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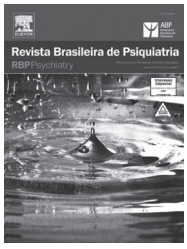

SPECIAL ARTICLE

Molecular medicine: a path towards a personalized medicine

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Abstract
Psychiatric disorders are among the most common human illnesses; still, the molecular and cellular mechanisms underlying their complex pathophysiology remain to be fully elucidated. Over the past 10 years, our group has been investigating the molecular abnormalities in major signaling pathways involved in psychiatric disorders. Recent evidences obtained by the *Instituto Nacional de Ciência e Tecnologia de Medicina Molecular* (National Institute of Science and Technology – Molecular Medicine, INCT-MM) and others using behavioral analysis of animal models provided valuable insights into the underlying molecular alterations responsible for many complex neuropsychiatric disorders, suggesting that "defects" in critical intracellular signaling pathways have an important role in regulating neurodevelopment, as well as in pathophysiology and treatment efficacy. Resources from the INCT have allowed us to start doing research in the field of molecular imaging. Molecular imaging is a research discipline that visualizes, characterizes, and quantifies the biologic processes taking place at cellular and molecular levels in humans and other living systems through the results of image within the reality of the physiological environment. In order to recognize targets, molecular imaging applies specific instruments (e.g., PET) that enable visualization and quantification in space and in real-time of signals from molecular imaging agents. The objective of molecular medicine is to individualize treatment and improve patient care. Thus, molecular imaging is an additional tool to achieve our ultimate goal.

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Medicina molecular: um passo em direção à medicina personalizada

Resumo
Os transtornos psiquiátricos estão entre as doenças humanas mais comuns. Os mecanismos celulares e moleculares subjacentes à sua complexa fisiopatologia ainda não estão totalmente esclarecidos. Nosso grupo está envolvido na investigação de anormalidades moleculares nas principais vias de sinalização das doenças psiquiátricas nos últimos 10 anos. Evidências recentemente obtidas pelo Instituto Nacional de Ciência e Tecnologia de Medicina Molecular (INCT-MM), utilizando análise comportamental de modelos animais, forneceram informações valiosas sobre as alterações moleculares subjacentes responsáveis por muitos distúrbios neuropsiquiátricos complexos, sugerindo que os "defeitos" nas vias de sinalização intracelular têm um papel importante na regulação do neurodesenvolvimento, bem como na fisiopatologia e eficácia do tratamento. Recursos do INCT nos permitiram iniciar pesquisas na área de imagem molecular. A imagem molecular é uma disciplina de investigação que visualiza, caracteriza e quantifica processos biológicos que ocorrem em níveis celular e molecular em seres humanos, e em outros sistemas vivos, através dos resultados de imagem dentro da realidade do ambiente fisiológico. A fim de reconhecer alvos, a imagem molecular aplica instrumentos específicos (PET, por exemplo) que permitem a visualização e quantificação em espaço e tempo real dos sinais dos agentes de imagem molecular, fornecendo medições de processos a nível molecular e celular. O objetivo da medicina molecular é individualizar o tratamento e melhorar a assistência ao paciente. Desse modo, a imagem molecular consiste em mais uma ferramenta para atingirmos nosso objetivo final.

Introduction

Psychiatric disorders are among the most common and destructive human illnesses; still, the molecular and cellular mechanisms underlying their complex pathophysiology remain to be fully elucidated. Over the past 10 years, our group has been investigating the molecular abnormalities in the main signaling pathways of psychiatric disorders. In 2008, this group developed a project that was funded by the National Institute of Science and Technology - Molecular Medicine (INCT-MM). Recent evidences obtained by us and others using behavioral analysis of animal models are providing valuable insights into the underlying molecular alterations responsible for many complex neuropsychiatric disorders, suggesting that "defects" in critical intracellular signaling pathways have an important role in regulating normal neurodevelopment, as well as in pathophysiology and treatment efficacy. These evidences are supported by the fact that the signaling pathway components are indirect targets of the most effective drugs for treating these disorders, such as antipsychotics, antidepressants, and mood stabilizers. Remarkably, the mechanisms by which these drugs exert their therapeutic actions are unknown, and the specific downstream effector molecule(s) that must be targeted for therapeutic efficacy remain to be determined, which is a crucial step for the development of novel and more effective medications to become possible. The surrogate in molecular medicine is to personalize the medicine for improving patient care.

These evidences have stimulated our interest in investigating genetic animal models, behavior and molecular basis of eletroconvulsotherapy, neurodevelopment, neuroprotection, and neuroinflammation, among others. Therefore, the challenging role of discovering how molecular alterations in signaling pathways are associated with psychiatric symptoms, provided by this research, seems to be a promising and attractive tool for new therapeutic approaches with greater efficacy and fewer undesirable side-effects.

