Technology in a Globalizing World

Multidisciplinary Science and Advanced Technologies

Kaushik Pal
Fernando Gomes
Thinakaran Narayanan
Editors

NOVA

TECHNOLOGY IN A GLOBALIZING WORLD

MULTIDISCIPLINARY SCIENCE AND ADVANCED TECHNOLOGIES

TECHNOLOGY IN A GLOBALIZING WORLD

Additional books and e-books in this series can be found on Nova's website under the Series tab.

TECHNOLOGY IN A GLOBALIZING WORLD

MULTIDISCIPLINARY SCIENCE AND ADVANCED TECHNOLOGIES

KAUSHIK PAL
FERNANDO GOMES
AND
THINAKARAN NARAYANAN
EDITORS

nova science publishers
New York

NOTICE TO THE READER

The Publisher has taken reasonable care in the preparation of this book, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained in this book. The Publisher shall not be liable for any special, consequential, or exemplary damages resulting, in whole or in part, from the readers' use of, or reliance upon, this material. Any parts of this book based on government reports are so indicated and copyright is claimed for those parts to the extent applicable to compilations of such works.

Independent verification should be sought for any data, advice or recommendations contained in this book. In addition, no responsibility is assumed by the Publisher for any injury and/or damage to persons or property arising from any methods, products, instructions, ideas or otherwise contained in this publication.

This publication is designed to provide accurate and authoritative information with regard to the subject matter covered herein. It is sold with the clear understanding that the Publisher is not engaged in rendering legal or any other professional services. If legal or any other expert assistance is required, the services of a competent person should be sought. FROM A DECLARATION OF PARTICIPANTS JOINTLY ADOPTED BY A COMMITTEE OF THE AMERICAN BAR ASSOCIATION AND A COMMITTEE OF PUBLISHERS.

Additional color graphics may be available in the e-book version of this book.

Library of Congress Cataloging-in-Publication Data

Names: Pal, Kaushik, editor. | Gomes, Fernando (Gomes Souza Junior), editor. | Narayanan, Thinakaran, editor.
Title: Multidisciplinary science and advanced technologies / Kaushik Pal (editor) Federal University of Rio de Janeiro, Brazil, Fernando Gomes (editor), Thinakaran Narayanan (editor).
Description: New York : Nova Science Publishers, [2020] | Series: Technology in a globalizing world | Includes bibliographical references and index. |
Identifiers: LCCN 2021002022 (print) | LCCN 2021002023 (ebook) | ISBN 9781536189599 (hardcover) | ISBN 9781536191981 (adobe pdf)
Subjects: LCSH: Technological innovations. | Engineering--Equipment and supplies. | Research.
Classification: LCC T173.8 .M84 2020 (print) | LCC T173.8 (ebook) | DDC 600--dc23
LC record available at https://lccn.loc.gov/2021002022
LC ebook record available at https://lccn.loc.gov/2021002023

Published by Nova Science Publishers, Inc. † New York

CONTENTS

Chapter 14

ANIMALS IN BIOMEDICAL RESEARCH: TOWARDS A MORE COMPASSIONATE APPROACH

J. Reis,[1,1] T. Oliveira[2], P. Faísca[3], D. Dantas[4], S. Silva[4] and O. Frazão[4]

[1]School of Agriculture, Polytechnic Institute of Viana do Castelo, Ponte de Lima, Portugal
[2]Department of Veterinary Medicine, University of Évora, Évora, Portugal
[3]Research Center for BioSciences & Health Technologies, Faculty of Veterinary Medicine, Lusófona University, Lisboa, Portugal
[4]INESC TEC - Institute for Systems and Computer Engineering, Technology and Science, Porto, Portugal

ABSTRACT

The development of novel biosensors and other medical devices often implies extensive animal testing before the final phases of product development are reached. Most products do not reach the market. On the other hand, natural disease in companion animals often mirrors disease processes in humans, being a more accurate representation of the complexity of disease processes than laboratory animals. These are poor

[1] Corresponding author: jmargaridareis@esa.ipvc.pt

models of genetic variability, environmental exposure influence and often, pathophysiology.

