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Paclitaxel–A Drug Successfully Developed from Natural Sources as Breast Cancer Therapy

Eri Amalia[1*], Tina Rostinawati[2], Yo ppi Iskandar[2], Iyan Sopyan[1], Sr iwidodo Sriwidodo[1], An is Yohana Chaerunisaa[1], Muhaimin Muhaimin[2]

[1]*Department of Pharmaceutics and Pharmaceutical Technology, University of Padjadjaran, Jatinangor, Indonesia*

[2]*Department of Biology Pharmacy, University of Padjadjaran, Jatinangor, 45363, Indonesia*

ABSTRACT

Background: The discovery of breast cancer drugs is continuously conducted through both computational and exploration of natural derivatives to obtain highly selective promising compounds with fewer adverse effects. Despite the difficulties experienced, six naturally derived drugs including paclitaxel have been successfully, continuously developed and approved for breast cancer therapy. Learning for its research would give insight on conducting similar research.

Methods: This article reviewed paclitaxel development stages, including different research types and outcomes, as well as current developments of the drug from reliable sources including science direct, pubmed, WHO, FDA, EMC, and PDB websites using the keywords "paclitaxel", "IC_{50} paclitaxel" and "paclitaxel encapsulation".

Conclusions: This research showed that paclitaxel development began in 1962 following current FDA guidelines. The anticancer activity of the Taxus extract and paclitaxel compound determined through *in vitro* examination was IC_{50}<50 µg/ml and < 20 µM against several cancer lines, respectively. Paclitaxel was powerful but with limitations in solubility, and its development had continuously been investigated, such as applying nanoparticle encapsulation for efficacy improvement. The development of anticancer drugs from natural sources is promising, e.g. for breast cancer therapy. The compound obtained from *in vitro* screening must be further examined by following the FDA guidelines. Similarly, continuous study of approved formulations of drugs is important to discover their superior therapeutic efficacy.

Keywords: Breast cancer; Natural source; Paclitaxel; IC_{50}, *In vivo; In vitro*; Clinical research; Encapsulation; Nanoparticle; Liposome

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Corresponding author: Eri Amalia, Department of Pharmaceutics and Pharmaceutical Technology, University of Padjadjaran, Jatinangor, Indonesia; E-mail: amalia@unpad.ac.id

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INTRODUCTION

Breast cancer is not contagious, however, the cases are increasing every year as 2.3 million women were diagnosed with this disease, and 685000 deaths were recorded globally in 2020 [1]. There are 33 drugs approved by the FDA for breast cancer treatment, but their cytotoxic characteristics have provided several unfavorable adverse effects, which currently could be minimized by the combination of several chemotherapy agents [2-5]. Accordingly, research on breast cancer is continuously conducted to discover alternative drugs urgently required by patients.

In general, stage 1 of drug discovery and development is completed in 3-5 years. Then, the hit compound proceeds into stage 2 (preclinical research lasting for 1-2 years, and stage 3 (clinical research which ranges from 6-7 years. Stage 4 is approximately 2 years of FDA reviews. In stage 5, post-market safety monitoring is continuously conducted to evaluate the safety and efficacy of the drug as demonstrated [6]. Years of the experiment are challenging for the research team, specifically in the initial stage where finding a promising compound with promising *in vitro* IC_{50} value is similar to tracing one needle inside a haystack. Therefore, learning from successfully approved drugs would be essential for researchers in order to effectively design and predict future experiments.

LITERATURE REVIEW

Methodology

This research was prepared by reviewing published journals about paclitaxel from 1970 to date using specific keywords “paclitaxel”, “taxol”, “paclitaxel encapsulation” on science direct and pubmed websites also other official websites. Inclusion criteria were related to specific keywords, while opinions and unrelated topics were the exclusion criteria [7-15].