Molecular medicine applied to psychiatry: animal models

Our group is composed by researches not only from the psychiatry field but also from the basic research. To develop our objectives we work with many different tools and expertise that are briefly described bellow.

Zebrafish

Zebrafish is a fish model for human diseases that is gaining increasing attention as a model for psychiatric disorders.[1] Several recent studies have used this model to study the neurodevelopmental basis of psychiatric disorders and the mechanisms involved in pharmacological treatment.[2] There are three main advantages of using zebrafish as a model for psychiatric disorder studies. First, the brain anatomy is conserved among fish and humans, but the patterns of zebrafish neural circuitry are simplified. Second, the emergence of motor behavior occurs just few days after fertilization, which makes it possible to measure the behavioral effects of pharmacological treatment and genetic manipulations in early stages. Third, because the development is external, it is easy to control environmental factors and to perform molecular-genetic and pharmacological experiments.[3,4]

There are three research projects using zebrafish being developed by this group:

The modulation of metabolism by neurotransmitters and the effects of antipsychotics on metabolic pathways

Dopamine and serotonin are neurotransmitters involved in fat metabolism regulation.[5] Furthermore, a common side effect of atypical antipsychotic treatment is the metabolic syndrome.[6,7] However, the mechanisms involved in this regulation are still unknown. We use zebrafish as a model to investigate fat metabolism regulation by neurotransmitters.

The main advantage of using zebrafish in these studies is that, over the first days after fertilization, the zebrafish larvae are nourished by the yolk sac. Thus, the larvae can survive for several days after hatching without being fed.[8] Therefore, it is possible to study the mechanisms involved in the metabolism without the intrusive variable of eating behavior. Our group is using genetic and pharmacological tools to study the neuronal receptors and intracellular pathways involved in metabolism. For this, the zebrafish larvae are chronically bath-treated with dopaminergic and serotoninergic agonists and antagonists. Additionally, specific genes related to neurotransmitter signaling are knocked down by morpholinos. Afterwards, the larvae are incubated in Nile red, which dyes phospholipids, cholesterol, and triglycerides. By microscopy, these larvae are observed in both yellow and red wavelengths, to evaluate the levels of phospholipids, cholesterol, and triglycerides, respectively. Furthermore, to investigate the receptors and intracellular pathways involved in metabolism regulation, the brains of the zebrafish larvae are dissected, and gene expression and protein phosphorylation are evaluated by RT-PCR and western blot.

With this model, we may elucidate direct neurophysiological functions in metabolism and the association of neuropsychiatric disorders and metabolic syndrome.

The neural circuitry and intracellular pathways involved in antipsychotic treatment and behavior

Dopaminergic receptors are the main target of antipsychotics.[9] However, studies of the mechanisms and location of antipsychotic effects are still needed. The complexity of the dynamics of neuronal receptors and neural circuitry poses a challenge for a full understanding of the systems involved in pharmacological treatment. Recently, many studies have shown that the neuroanatomical patterns of zebrafish larvae are analogous to human neuroanatomy, but reasonably simplified, showing that zebrafish are a relatively simple integrative system for research in neuroscience.[10,3]

Many studies have shown alterations in the intracellular pathways regulated by dopamine (such as, downregulation of DARPP-32 and Akt) in the frontal cortex of schizophrenics and bipolar disorder patients.[11-13] It was demonstrated that dopamine modulates the activity of Akt in adult mice striatum.[14] This Akt regulation modulates the motor behavior of mice.[14] Several studies have demonstrated that the dopaminergic machinery is expressed in the brain of zebrafish larvae as early as one day post-fertilization, and regulates larval motor behavior.[2] Therefore, we investigated which dopaminergic receptors modulate the Akt intracellular pathway in the brains of zebrafish larvae, as well as their functions in larval motor behaviour. It was demonstrated that dopamine regulates the Akt activity in a similar way to that occurring in the mammalian brain.[15] Dopamine specifically modulates the phosphorylation of Akt at threonine 308 in the telecenphalon of zebrafish larvae[15]. Furthermore, this modulation is through the dopaminergic receptor D2, which also modulates motor behavior through the regulation of Akt activity.[15] On the other hand, the dopaminergic D1 receptor increases motor behavior.[15] Afterwards, it was observed that both the decrease and increase of dopaminergic signalling can regulate the motor behavior of zebrafish larvae, suggesting an inverted-U shaped function of dopamine in the behavior, as it is postulated for attention deficit and hyperactivity disorder (ADHD).[15]

After the description of dopaminergic signalling and the different functions of diverse dopaminergic receptors in motor behavior, this group is studying the effects of both typical and atypical antipsychotics in intracellular pathways. It is likewise investigating the effects of antipsychotics on the larval motor behavior, as well as which neuronal circuits are involved. The use of zebrafish in this research will enable progress towards a holistic understanding of the mechanisms involved in antipsychotic treatment.