In this chapter, possible strategies for biomedical research that are based on a more compassionate approach are discussed, tapping on the enormous potential of comparative medicine for beneficing both humans and animals.

Keywords: animal experimentation, reduction, replacement, spontaneous disease models

1. Introduction

Research in the biomedical field has relied on animal use throughout the history of time [1, 2]. The methods used in this research have reflected the society and its values at each given timepoint [2]. Although the importance of Galen's work cannot be denied, it would now be unacceptable for most humans to allow, see or perform the vivisection of unanaesthetised living beings, in the absence of "pity or compassion" as he advised his students. But Galen lived in ancient Rome, where human and animal violent slaughtering were performed for the amusement of the people.

These days, the ethical doctrine applied to animal experimentation is dominated by utilitarianism, weighing the benefits for human health against the cost for the experimental animal [3]. Animal experimentation is permitted and regulated, but the scientific community has added responsibility in weighting potential benefit vs. harm caused.

The European and Portuguese legal frames recognize animals as sentient beings, capable, like humans, of feeling pain and anguish. No one that has observed a dog or a cat for a little time - and observation is essential in science - will deny that they are also able to feel and express joy and affection. The same applies to other species, often depending on the observer's knowledge and experience regarding the observed subject species.

The legislation also calls for implementing the 3Rs: replacement, reduction and refinement of animal use in experimental procedures.

However, a critical view upon the available data on animal use in experimentation and education may tell a different tale, at least when considering reduction and, in practice, effective replacement.

Apart from ethical and moral concerns, one may argue the use of provoked disease models has limited applicability in several situations and for several reasons. Resorting to spontaneous disease models may be more advantageous.

2. The Numbers: Are We Really Implementing the 3Rs?

Statistical data on the use of animals for scientific purposes in the Member States of the European Union is available in European reports assembling the data and for each Member State. The 2019 report informs the statistics of the use of animals for scientific purposes in 2015-2017, accounting for 10.664.749 animals used in 2017 vs. the previous report stating the use of nearly 11.500.000 animals in 2011 (no exact number is detailed). However, it should be noted that reporting rules were changed between these reports, so in some categories, it is difficult to compare. The numbers reported also do not include foetal forms of mammals, animals killed solely for organs and tissues (unless occasion was done by a method other than indicated by the Annex IV of Directive 2010/63/EU), and sentinels. Animals bred and killed without being used, apart from genetically altered animals with intended and exhibited harmful phenotype, and those having been genotyped with an invasive method before being killed are also not accounted for, even though they all fall under the directive ruling [4, 5]. These were estimated to amount to an additional 8 million [6]. Rodents (mice and rats) represent over 70% of the total number of animals and there seems to be a continuing trend of increase on the development of transgenic animals even if a slight use reduction is reported [4, 5]. This agrees with the USA's report, where an estimate of 11.000.000 to 23.000.000 animals are being used yearly in research, education, and testing, but there are no published statistics [6, 7].

In Portugal, there is no observable continuing reduction trend in the total number of animals used, as evidenced by Figure 1 [8]:

