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"NEW APPROACH METHODOLOGIES" FOR BIOMEDICAL RESEARCH

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Abstract

Animal testing is a global phenomenon but the national and European scientific scenario is changing, turning more and more to the promotion of substitute methods for the use of animals.

Significant progress has been made since the 1960s in replacing animal tests for diagnosing certain human diseases, for manufacturing biological drugs such as vaccines, and for testing their safety as they were produced. The initial driving force was the growing concern that drugs produced using animal materials could have contaminated animals of animal origin; however, the need for more accurate, faster, more efficient and less expensive testing also played a very important role.

Methods that replace techniques using live animals or methods of testing substances without the use of live animals are known as alternative methods. The term advanced technologies is often used as they are based on more sophisticated technologies and are more relevant to humans than the animal tests they replace.

Nowadays, alternative methods can include a range of techniques including cellular testing, tests using tissue from dead humans or animals, chemical-based analytical tests, computerized methods and human ethical studies.

Keywords: New Methodological Approaches, *NAMs*, alternative methods, SARS-CoV-2.

Introduction

Today more than ever, following the emergence of the emergency situation caused by the pandemic induced by the new Coronavirus, the importance of providing biomedical research becomes clear reliable, fast and effective answers. The New Methodological Approaches (NAMs) in vitro and in silico focused on human biology (human-based) and the study of patients, have led and are leading to new and interesting discoveries in the field of research concerning SARS-CoV-2 and the disease from it. caused. Some NAMs have already proven better than animal models at predicting human responses. If promoted and applied with an integrated approach, human-based NAMs allow, on the one hand, the development of safer and more effective drugs and vaccines for the human species and, on the other, the overcoming of animal testing[1].

For example, using post-mortem tissue from Covid-19 patients, a team of researchers from the Charité (Universitätsmedizin Berlin) discovered the mechanism by which the virus can reach the brain and how the immune system responds to invasion; the results show that the virus penetrates the brain through the nerve cells present in the olfactory mucosa[2]. Thanks to the latest technological frontiers, it is possible to carry out rapid tests by analyzing large amounts of data in a short time. For example, through multi-parametric single cell analyzes, it is possible to examine the immune profile of large population samples. All this allows us to study the human immune response in its unique complexity[3].

Large-scale studies of the genomes of human lung cells have made it possible to identify the genes and biological pathways potentially involved in the infection of lung cells, providing essential information on the mechanisms of infection and disease and therefore on potential therapeutic targets. Thanks to advanced genomic editing techniques applied to human cells in vitro, it was possible to identify which genes protect against infection and to characterize their respective biological functions[4].

In order to better understand how the virus spreads, improve the speed of diagnosis and consequently develop new therapeutic approaches, artificial intelligence can be applied by integrating and analyzing large amounts of data deriving from patients, identifying the most at risk according to the genetic and lifestyle characteristics. Advanced "machine learning" techniques have been used in various situations including the classification of different strains of the virus, the prediction of the survival of patients with severe forms of the disease and the discovery of new drug candidates for COVID-19 disease[5].

In the Netherlands, engineered three-dimensional cultures of patient-derived intestinal cells ("organoids") and advanced RNA sequencing techniques are being used to study the interaction between SARSCoV-2 and the human gastrointestinal system. Thanks to this model, it was discovered that SARS-CoV-2 is able to infect intestinal cells and reproduce in the intestine, using a specific receptor, present in large quantities on human intestinal cells. Researchers are further evaluating the differences between lung and intestinal infections by comparing lung and intestinal organoids infected with SARS-CoV-2[6].

Also in Italy, a research group from the University of Pisa is developing an innovative 3D lung model which, integrated with cells from sick or healed cells, allows to study the interaction between the pulmonary alveoli and the aerosol containing nanoparticles or virus.

These techniques, when used in an integrated way and combined with clinical and epidemiological studies, can provide reliable and relevant results for patients, avoiding the dangers of over-reliance on animal models, which are often unable to reflect the intricacies of human conditions[7-8].

The more we strive to promote new approaches centered on human biology, the sooner these methods will become the reference for biomedical and toxicological research, with significant benefit for patients awaiting treatment, for research and for animals. Often, unfortunately, this reality does not come out but we hear of scientists who claim animal experimentation as the only and essential way towards the progress of medical science.