Development of Paclitaxel as a Breast Cancer Drug

The discovery and development of paclitaxel required 30-32 years, from 1962 until the approval for ovarian and breast cancer therapy in 1992 and 1994, respectively, then it was used for other cancer types in the following years. According to numerous experiments were conducted stepwise to identify and evaluate its activity *in vitro* and *in vivo*, required for clinical research and post-market safety monitoring. Estimation showed that NIH’s investment cost reached $183 million from the development stage in 1977 until 1997 at the end of the Cooperative Research And Development Agreement (CRADA term, including the FDA post-market drug safety monitoring stage [16]. However, the longtime experiment was recompensed with the ability of the invention to help people improve their quality of life. Below are several research collected from published journals referring to the steps of paclitaxel development

Step 1: Drug Discovery

Drug discovery is the initial stage with the main activity including a Target identification and validation, research on basic knowledge of a disease in terms of its pathway and factors or enzymes involved during the development, as well as validating a potential target therapy; b Assay development (*in vivo* and *in vitro*; c High throughput screening of many extracts or compounds to a known specific target therapy; d Hit identification; and e Hit-to-lead, lead generation, and optimization [17].

Compound Screening

The discovery of paclitaxel as an anticancer started with a compound screening in 1962 when Arthur Barclay, United States Department of Agriculture (USDA) botanist, took bark samples of reddish Pacific yew *Taxus brevifolia*. The sample was sent to the Wisconsin alumni research foundation for further extraction and evaluation. The extract showed cytotoxicity against KB cells (Keratin-forming tumor cell line Hela) with IC_{50}<50 µg/ml [18]. Further hit identification was performed in September 1964 in collaboration between USDA and Research Triangle Institute (RTI), an organization where Monroe E. Wall and Mansukh C. Wani worked together. The pure crystalline substance responsible for its anticancer activity was successfully isolated in 1966 and named “Taxol” (“Tax” For Taxus and “Ol” for Alcohol). Additionally, paclitaxel structure identification was carried out by mass spectrometry, X-ray crystallography, and NMR spectroscopy. This became fruitful when the structure was discovered and published in 1971. Paclitaxel was found as a large molecule containing a complex structure in which two molecules are linked by a small side chain to form one unit with the formula $C_{47}H_{51}NO_{14}$. The structure-activity relationship research revealed that the side chain of carbon 13 is critical and responsible for the drug’s anticancer activity [19,20].

However, due to environmental issues in 1988, paclitaxel was developed through a semisynthetic method. Its preparation involved several stages beginning with redox, acetylation, and deacetylation reaction of 10-Deacetylbaccatine III (10-DAB) as the core structure obtained from *Taxus baccata* or English yew with an N-benzoyl-(2R,3S)-phenylisoserine methyl ester side chain. This semisynthetic compound was approved by FDA for treatment in 1994.

Target Identification and Validation

Susan Horwitz (who works at Albert Einstein college of medicine in New York’s Yeshiva university) and colleagues conducted further research from 1977 to late 1979 to determine and validate paclitaxel’s target. They stated that treatment of the Hela and mouse fibroblast cells with 10 µM of paclitaxel within 22 h was effective in stimulating more than 90% of the cells to form microtubule bundles. The drug’s mechanism of action was found different from previously approved anticancer agents who prevent microtubule formation, thereby causing the cells

to not divide and eventually die. Furthermore, paclitaxel binds to the N-terminal 31 amino acids of the β tubulin beta subunit and stimulates the formation of multiple microtubule assemblies in the absence of microtubule-associated proteins, rings, and additional guanosine 5'-triphosphate, while the cells are constantly attempting DNA replication. This condition makes the cells lose the ability to coordinate division and apoptosis is induced as indicated. The described mechanism of action leads to arresting of the cell cycle in G_2 and M.

