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Review Article

ANIMAL MODELS OF HUMAN PATHOLOGY - OUR EXPERIENCE

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Summary: Animal models are often criticized as not reflecting human pathology in all aspects of a disease. However, how closely an animal model resembles the human pathology or how an animal model is produced is not a matter of debate or judgment of a good animal model. It is important to recognize that any animal model has its own advantages and limitations that need to be taken into account. The choice of an animal model should be based on the scope and aims of a particular study and the characteristics and limitations of a particular model.
In our experience, work with animal models is a special branch of laboratory animal science that requires specific knowledge and attention. The aim of the present paper is thus to highlight the knowledge and experience we have obtained through work with chemically induced animal models and to draw attention to various factors that need to be taken into account when working with animal models. The characteristics of some chemically induced animal models that have been adopted and used at the Medical Experimental Centre (animal models of gastric, colorectal and mammary carcinogenesis, colitis and acute nephrotoxicity) are briefly introduced as examples and their similarities to the corresponding human disease discussed. The main factors that may seriously affect the validity of the results when using a particular animal model are also highlighted. Some experience-based recommendations when using animal models are mentioned at the end of the paper.

Key words: animal models; human pathology; carcinogenesis; animal welfare; ethics

Introduction

Animal models are often criticized as not reflecting human pathology in all aspects of a disease. However, how closely an animal model resembles the human pathology or how an animal model is produced is not a matter of debate or judgment of a good animal model. By definition, an animal model is "a living organism in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animal"(1). It is important to recognize that any animal model has its own advantages and limitations that need to be taken into account. The choice of an animal model should be based on the scope and aims of a particular study and the characteristics and limitations of a particular model (1,2).

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Animal models

Animal models of human pathology can be broadly divided on the basis of the mode of production into spontaneous, induced and transgenic (1,2).

Spontaneous animal models originate from naturally occurring mutations, identified and maintained in a particular strain. The best known spontaneous model is probably the nude mouse. Nowadays, there are several hundred strains with inherited disorders resembling similar conditions in humans (see http://www.jax.org). Some of them have been employed at the Faculty of Medicine. For example, Goto-Kakizaki (GK) rats, a spontaneous polygenic model for lean diabetes type II (Prof. Dr. Gorazd Drevenšek) and spontaneously hypertensive rats (SHR), a model for studies in hypertension and cardiovascular disorders (Prof. Dr. Ruda Zorc).

Transgenic animal models are developed with the use of genetic engineering technology, which, since the 1980s, has enabled the production of more than 10,000 transgenic animal models all over the world. The first transgenic mouse created in Slovenia, termed *Cyp51* (lanosterol-14α-demethylase) knockout mouse, was the work of Prof. Dr. Simon Horvat (3). According to the rules for strain nomenclature, (4) the correct designations of *Cyp 51* knock-out mice are B6.129SV-Cyp51<tm1Bfro>, B6.129SV-Cyp51<tm1.1Bfro>, B6.129SV-Cyp51<tm1.1Bfro>&Cyp51<tm1.1Bfro>Tg(Alb/cre)21Mgn etc.

Transgenic models are generally used to elucidate the role of a particular gene in an organism or in the pathogenesis of a specific disorder. For example, *Crem* (cAMP response element modulator) and *Cyp 51* knock-out mice are used to study cholesterol metabolism (Prof. Dr. Damjana Rozman) (5-8).

Induced animal models are usually healthy animals in which a certain pathological process, behavior or other condition is induced experimentally, either surgically or by the administration of biologically active substances, usually termed chemical agents (1,2).

In our experience, work with animal models is a special branch of laboratory animal science that requires specific knowledge and attention. The aim of the present paper is thus to highlight the knowledge and experience we have obtained at the Medical Experimental Centre (MEC) through work with animal models and to draw attention to various factors that need to be taken into account when working with animal models. Some experience-based recommendations when using animal models are mentioned at the end of the paper.

