equipped with actively shielded gradients at a maximum strength of 700
149 mT/m and an imaging coil with an inner diameter 60 mm. Dynamic contrast-enhanced MRI was
150 performed with intravenous administration of 0.025 mmoL Gd/kg (0.1 ml/kg) of Gd-EOB-DTPA
151 (Primovist, Bayer, Leverkusen, Germany). Three marmosets underwent MRI using T1-weighted
152 fast low angle shot sequences before and 65 weeks after continuous TAA administration using
153 the above protocol. Relative enhancement (RE) of signal intensity [35, 36] obtained from the
154 regions of interest in the liver and the gallbladder was significantly decreased post TAA
155 administration (Fig. 1c), indicating decreased uptake of Gd-EOB-DTPA in the hepatocytes in the
156 context of TAA-induced fibrosis. The protocols for inducing stable liver fibrosis and non-invasive
157 assessment of the hepatic lesion are an attractive option in preclinical research for novel
158 transplantation therapies.
159
160 **2. Veterinary management for marmosets in biomedical research**
161 **2.1 Research on veterinary management of marmosets at CIEA**
162 In the past 15 years, research in marmoset veterinary care has mainly involved clinical and
163 pathological studies as well as design of anesthetic protocols and microbiological surveys; the

latter two topics are presented in detail in the following sections. Our clinical and pathological surveys in the past five years (2017–2021) revealed that primary spontaneous diseases in marmosets leading to death or euthanasia were marmoset wasting syndrome (MWS), followed by duodenal dilation and neoplasms. This result indicates that gastrointestinal (GI) diseases are common in captive marmosets and a major health problem for the colony [37, 38]. MWS is clinically characterized by impaired weight gain, weight loss, muscle atrophy, and alopecia commonly accompanied with anemia and hypoalbuminemia [39, 40]. The etiology remains unknown, but MWS is associated with chronic lymphocytic enteritis [37, 38, 41]. Histological examination of MWS cases in our facility also showed considerable mononuclear cell infiltration in the lamina propria of small intestinal mucosa. Recently, our group described "duodenal dilation syndrome" as a novel GI disease characterized by proximal duodenal obstruction and dilation with chronic repetitive vomiting, chronic bloating, and exhaustion, which can cause fatal aspiration pneumonia [42]. Autopsy examination revealed a narrowing lumen of the distal duodenum due to an ulcer scar or abnormal flexure, suggesting an association with duodenal ulceration, duodenal-colonic adhesion, or cholangitis; however, the onset of the disease is not clear and similar cases has been found in other colonies [38]. We have established diagnosis methods for duodenal dilation using a combination of radiography and ultrasonography [38], and we will continue to investigate the etiology of the disease and treatment options. Neoplasms observed in marmosets at the CIEA included intestinal lymphomas and small intestinal adenocarcinomas, which are the commonly observed GI tumors in captive colonies [37, 38, 41],

as well as rare lung adenocarcinomas [42]. In addition, clinical procedures to maintain the health of the colony have been refined. For example, marmosets have a high risk of fatal blood loss because of the low whole blood volume; an adult marmoset of average weight (350 g) has an estimated circulating blood volume of 24.5 ml and only 4.9 ml (20% circulating blood volume) of acute blood loss can cause hemorrhagic shock [43]. We have established a protocol for whole blood transfusion, including cross-matching, for marmosets and have demonstrated its efficacy and safety in severe anemia and persistent hemorrhage cases [44].

2.2 Anesthesia and analgesia protocols in marmosets

Administration of anesthesia before surgical procedures is crucial to relieve animal pain and distress and performing stable experiments. Anesthetic and analgesic protocols should be optimized for specific animal species and experimental purposes. Diverse anesthetic and analgesic regimes for marmosets have been reviewed recently [45, 46]; in this section, we describe our procedures and some cautionary notes based on our experience at CIEA.

