

RESEARCH ARTICLE

Investigation of Antitumor Effects of Sorafenib and Lapatinib Alone and in Combination on MCF-7 Breast Cancer Cells

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Abstract

Background: Breast cancer evolution and tumor progression are controlled by complex interactions between steroid receptors and growth factor receptor signaling. Aberrant growth factor receptor signaling can augment or suppress estrogen receptor function in hormone-dependent breast cancer cells. Thus, we aimed to investigate antitumor effects of sorafenib and lapatinib alone and in combination on MCF-7 breast cancer cells. **Materials and Methods:** Cytotoxicity of the sorafenib and lapatinib was tested in MCF-7 cells by XTT assays. 50, 25, 12.5 and 6.25 μ M concentrations of sorafenib and 200, 100, 50 and 25 μ M concentrations of lapatinib were administered alone and in combination. Results were evaluated as absorbance at 450nm and IC₅₀ values are calculated according to the absorbance data. **Results:** Both sorafenib and lapatinib showed concentration dependent cytotoxic effects on MCF-7 cells. Sorafenib exerted cytotoxic effects with an IC₅₀ value of 32.0 μ M; in contrast with lapatinib the IC₅₀ was 136.6 μ M. When sorafenib and lapatinib combined, lapatinib increased cytotoxic effects of sorafenib at its ineffective concentrations. Also at the concentrations where both drugs had cytotoxic effects, combination show strong anticancer effects and killed approximately 70 percent of breast cancer cells. **Conclusions:** Combinations of tyrosine kinase inhibitors and cytotoxic agents or molecular targeted therapy has been successful for many types of cancer. The present study shows that both sorafenib and lapatinib alone are effective in the treatment of breast cancer. Also a combination of these two agents may be a promising therapeutic option in treatment of breast cancer.

Keywords: Tyrosine kinase inhibitor - sorafenib - lapatinib - breast cancer

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Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women. Biologic markers, such as hormone receptors including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) overexpression, tumor size, histological grade and subgroup status, lymph node involvement have prognostic and/or predictive value and are important factors in selecting appropriate treatment (Izadi et al., 2012; Liu et al., 2012; Macfarlane et al., 2012; Mackey et al., 2012; Zubeda et al., 2013; Cabuk et al., 2014). Estradiol exerts its effects by direct binding ER. ER is a phosphoprotein and belongs to a nuclear receptor superfamily. ER positive breast cancer cells produce growth factors that may influence the proliferation and responsiveness of breast cancer (Stoica et al., 2003). ER functions as a ligand dependent transcription factor and promotes variety of genes. Many of these gene products directly promote breast cancer cell proliferation, survival and tumor progression (Arpino et al., 2008; Osborne and Schiff,

2005). Nuclear ER induces the expression of different HER and other growth factor receptor ligands which are able to bind and activate epidermal growth factor receptor (EGFR) (Saeki et al., 1991; Salomon et al., 1995). Primary breast cancer also have strong association between ER and tyrosine kinase pathways. Therefore, therapeutics targeting therapy is important for breast cancer (Tozlu et al., 2006; Boulay et al., 2008). Several experimental studies demonstrated the role of EGFR pathways and vascular endothelial growth factor (VEGF)-dependent angiogenesis in cancer pathogenesis and progression (Kerbel, 2008; Hynes and MacDonald, 2009). Combined targeting of EGFR and VEGF dependent signaling was proven to be successful strategy in preclinical models (Ciardiello et al., 2000; Sini et al., 2005).

Sorafenib is a multikinase inhibitor that blocks several targets including C-RAF, B-RAF, c-KIT, FLT-3, RET, VEGFR-2, VEGFR-3, and platelet-derived growth factor receptor (PDGFR) (Takimoto and Awada, 2008). Sorafenib approved for the treatment of advanced hepatocellular and renal cell carcinoma (Escudier et al., 2007; Llovet et al., 2008).

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Lapatinib is a selective and reversible tyrosine kinase inhibitor (TKI) of both EGFR and HER-2 and inhibits key downstream signaling pathways mediating cell proliferation and survival (Rusnak et al., 2001; Coombes et al., 2013). Lapatinib approved for metastatic breast cancer (Geyer et al., 2006).

Preclinical evidence demonstrated that sorafenib has a dose-dependent synergistic effect in combination with other TKIs (Martinelli et al., 2010).

The aim of this study was to evaluate the cytotoxic activity of sorafenib and lapatinib alone and in combination on MCF-7[ER positive (ER+), PR negative (PR-), HER2 negative (HER2-) breast cancer cell lines.