Chemically induced animal models of human pathology at MEC

Chemically induced animal models are widely used models because they are relatively easy to induce, are reproducible and are widely available. Treatment can begin before exposure to the agents, during or after the induction period, or through all phases. Such protocols are usually used to assess the promotional or protective effects of the tested factor and, when followed closely, provide data that are fairly reproducible. Chemically-induced pathologies are also nowadays frequently used in genetically engineered mice and rats to study various basic mechanisms of induced diseases and to elucidate the role of a particular deleted or inserted gene in the pathogenesis of a particular disease.

In the following section, we briefly introduce the characteristics of some chemically induced animal models that have been adopted and used at MEC. Their similarities to the corresponding human disease and the main factors that may seriously affect the validity of the results when using a particular animal model are also highlighted in order to emphasize the enormous number of factors that need to be controlled when using animal models.

MNNG animal model of gastric carcinogenesis

MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) is an efficient direct carcinogen for the induction of gastric carcinoma in rodents (9). The optimal conditions for tumor induction are related to the concentration of MNNG in the drinking water and the duration of administration. A standard mode of application of MNNG is 100 μg/mL in drinking water for 6 months.

MNNG induces erosion, regeneration and benign hyperplastic adenomatous changes that precede the development of malignant adenocarcinomas, which infiltrate through the muscle layers and

involve the serosa (10). Carcinomas are most often found in the antrum of the glandular stomach and rarely in the corpus (Fig. 1a) (11). We have also found rare cases of squamous cell papillomas (Fig. 1b) in squamous gastric mucosa and malignant mesenchymal tumors (12).

The histological structure of induced adenocarcinomas in rat is similar to human gastric adenocarcinomas. Metastases of adenocarcinomas to the liver or the lymph nodes have only occasionally been noted in rats. Differentiated tumors (Fig. 1c) occur more frequently in rats, while human tumors show higher proliferation and metastases formation (10).

Since MNNG is degradable by light, the solution must be protected from light. The strain, sex and age of rats affect the incidence of gastric carcinoma. The use of inbred strains in carcinogenesis studies is recommended due to the many advantages they offer (the tumors are more readily transplantable, immunologic and molecular tests in genetically uniform animals are easier to perform etc.) (10,11,13).

DMH animal model of colorectal carcinogenesis

DMH (1,2 dimethylhidrazine) and its metabolite AOM (azoxymethane) are highly specific carcinogens that require metabolic activation for the induction of colorectal tumors. In short-term studies, colon carcinogenesis is usually induced by two s/c applications of DMH (150 mg/kg) or AOM (15 mg/kg) given one week apart and animals are scored for aberrant crypt foci (ACF) 8-12 weeks after the application. Tumor outcome depends on the total amount of carcinogen administered and the latency period. In long-term studies, DMH is administered weekly for 15 weeks in a relatively low concentration (20 mg/kg) and animals are scored for the number of colonic lesions 20 weeks later (Fig. 2a,b,c) (14-17).

Animals develop aberrant crypt foci (Fig. 2b), adenomas and various types of carcinomas, usually well differentiated adenocarcinomas (Fig. 2c) and, less frequently, mucinous, signet-ring cell type or undifferentiated carcinomas. Undifferentiated, mucinous and signet-ring cell carcinomas appear mostly in the proximal part of the colon and are usually surrounded by lymphoid tissue aggregates of intestinal mucosa. Tumors that are capable of metastasis are almost exclusively mucinous and signet ring cells carcinomas of the proximal colon. Adenocarcinomas of the distal colon have not been shown to metastasise (18). We have also observed a few cases of anal squamous cell carcinoma in CBA mice.

DMH/AOM colon carcinogenesis is a multistep process with morphological, histological and molecular features similar to those seen in human sporadic colon carcinogenesis, including similarities in response to some promotive and preventive agents (explained in detail in (18)).

In contrast to humans, metastases to the liver and lung are very uncommon in the DMH/AOM rat model. However, various rat and mouse strains differ in susceptibility to these carcinogens (19,20). The susceptibility for DMH/AOM-induced colorectal carcinogenesis is also sex (21) and age dependent (22,23). Since DMH is an indirect carcinogen, particular attention needs to be paid to the potential interference of a preventive compound with the metabolic pathway of DMH (24).

