Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

A Clinical Pharmacological Study on the Molecular Pharmacodynamic Mechanisms of Organoids and its Pharmacotherapeutic Significance in Biobanking: An Observational Descriptive Analytical Research

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Abstract

Background: Organoids are three-dimensional cell structures, grown *in vitro* from the stem cells. The organoids are formed of cells which differentiate, undergo spatially restricted lineage commitment, and acquire the specific tissue patterning to develop into several endoderm, mesoderm, and ectoderm-derived tissues. These organoids mostly tend to resemble the *in vivo* original organs, with the preservation of their genetic, phenotypic, and behavioral traits.

Objectives: The objective of this clinical pharmacological study was to perform an observational descriptive analytical research on the molecular pharmacodynamic mechanisms of organoids and its pharmacotherapeutic significance in biobanking.

Methods: The present study was an observational, descriptive analytical clinical research study of retrieved study literature derived from a thorough current investigative research database, research study literature, medical evidences, and review literature search, record and review from various available offline and online medical literature databases on organoids, for an evidence-based analysis of the molecular pharmacodynamic mechanisms of the organoids and their pharmacotherapeutic significance in biobanking, which was concluded on the basis of the extensive observational and evidence-based descriptive analytical derivations from the research on organoids.

Results: This evidence-based clinical pharmacological study comprehensively elaborated on the molecular pharmacodynamic mechanisms of the organoids and their clinical pharmacotherapeutic significance in biobanking.

Conclusion: With appropriate cellular composition, proper engraftment and vascularization into the host and adequately manifested functional activity, the efficacy and safety of organoid-based therapies can be properly instituted in different global institutes, hospitals, and medical health-care centers.

Key words: Biobanking, Clinical pharmacology, Evidence-based medicine, Molecular pharmacodynamics, Organoids, Pharmacology

Access this article online

www.ijss-sn.com

Month of Submission : 01-2022
Month of Peer Review : 01-2022
Month of Acceptance : 02-2022
Month of Publishing : 03-2022

INTRODUCTION

Organoids are three-dimensional (3D) cell structures, grown *in vitro* from the stem cells. These stem cells are mainly isolated from the biopsies or from the pluripotent stem cells (PSCs) that are extensively similar to the endogenous organs, in both their structural development and functional performance.

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The organoids are formed of cells which differentiate, undergo spatially restricted lineage commitment, and acquire the specific tissue patterning to develop into several endoderm, mesoderm, and ectoderm-derived tissues. These organoids mostly tend to resemble the *in vivo* original organs, with the preservation of their genetic, phenotypic, and behavioral traits. Organoids, which are derived from the adult stem cells (ASCs), can be developed directly from the diseased epithelium and matched normal tissues, and these organoids can also be genetically manipulated by CRISPR-Cas9 technology. These are not only complex structures but also possess unique capabilities of modeling human organ development and disease, showing wide similarities with the human organ system.[1-4]

Objectives

The objective of this clinical pharmacological study was to perform an observational descriptive analytical research on the molecular pharmacodynamic mechanisms of organoids and its pharmacotherapeutic significance in biobanking.

METHODS

Study Type

The present study was an observational, descriptive analytical clinical pharmacological study with evidence-based molecular pharmacodynamics research.

Study Period

The study period was 7 months, from February 2020 to March 2020 and May 2021 to January 2022.

Place of Study

This research study was done in the Departments of Pharmacology, Clinical Pharmacology, Molecular Pharmacology, Rational Pharmacotherapeutics, Pharmacovigilance, Evidence Based Medicine, Clinical Research, Clinical Medicine, Molecular Medicine, Rama Medical College Hospital and Research Centre, Rama University; Mamata Medical College and Hospitals; Departments of Stem Cell Therapy and Regenerative Medicine, GIOSTAR Institute of Regenerative Medicine Institutes, Hospitals, and Laboratories; and Fortis Hospitals.

