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THE SUITABILITY OF USING THE FERRET AS A MODEL ORGANISM IN BIOMEDICAL RESEARCH

ADECVAREA UTILIZĂRII DIHORULUI CA ORGANISM MODEL ÎN CERCETAREA BIOMEDICALĂ

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ABSTRACT

This paper reviews the literature data considering the use of the ferret as a model organism in biomedical research. The albino ferret is the most used variant from the ferret species as an animal model in biomedical research. Its use as a pet is also known. Similarities have been identified between ferrets and humans in terms of the physiology and morphology of the respiratory system, but also on the pathogenesis or evolution of some diseases on other organs and systems. Ferrets are intensively used in the study of respiratory diseases of viral nature, such as influenza and SARS-CoV, in hypersecretory respiratory disorders, such as cystic fibrosis, in the neuroendocrinology and neuroanatomy, and even in gastric ulcer research. Through genetic engineering, transgenic ferrets have been obtained, such individuals reproducing the studied pathology much more faithfully.

Keywords: *Mustela putorius* L., similarities in physiology and morphology, microbial etiopathology, transgenic animals

REZUMAT

Această lucrare revizuiește datele din literatura de specialitate cu privire la utilizarea dihorului ca organism model în cercetarea biomedicală. Dihorul albinotic este cea mai utilizată variantă de dihor folosit ca organism model în cercetarea biomedicală. Utilizarea sa ca animal de companie este, de asemenea, cunoscută. Similarități între dihori și oameni au fost identificate în privința fiziologiei și morfologiei sistemului respirator, dar și asupra patogenezei sau evoluției unor boli ale altor organe și sisteme. Dihorii sunt utilizați intensiv în studiul afecțiunilor respiratorii de natură virală, precum influența și SARS-CoV, în tulburări respiratorii hipersecretorii, precum fibroza chistică, în neuroendocrinologie și neuroanatomie, chiar și în studiul ulcerului gastric. Prin inginerie genetică, dihori transgenici au fost obținuți, astfel de indivizi reproducând mult mai fidel patologia studiată.

Cuvinte cheie: *Mustela putorius* L., similarități în fiziologie și morfologie, etiopatogenie microbiană, animale transgenice

The polecat *Mustela putorius* L. is a species included in the *Mustelidae* Family, one of the largest and most diverse of the *Carnivora* Order (2). It represents one of the three carnivores domesticated by humans, along with the domestic dog and cat, in a process that was started about 3000 years ago by the ancient Egyptians. The domestic ferret, also known as "the English ferret", is speciated as a member of the genus *Mustela*, subgenus *Putorius* var. *Furo* (41). It is believed to be a descendant of ancestors such as the Steppe Polecat (*Mustela evermanni*) from Siberia (5) or the European polecat (*Mustela putorius*) from Northern Europe and the United Kingdom (5,21). In the historian Pliny's writings dated from 23 to 79 AD, it is described that marmot-like animals (or marmots) were used by the Romans to hunt rabbits or they were kept in the vicinity of human settlements and on sea-going ships to combat rats and/or snakes (49). Acquired resistance to snake venom is known today for such animals (50). Even so, (33) described for the first time a treatment scheme, diagnosis, and symptomatology of a house ferret bitten by a rattlesnake (*Crotalus sp.*).

Since 1921, Pitt described the polecat *Mustela putorius* to resemble the domestic ferret (*Mustela furo*) in its general appearance,but with some differences, such as those of fur colouring. For example, the deep blackish-brown coloured fur characterizes the polecat, while the majority of ferret individuals are pink-eyed cream-coated albinos (40). It seems that the first albino ferret was described in 1551, being used at that time to hunt rabbits, for fur production, to control rodents' populations or as pets, and in biomedical research, since the second half of the XX[th] century. Most of the time, males were used in research, this aspect being determined by the aggressive manifestation related to oestrus in females, which can cause health disturbances, which could jeopardize the results of an experiment. Various research methodologies have been described, from basic procedures such as blood sampling to major invasive life-saving surgery. Some areas

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of interest for research were in *(1) the field of respiratory pathology,* including respiratory infections, human influenza and SARS-CoV infection, in the testing of new vaccines and therapeutic approaches in respiratory infections, in the study of hypersecretory respiratory diseases, *(2) neuroendocrinology and neuroanatomy research*, or *(3) digestive pathology*, including the study of gastric ulcer. Despite the relatively small number of animals used in the laboratory, ferrets have proven effective in studying diseases such as human influenza and the severe acute respiratory syndrome (SARS) coronavirus (CoV) (10,46). The aim of this paper is to debate these main areas of ferret use in biomedical research.