DSS animal model of colitis

Colitis is induced by DSS (dextran sulphate sodium) dissolved in the drinking water. Acute colitis is usually induced by continuous administration of 2-5% DSS for a short period (4-9 days). Chronic colitis can be induced by continuous treatment of low concentrations of DSS or cyclical administration of DSS; for instance, 4 cycles of DSS treatment for 7 days followed by 10 days of water (25,26).

The clinical manifestation of DSS colitis in the acute phase may include weight loss, diarrhea, occult blood in stools, piloerection, anemia, while clinical manifestations in the chronic phase of colitis do not usually reflect the severity of inflammation or histologic features found in colons. Macroscopic features include a shortened edematous colon. Typical histological changes of acute DSS-colitis are mucin depletion, epithelial degeneration and necrosis leading to the disappearance of epithelial cells (Fig. 3a). The latter is accompanied by neutrophil infiltration of the lamina propria and submucosa, cryptitis, crypt abscesses and phlegmonous inflammation in the mucosa (Fig. 3b) and submucosa. Shallow erosions also usually appear. Chronic changes consist of mononuclear leukocyte infiltration, crypt architectural

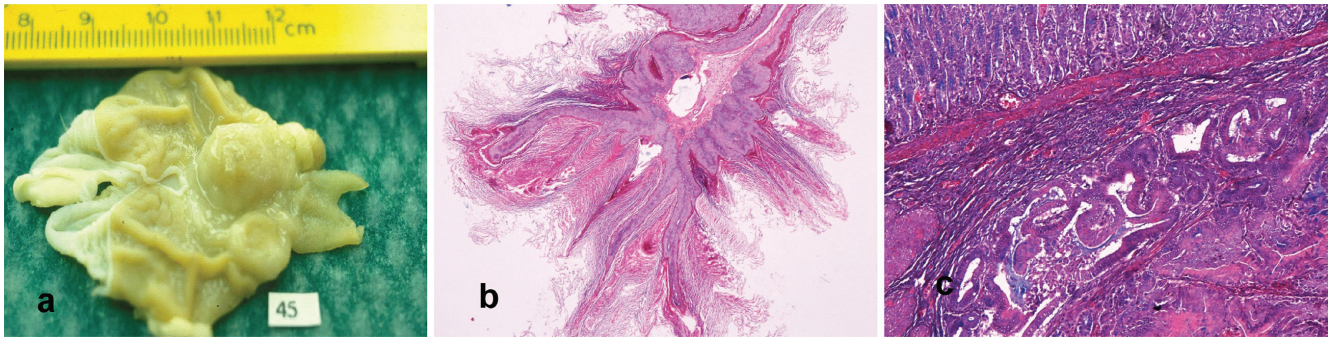

Figure 1: MNNG induced stomach carcinoma in Wistar rat that had been given MNNG solution for 6 months

a) On the right is a short segment of duodenum. A sessile (wide-based) tumor is situated in the antral region. On the opposite edge, there is a white area of squamous mucosa. b) Papillary squamous papilloma of the stomach. There is no sign of epithelial dysplasia. The tumor has a typical arborescent configuration. c) Well-differentiated adenocarcinoma of the stomach. The tumor is infiltrating the submucosa under the normal mucosa (upper edge) (Kreyberg stain).