Table 2 lists the anesthetic protocols, including premedication, emergency drugs, and postoperative analgesic doses for marmosets currently in use at CIEA. The small body of marmosets and their narrow airways pose challenges to the administration of anesthesia. Particular attention should be given to avoid vomiting, because of considerable risk of death from aspiration. Fasting the animals before anesthesia (at least 3 h) should be routinely performed and administration of antiemetics (e.g., maropitant) is recommended. To maintain stable respiration,

204 the use of anticholinergics (e.g., atropine) for the reduction of salivary and bronchial secretions,
205 keeping the tongue pulled out for preventing glossoptosis, and careful observation of breathing
206 during anesthesia are recommended. Fluid administration before and during anesthesia is
207 recommended for supporting the cardiovascular function and correction of fluid losses; for
208 example, 6–15 ml/kg of 2.5% dextrose and 0.45% sodium chloride solution is subcutaneously
209 administered before anesthesia at CIEA. Thermal support during and post anesthesia with a
210 heating device and an intensive care unit chamber is essential because the larger surface area to
211 volume ratio makes marmosets susceptible to hypothermia. In addition, a report indicated that the
212 administration of anesthetic agents might lead to hypoxemia [47]. Except for minor treatments,
213 inhalation anesthesia supplemented with oxygen and monitoring of saturation of percutaneous
214 oxygen (SpO_2) is recommended. At CIEA, a SpO_2 sensor probe for human neonates (e.g. TL-
215 260T multi-site Y probe, Nihon Kohden, Tokyo, Japan) is attached or clipped to the hand, foot,
216 calf, or tail, and a monitoring equipment for human (e.g. OLV-4201, Nihon Kohden) and small
217 animal medicine (e.g. BSM-3592, Nihon Kohden) are used. Other sensors of SpO_2 designed for
218 pediatric use or rodents are available for marmosets [46]. During a major surgery or long
219 anesthesia, in addition to respiration, SpO_2, and pulse, rectal temperature and electrocardiogram
220 are monitored. If anesthetic emergency, such as bradycardia (<120 bpm) or respiratory arrest, is
221 observed, the inhaled anesthetic concentration is lowered, and emergency drugs are administered
222 depending on the situation; Table 2 lists the emergency medications administered at CIEA. The
223 short oral cavity and visible larynx of marmosets make intratracheal intubation relatively easy,

and inhalation anesthesia with a ventilator should be performed in long-term surgeries to maintain a stable ventilation. At CIEA, feeding tubes (6–8 Fr) for human neonates (Atom Medical, Tokyo, Japan) as endotracheal tubes are intubated at a 4–5 cm distance from the incisors, and volume control ventilation is performed at 4–7 ml tidal volume for 30–40 times/min using a ventilator (SN-480, Shinano Manufacturing, Tokyo, Japan).

Induction with injectable agents allows smooth transition to anesthesia and provides adequate analgesia and stable maintenance of anesthetic level when combined with inhalation anesthetics. In the past, ketamine had been mainly used for induction at CIEA. Ketamine is a useful injectable anesthetic agent because of its rapid induction, analgesic effect as a N-methyl-D-aspartate receptor antagonist, and wide safety margin [46]. Combinations of ketamine and α_2-adrenergic receptor agonists, such as xylazine, medetomidine, and dexmedetomidine, induce sedation or general anesthesia in marmosets [7, 45, 46]. On the other hand, ketamine has been regulated as a narcotic agent with strict license-based restrictions in Japan since 2007. In our experience, administration of ketamine (30 mg/kg) caused adverse side effects, such as hypersalivation, vomiting, and respiratory arrest, during isoflurane inhalation anesthesia. Furthermore, a combination of medetomidine, an α_2-adrenergic receptor agonist, midazolam, a benzodiazepine, and butorphanol, an opioid (MMB), which has been widely used in mice and other laboratory animals [48, 49], is selected as an alternative induction agent (Table 1). Conveniently, butorphanol is known to have antiemetic effect [50, 51]. The preferred combination of MMB is medetomidine 0.04 mg/kg, midazolam 0.4 mg/kg, and butorphanol 0.4 mg/kg delivered via

244 intramuscular injection (IM); this combination was optimized for marmosets by arranging a dose
245 reported in ring-tailed lemurs [52] and patas monkeys [53]. The administration of atipamezole
246 0.2 mg/kg IM at the end of surgery reverses the effect of medetomidine and facilitates smooth
247 recovery from anesthesia. In our experience, MMB before isoflurane inhalation has been used in
248 more than 1,000 operation cases a year in the last 10 years with limited adverse effects, notably,
249 hypersalivation, vomiting, and apnea. Alfaxalone, which has been available in Japan since 2014,
250 and its combinations are also valid options for injectable anesthesia in marmosets [54, 55].
251 Postoperative analgesia must be provided for both humane and scientific purposes. Analgesic
252 regimens for marmosets reviewed in the literatures help appropriate pain management; however,
253 there is insufficient information on the evaluation of efficacy or pharmacokinetics of analgesic
254 agents in marmosets [46]. At CIEA, the analgesic protocol using non-steroidal anti-inflammatory
255 drugs (NSAIDs) is ketoprofen 1.2–2 mg/kg IM or meloxicam 0.1–0.2 mg/kg IM/per os
256 administered once daily for three more days post-surgery (Table 2). In cases where potent
257 analgesia is required, for example after a major surgery, opioids, butorphanol (0.02–0.2 mg/kg
258 IM), or buprenorphine (0.005–0.02 mg/kg IM/SC) are administered in addition to NSAIDs as a
259 multimodal approach.
260
261 **2.3 Microbiological surveys in marmosets**
262 Microbiological control is an essential process to maintain the health of the colony, reduce
263 biosafety risks, and obtain reliable scientific results. Although specific pathogen-free colonies