In this narrative review, we aim to analyze the state of art concerning the development and research of alternative approaches that allow us to study diseases from a human perspective, and also

to explain the effects of the infection and the disease of SARS-CoV-2 on the human body.

Animal experimentation

In recent years, one of the most complex and controversial issues by researchers and representatives of animal welfare organizations concerns the use of animals for scientific purposes.

The term "animal experimentation" in the scientific field refers to the wide range of experiments conducted with the aid of animal models for study and research purposes. In particular, animals are used to understand the origin and thus effectively prevent and treat a number of diseases and ailments that affect humans. Animal testing can be basic or applied: the first concerns studies of a fundamental nature, including those of physiology, designed to improve knowledge of the normal and abnormal structure, functioning and behavior of living organisms and the environment; investigations and analyzes aimed at improving or deepening knowledge on a particular subject, phenomenon or fundamental law of nature, rather than a specific practical application of the results[9].

Applied research, on the other hand, is descriptive and aims to find practical and specific solutions based on previous basic research; its main objective is not the advancement of theoretical knowledge, but that of exploiting the theoretical knowledge already acquired for practical purposes. The different terms such as "animal experimentation, animal research and in vivo research" have the same meaning and are therefore interchangeable. The term "vivisection" is instead used as a synonym for animal experimentation by organizations that oppose it[10]; however, this use is considered instrumental and improper by the scientific community. In fact, for most of them[11], research on animals is not vivisection, as the latter must be understood as the practice of anatomical dissection of live animals (Civil Court of Cassation, section III, sentence no. 14694 of 19 July 2016).

While animal welfare organizations have been contesting, for years now, the legitimacy of animal experimentation, claiming that it is cruel, of little scientific relevance, not adequately regulated, not in line with the times and that animals have an intrinsic right not to be used as guinea pigs and they believe that it is completely outdated, the scientific community continues to reiterate how inevitable it is, arguing that there are still no other methods available to acquire new knowledge in favor of the prevention and treatment of human and animal diseases.

However, the 4 ethical-moral assumptions and regulations require the scientific community to research and develop alternative or complementary methods that aim to avoid or reduce the use of experimental animals; they reconcile, in a balanced and shared way, different values, all worthy of being recognized, such as the well-being of men, the promotion of scientific research, the reduction of suffering for animals subjected to experimentation[8].

Inter-specific differences such as anatomy, structure and function of organs, metabolism and pathways of absorption, genetics, DNA repair mechanism, behavior and cell cycle, housing conditions and undiagnosed side effects in animals, are crucial factors that can make misleading and dangerous preclinical tests in applying data to humans.

The 3R model

In 1959, two British members of the University Federation of Animal Welfare published a book destined to become fundamental for the evolution of animal experimentation "The Principles of Humane Experimental Technique".

In fact, they described, for the first time, the principle of the 3Rs, inserted by the European Union in Directive 2010/63 / EU on the protection of animals used for scientific purposes. In this text, the two scholars presented an approach that the researchers should have adopted to implement a form of animal experimentation that is more attentive to the degree of suffering that scientific practice causes in the experimental subjects and that agreed both the quality of the experimental data and the conscious use of the experimental model used[12].

The 3R principle refers to three fundamental concepts: replace (*replacement*), reduce (*reduction*) and refine (*refinement*). According to these principles, the researcher should initially try to

replace, or replace, his animal model with an alternative model, try to reduce as much as possible the number of individuals used in a certain experimental protocol and finally, improve the experimental conditions to which they are subjected. the animals.

However, the impulse to tackle the theme of animal experimentation in a broader sense, placing it in a specific bioethical perspective, only came in the seventies, on the wave of publications destined to impose the animalistic theme in the philosophical field. The use of the term "alternative" dates back to these years to indicate a replacement option for a given system and not just one more possibility. It is therefore not surprising that, in 1978, Smyth re-proposed the model of the 3Rs in the volume "Alternatives to animal experiments", a diction whose ambiguity was also emphasized, as it would suggest the immediate possibility of abolishing animal experimentation, which, in fact, the 3R model does not propose[13]. It should be added that the alternative based on the 3R model represents a path that combines the scientific and economic interest with the more specifically ethical one of avoiding or at least reducing, as far as possible, the sacrifice of animals.