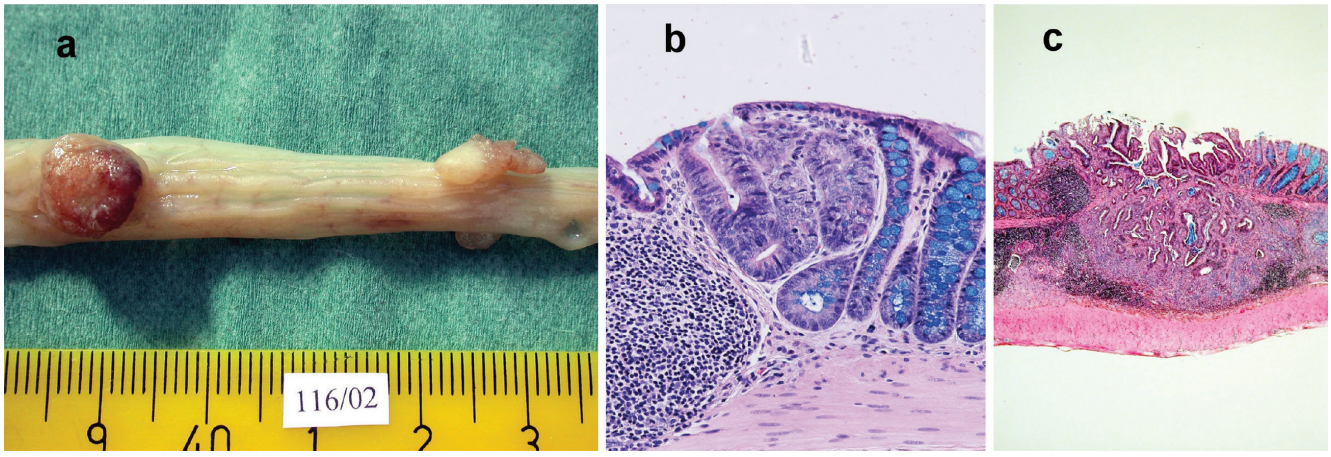

Figure 2: DMH induced colorectal tumors in Wistar rat. DMH has been injected s/c weekly for 15 weeks

a) The right tumor is a papillary adenoma while the left tumor is a sessile and ulcerated carcinoma. b) Dysplastic aberrant crypt focus situated in the vicinity of a lymphoid follicle. There is moderate epithelial dysplasia (Kreyberg stain). c) Well-differentiated colorectal adenocarcinoma infiltrating submucosa. Carcinoma invasion is accompanied by fibroplasia. The carcinoma is situated in the vicinity of a lymphoid follicle (Kreyberg stain).

disarray, a widening of the gaps between crypt bases and muscularis musosae, deep mucosal lymphocytosis, and transmural inflammation with lymphoid follicles (Fig. 3d). Re-epithelisation of rectal and distal colonic erosions by the squamous epithelium and moderate epithelial regeneratory atypia simulating dysplasia at the edge of chronic erosions are also found (Fig. 3c) (27).

The histological changes are the features of inflammatory bowel disease (IBD) in man, some of them of ulcerative colitis (regular rectal localization) and some of Crohn's disease (transmural inflammation with disseminated lymphoid follicles, focal lesions) (28,29).

Many factors can influence the susceptibility, onset, severity and responsiveness to DSS induced colitis, such as DSS (concentration, molecular weight, duration of DSS exposure), genetic (strain, substrain and gender) and microbiological (microbiological status and intestinal flora) factors of the animal, which is discussed in detail elsewhere (27).

MNU animal model of mammary carcinogenesis

MNU (N-methylnitrosourea) is a highly specific carcinogen for the mammary gland. Induction is usually i/p in a dose of 50 mg/kg (30-32). Animals develop mammary tumors (Fig. 4a) classified as adenomas and carcinomas. Carcinomas are invasive and non-invasive, and are termed *in*

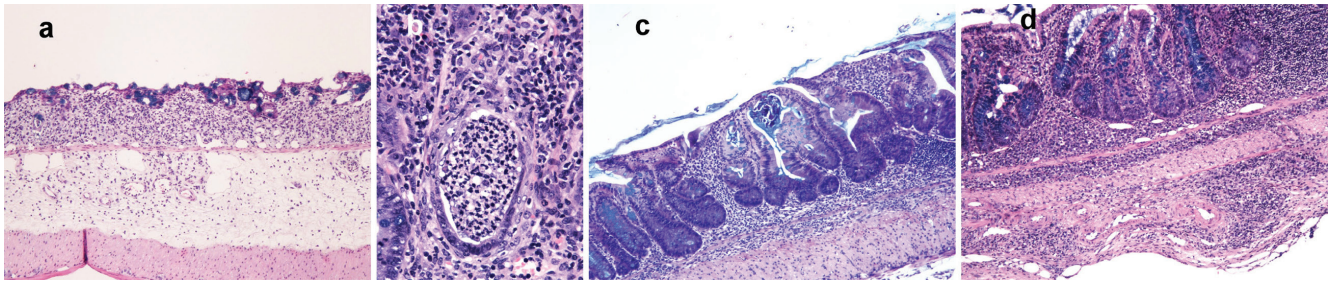

Figure 3: DSS lesions of colorectal mucosa in C57BL/6JOlaHsd mice. The mice have been given a 3% DSS solution for 5 days