Assay Development

There was unclear information regarding the exact period of paclitaxel assay development. However, Monks, et al. reported that the standard method applied by NCI to screen anticancer agents included preparing a series of five 10-fold dilutions for compounds and five 3-fold dilutions for extracts in duplicate wells. The concentrations of the test began from 10-4M for compounds and 250 µg/ml for extracts. Where, samples IC_{50} values were categorized as having intermediate cytotoxic activity provided the result showed a 20%-70% ratio of Test optical density/control optical density (T/C) in the above mentioned concentration series, or 50 µg/ml-175 µg/ml and 20 µM-70 µM for extracts and compounds, respectively. Extracts with IC_{50}<50 µg/ml and compounds with IC_{50}<20 µM could have high cytotoxic activity.

Presently, *in vitro* assay of paclitaxel has been performed in 395 cell lines. The lowest activity was found in EW-12 (Ewings sarcoma, bone tissue) with IC_{50} of 12.1 M, and the highest was in LC-2-ad (lung NSCLC adenocarcinoma, lung tissue) with IC_{50} of 1.26 nM. According to paclitaxel shows different activity in cell lines affected by the type of cells used during the research, length of incubation time (24 h, 48 h, 72 h, or others), the incubation media, and amount of cell seeding in a plate. The cells are also influenced by the viability of examination methods such as Sulforhodamine B, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sodium 3'-[1-[(phenylamino)-carbonyl]-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene-sulfonic acid hydrate (XTT), and clonogenic assays, etc.

The value of IC_{50} or half maximal inhibitory concentration expressed as molar concentration is important in drug discovery because it indicates the potency of a compound bound with a specific target in the biological system, and leads to 50% growth inhibition compared to the control or biological system without a compound.

Step 2: Preclinical Research

Preclinical research focuses on finding information about the compound's dosing and toxicity levels *in vitro* and *in vivo*, which can serious harm. The initial preclinical research conducted in the early 1970's involving NCI's murine cancer models showed negative results. However, the use of mouse xenograft screens developed by NCI in the mid-1970's showed positive results. In 1977, numerous laboratories worldwide investigated paclitaxel's activity against tumor models in mice. The mice received an implant of murine Madison 109 lung carcinoma (M109), M5076 sarcoma, or seven different human tumor xenografts, including A431 vulva; A2780 ovarian; LX-1, H2981, and L2987 lung; RCA and HCT-116 colon carcinomas; and M5076 sarcoma. The paclitaxel was administered IV for seven days. The research failed due to the insolubility character of paclitaxel which caused the compound not to reach distal site tumor models, thereby preventing its optimum activity. The research persistence, as reported in the published journal in 1992, produced good results after changing the previous vehicle mixture with cremophor/ethanol (50/50), except for the M5076 sarcoma. The administrations of daily injections for seven days reveal that paclitaxel exhibited flat dose-response levels that were only a fraction of its maximum tolerated dose. SCM109 model was used to analyze schedule dependency and dose-response of the compound.

Step 3: Clinical Research

Clinical research is conducted after fully reviewing preclinical research concluded with safety and toxicity information. The clinical trial includes phase 1 which determines drug safety and dosage in 20-100 healthy volunteers with diseases or certain conditions. Phase 2 involves finding information about the efficacy and side effects using several hundred volunteers with the diseases or conditions. Phase 3 is meant for efficacy and monitoring of adverse reactions in 300-3,000 sick volunteers. The clinical trial of paclitaxel began in 1983-1984 with phase 1 to assess the drug's safety and dose in patients of ovarian cancer and leukemia, followed by breast cancer. Five of the six clinical researches that Bristol-Myers Squibb (BMS) submitted to the FDA were conducted or funded by NIH.

Clinical Trial Phase 1 in Metastatic Solid Tumours and Breast Cancer

Research on clinical trial phase 1 began in 1983, one of which assessed 20 patients with metastatic solid tumors. Paclitaxel was mixed with intravenous fluid and administered over a period of 1 h daily for five days at three weeks intervals, with doses of 5 mg/m^2, 20 mg/m^2, and 40 mg/m^2. There was no significant adverse reaction at the lower dose, but at 20 mg/m^2 patients experienced alopecia and leukopenia. A higher dose increased the severity of the adverse effects. Based on the result, 30 mg/m^2 is recommended as starting dose for five days of treatment at three weeks intervals to continue in phase II.