A central feature of the 3R principle is that, before starting any type of experiment involving the use of animal models, it is necessary to proceed with an accurate analysis of the costs and benefits that takes into account, on the one hand, the suffering caused to experimental animals and, on the other hand, of the potential benefits that may derive from the experiment in question.

With the concept of *replacement*, we want to suggest to the researcher to investigate in depth the possibility of replacing the animal model with alternative methodologies. The two authors, Russell and Burch, described a number of alternative methods to animal experimentation based on non-living plants, microorganisms, chemical and physical systems. They introduced the concepts of partial replacement (relative replacement) and complete replacement (absolute replacement). In the first case, we refer to the animal species replaced by another species characterized by a relatively less complex nervous system than the original one, or, to those in which in a particular phase of the experimental protocol, the animal has been replaced by a non-sentient model. In the second case, however, the animal model is completely eliminated from the experimental protocol.

The second step concerns the *reduction* of the number of subjects used in a given experimental protocol. Russell and Burch described this concept as a reduction in the number of animals used, such as to obtain a quantity of numerically significant data of sufficient precision. In this type of approach, the use of statistics is of fundamental importance: an accurate experimental design, in terms of sample size and power of the selected statistical test, is essential to determine the minimum necessary number of subjects to use. Another way to generally reduce the number of experimental subjects used by different laboratories should, in theory, be to harmonize as much as possible, and internationally, the standard protocols required for toxicity tests. This would significantly reduce the need to repeat the same tests in different countries, automatically lowering the number of animals used in this particular experimental practice.

The third R, *refinement*, foresees a planning of the researches with sophisticated programming tools, in order to reduce to the minimum possible, the suffering, the stress and the damages suffered by the animals; the establishment of best practices for experiments; the housing of animals in environments suitable for each species, within the framework of a more efficient Animal Care, which is indispensable for the reliability of in vivo experimentation.

The scientific community, therefore, now has extremely wide possibilities of study available, which in the future could make it possible to gradually reduce the use of animals and improve the conditions for experimentation. This action begins when every possible effort has been made to find alternatives to the originally chosen animal model and to reduce the number of individuals used in a specific experimental design.

However, what appears particularly relevant in this proposal is the reference to a necessary and active effort to improve the welfare state of the experimental animal, beyond a simple minimization of the state of discomfort[14]. A judgment on the degree of well-being of laboratory animals and how this can be affected by certain captive conditions must be based on an accurate knowledge of the

animal species involved. Depending on the animal species and its normal social organization, environmental factors such as, for example, size and structure of the cage, light (intensity, wavelength, photoperiod, frequency), sounds, ventilation, are as important as the presence or absence of subjects of the same species, their sex and the predictability and controllability of the environment. If the goal is to improve the general conditions of well-being of an animal used in experimentation, one of the possible ways is, for example, to provide for the preparation of a stimulating and varied environment.

It may happen that the 3Rs conflict with each other as, for example, if alternative methods are to be validated and there is a need to compare the alternative method proposed with the corresponding and traditional in vivo version of this technique. This represents a conflict between the concepts of replacement and reduction.

On the other hand, a similar situation also arises when it is necessary to verify the validity of some improvement techniques of a given experimental protocol, with the aim of reducing the degree of animal suffering. In this case, the concepts of improving the experimental procedure and reducing the number of experimental subjects come into conflict. The use of remote telemetry methods, which are implanted subcutaneously or in the visceral cavity of the animal, allows to detect physiological parameters using animals free to move, and not limited by permanent catheters, or blocked by measurements that involve the immobilization of the subject experimental. These methods therefore represent an improvement of the experimental conditions for the animal used.

European standards

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010, regarding the protection of animals used for scientific purposes, is currently implemented by all 28 member states of the European Union, including Italy. It, designed to eliminate the disparities between the laws, regulations and administrative provisions of the Member States on the matter, under point 2 emphasizes that animal welfare is a value enshrined in Article 13 of the Treaty on the Functioning of the European Union and its content it has already been approved by the animal rights organization Eurogroup for animals, which includes the main animal rights groups of various European countries, including the Italian anti-vivisection league (LAV).