Study Procedure

In this qualitative clinical research study, a multivariate analysis of the retrieved study literature derived from a thorough current investigative research database, research study literature, medical evidences, and review literature search, record and review from various available offline medical literature databases on organoids was performed, such as, any or all types of original research studies, systematic reviews, meta-analyses, case reports, case series, narrative reviews, study series, parallel studies and similar kind of studies or reviews, which were either qualitative, or quantitative, or both qualitative as well as quantitative, within institutes, hospitals, laboratories, medical health-care centers, medical libraries and archives, as well as, various available online medical literature databases, like, any or all types of original research studies, systematic reviews, meta-analyses, case reports, case series, narrative reviews, study series, parallel studies and similar kind of studies or reviews, which were either qualitative, or quantitative, or both qualitative as well as quantitative, on organoids, including published medical articles and archival literature, obtained from various global electronic medical search engines and databases such as Google Scholar, EMBASE, MEDLINE, Cochrane Library, PubMed, review of proceedings from selected scientific meetings, medical conferences, medical congress, medical summits, clinical trial registries, bibliographies of retrieved citations and reference lists, and expert recommendations, were also searched, to record and review, with thorough observational descriptive analysis of the molecular pharmacodynamic mechanisms of organoids and their pharmacotherapeutic significance in biobanking. A multi-variate evidence-based medical research study was conducted, with comparative analysis of the global heterogenous multi-disciplinary experimental and analytical study literature on the molecular pharmacodynamic mechanisms of organoids and their clinical pharmacotherapeutic significance in biobanking, by recording, reviewing, analyzing, and comprehensively analyzing the molecular pharmacodynamic mechanisms of organoids, from the deduced research study findings. This study was concluded on the basis of the extensive observational and evidence-based descriptive analytical derivations from the research on organoids.

RESULTS AND DISCUSSION

This thorough qualitative observational and evidence-based descriptive analytical clinical pharmacological study on organoids, elaborated on the following molecular pharmacodynamic mechanisms of organoids and their clinical pharmacotherapeutic significance in biobanking, with the following certain selective analytical elaborations:

Organoids can be derived from: (i) PSCs, such as embryonic stem cells and induced PSCs (iPSCs), or (ii) ASCs. iPSCs-derived organoids included development from optic cup, intestine, stomach, liver, lung, thyroid, and kidney. Each germ layer (endoderm, mesoderm, and ectoderm) is represented among this set of organs. iPSCs are expanded and subsequently differentiated through a multi-step protocol, that moves toward a fully differentiated structure. Specific cocktails of growth factors are required for each step. The differentiation process usually takes about 2–3 months, which depends on the specific type of organ.

The structure of iPSCs-derived organoids is complex and may contain mesenchymal, as well as epithelial and endothelial components.

Another Air-Liquid Interface (ALI) method was introduced allowing for the preservation of both epithelium and matched *in vitro* stromal microenvironment. The ALI method employs a Boyden chamber-like structure where primary tissue is seeded in extracellular matrix (ECM) gel in an inner Transwell dish which is exposed to air to enhance oxygenation. Culture medium is added to the outer dish and can diffuse through the permeable Transwell into the inner dish. ALI method has been applied in PSCs derived organoid culture. The continuous patterning and dynamic morphogenesis of hepatic, biliary and pancreatic structures, invaginating from ALI culture of anterior and posterior gut spheroids differentiated from human PSC. Adapted ALI culture of human cerebral organoids and neocortical organoid derived from PSCs were also developed.