The role of neurotransmitters in brain development

Several studies have suggested that abnormalities during neurodevelopment could be involved in neuropsychiatric disorders.[2] Imbalances of both dopamine and serotonin are associated with many neurodevelopmental disorders, such as schizophrenia, autism, and ADHD.[2,16] Furthermore, dopaminergic and serotoninergic receptors are the main target of the pharmacological treatments for these diseases.[9] Interestingly, both neurotransmitters modulate intracellular pathways involved in neurodevelopment (e.g., Akt).[2,16] However, the role of both neurotransmitters in neurodevelopment still remains to be fully understood.

On that ground, we have studied the role of different dopaminergic receptors in the development of the GABAergic system, which is altered in brains of schizophrenic and autistic patients.[17,18] We used the zebrafish model to do this, since its development is external, allowing for better control of environmental factors and pharmacological manipulation. The zebrafish larvae were chronically bath-treated with dopaminergic agonists and antagonists in a specific developmental window (3 to 5 days post-fertilization). It was shown that dopamine modulates the number of GABAergic neurons through dopaminergic D2 receptor.[15] The theoretical inverted-U shaped model for dopamine is also observed in the development of GABAergic system in the telencephalon of zebrafish larvae, suggesting a fine-tuned function of dopamine in neurodevelopment.[15] Furthermore, a correlation was observed between alterations in motor behavior and changes in the GABAergic development caused by dopamine abnormalities.[15]

Thus, we are using zebrafish as an integrative model to understand the developmental basis of psychiatric disorders, which in addition to the studies using other models, including humans, will facilitate translational research.

Caenorhabditis elegans: a model for studying neuropsychiatric and metabolic disorders

Obesity has emerged as a major health problem implicated in several human diseases.[19-21] Studies aimed at understanding the physiological mechanisms of body weight regulation have revealed that the central nervous system (CNS) plays

a critical role in energy homeostasis.[22] The CNS integrates internal and external cues of energy demand and availability to coordinate behavioral, physiological, and metabolic responses of an organism over time.[22-24] However, the neural circuits and molecular mechanisms underlying the perception of and responses to different energetic states remain poorly understood. A number of findings point to the monoamines as central regulators of energy balance. For instance, antipsychotics having monoamine receptors as their targets, which are used for treating schizophrenia, lead to major metabolic disturbances. Thus, while a range of data highlight a relationship involving mental disorders and energy balance, the precise molecular background remains unresolved.

Caenorhabditis elegans provides a genetically tractable system for rapid and functional analysis of various fat and neuronal regulatory pathways in the context of an intact animal.[25-28] As in mammals, *C. elegans* neural sensory mechanisms gauge environmental conditions and coordinate myriad behavioral and physiological responses.[29,30] Its conserved gene systems reveal many parallels with vertebrates and, together with a simple nervous system composed of 302 neurons, appears as an attractive model for study of neuronal control of metabolism.

Using vital dyes as Nile red and BODIPY-labeled fatty acids or biochemical approaches as thin-layer-chromatography and gas-chromatography/mass-spectrometry or label-free images, based on Raman scattering microscopy, it is possible to assess worm's metabolic state as reflected in triglyceride content.

Following this strategy, our group investigated the effects of dopamine signaling on *C. elegans* fat content and found that increased dopamine signaling is associated with fat reduction (Figure 1). Analyses of dopaminergic receptors loss of function mutants revealed an intricate relationship that ultimately results in fat breakdown via fat oxidation pathways.

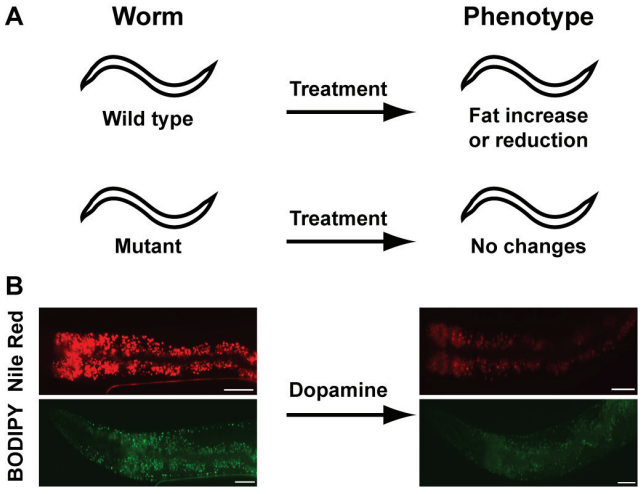

Figure 1 A straightforward strategy for fat measurement in *Caenorhabditis elegans*.