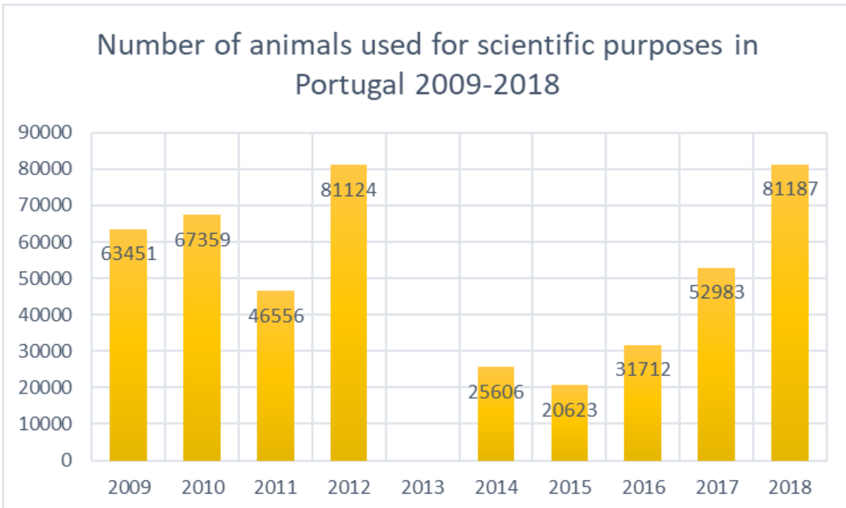

Figure 1. Number of animals used yearly for scientific purposes (research and education) in Portugal, in the period from 2009 to 2018. There is no data available for the year of 2013, when the directive transposition to national law occurred. Portugal has a population of a little over 10 million people.

The observed abrupt reduction between 2012 and the following years was consequence of the economic recession and austerity policies that heavily affected research dependent on national funds. Of the total number of animals, only a small part was used in education and training (less than 0.5% in 2018). In the year of 2018, roughly 65% of the animals were used for basic research, almost 73% were mice, and they were used with the purpose of studying mainly the immune system (31%), in oncology (13%) and for studying the nervous system (11%), amongst others.

3. Why Worry about Not Enough Reduction in Animal Use?

Apart from ethical and moral concerns regarding the millions of lives of sentient beings sacrificed yearly, that should be enough to make the scientific community reflect, there are economical and scientific reasons for a more active replacement of laboratory animals.

Limitations in the use of genetically altered animals may arise from not reproducing accurately the pathophysiology of the disease processes. For example, FAD (familial Alzheimer's disease). Mice are a model of familial Alzheimer's disease. These mice overexpress amyloid-beta precursor protein, at levels much higher than the physiological ones; however, in human Alzheimer's disease there is a reduction of amyloid elimination, and not to an increase in its production [9].

On the other hand, the genetic homogeneity of laboratory animals and the secluded facilities of a laboratory are not good reproductions of the genetic heterogeneity of the human population, nor of the variety of environmental stimuli humans are exposed to. Also, the life cycle of mice is short, and a young mouse can hardly be a good model for an aging human, irrespective of genetic modification. The short generation cycle makes faster science but not necessarily better science.

Still, research funds are largely invested in such research models, often overlooking alternatives. And sometimes, and for the reasons mentioned above, drugs that seem promising in mice fail market approval and prove ineffective. Fewer than 10% of candidate drugs are approved for the market, even if preclinical trials are successful. Numbers are lower when considering oncology drugs, unless nanomedicine based [11]. Nonetheless, care must be taken not to introduce in the market drugs or implantable devices that are not evidenced-based (keeping in mind that animal experimentation does not equal to evidence). Concerning, for example, hip and knee implants, small changes in design and production have led to revision rates as high as two to ten-fold the standard revision rate [12].

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

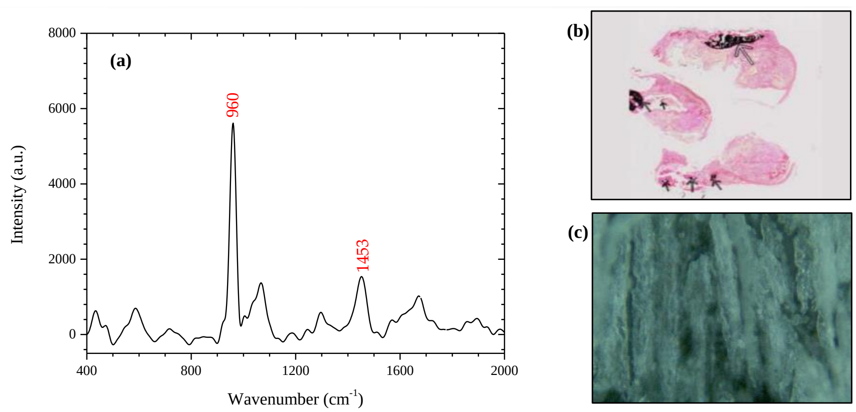

Figure 2. (a) Raman spectrum of the benign and malign samples with two band representation and the corresponding (b) histological and (c) microscopic images: the 960 cm^{-1} calcium-phosphate stretch band-microcalcification type II and the 1453 cm^{-1} band associated with the structural protein modes of tumors.