a) Early DSS lesion. There is severe depletion of the epithelial cells and mucinous depletion of the colorectal mucosa. Mild mononuclear and neutrophil inflammatory cell infiltration is present in the submucosa (Kreyberg stain). b) Crypt abscess as an early DSS lesion of the colorectal mucosa. There is mucinous depletion in the epithelial cells and dense mixed inflammatory infiltration of the lamina propria (Kreyberg stain). c) In addition to severe inflammatory changes, there are moderate epithelial atypia (nuclear stratification, numerous mitoses, budding) indistinguishable from dysplasia, characteristic of neoplasia (Kreyberg stain). d) Transmural inflammatory infiltration in a chronic phase of DSS colitis. There is mucinous depletion, deep lymphocytosis, architectural crypt anomalies (Kreyberg stain).

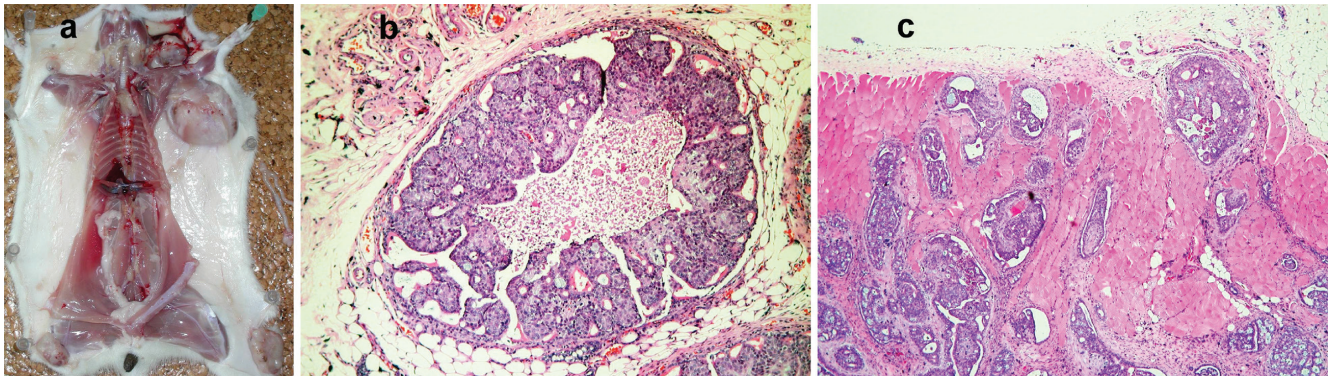

Figure 4: MNU induced mammary tumors in Sprague Dawley female rats. The first palpable tumors were observed 9 weeks after two i/p applications of MNU (50mg/kg)

a) Tumors are mainly localized in axillary and inguinal regions. b) Mammary in situ carcinoma. The tumor is surrounded by intact ductal wall. c) Mammary invasive carcinoma. Moderately differentiated carcinoma infiltrates throughout the skeletal muscle wall.