(A - B) Animals exposed to dopamine have their fat content measured through fluorescence methods. (A) Illustration of strategy to uncover molecular machinery related to fat regulation by certain compound. (B) Dopamine exposure reduces fat stores as visualized by Nile red and BODIPY-labeled fatty acids.

The idea that dopamine may influence fat stores through modulation of cellular bioenergetics brings forth a new aspect of dopamine signaling to be explored. Indeed, treatment with a dopamine D2 receptor agonist has recently been found to increase resting energy expenditure in women.[31,32]

We aim to widen our data searching for the bioenergetic outcomes that follows concurrent manipulation of dopaminergic and serotoninergic systems in *C. elegans*. Therefore, new roles for monoamine signaling in the regulation of peripheral metabolism may be found and assist in studies of humans.

Clinical and neuropsychological models

Many thematic clinical services have been organized to support clinical research: affective, schizophrenia, anxiety disorders, obsessive-compulsive disorder, and children, elderly, and women mental health. These services are structured in order to allow a complete clinical and neuropsychological characterization, beyond psychiatric diagnosis, of the enrolled patients. As an example of this approach we can refer to suicidal behavior studies. It is well established that suicidal behavior runs within families and that this familial transmission is independent of the transmission of psychiatric disorders per se and linked to a serotonergic dysfunction. Taken together, these data show that a phenotypic stratification, taking into account the suicidal behavior history, is of pivotal importance when performing association studies between psychiatric disorders and genetics, particularly serotonin related genes. Interestingly, suicidal behavior is a complex phenotype itself, and a fully characterization of suicidal behavior is necessary because a gene may be associated with some types of suicidal behavior (e.g., impulsive or violent) but not with others. Using this approach, we found an association between a functional polymorphism of the serotonin transporter gene and violent suicide behavior, regardless of psychiatric diagnosis, but it was not associated with a psychiatric diagnosis itself,[33,34] suggesting that suicidal behavior could be a confounding factor when studying other serotonergic-related genes in the context of psychiatric disorders with high risk of suicidal behavior.

Development and use of neuropsychological assessment resources

Neuropsychological assessment is an important tool applied to the characterization of behavioral and cognitive functions related to brain development under normal and pathological conditions. The use of this clinical resource is important both for diagnostic and therapeutic interventions. The Laboratory of Neuropsychological Investigation (LIN) of INCT-MM aims to:

a) adapt and develop neuropsychological tasks for use in research and clinical practices concerning developmental psychopathology issues across the life span;
b) pursue neuropsychological and personality assessment for characterization of cognition and behavior aspects of neuropsychiatric diseases;
c) contribute to studies concerning the relationship between molecular biology and cognitive phenotypes.

Development and adaptation of neuropsychological tasks

Due to the lack of neuropsychological tasks adapted to Brazilian subjects, several neuropsychological tests and

scales, which have been worldwide used in cognitive and personality assessments, were selected and submitted to psychometric studies. These tasks were selected and submitted to cross-cultural adaptation and studies concerning its psychometrics properties in Brazilian subjects.[35] Tasks presenting appropriate psychometric properties are used for both research and clinical practices.

Neuropsychological assessment of neuropsychiatric diseases

Cognitive and behavioral symptoms are core manifestations in neuropsychiatric diseases. Some of these characteristics are important for diagnostic proposes (e.g., cognitive decline in elderly people or in learning disabilities in childhood). One important research objective is to assess different cognitive domains in neuropsychiatric disorders in childhood and adolescence (e.g., ADHD, Learning Disabilities, Infantile Bipolar Disorders, and Developmental Coordination Disorder), adulthood (e.g., Bipolar Disorders, Obsessive Compulsive Disorder, Schizophrenia, ADHD, and Anxiety), and elderly (e.g., Dementia, Pseudodementia, Mild Cognitive Impairment). In the Psychiatric Unit ambulatories of the Clinical Hospital at UFMG, patients are submitted to a comprehensive neuropsychological assessment which serves both for clinical and research proposes. The assessment encompasses areas, such as intelligence, memory, attention, executive functions, personality, and motor behavior.

Neuropsychological assessment has been an important source of knowledge concerning frequent symptoms presented by subgroups of patients. Therefore, we are interested in the relationship between some clinical manifestations in psychiatric disorders and cognitive processes. For instance, we have studied in a sample of adults diagnosed with bipolar disorder (during an euthymic phase) the relationship between executive functions and suicide attempts,[36] psychotic symptoms and episodic memory,[37] and anxiety and impulsive behavior. Another issue of interest is the neuropsychological predictors of success in pharmacological and non-pharmacological interventions. In this research field, we are conducting a study concerning the neuropsychological predictors of success in psychoeducation of bipolar patients and treatment adherence in schizophrenic patients.