MATERIAL AND METHODS

The use of the ferret as a model organism in the fields of respiratory pathology, neuroendocrinology and neuroanatomy, and digestive pathology represents a subject of interest in this review debate. To accomplish this aim of the paper, 52 scientific papers selected by the criteria of scientific relevance for the subject matter were revised.

RESULTS AND DISCUSSIONS

1. The respiratory pathology research

Investigations of airway physiology and pathophysiology in the ferret have led to the identification of several similarities in lung physiology and morphology between ferrets and humans. Due to the similarities in size of the trachea and the morphology of the larynx of the adult ferret to that of children, the ferret constitutes an experimental model of endotracheal intubation manoeuvres, which are carried out in a manner similar to those in neonates (13, 29). Thus, a study of (29) established that an adult ferret can be intubated up to 10 times per training session without resulting in the trauma of the upper respiratory tract, the average number being 8.1 intubations. A total of 170 intubations were completed on ferrets over a 12-month period that could be safely performed on each ferret without causing excessive trauma.

In addition to such morphological similarities of the respiratory tract that makes it possible to use the ferret as an experimental model for various techniques and usages, there is also a similarity of viral respiratory etiopathology in ferrets and humans represented by the sensitization based on similarities in the expression of some cellular makers or the existence of the same types of mucosal receptors for influenza-viruses (24, 39). Since the 1930s, ferrets have been used in studies of human influenza (36, 41, 47), and various biomedical researches (12, 32) showing that ferrets are the most susceptible to infection with human and avian influenza viruses in which the specific clinical signs of infection (fever, nasal discharge, lethargy, and sneezing) are established. A high degree of morbidity was also found, the disease is transmitted through the release and contact with respiratory contaminating droplets; in this case, the disease may easily evolve in the entire population if measures of prophylaxis are not respected. The ferret's susceptibility to such diseases is higher than that of the carnivores commonly used in these experiments. In experimental models of influenza infection, ferrets are resistant to infection with the same strain for 5 weeks after primary infection, and newborn ferrets are protected by antibodies excreted in milk. Ferrets identified as seronegative for the tested viruses are used to assess the efficacy of influenza vaccines or sera. This protocol helps ~~aims~~ to eliminate additional co-infectious factors that cannot be assessed in human population, where most adults have been exposed to at least one, but to many strains of circulating subtypes of influenza viruses (34, 43).

Ferrets are experimental animal models for the study of SARS infections (11), of the way and ability of SARS-CoV-1 or SARS-CoV-2 infecting through aerosols (7,31) or for the study of the respiratory pathology caused by these viruses (28,44). Preliminary studies have shown that ferrets reproduce disease symptoms and pathology similar to that seen in humans infected with SARS-CoV. Ferrets infected intranasally with SARS-CoV using a high viral titre (up to 106 TCID50 – Median Tissue Culture Infectious Dose, U/mL) showed classic SARS symptoms, including death in some cases, while cats did not manifest clinical symptoms. Therefore, these results suggest that the ferret could be used for preclinical evaluation of therapeutic efficacy in SARS-CoV infections (35).

Experimental infections with other viruses that affect humans are also known, including here the Simian Virus 5, canine or human parainfluenza viruses, togaviruses such as rubella virus, and paramyxoviruses that cause mumps or measles in children (16,18). Rubella virus infection in early pregnancy in women often induces congenital rubella syndrome (CRS) in children. Because the pathogenesis of the virus has not been sufficiently studied, pregnant female ferrets inoculated intranasally and subcutaneously with wild-type rubella virus TO-336 or vaccine strain TO-336 on the third day of gestation were used as an animal model. The dominant disorders induced in foetuses obtained after abortion or hysterectomy were cleft palate, iris hypoplasia, spinal deformity, visceral ectopia, and coccygeal hypoplasia. Detection of the virus in the placenta and foetal organs indicate a persistent and systemic infection occurring in the foetus, even in those without alterations (37).

Cystic fibrosis and chronic bronchitis are among the most intensively studied hypersecretory respiratory disorders in ferrets. Common characteristics of bronchial mucus, goblet cell function, and submucosal glands in the tracheobronchial tree are observed in both humans and ferrets. In order to elucidate the physiopathology of this serious disease produced by an autosomal recessive disease, in 2008, transgenic ferrets were obtained through genetic engineering that reproduce cystic fibrosis with multi-organ involvement, as it happens in human pathology, thus succeeding in the prospect of developing gene and cell therapies (27, 48, 51).