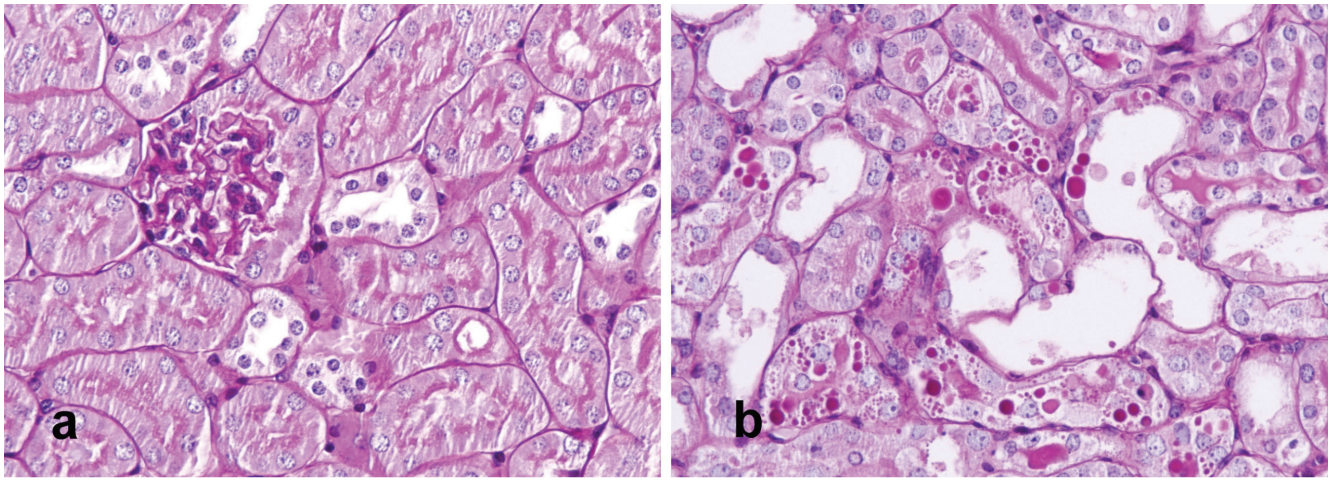

Figure 5: Early cisplatin induced kidney cortical lesions in Balb/c male mouse (5th day after i/p cisplatin application - 18 mg/kg)

a) Normal kidney cortex in Balb/c male mouse. Microvilli of proximal tubuli and basal membranes are intensively stained (PAS stain).b) There is severe necrosis and apoptosis of ductal epithelial cells, with dilatation of ducts and disappearance of cells (PAS stain).

situ ductal carcinomas (Fig. 4b). Histologically, carcinomas are composed of papillary, cystic, cribriform, solid or rarely tubular patterns. A massive stromal response, demonstrated by inflammatory infiltration and fibrosis, has frequently been observed. Invasive carcinomas (Fig. 4c) may ulcerate the skin, contain large areas of necrosis and hemorrhage and invade the local neighboring tissues, such as muscle, salivary gland or lymph nodes. Metastases in a local lymph node and in the liver or spleen are rare (33).

There is substantial evidence suggesting that the MNU animal model mimics human breast cancer in terms of the rat tumor's histopathology, mammary ductal epithelial cells origin, dependence on ovarian hormones for tumor development, and altered expression of TGFB, erbB2 and cyclin D1 (34). The MNU induced breast cancer model has therefore been used extensively to evaluate preventive and therapeutic agents for human breast cancer (35-38) and to study malignant progression (39).

It has been shown that the tumor incidence, latency period and tumor multiplicity are strain (40), dose and age (40-42) dependent, irrespective of the route of administration (42). Mammary carcinogenesis can be influenced by strain, diet, age, dose, time of day and year of carcinogen administration, and immune and endocrine system status, as well as other still unknown factors that may cause changes in tumor induction in rodents under identical conditions (43).

Cisplatin animal model of acute nephrotoxicity

Cisplatin is an effective antitumor drug with nephrotoxic activity, which is a serious problem in human medicine. Depending on the dosage of cisplatin, animals may develop various clinical and pathohistologic features of acute kidney injury. After cisplatin administration, animals often show dehydration and weight loss, a decreased number of white blood cells and hemoglobin content and bone marrow cellularity, significantly increased levels of serum creatinine and blood urea. Treatment with high doses of cisplatin usually elicits severe acute toxicity, followed by death within 3-5 days after administration (44).

Histologically, cisplatin nephropathy in rodents is characterized by early degenerative changes in the proximal tubule and consists of hydropic degeneration, cytoplasmic vacuolization and tubular dilatation. However, frequent histological features also include apoptotic and necrotic cells found in the tubules of the corticomedullary area, predominantly in the pars recta of the proximal tubule (Fig. 5) (45).

The onset, severity and mortality rate of cisplatin induced nephrotoxicity depend on the vehicle, concentration and duration of cisplatin exposure, application route, genetic background of animals (strain and substrain, gender), age and other conditions (such as food and water access).