Molecular basis of cognitive and behavioral symptoms in neuropsychiatric population

The main research focus of LIN-INCT in the next years will be related to the increase of studies concerning molecular basis of cognition and behavior. For this propose, the integration between neuropsychological assessment and molecular genetics is been used and we pretend to include the neuroimaging methods.

An important research issue of LIN-INCT is the identification of cognitive endophenotypes in psychiatric disorders. An endophenotype must meet certain criteria, such as association with a genetic polymorphism, inheritance, and disease association parameters. In this line of research, LIN-INCT is collaborating in studies concerning the relationship between some genetic polymorphisms related to neurotransmission and specific cognitive manifestations. For instance, we have carried out studies on the relationship between the functional polymorphism of 5-HTTLPR and impulsive behavior in obsessive compulsive disorder,[38] bipolar disorder, and normal subjects.[39] Regarding the polymorphism val66met of BDNF, we have studied its relationship with decision-making process in obsessive compulsive disorder[40] and executive functions in bipolar disorder patients.[41] Studies involving patients' relatives and normal health controls are in progress at the INCT of Molecular Medicine, and LIN-INCT is collaborating in these studies whose results will add information concerning cognitive and behavioral endophenotypes in psychiatric diseases.

Pharmachological assays and Models

Peptide toxins as drug prototypes and pharmacological tools

Another line of research developed by National Institute of Science and Technology – Molecular Medicine (INCT-MM) aims to reveal the pharmacological actions of some peptides, particularly the peptide toxins purified from the venom of the Brazilian spider *Phoneutria nigriventer*. Some of these toxins were proven to be useful for pain treatment, as well as for preventing neuronal death related to ischemic insults and cardiac arrhythmias. The proposed actions of these toxins in pain treatment and to prevent neuronal death were based in previous studies conducted by our group, which showed that these toxins are calcium channel blockers. Previously, we have shown that the venom also has a toxin (Tx3-1) that blocks A-type K+ currents and protects against cardiac arrhythmias.[42]

It has been shown that some fractions of the venom has an inhibitory action on voltage-gated calcium currents, on neurotransmitter release, and on synaptic vesicle exocitosis.[42] The spider-care facilities, venom extraction, and pharmacological fractions purification were based on previous work.[43] This work is performed in collaboration with a governmental health foundation in Brazil called Fundação Ezequiel Dias (FUNED), however all the pharmacological assays are done at INCT-MM.

Phα1β (also known as Tx3-6) is one of the peptide toxins obtained from *P. nigriventer* venom. It has been shown that this toxin is capable of blocking N-type voltage-gated calcium channels. Subsequently, we demonstrated that Phα1ß exerts antinociceptive action both in acute and in chronic pain in rat models.[44] By comparison, Phα1β showed more long lasting antinociceptive effect than ω-conotoxin MVIIA. Furthermore, we have shown that Phα1β induces in rodents fewer side effects than ω-conotoxin MVIIA. ω-conotoxin MVIIA is the prototype for Ziconotide (Ellan Pharmaceuticals), which is the first toxin, calcium channel blocker, approved by the FDA for pain treatment in humans.

Phoneutria toxins Tx3-3 and Tx3-4 are potent blockers of voltage-gated calcium channels; however, different from Phα1β, they exert markedly inhibitory effect on P/Q-Type voltage-gated calcium channels. While tested in an *in vitro* model of ischemic injury, Tx3-3 and Tx3-4 were able to prevent neuronal death in the hippocampal CA1 region,[45] as well as in the retina.[46] In both cases, the neuroprotective effect was related to inhibition of glutamate release by tissues, thus interrupting the neurotransmitter-mediated excitotoxic effect caused by the ischemic insult.

Pain is a clinical symptom prevalent in ~20% of adults. Pain causes physical and emotional stress, in addition to being directly responsible for loss of workers' productivity. The USA govern spends ~U$60 million annually treating pain, and the

International Association for the Study of Pain (IASP) estimates that half the people do not receive adequate pharmacological treatment. Not less important than a public health problem, stroke is now ranked as the second global cause of death. The reasons cited above are some of the driving forces that trigger this branch of research at the INCT-MM. Although the pharmacological data on *P. nigriventer* toxins are promising, other studies should be performed for better determining the molecular targets of these toxins, pharmacokinetics features, clinical efficacy, and safety of these drugs. Taking advantage of PET techniques, at the INCT - Molecular Medicine, new functional *in vivo* analysis will be done for testing molecular targets, as well as pharmacokinetics studies of toxins and natural products against neurodegenerative disorders. National Science and Technology Institutes are a recent initiative of the Brazilian government for funding applied research, allowing the results from basic research to be applied to solve health problems.