It provides for the prohibition of the use of primates without prejudice to the possibility of derogation; the experimentation must be authorized by the competent authorities who must certify the necessity, granting the authorization only if there are no alternative methods that can be used; the project must be made public; facilities that use animals will be subject to annual inspections and stray and wild-caught animals may not be used. It should also be kept in mind the European Commission Recommendation of 18 June 2007 relating to Guidelines for the accommodation and protection of animals used for experimental or other scientific purposes 2007/526/EC (published in the Official Journal of the European Union L197, of 30/07/2007).

The Decree is also concerned with regulating animal testing so that it is carried out by qualified persons, that suffering is minimized and that the animals themselves are raised and housed in adequate conditions.

The procedures must ensure that the experiments comply with the justification principle and that the experiments are carried out with the fewest animals, with the animals with the lowest neurological development, with methods that involve the lowest level of pain, possibly with anesthesia, and that, in any case, experimental protocols are chosen with greater probability of providing satisfactory results.

Alternative methods

On the basis of the above-mentioned Directive, an intense research and validation activity (determination of the reliability and reproducibility of a method) has been developed at European level, aimed both at identifying new in vitro methods that could be used for regulatory activity and to modify some of the existing in vivo methods to reduce the number of animals used and minimize suffering and harm.

The main alternative methods concern the use of cell cultures, the use of in silico methods and the use of invertebrate animals that are less developed from the evolutionary point of view and therefore considered less sensitive.

The MEIC (Multi Evaluation of in vitro Cytotoxicity) study, conducted between the 1980s and 1990s, was performed to verify the predictive capacity of cell cultures compared to animals in cytotoxicity tests, based on some known toxicity data of some substances on man. This study demonstrated that a battery of three assays on human cell cultures was more predictive, economical and practical than animal studies. The predictive index among this test battery was 83% compared to the results obtained using rats and mice, the index of which was 65%.

Cell culture is a promising alternative to the use of animals. This technique can be used efficiently for the screening of highly toxic compounds at an early stage of testing. Cell cultures are simplified systems that mimic physiological conditions. Cells grown in vitro are used both for the study of biological phenomena such as growth and differentiation, and for the study of the structure and function of genes by manipulation. Cell cultures offer many advantages such as the possible study of toxic effects on human cells combined with a high reproducibility and also lead to a reduction in costs compared to in vivo experimentation as they can also be obtained in a short time and requiring reduced quantities of xenobiotics.

The term in silico, on the other hand, is used to indicate phenomena of a chemical biological nature reproduced in a computer mathematical simulation, rather than in vitro or in vivo. However, it should be noted that the in silico simulations of particular phenomena, especially those involving biological macromolecules such as proteins, nucleic acids, polysaccharides, have limitations due to the inevitable hypotheses and simplifications adopted given the complexity of the simulated systems in the physical-mathematical model used for the simulation. An experiment conducted with the in silico method allows both to be able to interpret in more detail the knowledge that can derive from an experimental investigation thanks to the application of the most modern calculation methods, and to investigate the properties of a vast series of molecules that can be considered valid candidates for various technological applications such as the study of new drugs. Several 'virtual human' models called in silico have been built up to now to study known human reactions. Some examples include computer models to study human metabolism, plaque accumulation and cardiovascular risk, and to assess drug toxicity. For example, protease inhibitors for HIV patients were computer designed and tested in human tissue cultures and computer models, bypassing animal testing due to the urgent need for treatment. A new cardiovascular drug was developed and approved in 1997 based on data from a virtual heart, the data obtained on animals being inconclusive[15]. Exactly simulating real animal experiments on a computer is not easy, because the biological responses are very complex. Therefore, the results obtained with these simulated models cannot be very accurate.

Finally, invertebrates can replace the more commonly used laboratory animals. The most commonly used invertebrate species are Drosophila melanogaster, a fruit fly, a classic model used to detect mutagenicity, teratogenicity and reproductive toxicity, and Caenorhabditis elegans, a nematode worm whose body is completely transparent. These organisms have a short life cycle and can be studied in large numbers, a distinct advantage over vertebrates. Despite some obvious drawbacks such as the lack of an adaptive immune system, which is a deterrent to their use in certain types of research such as vaccine development, these organisms have potential as alternatives to the use of conventional animals. Likewise, the fruit fly can be useful in identifying new virulence factors or pharmacologically active compounds[16].