Complementary to PSCs-derived organoids recapitulate development *in vitro*, ASCs-derived organoids model adult tissue repair, and can be established only from regenerative tissue compartments. There are different culture methods for ASCs-derived organoids. In the WENR method, epithelial organoids are derived from tumor biopsies directly in Matrigel with cocktail growth factors, with long-term expansion but no tumor micro environment. In the air-liquid interphase (ALI) method, tumor biopsies are cultured in ALI in the entire tumor microenvironment as a cell suspension of all cell types, including immune cells and other non-epithelial cell types, but with limited expansion. ASCs-derived organoids were successfully developed from Lgr5-positive intestinal stem cells in culture conditions modeling the stem cell *niche* of intestine. By providing the Wnt agonist R-spondin (RSPO), epidermal growth factor (EGF), and the bone morphogenetic protein inhibitor Noggin, and embedding the cells in an ECM-providing basement membrane extract (WENR method, Wnt3a+EGF+Noggin+RSPO1), Lgr5-positive stem cells are able to self-organize, proliferate and form differentiated crypt-villus like organoids. Since then, by modifying cocktails of growth factors and cell isolation procedures, cultures of patient-derived organoids have been successfully established for various human tissues by biopsy or resection, including the esophagus, stomach, colon, liver, pancreas, salivary gland, fallopian tube, ovary, prostate, breast, airway, taste buds, endometrium, kidney, bladder, thyroid, biliary tract, oral mucosa, and glioblastoma. A counterintuitive phenomenon is found that normal epithelium organoids often outgrow tumor organoids, which, in some instances, can be prevented using cancer-specific selection methods. For example, tumor organoids from colorectal cancer (CRC) can be selectively expanded on withdrawal of Wnt3a and RSPO1. Nearly all CRCs harbor activating mutations in the Wnt pathway or fusion of RSPO1 genes, allowing for the expansion of cancer cells without Wnts and RSPO, while normal epithelial cells arrest. Another approach to culture tumor cells selectively is to stabilize wild-type P53 by adding the MDM2 inhibitor Nutlin-3. Tumor cells are not affected by Nutlin-3 due to a loss of TP53, while normal cells in culture present cell cycle arrest and death, allowing for the selection of tumor cells. In general, patient derived organoids (PDOs) using WENR method can be derived from any epithelium of normal tissues as well as malignant or otherwise diseased tissues within approximately 7 days after embedding the cells into ECM matrix. PDOs can be expanded long term and cryopreserved while remaining genetically stable, making organoids an ideal tool for disease modeling. In addition, this type of organoid culture allows the direct parallel expansion of diseased cells and matched normal cells from individual patients, which allows for the generation of living tumor organoid biobank and facilitates its potential application in personalized therapy. However, nearly all PDOs types represent only the epithelial parts of organs, and there is an absence of stroma, nerves, and vasculature. Adopting ALI method, researchers can generate ASCs-derived organoids from various murine tissues including small intestine, colon, stomach, and pancreas, and then extending to culture clinical tumor samples, accurately recapitulating stem cell populations and their multi-lineage differentiation. The ALI model preserves tumor microenvironment with tumor parenchyma and stroma, including functional tumor infiltrating lymphocytes (TILs), providing a promising model for immunotherapy research for patients with cancer.

The organoids are proved amenable to all standard laboratory techniques, as well as to genetic modification. Organoids can be fast expanded, cryopreserved and applied to high-throughput analyses. Organoids are a promising research model bridging the gap between cell lines and patient-derived xenografts. Organoids have medium cost and heterogeneity. Organoids match normal control, have stable genetic and phenotypic features and cause genetic modification. Organoid cultures cannot mimic interactions with vasculature and stroma.

The dynamic organs undergo renewal by the process of constant growth and differentiation, with the continual growing tissue maintenance and cell differentiation by the stem cells. Organoids, which are derived from the ASCs or PSCs, can be grown with similarities with the original organs. The organoids have already been utilized in the medical and surgical clinics, an example including its clinical utilization in the treatment of inflammatory bowel disease in personalized medicine. The current organoid technology and the organoids have found their wide-spread applications

in basic and medical research, involving the modeling of human development and diseases, for example, genetic, infectious and malignant diseases, drug development, development of new treatments, personalized treatment and regenerative medicine, including biobanking of patient derived organoids for many cancers and cystic fibrosis, and also have shown potentials, yet beyond.

Although the organoids have extensive use in basic research, their translational biomedical application mostly involves drug testing and initial cell replacement strategies.

The regulatory frameworks and general guidelines required for organoids and their clinical applications, for example, drug testing using organoids in Europe, include "Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches" by the European Medicines Agency, the regulatory requirements for cell and gene-based therapies, and good manufacturing practices (GMP) of a pharmaceutical drug, for the clinical use of organoids.

In regenerative medicine, organoids have huge potential applications, provided the following limitations are overcome:

(i) Clonal variation, developmental stage, pluri-/multi-potency, and chromosomal stability of stem cells;
(ii) Reproducibility, accuracy, and scalability of the methodologies proposed;
(iii) Meaningful functional assessment of resulting organoids; and
(iv) Preclinical validation.

Organoid technology can be employed for novel approaches in several surgical operative procedures, like the replacement of severely damaged organs, such as the pancreas, in patients with type 1 diabetes. The human pancreas organoids is a cell-based medicinal product, produced pharmaceutically, with present GMP compliant guidelines. The regenerative process by the human pancreas organoids is an intricate mechanism. The presently practiced methods of replacement and regeneration of certain organs, like pancreas, are obviously prolonged, complex, expensive, and lack reproducibility. Therefore, improvements in the overall bioprocess design, serum-free culture media, and the starting materials, while in compliance with the quality and global regulatory guidelines, and other specifications, would result in an improvisation in the critical quality attributes of the entire process of replacement and regeneration of organs, including the organoid technology, itself. The feasibility of human pancreas organoid generation from the human pancreas has been refined by GMP-compliant regulations in the in-process controls and media formulations, through replacing RSPO1 conditioned medium, with recombinant RSPO1 protein, thus developing a cryopreserved human pancreas organoid, for further use in allogeneic therapies for type 1 diabetes.