Molecular Medicine going through Molecular Imaging

Molecular imaging is a new research discipline that visualizes, characterizes, and quantifies biologic processes taking place at the cellular and molecular levels within humans and other living systems. These processes reflect cellular and molecular pathways and mechanisms of disease and can be studied in their own physiological environment. In contrast to the classical form of diagnostic imaging in which the recognized findings show the end effects of these cellular/molecular changes, the molecular imaging relies on the visualization of these changes that form the basis of disease in a living subject without any level of invasiveness. The molecular imaging uses endogenous molecules and exogenous probes to visualize, characterize, and measure biological processes in living systems. The interactions of the molecules/probes with molecular/cellular targets are characterized by two types: a receptor-ligand binding where the final product of a specific gene expression in particular cells or subcellular compartments is the target or an enzyme mediated trapping of labeled substrate. In order to recognize targets, molecular imaging applies specific instruments (e.g., PET) that enable visualization and quantification in space and in real-time of signals from molecular imaging agents. Molecular imaging provides measurements of processes at molecular and cellular levels and it is a key element of molecular imaging data and image analysis, especially for inter- and intra-subject comparisons. The molecular imaging acts as complementary tool in all activities of our research projects.

PET and microPET technologies

PET

Positron emission tomography (PET) is a non-invasive functional imaging technique with good resolution, high sensitivity, and accurate quantification. PET provides quantitative information of physiological, biochemical, and pharmacological processes in humans and living subjects without disturbing the biological system (PET tracers are in picomolar concentration). PET has been applied as a clinical and medical research imaging methodology in the field of oncology, cardiology, and neurology.[47-49] Furthermore, PET plays an important role in the process of drug development and evaluation.[50-52]

PET requires a tracer that is labeled with a positron emitting radioisotopes and a PET camera for imaging the subject. Development of new imaging probes for PET requires a multidisciplinary approach: target selection, organic synthesis, radiolabeling, in vitro and *in vivo* evaluation, and kinetic modeling of the radiolabeled compounds.[53] PET is a quantitative imaging tool that measures the regional concentration of the probe with a high level of accuracy at the picomolar level. Changes in tissue radiotracer concentration with time (time-activity curves – TAC, with dynamic acquisitions) and standardized uptake value (SUV – with static acquisitions) are commonly used parameters to measure PET radiotracer concentration in a specific compartment.[53,54] The kinetic modeling of PET data may enable the quantification of receptor density, enzyme activity, metabolic rate, and perfusion.[55]

Positron-emitting radionuclides are elements (e.g., ^{11}C, ^{13}N, ^{15}O) that can replace the stable analogues in drugs and biomolecules. Thus, it is possible to synthesize PET probes with the same chemical structure as the parent unlabeled molecules without altering their biological activity. The choice of the PET radionuclide is defined by its physical and chemical characteristics availability, the timescale for the studied biological process,[56] and its physical half-life. If the tracers need to be transported from the radiopharmacy sites to the imaging sites, or if significantly long labeling procedure is required, it may not be possible to use short lived isotopes (e.g., C-11). Tracers labeled with very short half-life (e.g., O-15) should be prepared very close to the imaging site.

MicroPET

MicroPET is a miniaturization of clinical PET for small animal studies. The first microPET for rats was developed at the Crump Institute at UCLA, in collaboration with CTI PET Systems (Knoxville, TN).[57] Although the prototype had a limited axial field-of-view (FOV) and compromised sensitivity,[58] it was applied to monitor the functional status of the dopaminergic system in a rat model of Huntingdon's disease following the grafting of fetal striatum implants.[59] PET scans were performed using the postsynaptic markers (^{11}C) raclopride and (^{11}C) SCH-23390, and the investigators were able to assess graft viability non-invasively and to correlate dopaminergic function with behavioral assays.

The latest developments in small animal scanner technology represent important and exciting advances in the field of PET, arousing great interest. Technological development has now reached a great deal of improvement in imaging acquisition and resolution. For instance, the microPET recently installed in our institution is equipped with a LYSO/LGSO detector, improving intrinsic resolution of the system with insignificant dead time loss. In addition, it has a great spatial resolution of 1.35 mm with Filtered Back Projection (FBP) reconstruction. Likewise, data from this scanner is acquired in list mode and, given the almost infinitely fine sampling across the detector plates, can be reformatted into sinograms of a chosen size.