Limitations and modifying factors of animal models

As demonstrated in the previous section, there are many factors that can influence the course of a disease in a particular chemically induced animal model and thus affect the characteristics of the model and the validity of the results. In chemically induced animal models most common modifying factors are the route of application and the dose of chemical agent used. Rarely mentioned but very important are also the preparation of the agent (pH, light, shelf life) and the application of the prepared solution, which should be fresh, sterile and properly protected. If the compound is sensitive to light and is not properly protected, then the concentration of the solution is not under control and, consequently, the outcome of the study may be significantly affected, i.e., unreproducible. When transgenic models are used, it is important to be aware that they were created in a laboratory and may therefore contain unknown mutations in other parts of the genome. In transgenic or spontaneous models, the genetic background of animals plays an important role in the characteristics of the model. The breeding and naming of a transgenic or spontaneous model is thus a factor that may significantly affect the characteristics of a model (4,46,47). To avoid misinterpretation of the results, one should closely follow the rules for strain nomenclature of the International Committee on Standardized Genetic Nomenclature for Mice and Rats (4,47).

Anyone who uses animal models should be aware that environmental conditions, microbiological factors, such as microbiota and the micobiological state of the animal (i.e., germ free, SPF, conventional) and genetic factors, such

as species, strain, gender and very often also the age of the animals, are important factors that can influence the characteristics of an animal model and affect the variability and the validity of the results.

Advantages of animal models

Although all animal models have embedded limitations, they are valuable tools for understanding various aspects of a disease, including the pathogenesis, pathophysiology of the disease and for discovering novel therapeutic targets and drugs. Research on laboratory animal models also has advantages.

In contrast to clinical or epidemiological studies, research on laboratory animals is carried out in standardized conditions. Environmental conditions (i.e., light/dark period, temperature, humidity, diet, water, bedding, housing conditions, equipment etc.) and microbiological conditions are controlled and known. Laboratory animals are kept in highly controlled environments with limited exposure to pathogens (48). Their microbiological status is regularly monitored. The genetic characteristics of animals are usually known, particularly when using inbred or other isogenic strains. The use of genetically identical animals enables highly reproducible experiments to be performed and the role of genetic factors in the pathogenesis of a particular disorder to be elucidated (49-51).

Experience based recommendations for the responsible use of animal models

Many questions about human disorders have been solved thanks to animal models and many advances have been incorporated into human health care. However, although animal models are indispensable tools in biomedical research, their use in research is not an automatic right. Scientists have a moral, ethical and legal obligation to conduct experiments on animals responsibly. This means that scientists should be well-trained and well-informed before performing an experiment on animals. There are many protocols in the literature for establishing a particular animal model. Searching for literature about a particular model is therefore an essential task. Most animal models were developed several decades ago. This means that a lot of information about both the characteristics and the limitations or other modifying factors of a particular model can be found before its use. Anyone wishing to establish a particular model in a laboratory and without any experience with animal models, is recommended to consult experts with adequate experience of animal models. Those who already have experience with animal models know that establishing an animal model in a laboratory for the first time requires experience, a thorough examination of the literature and preliminary experiment. As indicated above, there are many factors that can influence the characteristics of a particular animal model. The aim of preliminary experiment is to establish a reliable and reproducible animal model with the same characteristics, regardless of the laboratory in which the model is used. Preliminary experiment should be performed on a sufficient number of animals to obtain statistically significant results and ascertain the characteristics of the chosen model and should closely follow the protocol used. Sometimes even small discrepancies, such as age, sex, sub-strain of the animals, infection with potential pathogenic microorganism or merely application at an inappropriate time of the day, may result in failure to establish a reliable model. It is important to bear in mind that studies meet ethic and scientific criteria only when they are performed on reliable and reproducible animal models. The establishment of a reliable and reproducible animal model in the laboratory is thus a prerequisite for further research. After the establishment of a reliable and reproducible animal model, responsible research on animal models, which includes careful preparation, observation and monitoring of the animals during the research, can commence.

During experiments, careful observation of the animals is of great importance. This includes regular monitoring of the animals' weight, diet and water consumption (at least on a weekly basis). For example, monitoring DSS consumption is necessary, especially when animals are exposed to various therapeutic strategies, which may lower the consumption of DSS (increased fluid intake or thirst) (52). Changes in body weight and diet and water consumption are important measurable data that can show alterations in an animal's health.