have been established in barrier environments [56, 57], marmosets are commonly raised in conventional environments. Marmosets are susceptive to various human pathogens; for example, fatal outbreaks of measles [58] and herpes simplex viruses [59] have been reported. Emphasis should be placed on preventive medical practices against human pathogens, including mandatory health certificates for staff and visitors, showing measles antibody levels and tuberculosis-free status, and restricting admission of individuals suspected of having infectious diseases. Zoonotic risks from marmosets to humans are low in established laboratory animal colonies, as marmosets are not natural hosts of herpes B virus, which is a serious zoonotic pathogen transmitted from macaques to humans [41]. Nevertheless, major zoonotic pathogens that have serious risks among humans and marmosets should be monitored because pathogens can be transmitted by indirect or direct contact with infected humans, NHPs, or other animals. At CIEA, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., and intestinal parasites have been examined in quarantine and periodical examinations. No positive cases of these bacteria and pathogenic parasites, including *Entamoeba histolytica,* have been found since the establishment of colony.

However, a major source for concern is GI tract diseases, a usual finding in captive marmosets. Opportunistic microbial infections are suspected causes of intestinal lesions; marmoset facilities worldwide have conducted investigations to understand disease causation, and several pathogens related to diseases have been reported [40, 60-62]. However, there is limited information, and microbes harbored by animals depend on their origins and housing environments. A survey at the CIEA marmoset colony aimed to identify pathogens associated with intestinal diseases and

284 improve veterinary care practices; the rest of this section highlights our main results.

285 Table 3 lists the commonly isolated protozoan, bacterial, and viral pathogens from the
286 marmosets at CIEA. Trichomonad protozoa are prevalent intestinal parasites in the colony, and
287 their association with bowel diseases has been evaluated [63]. Trichomonas is a flagellate
288 protozoan parasite that infects the digestive tract and reproductive organs of various mammals,
289 including members of the Callitrichidae family [40]. Identification of protozoan species and
290 reports on pathogenicity in marmoset colonies are largely limited. In our survey [63],
291 morphological characterization and 18S rRNA gene analysis of marmoset fecal samples identified
292 *Pentatrichomonas hominis*, a non-pathogenic opportunist in the large intestine of various
293 mammalian hosts, including NHPs [40, 64]. In this study, the positive rates of trichomonad
294 trophozoites in normal and diarrheal feces were similar, indicating that *P. hominis* was not the
295 primary cause of diarrhea or colitis. On the other hand, there tended to be large numbers of the
296 protozoa found in diarrhea feces. Some diarrheal cases with large numbers of this protozoa have
297 been treated successfully with metronidazole, an antitrichomonal and antibacterial agent,
298 suggesting a possibility that *P. hominis* is likely associated with diarrhea, and treatment with
299 metronidazole in diarrhea cases with elevated trichomonad levels can be effective. In subsequent
300 analysis of the nucleotide sequences, including the internal transcribed spacer regions, we
301 revealed low genetic divergence of *P. hominis* within our colony and other reported mammal
302 hosts, suggesting that *P. hominis* can be transmitted among marmosets and other mammals.