PET and microPET applications

Molecular biology

It has now become clear that small animal PET can be employed in three types of application with a great deal of laboratory cost reduction. First, the acquisition of dynamic data in microPET provides a complete description of the

ligand binding kinetics in a single animal (in contrast to *ex vivo* dissection methods in which each animal can only contribute to a single time point). Second, considerably cost reduction in the number of animals used to > 50% (e.g., in occupancy studies, a minimum of 30 animals is required for a saturation curve produced using post-mortem dissection, while using microPET the number of animals drops to 12).[60] Finally, whole body biodistribution studies can also be rationalized using animal PET, as the need to pre-determine sacrifice times and target organs for dissection is removed.

Recently, particular interest has been seen in the imaging of gene expression *in vivo* using PET. The technique was first applied by Tjuvajev and co-workers using (^{124}I)-5-iodo-29-fluoro-1-beta-D-arabinofuranosyl-uracil (^{124}I-FIAU) to image the transfer and expression of the herpes virus type 1 thymidine kinase (HSV1-tk) reporter gene.[61] Then, researchers from the UCLA have reported a number of advances in this area.[62] The principle of gene imaging using PET has now been proven and will be an important component of the applications in small animal PET imaging.

Clinical use

Molecular imaging is doubtless a very important diagnostic tool in patients with neurological and oncological diseases. The ultimate goal is to personalize patient care by characterizing specific disease processes in different individuals. By accurately characterizing tumor properties or biological processes, molecular imaging plays an essential role in guiding cancer patient management: diagnosing, staging, assessing therapeutic targets, monitoring therapy, and evaluating prognoses (17-19). Molecular imaging is a very important diagnostic tool in early assessment, risk stratification, evaluation, and follow-up of patients with neurological diseases (e.g., Alzheimer's disease, movement disorders, seizure disorders, and psychiatric disorders; Figure 2).

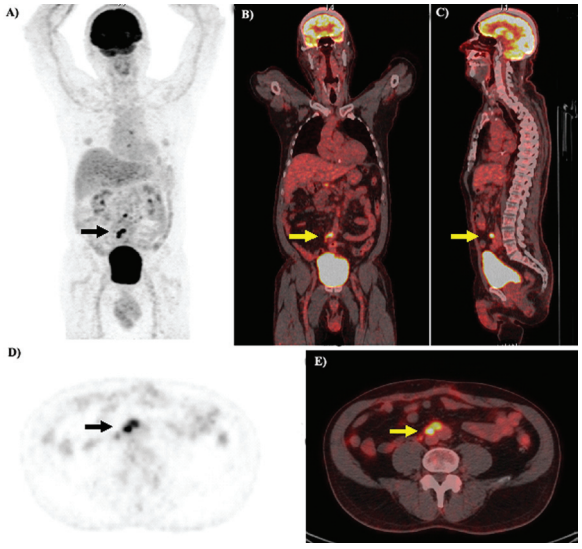

Figure 2 Typical example of ^{18}F-FDG PET/CT clinical application in Oncology.

A patient with a history of colon cancer, who underwent a tumor resection and chemotherapy, and had a suspected lymph node (LN) involvement in the retroperitoneal region on CT scan. ^{18}F-FDG PET/CT scan revealed several LNs with high uptake in the retroperitoneal region (arrows) and in the mediastinum. A) MIP image; B) Coronal fused image; C) Sagittal fused image; D) Axial slice of PET; and E) Axial fused image.

Molecular imaging has great potential to contribute to the development of new therapies in oncology and neuropsychiatry, as it plays an important role in diagnosis and response monitoring. PET radiotracers are now being developed as early response markers to assess the impact of novel therapies.^{18}F-FDG, an analogous of glucose, is the most advanced and used biomarker. However, several other probes have been shown promising results for this purpose (e.g., ^{18}F-Fluoro-thymidine, ^{18}F-Fluoromisonidazole etc). Molecular imaging in oncology could guide patient management and drug selection through response monitoring and tumor-specific target identification (Figure 3).

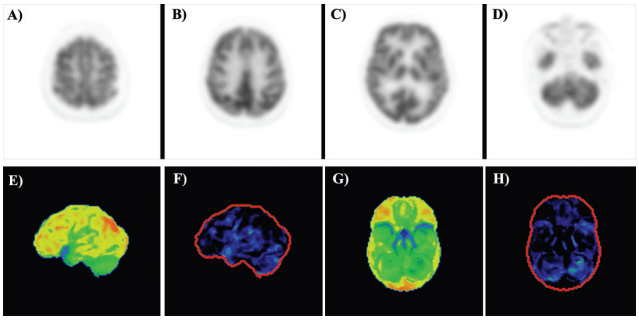

Figure 3 An example of ^{18}F-FDG PET/CT application in Neurology. A female patient with mild cognitive impairment (MCI) who had a PET/CT scan performed.