Human-iPSC-derived retinal organoids have been developed, according to GMP standards, for the generation of transplantable photoreceptor cells for future clinical applications. Liver buds have also been developed, in accordance with GMP standards, for transplantation processes. Cell production, which occurs under fully defined, xeno-free conditions, and which is beneficial for therapeutic applications among patients suffering from different diseases, have improved reproducibility, without causing any xeno-mediated disease or immune rejection.

Organoids is an ideal *in vitro* tool for the identification of novel stem cell markers. Organoid culture allows for the generation of specific cell types that were previously impossible in 2D cultures. For disease modeling, organoids can be genetically engineered to model genetic and malignant diseases by using CRISPR-Cas9. Normal organoids can be transplanted to wounds for tissue repair. Tumor-derived organoids can be used for basic research by genetic modification and modeling rare cancer. For translational research, tumor derived organoids can be used for biobanking, genetic repair, and drug screening studies, both for personalized medicine and drug development, as well as immunotherapy research. Human and murine organoids have been orthotopically transplanted into mice to model disease or to show tumorigenic potential.

Because of their characteristics, organoids have enormous potential for drug development and precision medicine, which aims to increase cost effectiveness and risk-benefit ratios of therapies by more precisely targeting therapies to individual patients. Biobank research on patient-derived organoids has already led to successful personalized treatment of cystic fibrosis. To facilitate such research, organoids are cultivated from patient-derived stem cells and stored in tissue repositories called "biobanks." Biobanks facilitate multidisciplinary research aimed at a variety of purposes such as drug screening, drug development and disease modeling, as well as enabling large-scale data sharing. Organoids are applied for translational research, and the living organoid biobanks are a tool for personalized treatment and drug development. Organoids can be efficiently established from patient-derived normal and tumor tissue samples, which can be cryopreserved and stored in living organoid biobanks. PDOs resemble the tumor epithelium they were derived from both phenotypically and genetically. Combined molecular and therapeutic profiling of PDOs may help predict treatment response and contribute to personalized cancer treatment and drug development. Among the organoid biobanks, a colon cancer derived biobank of 22 lines, is of significant mention, where all the samples had

performed RNA sequencing and whole genome sequencing analysis. The molecular characteristics of PDOs cover all five consensus molecular subtypes of CRC. The mutations in the organoids were largely concordant with the original tumors, which was validated in a set of organoids established of colorectal metastases. High-throughput screening of a panel of 83 compounds found that there are differences in drug sensitivity among the organoid lines that in some cases correlated with specific mutation. For example, RNF43-mutant organoids were sensitive to WNT secretion inhibitors, and KRAS-mutant organoids were resistant to the epidermal growth factor receptor (EGFR) inhibitors, including cetuximab and afatinib. Later, in a biobank of 35 organoid lines from CRC, the organoid models reproduce most of the genetic and transcriptomic characteristics of the donors, but determined less complex molecular subtypes for the absence of stroma. Drug screening with therapeutic compounds representing the standard of care for CRC, combined with molecular profiles, helped identify a signature outperforming RAS/RAF mutation which has predictive value for sensitivity to the EGFR inhibitor cetuximab. Drug response in organoids and clinical response was also observed to prove that the *in vitro* organoid response correlates with the *in vivo* response. A clinical study of PDOs derived from metastatic gastroesophageal and CRC showed a strong correlation (100% sensitivity, 93% specificity, 88% positive predictive value, and 100% negative predictive value) between the *in vitro* organoid response to a set of targeted therapies and chemotherapies and the response of the tumor in patients. Another study adopted organoids for colon cancer chemoprediction showing that PDOs test predicted more than 80% of patients' response treated with irinotecan-based therapies. Together, these studies indicate the potential of tumor-derived organoids to predict patients' responses. Recently, two studies showed the applications of PDOs derived from rectal cancer to predicting patient responses to neoadjuvant chemoradiation therapy. A rectal cancer derived biobank (n=80) was generated and PDOs' sensitivity to 5-FU, irinotecan, or radiation were tested. They incorporated a correlation between *in vitro* responses in organoids and the histopathologically determined tumor regression scores after surgical resection to define prognostic cut-offs. Using these parameters, the *in vitro* responses could predict clinical responses with an impressive area under the curve of 0.88 and an accuracy of 84%. In the other study, 65 PDO lines from rectal cancer were established to test responses to neoadjuvant chemoradiation therapy, including the standard FOLFOX chemotherapy and radiation. The PDO responses significantly reflected the patients' progression-free survival.