Careful evaluation and recording of the clinical status of animals on a daily basis, including

weekends or holidays, is necessary. It is advisable to create a list of all expected alterations in the clinical status of an animal model before starting the experiment. A list of the scale or grade of observed and monitored changes in animals' health or behavior can serve as a guide in daily monitoring of animals in research. Monitoring and recording of all expected and unexpected occurrences in animal models by an experienced person with special skills and care are advised on a daily basis.

Avoiding any actions that can cause unnecessary stress to the animals is required. Stress can influence many parameters in the body, including the immune system, carcinogenesis, inflammatory diseases etc. and consequently affect the animals' welfare, as well as the scientific results. The creation of instructions about humane endpoints and actions for avoiding unnecessary suffering of animals is recommended. These instructions on humane endpoints should take into consideration all aspects of the research, i.e., the aim of the study, use of the optimal model, conditions under which the experiment is performed and animal welfare. For example, in an animal model of mammary carcinogenesis, the weight or expanse of individual or total tumor mass per animal is important data. It can affect animal behavior or even the animal's health. The influence on an animal's welfare can be even greater in the case of malignant necrotizing tumors that ulcerate. Such animals usually have an increased number of neutrophils, an indication of an inflammatory response. Thus, not only animal welfare or humane endpoints but also the validity of the scientific results can be confounded because of the unsuitable state of an animal or of factors other than those investigated (33,53).

Finally, at the end of an experiment thorough examination of each animal (autopsy) and accurate identification and interpretation of all observed alterations that have occurred in a particular model is required for the responsible conduct of an animal experiment. All expected and unexpected macroscopic lesions should be recorded and taken for further analysis. Particular attention should to be paid to unexpected lesions, i.e., lesions that are not a characteristic of a particular model. Some clinically healthy animals may possess sporadic inherited malformations or even hidden disorders that cannot be seen before careful autopsy. In such cases, identification of a lesion and its cause, as well as evaluation of its potential influence on the results of the study, is needed to avoid misinterpretation of the obtained results (54). It is advisable that, at autopsy, not only the organ/tissue of interest but various organs and tissues are taken for further analysis. Although animal models do not reflect corresponding human disorders in all aspects of the disease, most of the obtained results are needed adequately to characterize the animal model used and to corroborate the validity of the research performed.

In conclusion, although animal models do not represent the complexity of human disease, they are valuable and indispensable tools, which provide a wide range of options for investigating mechanisms and therapeutic options. However, the validity of the obtained results greatly depends on the quality and specificity of the experimental question asked and the responsible use of animals. From the point of view of animal welfare, humane endpoints, ethical considerations and scientific validity, it is very important to have enough information and knowledge about animal models to design the experiment responsibly and to evaluate the results properly.

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ANIMAL MODELS OF HUMAN PATHOLOGY - OUR EXPERIENCE

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Summary: Animal models are often criticized as not reflecting human pathology in all aspects of a disease. However, how closely an animal model resembles the human pathology or how an animal model is produced is not a matter of debate or judgment of a good animal model. It is important to recognize that any animal model has its own advantages and limitations that need to be taken into account. The choice of an animal model should be based on the scope and aims of a particular study and the characteristics and limitations of a particular model.

In our experience, work with animal models is a special branch of laboratory animal science that requires specific knowledge and attention. The aim of the present paper is thus to highlight the knowledge and experience we have obtained through work with chemically induced animal models and to draw attention to various factors that need to be taken into account when working with animal models. The characteristics of some chemically induced animal models that have been adopted and used at the Medical Experimental Centre (animal models of gastric, colorectal and mammary carcinogenesis, colitis and acute nephrotoxicity) are briefly introduced as examples and their similarities to the corresponding human disease discussed. The main factors that may seriously affect the validity of the results when using a particular animal model are also highlighted. Some experience-based recommendations when using animal models are mentioned at the end of the paper.

Key words: animal models; human pathology; carcinogenesis; animal welfare; ethics