Materials and Methods

Cell Lines and Reagents Human cancer (MCF-7) lines were obtained from ATCC cell collection. The cells, which are adherent cell lines and grow as monolayers, were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin in 75 cm² polystyrene flasks (Corning Life Sciences, Tewksbury, MA) and maintained at 37°C in a humidified atmosphere with 5% CO₂. Growth and morphology were monitored and cells were passaged when they had reached 90% confluence. Cell-culture supplies were obtained from Life Technologies (Darmstadt Germany). All other chemicals, unless mentioned, were purchased from Sigma Chemical Co (St. Louis, MO).

XTT viability assay

After verifying cell viability using trypan blue dye exclusion test by Cellometer automatic cell counter (Nexcelom Inc., Lawrence, MA), cells were seeded at approximately 1×10⁴ cells/well in a final volume of 200 µl in 96-well flat-bottom microtiter plates with or without various concentrations of the Sorafenib and Lapatinib. Plates were incubated at 37°C in a 5% CO₂ incubator for 24, 48, and 72h. Media was not refreshed during this time. At the end of incubation, 100µl of XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) (Roche Applied Science, Mannheim, Germany) was added to each well, and plates were incubated at 37°C for another 4h. Absorbance was measured at 450nm against a reference wavelength at 650nm using a microplate reader (DTX 880 Multimode Reader, Beckman Coulter, Miami, FL). The mean of triplicate experiments for each dose was used to calculate the IC₅₀ values.

Statistical analysis

The results of the study were expressed as mean±SD and data was analyzed by using 1-way analysis of variance test followed by Dunnett's t-test for multiple comparisons. Values with p<0.05 were considered as significant.

Results

In order to determine cytotoxic effect of sorafenib and lapatinib, 50, 25, 12.5 and 6.25µM concentrations

of sorafenib and 200, 100, 50 and 25µM concentrations of lapatinib administrated alone and in combination. Sorafenib caused concentration dependent cytotoxic effect of MCF7 breast cancer cells (p<0.05) (Figure 1). While 50 and 25µM concentrations of sorafenib caused statistically significant cytotoxicity, there was no cytotoxic effect of 12.5 and 6.25µM concentrations of sorafenib (Figure 1). IC₅₀ value of sorafenib was 32.02µM (Figure 3A). Similarly lapatinib also caused concentration dependent cytotoxic effect of MCF7 breast cancer cells (p<0.05) (Figure 2). While 200 and 100µM concentrations of lapatinib caused statistically significant cytotoxicity, there was no cytotoxic effect of 50 and 25µM concentrations of lapatinib (Figure 2). IC₅₀ value of lapatinib was 136.64µM (Figure 3B).

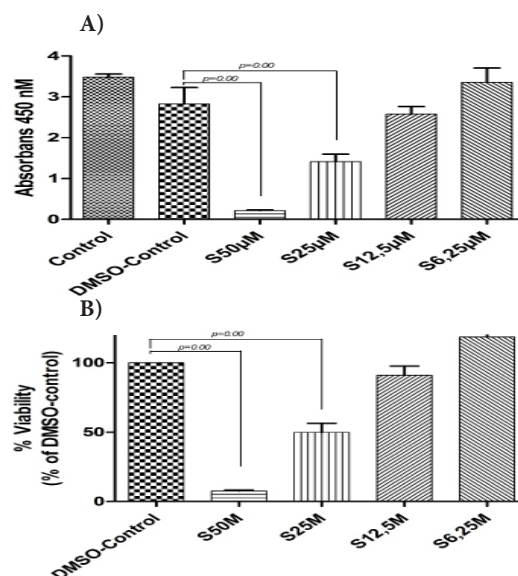


Figure 1. Cytotoxic Effect of Sorafenib in 50, 25, 12.5 and 6.25µM Concentrations on MCF-7 Breast Cancer Cells. A) Data express as absorbance values; B) Data express as percentage of DMSO control

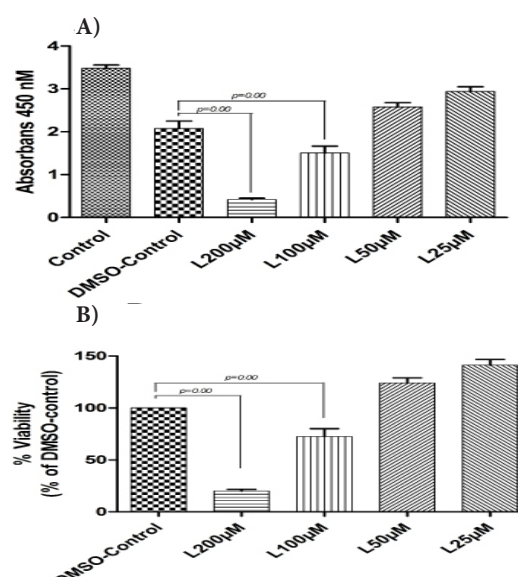


Figure 2. Cytotoxic effect of Lapatinib in 200, 100, 50 and 25µM Concentrations on MCF-7 Breast Cancer Cells. A) Data Express as absorbance values; B) Data express as percentage of DMSO control