303 Enteropathogenic *Escherichia coli* (EPEC) is a common bacterial pathogen in the GI tract of

304 marmosets (Table 3). EPEC positive for the attaching and effacing virulence gene, *eae*, is a
305 recognized cause of typhlocolitis in marmosets [65-67]. Hayashimoto et al. [66] revealed the
306 prevalence of EPEC in bloody diarrhea cases at the CIEA colony, and experimental infection of
307 an EPEC strain (R811) isolated from a marmoset in our facility caused hematochezia with
308 attachment of gram-negative bacilli to epithelial apical membranes and desquamated epithelial
309 cells in the cecum. The recommended treatment of hemorrhagic typhlocolitis at CIEA is with
310 appropriate antibiotic choices (e.g., enrofloxacin). It should be noted that asymptomatic carriers
311 of EPEC have also been found [66], and management of EPEC in the colony requires further
312 assessment.
313 *Clostridioides* (*Clositridium*) *difficile* has also been implicated in GI diseases in the CIEA
314 marmoset colony. *C. difficile* is a gram-positive spore-forming anaerobic bacillus found naturally
315 in the GI tracts of various mammals as well as in soils and the environment [68]. Elevated
316 concentrations of these bacteria produce toxins that cause diarrhea and colitis in the host organism
317 because of an imbalance in intestinal microbiota, and fatal pseudomembranous enterocolitis cases
318 associated with *C. difficile* infection have been reported in common marmosets and related species
319 [69, 70]. At CIEA we have used an immunochromatography kit (C. DIFF QUIK CHEK®, Alere,
320 Orland, FL) to detect *C. difficile* toxins. The clinical presentations of *C. difficile* enteritis include
321 diarrhea with mucus, acute weight loss, anorexia, and no feces. When signs are observed in the
322 colony, diagnostic screening is performed, and positive cases are treated with appropriate
323 antibiotics, commonly vancomycin or metronidazole. Fecal transplantation can also be a

designated treatment strategy for *C. difficile* infection in marmosets [71].

Among rarely occurring diseases, sepsis and pneumonia cases caused by *Klebsiella pneumoniae* were prevalent in the early years of the breeding colony, in the 1970s and early 1980s; vaccination with formaldehyde-killed bacteria was conducted to manage infection [72]. In addition, a sepsis case (non-traumatic gas gangrene) caused by *Clostridium perfringens* Type A has been reported in the colony [73]; sepsis is rare as *C. perfringens* is generally considered commensal.

Although current knowledge on viruses endemic to marmosets is limited, Callitrichine herpes virus 3 (CalHV-3) is a recognized agent that may induce intestinal lymphoproliferative disease or lymphoma [74, 75]. CalHV-3 is a lymphocryptovirus of the *Gammaherpesvirinae* subfamily and closely related to the human Epstein–Barr virus [75]. Seroprevalence of CalHV-3 was 37% and 47% in two captive colonies and 50% in individuals recently captured from the wild, indicating that marmosets are natural hosts for CalHV-3 [76]. We surveyed the prevalence of CalHV-3 in the CIEA colony using polymerase chain reactions to amplify DNA samples from peripheral blood and enlarged lymph nodes of marmosets, with primers targeting major internal repeats designed by Fogg et al. [76]. The three samples from the enlarged lymph nodes and 63% (15/25) of the blood samples tested positive. This result suggests that the virus is endemic to our marmoset colony and may be responsible for the lymphoproliferative disease.

Concluding remarks

The common marmoset is currently emerging as the NHP species of choice for biomedical research. There is an increasing demand worldwide for marmosets in neuroscience projects to elucidate the organization of brain circuits and as models for neurological disorders; genome editing technologies applicable in translational studies are particularly advantageous [77-79]. The recent successful use of marmosets in biomedical studies is an extension of basic research projects for breeding, care, and experimental use since the 1970s. The development of experimental disease and preclinical marmoset models reviewed in this report, has expanded research applications using this species. A parallel advancement of experimental procedures, such as MRI, anesthesia, and veterinary care and management, including microbiological control of the colony ensued. To sustain research using the marmoset paradigm, we will continue refining experimental methods and improving veterinary care as well as practicing the principles of 3Rs (replacement, reduction, and refinement) for animal experimentation.

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369

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Figure legends

Fig. 1. Hepatic fibrosis induced by TAA in marmosets.

a. Nodular liver surface of a marmoset subcutaneously injected with TAA at a dose of 30 mg/kg twice a week for 15 months. Scale bar (black): 2 mm. b. Liver biopsy specimen with Masson trichrome stain of a TAA-treated marmoset identical to a. Fibrous lesions containing blue-stained collagen were largely located around hepatic lobules. Scale bar (black): 500 μm. c. Relative enhancement (RE) of signal intensity by dynamic contrast-enhanced MRI using Gd-EOB-DTPA, a hepatocyte-targeted contrast agent, before and 15 months post continuous TAA treatment. RE in liver and gallbladder at time points after Gd-EOB-DTPA injection was significantly decreased post TAA treatment in marmosets (n = 3). Statistical analysis was conducted by Bonferroni's multiple comparisons test following two-way ANOVA. *$P < 0.05$, **$P < 0.01$.