Meanwhile, 13 research conducted the clinical trial phase I of paclitaxel in a patient with breast cancer from 1987-1994. These showed that the maximum tolerated dose for paclitaxel was 250 mg/m^2, 225 mg/m^2, and 140 mg/m^2 for 3 h, 24 h, and 96 h, respectively a tendency of hypersensitivity reaction was discovered due to chremophor application in the drug formula.

Clinical Trial Phase 2 in Breast Cancer

Clinical trial phase 2 of paclitaxel to assess the efficacy and side effects began in 1985 in patients with ovarian cancer. Several research also evaluated the drug's efficacy on breast cancer, one of which administered a 250 mg/m^2 dose to 25 patients for 3 h. Another one administered 135 mg/m^2 and 174 mg/m^2 through i.v infusion over 3 h, then 175 mg/m^2 was approved as the safe dose to be used for further research.

Clinical Trial Phase 3 in Breast Cancer

Clinical trial phase 3 was first conducted in patients with metastatic breast cancer in several study from 1999 to 2000 with more than 1279 patients. The subjects received a random intravenous infusion of 200 mg/m^2 paclitaxel over a 3 h period for eight cycles (24 weeks) or standard Cyclophosphamide-Methotrexate-Fluorouracil-Prednisone (CMFP) treatment. The research showed a good result that the drug exerted less myelosuppression and control of infections compared with standard CMFP treatment. Moreover, paclitaxel can manage the growth of breast cancer similarly to CMFP as well as patients' quality of life. Interestingly, it ensures longer survival of patients compared to CMFP. Other study also showed paclitaxel's effectiveness compare to doxorubicin in dose of paclitaxel 175 mg/m^2/24 h, doxorubicin 60 mg/m^2/24 h infusion and its combination; also study at dose paclitaxel 200 mg/m^2/3 h and doxorubicin 75 mg/m^2/3 h infusion. Clinical study of paclitaxel as adjuvant therapy was conducted in randomized design of 3170 patients also showed beneficial effect of paclitaxel in decrease of cancer reoccurrence and risk of death for 22% and 26%, respectively.

Step 4: FDA Drug Review

Subsequent to several clinical research conducted with promising results, the NCI as part of the National Institutes of Health (NIH) successfully reached an agreement with Bristol-Myers Squibb (BMS) in 1991 under CRADA. After BMS obtained the patent, the company took every responsibility to supply paclitaxel for further clinical trials. Initially, the clinical trial was supported and funded by NIH through less than 500 patients in 1989 and after the agreement until the end of the CRADA term, up to 28,882 patients enrolled in the activity. Out of six clinical researches submitted for registration, five were funded by NIH, and one by BMS. In the FDA drug review stage, BMS was responsible for preparing a dossier to be submitted for approval from the FDA. In December 1992, paclitaxel injection was successfully approved by FDA to treat metastatic ovarian cancer. Later in June 1994, it was approved for metastatic breast cancer and as second-line therapy for AIDS-related Kaposi's sarcoma in August 1997. The injection is a clear, colorless slightly yellow viscous solution. Each ml contains 6 mg paclitaxel, 527 mg of purified cremophor EL (polyoxyethylated castor oil), and 49.7% (v/v) dehydrated alcohol, USP, and it is also available as 5 ml, 16.7 ml, and 50 ml sizes in the vial.