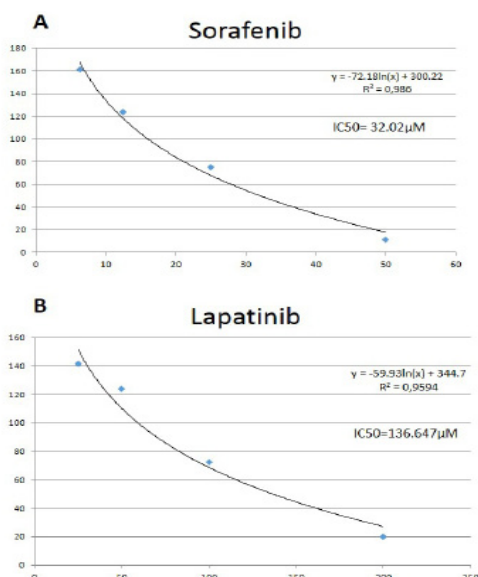


Figure 3. Calculation IC_{50} Values of A) Sorafenib and Lapatinib B)

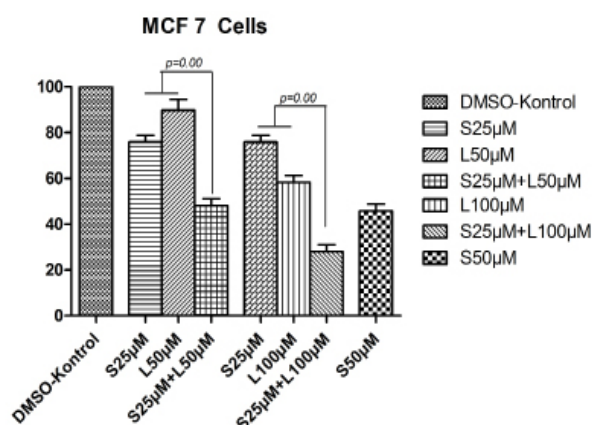


Figure 4. Cytotoxic Effect of Sorafenib and Lapatinib Alone and in Combination on MCF-7 Breast Cancer Cells

In order to investigate interaction between sorafenib and lapatinib, these two drugs have been administered in combinations of different concentrations. When partially effective concentration of sorafenib (S 25 μM), which killed 24.16% of MCF-7 cells, combined with ineffective concentration of lapatinib (L 50 μM), it has been observed that cytotoxic effect of combination was statistically significantly higher when compared to sorafenib (S 25 μM) alone. Combination of sorafenib and lapatinib (S25 μM +L50 μM) killed %51.88 of MCF-7 cells. When partially effective concentration of sorafenib (S 25 μM) combined with also partially effective concentration of lapatinib (L 100 μM) which killed %41.74 of MCF cells, it has been observed that cytotoxic effect of combination was statistically significantly higher when compared to both sorafenib and lapatinib alone ($p < 0.05$) (Figure 4).

Discussion

Angiogenesis is required for tumor growth, invasion and metastasis in several malignancies, including breast

cancer. The VEGF and EGFR are key mediators of angiogenesis and have been shown to be a valid target for targeted therapy in several tumors (Brady-West and McGrowder, 2011). EGFR is a potent stimulating factor of cell-growth-activating pathways and cross talk between ER and growth factor receptor has been shown (Bonelli et al., 2010; Brady-West and McGrowder, 2011). ER can directly or indirectly activate EGFR (Lee et al., 2000). Besides, several number of studies have shown the linking triple negative breast cancer (TNBC) to EGFR expression, with percentages ranging from 42 to 71% (Cheang et al., 2008; Collins et al., 2009; Meche et al., 2009). And also activation of the EGFR and the VEGFR pathways play a key role in the development, progression, metastasis of many type of cancers. Moreover, some of patients benefit from treatments with drugs targeting the EGFR or the VEGFR pathways (Llovet et al., 2008; Chan et al., 2010; Brady-West and McGrowder, 2011). Thus, EGFR and VEGFR pathways are a promising therapeutic target for many cancer types (Kong et al., 2008). Viale et al. (2009) reported that disease-free survival (DFS) overall survival (OS) rates worsened in patients with TNBC having EGFR expression when compared to those with tumors without EGFR expression (Viale et al., 2009). Simonelli et al. (2013) showed that combination of TKIs are feasible, a suitable strategy in the treatment of cancer (Simonelli et al., 2013).