For pancreatic cancer, a much larger PDAC biobank (n=114) was generated and exposed a subset of these organoid lines to the standard-of-care chemotherapies. Their sensitivities paralleled clinical responses in patients. Besides, gene expression signatures of chemosensitivity based on organoids were developed to help predict responses to chemotherapy in both the adjuvant and advanced disease settings. By high throughput drug screening, they nominated alternative treatment strategies for chemo refractory PDO. Another study also used PDOs (n=30) to identify novel therapeutics to target pancreatic tumor cells in a biobank covering different histological subtypes, including PDACs, acinar cell carcinoma, cholangiocarcinoma, adenosquamous-PDACs, intraductal papillary mucinous neoplasm-derived PDACs and papilla of Vater adenocarcinomas. PDOs were exposed to 76 therapeutic agents currently not exploited in the clinic. The Protein Arginine Methyltransferase 5 inhibitor, EZP015556, was shown to target methyl thioadenosine phosphorylase (MTAP)-negative tumors, but also appeared to constitute an effective therapy for a subset of MTAP-positive tumors, indicating the importance of personalized approaches for cancer treatment. A liver tumor biobank (n=13) containing hepatocellular carcinoma and cholangiocarcinoma, as well as the rarer lymphoepithelioma-like cholangiocarcinoma was also developed. In drug screening experiments with 29 compounds, the extracellular regulated protein kinases inhibitor SCH772984 was found to effectively inhibit the growth of tumor organoids, which was validated *in vivo* using xeno transplanted organoid lines in mice, highlighting SCH772984 as a possible therapeutic agent. Biliary tract carcinomas-derived organoids biobank was also established, covering intrahepatic cholangiocarcinoma, gallbladder cancer, and neuroendocrine carcinoma of the ampulla of Vater. Gene expression profiling of the organoids indicated that SOX2, KLK6 and CPB2 could be potential prognostic biomarkers. Drug screening using a compound library of 339 drugs showed that the antifungal drugs, amorolfine and fenticonazole, significantly suppressed the growth of biliary tract carcinomas organoids with little toxicity to normal biliary epithelial cells. An organoid biobank of high-grade serous ovarian cancer (HGSC) (n=33) was established. Up to 50% of all patients with HGSC have DNA repair defects, typically mutation of BRCA1 or BRCA2. These patients were thought to benefit from treatment with poly (ADP-ribose) polymerase (PARP) inhibitors.[1-4]

CONCLUSIONS

The present study well-elaborated and analyzed the distinctive molecular pharmacodynamic mechanisms of organoids and their pharmacological functioning. This study also described, in details, the significance of organoids in biobanking. To conclude, with appropriate cellular composition, proper engraftment and vascularization into the host and adequately manifested functional activity, the efficacy and safety of organoid-based

therapies can be properly instituted in different global institutes, hospitals, and medical health-care centers. The forthcoming human leukocyte antigen-homozygous iPSC initiatives would hugely improvise the clinical applications of organoid technologies. This would also accelerate the development of patient compatible regenerative therapeutic approaches, through large-scale production of organoids and organoids biobanking. The blending of organoid technology with 3D bioprinting and vascularization approaches might produce macrostructures with the desired cellular composition, thus, complementing successful transplantation. A proper co-ordination of all these strategies of clinical biotechnology would delineate the most successful clinical pharmacotherapeutic utilization of organoids and organoid technologies.

ACKNOWLEDGMENTS

My sincere acknowledgments to the Departments of Pharmacology, Clinical Pharmacology, Molecular Pharmacology, Rational Pharmacotherapeutics, Pharmacovigilance, Evidence Based Medicine, Clinical Research, Clinical Medicine, Molecular Medicine, Rama Medical College Hospital and Research Centre, Rama University; Mamata Medical College and Hospitals; Departments of Stem Cell Therapy and Regenerative Medicine, GIOSTAR Institute of Regenerative Medicine Institutes, Hospitals, and Laboratories; and Fortis Hospitals, for the completion of this research project.

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How to cite this article: Hazra M. A Clinical Pharmacological Study on the Molecular Pharmacodynamic Mechanisms of Organoids and its Pharmacotherapeutic Significance in Biobanking: An Observational Descriptive Analytical Research. Int J Sci Stud 2022;9(12):58-63.

Source of Support: Nil, **Conflicts of Interest:** None declared.