The study revealed almost homogeneous ^{18}F-FDG uptake throughout the cerebral cortex, basal ganglia, and cerebellum. The parametric analyses showed lower Z-score values on temporal lobes and cerebellum (adjusted to pons) compared with the normal database for her age. **A through D:** Axial slices of PET; **E and G:** Parametric analysis; **F and H:** Z-score analyses.

Drug discovery

The need for innovative therapies has increased due to the growing incidence of chronic illnesses, such as cancer, metabolic disorders, and neurological and psychiatric diseases. Regardless of a huge variety of drug targets in cancer and brain illnesses, the challenges of discovering and developing new treatments are appreciable. The high cost of the drug development process is unquestionable and successful drug discovery and development is limited to the drug targets and to the best molecules towards those targets we choose. Molecular imaging is playing an increasingly important role in our efforts to direct these challenges. Molecular imaging has potential applications throughout the drug discovery and development process. Its application can be divided in basically two levels of use: the preclinical and clinical settings.

1) Preclinical setting: molecular imaging can help pharmaceutical companies to validate drug candidates by *in vivo* assessment of accurate titration of therapeutic dose and, using a labeled form of the compound, quicker characterization of the drug's pharmacodynamics and pharmacokinetics in selected animal models. This knowledge will certainly improve data quality, reduce number of animals used, and decrease the work-up time for new drugs.

2) Clinical setting: molecular imaging can also help reduce cycle times during drug development in Phase I clinical trials (clinical testing strategies can be markedly shortened) accelerating dose selection and enabling a focused approach to Phase II; in Phases II and III clinical trials, molecular imaging biomarkers that recognize therapeutic response and/or disease progression can potentially serve as surrogate endpoints for registration and thereby speed drug assessment and approval. Thus, molecular imaging has been used to stratify patients that will enable enrichment of proof of concept clinical studies, leading to shorter and potentially more successful trials. Such stratification is a key goal of efforts to improve the efficiency of drug development and, ultimately, deliver the right therapy to the right patient at the right dose.

Drug development in psychiatry

The process of discovering and bringing a drug to market is both time-consuming and money-consuming. In fact, the average cost for a new drug to be released is estimated in US$ 1 billion, and the entire process usually takes 12-15 years. Even then, less than 10% of the drugs that enter a clinical trial actually reach the market.[57]

The use of small-animal PET (saPET) in drug development offers many advantages over traditional *ex vivo* techniques. A great benefit in the use of saPET is the fact that the animal remains alive, enabling longitudinal studies in which each animal can be used as their own control. Because saPET is non-invasive, it is feasible to analyze the same animal repeated times and in different therapeutic conditions, increasing the results reliability by reducing intersubject variation. Another possibility is the use of animals of the same strain, which confers a relatively low interanimal variation when compared to human subjects.[58,63,64]

The kinetics of drug absorption, distribution, metabolism, and excretion can be estimated with great precision because saPET permits the spatial and temporal detection of picomolar concentrations of a radiolabeled compound with great sensitivity. The brain penetration of a new drug and overall pharmacokinetics can be then assumed.[59,60,65]

The compound's pharmacodynamic effects can be monitored by the usage of different tracers, given that many physiological and biochemical parameters can be measured, such as blood flow, oxygen, and glucose metabolism. Furthermore, PET-based receptor occupancy studies may elucidate the mechanism of drug action and its ability to alter the biological process of disease.[63,66,67]

One of the greatest saPET applications is elucidating the pathophysiology of many diseases. The use of animal models of human disease and knock-out or knock-in animals may help in understanding the molecular basis and pathogenesis of disease, and also may lead to a prediction of the effective dose of a compound.[65]

Small-animal molecular imaging can also be used to analyze gene expression by using radiolabeld molecular probes introduced into target tissue. With the use of mathematical models, PET can measure protein synthesis and transport, allowing the analysis of biological changes deriving from gene modification.[66]

In collaboration with pharmaceutical companies this great potential of saPET in drug discovery will be explored. INCT-MM has a LabPET 4 PET Scanner system that will be used in the development of new tracers, and for this purpose it will be installed in a facility adjacent to the cyclotron located at the main Campus of UFMG.

Discussion

Molecular imaging represents a complementary tool for Molecular Medicine providing a key analysis pathway between the molecular mechanisms involved in the pathophysiology of disease and therapies targeted to those, resulting in better, faster, and cost-effective decision making. Molecular imaging helps prompt drug development towards the best drug candidate, therefore, enhancing safe drug delivery to people who need them. Previously, we have developed genetic, psychological and animal models to support the research in psychiatry and basic science. Our infrastructure allowed us to create and maintain a post-graduation program in Molecular Medicine, where we are promoting the necessary training of specialized human resources in the area, particularly in molecular imaging.

Disclosures

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* Modest
** Significant
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