The main NAM

Advances in biotechnology and micro engineering have allowed the development of New Methodological Approaches (NAMs). Today, NAMs make it possible to study human conditions and diseases in a human-based perspective, i.e. based on human biology and in physiologically relevant conditions, playing a fundamental role in biomedical and toxicological research[17].

On the one hand, human-based NAMs allow the development of safer and more effective drugs and

vaccines for the human species and on the other the overcoming of animal testing. There is a wide variety of scanning technologies that can reveal processes in vivo, so non-invasive, in humans. The images produced at very high resolution are particularly useful in the study of the brain, neurodegenerative diseases such as Parkinson's disease and Alzheimer's. Some examples are Magnetic Resonance Imaging (MRI), Functional Magnetic Resonance Imaging (fMRI), Computed Axial Tomography (CT) with three-dimensional reconstruction, Tomography and Positron Emission (PET). These technologies, combined with each other or with other techniques, allow to study in a non-invasive way not only the anatomical structures but also the biochemical composition and metabolism of the various organs. The scientific literature of the last decade describes the use of the latest neuroimaging techniques to detect brain features related to Alzheimer's disease and metabolic syndrome and also to investigate the associations between established dietary models or nutritional interventions and Alzheimer's disease, focusing in particular on observational and intervention studies in humans[18].

Human tissues can be reconstituted in vitro starting from single cell types, aiming to reproduce the original architecture of the tissue in vivo. Thanks to recent developments in 3D printing techniques, it is possible to reconstitute different human tissues with a high level of precision. Examples of 3D reconstituted tissues are the epidermis, the corneal, oral and gingival epithelium, the vaginal epithelium and the respiratory tract. Tissue engineering is the science that studies the possibility of regenerating organs and tissues of the human body. Although it was originally born for therapeutic purposes (regeneration / replacement of damaged tissues or organs), today it is assuming great importance in the development of human-based experimental models for scientific research. Human tissues and organs Raw materials of human origin can be obtained and used in various ways, from post mortem to in vivo donation (eg DNA, blood). Intact slices of human tissue, obtained from patients undergoing surgery or biopsies, can be kept in the laboratory so that they preserve their function. Tumor biopsies, for example, can be used to see if a drug has bound to its intended molecular target. The comparison between donated, healthy and diseased organs can provide important information on pathological processes[19].

Human stem cells are also of enormous use in research. Pluripotent ("immature") stem cells are able to differentiate into the different cell types of the body. Since this type of cell is normally found in the embryo in the early stages of development, until a few years ago, it would have been necessary to destroy human embryos to obtain human pluripotent stem cells; this greatly limited its use for ethical reasons.

Thanks to a research group of the University of Kyoto, today these cells can be obtained directly from the patient's cells, for example those of the dermis and differentiated into a large number of cell types in the human body (such as neuronal cells, pancreatic, cardiac and hepatic), without having to resort to embryos. Furthermore, with pluripotent stem cells it is possible to obtain virtually all cell types from the same patient: they have the same genes and mutations as the patients they come from, and the researchers can use them to recreate disease in the laboratory and study how patient's genetics and environmental conditions contribute to his disease[20].

Instead, an organoid is an in vitro 3D cell cluster, a simplified and reduced-scale version of an organ, which follows its architecture and function. An organoid has a multicellular structure where the cells, when subjected to adequate biochemical stimuli in vitro, differentiate, self-assemble and self-organize into tissues, summarizing what happens in the embryo in the first weeks of development. They can be used in the study of the regulatory mechanism of organogenesis, in the modeling of human disorders (infectious diseases, hereditary diseases, neoplasms), in toxicity and pharmacological efficacy tests.

Organoids can be produced from induced pluripotent stem cells obtained from patients. Modular multi-compartmental fluidic bioreactors These are advanced in vitro systems which, thanks to the presence of a fluidic circuit and a peristaltic pump, allow dynamic interaction between cultures and cell co-cultures, housed in chambers or modules, connected to each other. Each module represents an organ of the human body and by connecting the modules to each other in series or in

parallel through a fluid circuit that mimics the blood flow, it is possible to model the interaction between organs and systems similar to what happens in vivo[21].