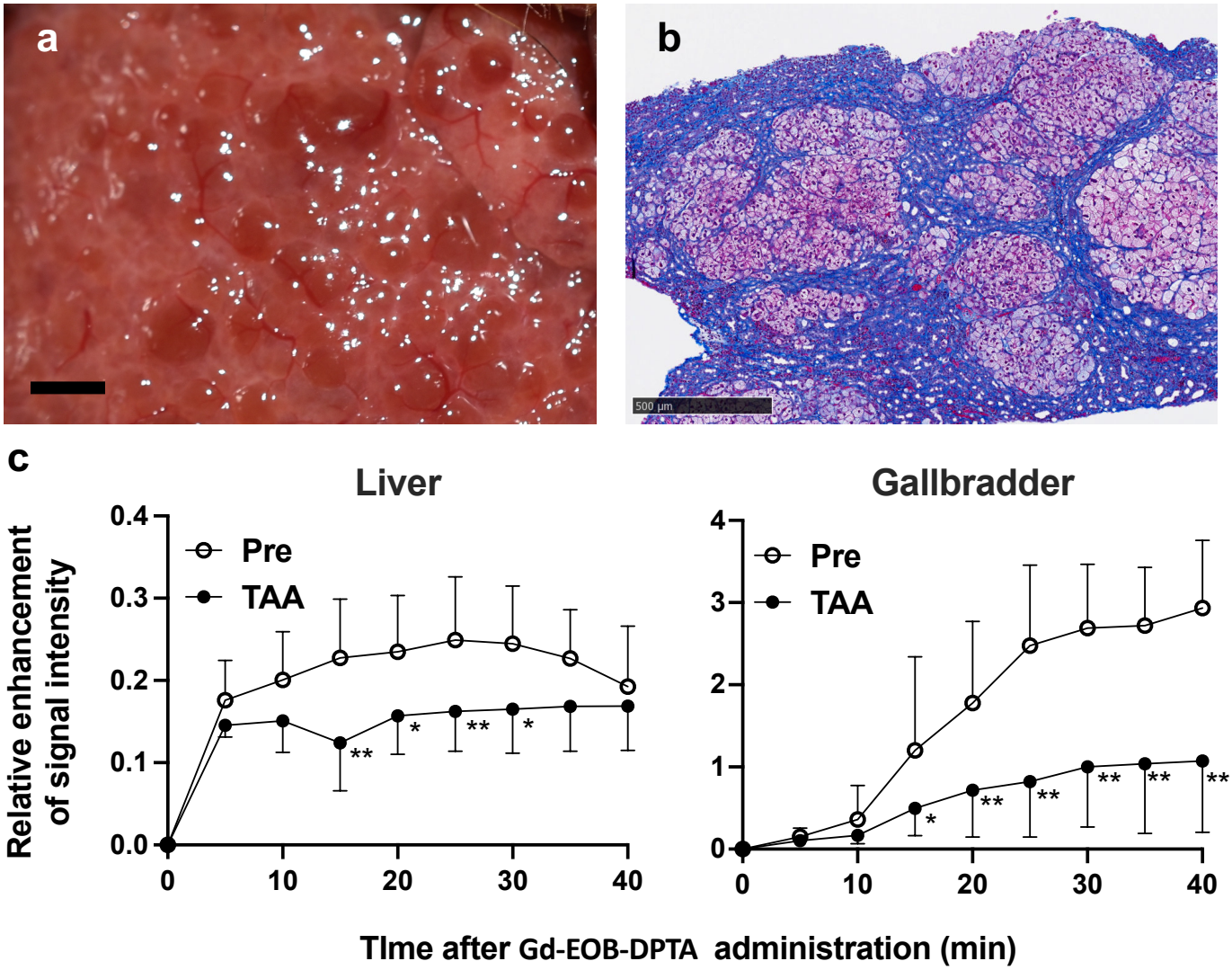

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Table 1. Examples of experimental (non-GM) disease models using marmosets involved at Central Institute for Experimental Animals

