Molluscs as Models for Translational Medicine

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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This paper describes the advantages of adopting a molluscan model for studying the biological basis of some central nervous system pathologies affecting humans. In particular, we will focus on the freshwater snail *Lymnaea stagnalis*, which is already the subject of electrophysiological studies related to learning and memory, as well as ecotoxicological studies. The genome of *L. stagnalis* has been sequenced and annotated but the gene characterization has not yet been performed. We consider the characterization of the gene networks that play crucial roles in development and functioning of the central nervous system in *L. stagnalis,* an important scientific development that comparative biologists should pursue. This important effort would add a new experimental model to the limited number of invertebrates already used in studies of translational medicine, the discipline that seeks to improve human health by taking advantage of knowledge collected at the molecular and cellular levels in non-human organisms.

MeSH Keywords: Central Nervous System • Gastropoda • *Lymnaea stagnalis*

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Background

In recent decades, basic and applied biomedical research has made huge progress and provided a large amount of information. Unfortunately, these great efforts and knowledge gained have not been quickly followed by the desired and expected therapeutic results [1]. This has great significance for several diseases affecting the central nervous system for which, at present, there is no effective treatment (e.g., Alzheimer's disease and other forms of dementia). Traditionally, the process of drug discovery begins with the identification of one or more target proteins that are potentially implicated in a specific human disease. The identification of target proteins is followed by the search for one or more chemicals that can alter the function of the target proteins. This last phase consists of the screening of many chemical compounds to evaluate their effects in the animal models usually used for studying the pathology of interest. The process of identifying a single promising compound among thousands of candidates may take a long time and tens of millions of dollars, with no guarantee of success. The animal models used most frequently in this screening process are small mammals (i.e., rats and mice) and primates. This approach, which is not always effective, led to many protests and fueled much scientific, ethical, economic, and social discussion, as well as leading to profound changes in the law associated with ethical restrictions in animal experiments. The high cost of these studies and the increasing difficulties in obtaining permits for experimentation prompted researchers to look for other strategies. Many researchers have attempted to solve the problem by using *in vitro* cell systems that have lower operating costs and higher speed in collecting data. Unfortunately, the obtained results were often limited and inconclusive in elucidating the basis of diseases and identifying effective therapeutic strategies [2]. On the basis of these considerations, the need to identify alternative and reliable models that have fewer ethical restrictions is both important and urgent.

Despite the many advances in biological and biomedical sciences, the understanding of incurable diseases remains inadequate. The disparity between the huge amount of information on the molecular and cell biology and the modest capacity to intervene in important human neurological disorders such as Alzheimer's disease may appear incongruous. Researchers have in part clarified how cells work individually but they also clearly showed how extremely sophisticated and complex are the processes that allow the survival or alter the proper functioning of multicellular organisms [1]. In this complex picture more effective and ethical approaches in biomedical research are required, exceeding the practical and conceptual limitations of experiments on mammals and on cell cultures.

Development of Alternatives to Mammalian Models

The evolutionary process that prompted the diversity among species also promoted the conservation of numerous key physiological processes that are well preserved across species, including humans [3,4]. Unlike mammals, invertebrates frequently have short generation times, numerous offspring, and can be more easily experimentally manipulated. If detailed genetic information is available, powerful genetic tools allow a fine dissection of the metabolic pathways, allowing to understand the basic mechanisms of physiology and pathology in the analyzed organism. On this basis, it is not surprising that some of the most advanced research on invertebrates [5] in the biomedical field provide a quick and efficient way to develop treatments for human diseases [6]. Today, the most common and best characterized invertebrate models are the insect *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. These organisms are valid alternative models for the study of the biological basis of many processes, especially in human diseases, and open up interesting perspectives concerning both the validation of the mechanism of action of existing drugs and the preclinical studies of drugs in development. However, their anatomical features, the peculiarities of the genome [7], and the very short life cycle limit their effectiveness in studies involving aging and neuro-degeneration or chronic diseases. Aside the two most diffused invertebrate models, other invertebrates have been the subjects of basic and applied biomedical researches. For instance, the sea squirt *Ciona intestinalis* is a chordate and a useful model of neuroendocrinology experiments. This ascidian has many hormones (i.e., gonadotropin-releasing hormone, insulin, and insulin-like growth factor) analogues to those found in higher animals [8]. The small genomes of insects such as *Apis mellifera, Acyrthosiphon pisum* and *Bombyx mori,* allow the utilization of these animals as models in studies on DNA methylation in order to understand the general relevance of this important epigenetic mechanism [9–11]. It is well known that the tumor suppressor gene *p53* is one of the best studied genes in human cancer research [12]. In this context, the discovery in the bivalve *Mya arenaria* of human homologues for *p53* (*Map53*) and *p73* (*Map73*) genes makes this mollusc a model of interest in the study of human cancer [13].