Step 5: FDA Post-Market Drug Safety Monitoring

Stage 5 evaluates a product's safety over the months and even years that constitute its lifetime in the marketplace by thousands of volunteers with a disease or certain condition. FDA reviews every problem with the prescription reported and additional caution can be included in the dosage or usage information. The post-market surveillance conducted in 812 patients identified possible adverse effects of paclitaxel which affect several organs as well as the disorders initiated including bone marrow and its related disease or condition, namely neutropenia, leukopenia, thrombocytopenia, anemia, infection, bleeding, red cell transfusions, and platelet transfusions. Furthermore, hypersensitivity reaction, cardiovascular abnormalities (vital sign changes such as bradycardia and hypotension, and significant cardiovascular event), abnormal ECG, peripheral neuropathy, myalgia/arthralgia, and gastrointestinal problems (nausea, vomiting, diarrhea, mucositis, and alopecia) were discovered. Hepatic disorders (bilirubin, alkaline phosphatase, and AST or SGOT elevation) and experience of hypersensitivity at the injection site were also reported.

DISCUSSION

Future Perspective of Paclitaxel

Paclitaxel is an example of successfully developed anticancer drugs from natural sources. Its limitation is due to the insolubility in water and efflux through the activity of multidrug MDR protein 7 (ABCC10) which causes the drug's low bioavailability in blood and less effectiveness when administered orally. The available micelle paclitaxel in cremophor EL as a co-solvent remains the leading drug for paclitaxel. Nevertheless, it is continuously developed to optimize its effectiveness in both formulation development and drug combination aspects.

In terms of formulation development, the drug's encapsulation in suitable lipid based carriers of liposome or polymers micelle could be the alternative to increase its efficacy. One of the successful developments is albumin-bound paclitaxel (nab-paclitaxel or abraxane) with a nanoparticle size of 130 nm. This lyophilized product was approved by FDA on January 2005 for the treatment of metastatic breast cancer, particularly in patients with conventional treatment failure, those experiencing reoccurrence, and people who have severe hypersensitivity to the conventional injections. The pharmaceutical formulation is designed to contain 100 mg of paclitaxel bound to 900 mg of human albumin, also containing sodium caprylate and sodium acetyltryptophanate. Human albumin structure, refer to PDB: 1N5U) has primary binding site at interface of subdomain IIA and IIIA. It has function as a natural drug carrier in the product and it is also available in the human body, thereby allowing it to transfer paclitaxel effectively into cell targets. Meanwhile, sodium caprylate and acetyltryptophanate are intended as stabilizers to albumin, this compound will strongly bind to albumin to avoid thermal

degradation during the process. The outstanding discovery inspires research into other albumin-binding anticancer drugs that will successfully enter phase I/II of clinical trials currently. Another development of paclitaxel encapsulation was reported in several carriers as a) Lipid based encapsulation, where the formula containing 1% (w/w) paclitaxel, 55%monoolein, 27.5% tricaprylin, and 16.5% polysorbate. Interestingly, the formula have been examined on a laboratory scale in mice to determine the effective oral dosage form of paclitaxel; b) Liposome formula with various combinations of lipids as seen in e.g. paclitaxel encapsulated in cationic liposome of DOTAP (2,3 dioleoyloxypropyltrimethylammonium chloride) and neutral lipid DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine in 3:50:47 molar ratio; c) Polymeric micelle of diblock copolymer surfactant, e.g. preparation of nanosize of paclitaxel micelle in diblock copolymer of methoxy poly(ethylene glycol)-block-poly(caprolactone); d) Polymeric lipid hybrid nanoparticles, where the structure of nanoparticle composed of poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCL-PEG-PCL) amphiphilic copolymers as the hydrophobic core, a lipid monolayer of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (DSPE-PEG2000) conjugated with folic acid for targeted delivery ability.

CONCLUSION

Natural sources including plant, marine-derived materials, and microbes are possibly developed as a breast cancer drug. The promising compounds needed must be meticulously investigated from different aspects by referring to FDA guidelines, and further developed to obtain data on drug safety and efficacy for the preparation of dossiers submitted to the FDA for review and approval. Among several experiments, *in vitro* assay is crucial to assess drug activity against diseases such as breast cancer. From 30 years of paclitaxel development, it was discovered that persistence, hard work, and diligence are assets for research teams in developing a drug. Similarly, collaboration and support from academia, the pharmaceutical industry, and the government will be required to achieve the mutual goals of finding a drug required by breast cancer patients to save and improve their quality of life. More importantly, continuous study and improvement formula of the potent chemotherapeutic agents are required to obtain leading formula with superior therapeutic efficacy and less adverse effect.