Category	Disease model	Methods	Research purposes	References
Central nervous system	Parkinson's disease	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administraion 6-hydroxydopamine injection in the brain	Behavioral pharmacology, preclinical study in drug development, MR imaging	25-31
	Spinal cord injury	Contusive injury or hemisection	Pathophysiology, stem cell therapy, preclinical study in drug development, MR imaging	32-34
	Multiple sclerosis (Experimental autoimmune encephalomyelitis, EAE)	Recombinant human myelin-oligodendrocyte glycoprotein extracellular domain (rhMOG) immunization	Pathophysiology	-
	Cerebral ischemia	Middle cerebral artery occlusion	Stem cell therapy	-
Infectious disease	Human T-cell Leukemia Virus Type1 (HTLV-1)	Infection and immune supression	Pathophysiology	21
	Influenza A	Infection	Pathophysiology	20
Others	Myocardial infarction	Ligation of left anterior descending coronary artery	Stem cell therapy	-
	Hypertrophic scar	Skin incision	Preclinical study of nucleic acid-targeted drug	35
	Diabetes mellitus (Type I)	Partial pancreatectomy and streptozotocin (STZ) administraion	Stem cell therapy	36
	Liver fibrosis	Thioacetamide administration	Stem cell therapy	37
	Glaucoma	Spontaneous (aged)	Pathophysiology	22

-: unpublished.

Table 2. Anesthesia and analgesia protocols for marmosets at Central Institute for Experimental Animals

Anesthesia protocols

Procedure	Inductive anesthetics, analgesic, premedication[a)]		Maintain anesthesia
Brief treatment	Isoflurane	4%–5% (induction, mask or box) , 1%–3% (maintain, mask)	
	Ketamine	15–50 mg/kg, IM	
	Ketamine + xylazine	15–50 mg/kg + 1.2–4 mg/kg IM	
	Alphaxalone	8–12 mg/kg, IM	
Minor surgery	MMB mixture		
	Medetomidine[b)]	0.04 mg/kg, IM	
	Midazolam	0.4 mg/kg, IM	
	Butorphanol	0.4 mg/kg, IM	Isoflurane 1%–3% (mask)
Cesarean section	Isoflurane	4%–5% (mask or box)	
	Lidocaine	1.5 mg/kg, SC (local)	
	Butorphanol (after delivery)	0.03 mg/kg, SC	
	Ketoprofen	1.2 mg/kg, IM	
Major surgery	Ketamine	30 mg/kg, IM	
	Midazolam	0.2 mg/kg, IM	
	Butorphanol	0.03 mg/kg, IM	Isoflurane 1%–3% (tracheal intubation and artificial ventilation)
	Ketoprofen	1.2 mg/kg, IM	
	Atropine[c)]	0.05 mg/kg, IM	
MRI imaging	Alphaxalone	12 mg/kg, IM	
	Atropine[c)]	0.05 mg/kg, IM	

Emergency drugs

Indication	Drug	Dose
Bradycardia	Atropine	0.05–0.1 mg/kg IM/VI
Arrhythmia	Lidocaine	0.3 mg/kg IV
Cardiac arrest	Epinephrine	0.01–0.1 mg/kg IM/VI
Respiratory arrest	Dimorpholamine	0.5–1.0 mg/kg IM

Post operateive analgesic

Analgesic		Dose
NSAIDs	Ketoprofen	1.2–2.0 mg/kg, IM
	Meloxicam	0.1–0.2 mg/kg, IM/PO
Opioids	Butorphanol	0.02–0.2 mg/kg, IM
	Buprenorphine	0.005–0.02 mg/kg, IM/SC

[a)] Pre-anesthetic fasting for at least 3 hours or maropitant 0.1 ml/kg, IM for preventing vomiting in urgent cases. Dextrose 2.5% and 0.45% sodium chloride solution 6–15 ml/kg, SC for hydration.

[b)] Reversal by atipamezole 0.2 mg/kg, IM post-surgery.

[c)] Anticholinergic for the redcution of salivary and bronchial secretion.

Table 3. Microorganisms harbored in common marmosets surveyed at the Central Institute for Experimental Animals

	Microorganisms	Relation with disease
Protozoa	*Pentatrichomonas hominis*	Commensal or diarrhea
Bacteria	Enteropathogenic *Escherichia coli* (EPEC)	Bloody diarrhea
	Clostridioides difficile	Diarrhea, pseudomembranous colitis (severe)
	Clostridium perfringens	Sepsis (rare)
	Klebsiealla pneumoniae	Sepsis, pneumonia (in old-time)
Virus	Callitrichine herpesvirus 3	Lymphoproliferative disease