New Directions of Translational Medicine

Invertebrates are a valid alternative model in the study and characterization of human disease processes and in the discovery and development of new drug therapies. Invertebrate models have many advantages compared to mammalian models, including significant experimental efficiency due to the reduced time needed for experiments and the low costs

required for their care. Moreover, conservation of fundamental gene networks in invertebrate models allows researchers to benefit from projects focused on humans. The Encyclopedia of DNA Elements (ENCODE) project (www.encodeproject.org) has provided, for the first time, many functional and regulatory elements in the genome (e.g., protein, RNA, and networking genes, including their adjustment), that are the basis of metabolic pathways and processes, especially in humans. The availability of such data has had a significant impact on other model organisms, leading to the modENCODE project (*www.modencode.org*) aimed at identifying the functional elements and gene networks in *D. melanogaster* and *C. elegans* genomes [14–16]. Thanks to modENCODE it is possible to improve knowledge about gene functional annotations in other organisms whose genomes have been completely sequenced and annotated. Among them, attention should be paid to the genome of the gastropod mollusc *Lymnaea stagnalis* widely used to study the molecular mechanisms related to learning and memory as well as neurodegenerative diseases [17,18]. Numerous studies suggest that *L. stagnalis* could be a good and innovative model in biomedical research for the analysis of the genetic and molecular basis of human central nervous system diseases and for the identification, characterization, and development of new, safe, and effective therapeutic strategies. In 1997, an interesting review by Kemenes [19] reported the presence in *L. stagnalis* motor neurons of signaling pathways associated with specific behaviors similar to other invertebrate and vertebrate models. Recently, neuronal aging research in *Lymnaea* demonstrated that its neurons and its behaviorally characterized neuronal circuits show mechanisms analogous to that described in the etiology of age-associated dysfunctions and diseases of human and mammalian brains [20]. Although quite distant evolutionarily from humans, molluscs have already been proposed as models for translational medicine (e.g., in studies on human cancer) [21–23].

Compared to the established models *D. melanogaster* and *C. elegans*, *Lymnaea* has many benefits and is potentially important as a model in biomedical research due to the large size of its neurons, data from electrophysiological studies, and its already characterized neuronal circuits. *Lymnaea* also offers remarkable convenience, a large amount of biological material that could be molecularly and morphologically analyzed, and the possibility of performing behavioral tests [14]. More importantly, while *D. melanogaster* and *C. elegans* have a life cycle that lasts 2–3 weeks, *Lymnaea* develops in 2 weeks inside the eggs and then has an average life span of 9–12 months. This last factor becomes particularly interesting and useful in studies on chronic human pathologies, especially neurodegenerative diseases. The direct development and longevity of *L. stagnalis* allow the identification of age "stages" that could be connected to age-related modifications that may involve genetic, molecular, and cellular mechanisms, which usually take time to manifest their effects (as is also the case in human diseases such as Alzheimer's, Parkinson's, and other neurodegenerative diseases).

Conclusions

Because of historical, economic, and cultural/ethical advances, the use of mammals in biomedical research is increasingly expensive and restricted. This has created a pressing demand for alternative models that are less expensive but still effective to study the basis of human physiology and pathology. This now seems possible thanks to the great advances in comparative genomics that made possible the use of animal models that are cheap, simple, and easy to handle but in some situations just as effective as mammalian models due to the availability of their genomes and molecular tools important in analyzing gene networks. In this context, the neurobiological and genetic characterization of *L. stagnalis* may offer to translational medicine a powerful new tool to study age-related diseases of the nervous system and identifying new molecular targets for the development of innovative therapeutic strategies. In addition to the scientific implications, such a model has great ethical and economic value. Invertebrate models would limit as much as possible the use of several mammalian models, that would be involved only for the necessary validation of the results obtained from invertebrates. This would reduce by several orders of magnitude the costs of numerous pharmacological studies.

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