Another NAM is the micro-physiological system, a system of cell cultures each representing a tissue or organ interacting with each other at different levels on a microchip, through a microfluidic circuit, under strictly controlled conditions. Thanks to integrated sensors, real-time monitoring of cellular responses to mechanical or chemical stimuli is possible with more precise control of the cellular environment than conventional methods. The system allows to mimic the interactions between cells, tissues and even different organs and systems (human on a chip), and to provide adequate mechanical, structural and biochemical stimuli, reproducing at all levels what happens in vivo. The use of cells taken from the individual patient for the assembly of customized human on a chip represents one of the greatest promises for the near future of medicine.

The "omics" sciences deal with the study of biological molecules (nucleic acids, proteins, enzymes) in certain biological samples (serum, urine, CSF, saliva, tissues). They analyze, in their together:

- DNA genes (genomics) and their functions (functional genomics);
- DNA transcripts, i.e. RNA (transcriptomics);
- proteins (proteomics);
- metabolites within an organism (metabolomics).

They also study the interactions between these molecules (interactomics) and between these molecules and the factors environmental (exposure), nutrients (nutrigenomics), epigenetic factors (epigenomics), etc.

The purpose of this holistic approach is to be able to understand, working with integrative approaches, higher-level operating principles that collectively define systems biology. They make use of the use of comparative genetic analysis techniques or variations in the number of copies of certain stretches of DNA or DNA sequencing, cytometry, analysis techniques a single cell, mass spectrometry and computational methods that analyze data of tens, hundreds or thousands of molecules/samples[22]. Human systems can be simulated using highly sophisticated computer programs. These are created using data obtained from people. Computer simulations have been developed, for example, to predict the behavior of a drug in the digestive system. These simulations are likely to predict these effects in humans more accurately than animal models and in a much more efficient way. Read-across uses relevant information on analogous ("base") substances to predict the properties of "target" substances. If read-across is applied correctly, experimental testing can be reduced as not every target substance needs to be tested. One of the most important characteristics of a drug is its pharmacokinetics, that is, how the drug is absorbed, distributed, metabolized, excreted by the body.

Unfortunately, the pharmacokinetic data provided by traditional preclinical models, whether in vitro or in vivo (animal models) are often not reliable, as they are not relevant for human biology. Not surprisingly, the major cause of failure in drug development is attributed to the inability to obtain early pharmacokinetic data relevant to humans. Too low concentrations of the drug at the target organ level, for too short a time, can cause ineffectiveness while too high concentrations, for too long, could induce toxic effects.

An experimental approach useful for overcoming these problems is microdosing, which consists in administering extremely small, non-pharmacologically active doses of a certain drug to healthy volunteers to establish its pharmacokinetic profile in humans. It is based on ultra-sensitive analytical technologies capable of measuring infinitesimal quantities and concentrations (of the order of the picogram or femtogram) of drugs and metabolites; the technologies most used for this purpose are liquid chromatography in association with tandem mass spectrometry, ultrasensitive accelerator mass spectrometry and positron emission tomography (PET)[23].

Cellular models and SARS-CoV-2 infection

At this time, in many laboratories around the world, organoids are used for attempt to explain the effects of SARS-CoV-2 infection and disease on the human body.

The data collected from hospitalized patients and those from autopsy reports clearly show that the virus attacks all organs, often with such aggression

as to cause serious damage. SARS-CoV-2 has been found not only in the lungs and larynx, but also in the tissues of the heart, liver, brain and kidneys. What researchers around the world are trying to understand is whether the observed damage is directly caused by the virus or whether it is due to complications of the infection.

In fact, many severely affected individuals already had it comorbidities (hypertension, cardiovascular or kidney problems and diabetes), but several people infected they had no particular previous health problems. To try to give an answer to this question, researchers must have access to the most advanced technical solutions and cellular models closer to reality and the organoids faithfully reproduce the morphology original of the fabrics studied.