2. The neuroendocrinology and neuroanatomy research

Shifting from natural protein feeding to captive high-carbohydrate diets resulted in the development of pancreatic insulinomas (9). Moreover, the use of ferrets as pets often required their castration, the hormonal deficiency produced being associated with adrenocortical hyperplasia and the appearance of adrenocortical neoplasms (45). Endocrine disorders and tumours are most frequently observed in ferrets starting at the age of 3-5 years (3). Even if the pathogenesis of tumoral conditions in ferrets is not completely known, these animals are considered a model with potential for the study of multiple endocrine neoplastic syndromes (MEN). Unlike rodents, in which the cerebral cortex lacks gyri, in ferrets, similar to humans, there are more gyri and grooves. Thus, the use of ferrets in the research of cortical projection areas makes it possible the study of the factors that influence the development and organization of cortical structures ante- and postpartum (17,30). These animal models of complex cortical development are useful for understanding and identifying those neurodevelopmental challenges that preterm infants face. Through non-invasive MRI techniques, brain lesions and changes in brain development can be identified so that the ferret born prematurely would show a similar sequence of brain development to that of the premature infants. Ferrets are born lissencephaly, showing a thin cortical plate and relatively large ventricles. Cortical folding and white matter maturation occur in the first month of life. Thus, by the third week of postnatal life, the ferret brain undergoes a similar, albeit less complex, pattern of maturation as that observed in the human brain during the second half of gestation (4). The ferret is also considered an animal model for studying the distribution of cerebral arteries and veins, with the aim of the possibilities evaluation for the intervention in cerebral areas affected by ischemia or infarctions (1).

Ferret is used as an animal model in the study of Reye Syndrome, too. Reye's syndrome is a serious condition found in children between the ages of 6 and 12 and which is induced by the administration of aspirin but also by other anti-inflammatory drugs, mainly affecting the children's brain and liver. Hyperammonaemia was produced in young male ferrets either by feeding them with small amounts of arginine-deficient diet after an overnight fast, or by an intraperitoneal injection of urea (14). In ferrets, symptoms of the disease are manifested only after the administration of a diet unbalanced in arginine, followed by infection with human influenza type B virus and treatment with aspirin. Affected ferrets develop symptoms of encephalopathy progressing to coma and death, similar to children (14, 15, 38). Complications of Reye's syndrome can also affect the inner ear, both in children and ferrets. (42) suggest that hyperammonaemia and virus-induced metabolic disturbances may alter the micro homeostasis of the inner ear in patients with Reye's syndrome and lead to hearing loss.

3. The study of gastric ulcer

Both in humans and animals, the gastric ulcer is caused by *Helicobacter sp*. Or *Campylobacter sp.* (19,52). Although the infection shares many common symptoms, the ferret does not exhibit the polymorphonuclear cell response seen in chronic active gastritis typically described in humans with *Helicobacter pylori* infection. The damages produced in ferrets closely resemble the diffuse antral gastritis seen in some adults with gastritis, as well as the symptoms seen in children infected with *H. pylori*. The same, infection with *H. pylori* is strongly suspected of developing Irritable Bowel Disease (IBD) which is frequent in dogs, cats, ferrets, and humans (6,20,22,23,25,26). Having unclear and complex causes, disease surveillance is done by controlling the diet and, in severe cases, the medication with prednisolone can reduce the chronic inflammation of the guts (8,20,22). As in humans, IBD may produce in time malnutrition and nutrient deficiencies, it reduces the gut motility, which can progress to lymphoma, hormonal and metabolic disorders (20, 23).

CONCLUSIONS

The polecat *Mustela putorius* L. can be considered a model organism in biomedical research, considering various similarities to humans in terms of physiology and morphology of the respiratory tract, various microbial etiopathologies for respiratory and digestive systems, or in the field of neuroendocrinology and neuroanatomy research. Similarities with humans in lung physiology and morphology make the ferret a model organism for experimental endotracheal intubation. Moreover, due to the possible the same types of mucosal receptors or similarities in the expression of some cellular markers, a correspondence between clinical symptoms and etiopathological mechanisms can be concluded for various microbial infections in humans and the ferret.

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