Spontaneous disease models are not without limitations. For some of the diseases that afflict human patients (coronary heart disease, Parkinson's disease, for example), spontaneous disease models in animals are not yet identified. Genetic and metabolism differences should always be considered too. Significant caseloads also take longer to build than a generation cycle in mice. The heterogeneous nature of the clinical cases upon their presentation poses additional challenges in sample characterization and for results interpretation. However, in human disease, all patients are different, also. Often humans, like veterinary patients, have co-morbidities. Some are young, and some are old. This lack of uniformization should be looked upon as an advantage if it is clearly characterized and reported. Negative outcomes publication should be regarded as necessary, if not more so, as positive results. One can learn a great deal from errors.

CONCLUSION

The scientific community must play an active role in effectively implementing animal reduction in research and take a more critical perspective on animal use. The resource to alternatives to animal experimentation and further investigation of natural disease in companion animals present opportunities for better quality science and a more compassionate stand. Full progresses also call for a multidisciplinary approach, not limited to veterinary medicine and medicine, but with teams that are able to join knowledge from scientific areas as distinct as physics, chemistry, pharmaceutics, and mechanical engineering, among others. Once the scientific jargon barrier is crossed, evolution can be remarkable.

ACKNOWLEDGMENTS

Joana Reis would like to thank her family and Prof. Rita Payan Carreira for the support, wisdom and willingness to discuss these matters. The authors would also like to acknowledge the FCT financial support through project ENDOR-Endoscope based on New Optical Fibre Technology for Raman Spectroscopy (POCI-01-0145-FEDER-029724).

REFERENCES

[1] Hajar, R. (2011). Animal testing and medicine. *Heart Views* 12: 42. doi: 10.4103/1995-705X.81548.

[2] Franco, N. H. (2013). Animal experiments in biomedical research: a historical perspective. *Animals*. 3:238-273. doi.org/10.3390/ani 3010238.

[3] Graham, M. L., Prescott, M. J. (2015). The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease.

European Journal of Pharmacology. 759:19-29. doi.org/10.1016/j.ejphar.2015.03.040.

[4] Relatório da Comissão ao Conselho e ao parlamento Europeu (2013) - *Sétimo relatório de dados estatísticos sobre o número de animais utilizados para fins experimentais e outros fins científicos nos Estados-Membros da União Europeia* [Report from the Commission to the Council and the European Parliament (2013) - Seventh report of statistical data on the number of animals used for experimental and other scientific purposes in the Member States of the European Union].

[5] Relatório da Comissão ao Conselho e ao parlamento Europeu (2019) - *Relatório de 2019 relativo a dados estatísticos sobre a utilização de animais para fins científicos nos Estados-Membros da União Europeia em 2015-2017* [Report from the Commission to the Council and the European Parliament (2019) - 2019 report on statistical data on the use of animals for scientific purposes in the Member States of the European Union in 2015-2017].

[6] Busquet, F., Kleensang, A., Rovida, C., Herrmann, K., Leist, M., & Hartung, T. (2020). New European Union statistics on laboratory animal use-what really counts!. *ALTEX-Alternatives to animal experimentation*. 37:167-186.

[7] Goodman, J., Chandna, A. & Roe, K. (2015). Trends in animal use at US research facilities. *Journal of Medical Ethics*. 41:567-9. doi: 10.1136/medethics-2014-102404.

[8] Dados estatíticos relativos à utilização de animais experimentais em Portugal [Statistical data on the use of experimental animals in Portugal] [Internet]. [cited 2020 7]. Available from: http://srvbamid.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?generico=1149097&cboui=1149097.