The main strength of these cellular models is given by the possibility of overcoming the bidimensionality linked to the cultivation of cells in the plate, allowing virologists and microbiologists to reconstruct three-dimensional models that add all the variants to the equation linked to the supply of nutrients, oxygenation and also to the physical stimulations undergone by an organ real.

In fact, they are nothing more than mini-organs capable of assuming the morphology of the organ of starting with the advantage of being able to be designed and built including various cell types. Of course, the revolution brought about by organoids is still too recent to think of regardless of animal models, especially in reference to an unknown pathology such as that elicited by the SARS-CoV-2 virus, but their contribution to understanding the dynamics of this pathology is essential to be able to design - even in a short time - more and more clinical studies targeted in the search for new therapeutic opportunities[24]. A group of researchers from Kyoto University has created bronchial organoids composed of four cell types (basal, bronchiolar, ciliated and goblet cells) and their model predicted also a high level of expression of the ACE2 receptor and TMPRSS2, necessary for binding with the "Spike" protein of the virus, with the advantage of reproducing all the main cell types of the airways.

Once the organoid was infected with the SARS-CoV-2 virus, they observed that the most affected cells are the basal ones, while in the bronchiolar cells the virus struggles more to enter. This is an important achievement that could direct research towards the use of effective molecules in the treatment of the disease.

A mini model of the lung was developed at Weill Cornell Medicine in New York City using induced pluripotent stem cells in an attempt to study the effect on affected cells from the virus of different types of drugs. The researchers tested the effect of an antimalarial drug, an immunosuppressant and a tyrosine kinase inhibitor on infected lung cells, observing a positive result especially for drugs that shut down the massive response immune[25].

In a work published in the journal Clinical Immunology an Italian research team from the University of Modena explained the immunological mechanism of vasculitis, i.e. inflammation of the blood vessels, revealing that the precipitation of antigen-antibody complexes in tissues (and in particularly in those of the blood vessels) is accompanied by the activation of the complement pathway and induces a severe state of inflammation compatible with that found in some patients with COVID-19[26].

What was deduced from the tissue analysis is partly confirmed by the experiments on organoids of a biologist expert in stem cells at the Institute of Bioengineering of the Catalonia: thanks to the use of organoids derived from induced stem cells, it has shown how the SARS-CoV-2 virus can attack the endothelium to be released into the blood and circulate within the body, also infecting particularly delicate organs such as the kidney[27].

Certainly, each of these researches has a not insignificant value, especially in perspective to find therapeutic formulations that act effectively to contain the disease, but it is necessary to persevere in the investigation of the ways in which the virus enters cells.

The damage observed in the various studies mentioned above is probably due to the combination viral infection and a disproportionate immune response and it is for this reason that it is necessary use organoids, trying to better understand the relative weight of the two components in addition large picture of a still unknown and certainly dangerous disease.

Conclusions

The use of animals in scientific research is a very controversial topic.

Advocates of animal testing argue that animal testing is essential in the development of new treatments and for the prevention of human disease; that the major achievements of medicine have only been possible thanks to animal experimentation; that the complexity of the human organism can only be assimilated by the complexity of the animal.

In fact, the role that some animal experiments have played has been significant, just think of the identification of insulin or the discovery of its action potential. However, the limiting factor is the choice of an animal model that allows the reproduction of a specific human biological function, as well as preserves intact the characteristics under investigation.

Human-based research encompasses a wide range of innovative methodologies and biotechnologies or New Methodological Approaches (NAMs), relevant to humans as they use current advanced knowledge on human biology, to study diseases and develop safe and effective drugs in man, recognizing the importance of species-specificity.

The usefulness of human-based methods for understanding human pathologies and predicting human responses have already been demonstrated, in some cases even better than traditional in vivo models can do. Despite this, their potential is limited by regulatory restrictions and they are still poorly considered in the context of basic research, which is still dominated by traditional approaches.

Replacing animal testing with modern human-based approaches is essential to prevent harm to human volunteers in clinical trials. Outdated regulations requiring preclinical animal testing were intended to protect people in an age where today's possibilities and technologies did not exist.

In the light of current knowledge, it is now recognized that the unreliability of animal test data not only risks making us trash potential treatments but also exposes human volunteers to serious risks.

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