Preclinical studies with breast cancer cell lines suggest ER-positive breast cancer inhibited by tamoxifen or by hormone deprivation inhibiting with EGFR and HER2 signaling pathways (Mayer and Arteaga, 2010). Moreover, lapatinib restores ER status. (Rusnak et al., 2001).

Some reports showed that EGFR inhibition in ER-positive breast cancer could response the treatment (Finn et al., 2009). Recently, some reports have suggested lapatinib may have an important effect on proliferative process in ER-positive breast cancer cells. It can inhibit proliferation via inhibition of cross-talk. And also it showed that the addition with or without chemotherapy increased progression free survival (PFS) in the HER2-subgroup (Young et al., 1999; Coombes et al., 2013). Johnston et al. (2009) in their study, the patients with postmenopausal ER-positive metastatic breast cancer with any level of HER2 was reported were treated with letrozole+lapatinib. In that trial, addition of lapatinib to letrozole resulted in an increase in PFS (3.1 months vs 8.3 months) in patients with HER2-cancers (Johnston et al., 2009).

In some breast cancer and in MCF7/HER2-18 cells, tamoxifen resistance may arise through altered effects of on ER activated transcription. Shou et al. (2004) reported that pretreatment with gefitinib, pure Erb B1 inhibitor, restored ER (Shou et al., 2004).

Molecularly targeted therapy has been successful according to single agent. Dasatinib enhanced the effect of cytotoxic agents and molecularly targeted agents (Park et al., 2012). It was shown that lapatinib might inhibit the function of ATP-binding cassette (ABC) transporters by binding to their ATP-binding sites. Lapatinib significantly potentiated the effects by inhibiting ABC (Dai et al., 2008). Dual EGFR and HER-2 directed treatment model

had enhanced the cytotoxic effects in glioblastoma T98G cells, colorectal carcinoma HCT8 cells and MDA-MB-231 cells (Erlichman et al., 2001). Polli et al. (2008) showed that low concentrations of lapatinib was able to show its effect (Polli et al., 2008).

Some cancers such as renal and hepatocellular carcinoma benefit from the treatment with drugs targeting the VEGFR pathways. The multikinase inhibitor sorafenib suppresses the angiogenesis and promotes autophagy in tumor cells. Bareford et al. (2011) displayed that sorafenib and cytotoxic agents act synergistically to enhance tumor killing on MCF 7 cells. They suggest that combination therapy may be a future therapeutic option in the treatment of solid tumors (Bareford et al., 2011).

Cross-talk between the EGFR-dependent autocrine pathway and of VEGFR-dependent signaling in cancer cells has been shown by the study of Martinelli et al. (2010). Moreover, they evaluated the *in vitro* and *in vivo* antitumor activity of anti-EGFR drugs, such as erlotinib, cetuximab and sorafenib. Martinelli et al. (2010) displayed the anti-proliferative effects of sorafenib on non-small cell lung cancer and colorectal cells (Martinelli et al., 2010).

Simonelli et al. (2013) used sorafenib and lapatinib combination in 30 patients with refractory solid tumors and reported that combination of sorafenib and lapatinib achieved in stabilization of disease. The disease control rate overall was 63%. This study has showed that sorafenib and lapatinib combination is a feasible approach in solid organ tumors (Simonelli et al., 2013).

In the present study, in order to determine whether sorafenib and lapatinib have cytotoxic effect on breast cancer cells and we used the different concentration of sorafenib and lapatinib on MCF-7 breast cancer cells. Sorafenib showed concentration-dependent cytotoxic effect on MCF-7 cells and IC_{50} value of sorafenib was $32.02\mu M$. Especially, $50\mu M$ concentration of sorafenib was highly effective. Cytotoxic effect of lapatinib was seen at $200\mu M$ and $100\mu M$ concentrations and IC_{50} of lapatinib was $136.64\mu M$. We showed that combination of ineffective concentration of sorafenib ($25\mu M$) and effective concentration of lapatinib ($100\mu M$) caused significantly higher cytotoxic effect than lapatinib ($100\mu M$) alone. The combination of ineffective concentration of sorafenib ($25\mu M$) and lapatinib ($50\mu M$) caused cytotoxicity as much effective as $50\mu M$ of sorafenib, also showed significantly higher cytotoxic effect than $25\mu M$ of sorafenib.

In conclusion, the combination of TKIs and cytotoxic agents or molecularly targeted therapy has been successful many types of cancer. It has been under investigation in last decade and one of the hot topics in cancer research. Combination therapies are not only important because of the increase in affectivity but also because of decreasing the side effects of cancer treatments which is one of the most important limitation of some of the valuable drugs. The present study shows that both sorafenib and lapatinib alone are effective in the treatment of breast cancer. Also combination of these two agents may be one the promising therapeutic options in treatment of breast cancer.

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