[9] Ransohoff, R. M. (2018). All (animal) models (of neurodegeneration) are wrong. Are they also useful?. *The Journal of Experimental Medicine*. 21: 2955. doi: 10.1084/jem.20182042.

[10] Wong, C. H., Siah, K. W., & Lo, A. W. (2019). Estimation of clinical trial success rates and related parameters. *Biostatistics*. 20:273-286. doi.org/10.1093/biostatistics/kxx069.

[11] He, H., Liu, L., Morin, E. E., Liu, M., & Schwendeman, A. (2019). Survey of clinical translation of cancer nanomedicines - Lessons learned from successes and failures. *Accounts of Chemical Research*, 52: 2445-2461. doi.org/10.1021/acs.accounts.9b00228.

[12] Pijls, B. G., & Nelissen, R. G. H. H. (2016). The era of phased introduction of new implants. *Bone and Joint Research.* 5: 2015-7. https://doi.org/10.1302/2046-3758.56.2000653.

[13] Kramer, M., & Font, E. (2017). Reducing sample size in experiments with animals: historical controls and related strategies. *Biological Reviews*. 92:431-445. doi.org/10.1111/brv.12237.

[14] Oliveira, M. T., Lucena, S., Potes, J., Queiroga, M. C., Rehman, S., Dalgarno, K., Ramos, A. & Reis, J. (2015): *Ex Vivo* Model for Percutaneous Vertebroplasty. *Key Engineering Materials*. 631: 408-413. doi.org/10.4028/www.scientific.net/KEM.631.408.

[15] Pamies, D., Barrera, P., Block, K., Makri, G., Kumar, A., Wiersma, D., & Harris, G. (2017). A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity. *ALTEX-Alternatives to animal experimentation*. 34: 362-376. doi: 10.14573/altex.1609122.

[16] Simpson, S., Dunning, M. D., de Brot, S., Grau-Roma, L., Mongan, N. P., & Rutland, C. S. (2017). Comparative review of human and canine osteosarcoma: Morphology, epidemiology, prognosis, treatment and genetics. *Acta Veterinaria Scandinavica*. 59:71. doi: 10.1186/s13028-017-0341-9.

[17] Abdelmegeed, S. M. & Mohammed, S. (2018). Canine mammary tumors as a model for human disease. *Oncology Letters* 15:8195-205. doi: 10.3892/ol.2018.8411.

[18] Cancer Genome Atlas Network (2012). Comprehensive molecular portraits of human breast tumours. *Nature* 490: 61-70. doi.org/10.1038/nature11412.

[19] Nyante, S. J., Lee, S. S., Benefield, T. S., Hoots, T. N. & Henderson, L. M. The association between mammographic calcifications and breast cancer prognostic factors in a population-based registry cohort. *Cancer* 2017;123:219-27. doi.org/10.1002/cncr.30281.

[20] Grimm, L. J., Miller, M. M., Thomas, S. M., Liu, Y., Lo, J. Y., Hwang, E. S., Hyslop, T. &. Ryser M. D. (2019). Growth dynamics of mammographic calcifications: differentiating ductal carcinoma in situ from benign breast disease. *Radiology*. 292:77-83.

[21] Reis, J., Oliveira, T., Pereira, A., Infante, P., Leal, N., & Faísca, P. (2019). Microtomographic characterization of calcifications in canine mammary tumours. *Veterinary and Comparative Oncology*. doi: 10. 1111/vco.12545.

[22] Surmacki, J., Musial, J., Kordek, R. & Abramczyk, H. Raman imaging at biological interfaces: applications in breast cancer diagnosis. *Molecular Cancer* 2013, 12, 48. doi: 10.1186/1476-4598-12-48.

[23] Gosling, S., Scott, R., Greenwood, C., Bouzy, P., Nallala, J., Lyburn, I. D., Stone, N. & Rogers, K. (2019). Calcification Microstructure Reflects Breast Tissue Microenvironment. *Journal of Mammary Gland Biology and Neoplasia*. 24, 493-541. doi.org/10.1007/s10911-019-09441-3.