AUTHOR CONTRIBUTIONS

Conceptualization: EA, MM; Investigation: EA; Data curation: EA, TR, YI; Resources: AYCh; Writing original draft preparation: EA, IS, SS; Review and editing: M.M. All authors have read and agreed to the published of the manuscript.

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Data sharing is not applicable.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Amalia E, Diantini A, Subarnas A (2019) Overview of current and future targets of breast cancer medicines. J Pharm Sci Res. 11(6):2385-2397.
2. Fisusi FA, Akala EO (2019) Drug combinations in breast cancer therapy. Pharm Nanotechnol. 7(1):3-23.
3. Maughan KL, Lutterbie MA, Ham PS (2010) Treatment of Breast Cancer. Am Fam Physician. 81(11):1339-1346.
4. Botchkarev VA (2003) Molecular mechanisms of chemotherapy-induced hair loss. J Investig Dermatology Symp Proc. 8(1):72-75.
5. Mathur S, Hoskins C (2017) Drug development: Lessons from nature (review). Biomed Rep. 6:612–614.
6. Williams GH, Stoeber K (2012) The cell cycle and cancer. Med Cell Biol. 226:352-364.
7. Branzei D, Foiani M (2008) Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol. 9(4): 297-308.
8. Staton RA, Gernert KM, Nettles JH, Aneja R (2011) Drugs that target dynamic microtubules: A new molecular perspective. Med Res Rev. 31(3):443-481.
9. Thorn CF, Oshiro C, Marsh S, Boussard TH, McLeod H, et al. (2011) Doxorubicin pathways: Pharmacodynamics and adverse effects. Pharmacogenet. Genomics. 21(7): 440-446.
10. Shetty N, Gupta S (2014) Eribulin drug review. South Asian J Can. 3(1):57-59.
11. Zhu L, Chen L (2019) Progress in research on paclitaxel and tumor immunotherapy. Cell Mol Biol Lett. 24(40): 1-11.

12. Snyder JP, Nettles JH, Cornett B, Downing KH, Nogales E (2001) The binding conformation of taxol in β-tubulin: A model based on electron crystallographic density. Proc Natl Acad Sci USA. 98(9):5312–5316.
13. Hughes JP, Rees S, Kalindjian SB, Philpott KL (2011) Principles of the early drug. Br J Pharmacol. 162(6): 1239-1249.
14. Monks A, Scuderio D, Skehan P, Shoemaker RH, Paull KD, et al. (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst. 83(11):757-766.
15. Weaver BA (2014) How taxol/paclitaxel kills cancer cells. Mol Biol Cell. 25(18):2677-2681.
16. Patt D, Gauthier M, Giordano S (2006) Paclitaxel in breast cancer. Oncologist. 2(1):11-21.
17. Fan Y, Chen S, Li, Chen WJ, Wang H, Kong H (2020) Synthesis of taxol and docetaxel by using 10-deacetyl-7-xylosyltaxanes. Chem Biodivers. 17(2):1900631.
18. Parness J, Horwitz SB (1881) Taxol binds to polymerized tubulin *in vitro*. J Cell Biol. 91(2):479-487.
19. Schatten G, Schatten H, Bestor TH, Balczon R (1982) Taxol inhibits the nuclear movements during fertilization and induces asters in unfertilized sea urchin Eggs. J Cell Biol. 94:455-465.
20. Zhang L, Zhu D, Dong X, Sun H, Song C, et al. Folate-modified lipid–polymer hybrid nanoparticles for targeted paclitaxel delivery. Int J Nanomed. 10:2101-2114.