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LAPATINIB INDUCED-CHANGES IN CACO-2 INTESTINAL MONOLAYER VIA ERBB1 INHIBITION

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ABSTRACT

Lapatinib (LAP) is an orally administered dual ErbB1 and ErbB2 tyrosine kinase inhibitor for ErbB2-positive breast tumours, but is associated with diarrhoea. Incidence of lapatinib-induced diarrhoea (LID) is as high as 58-78% in treated patients. Although short-term diarrhoea is tolerable, prolonged diarrhoea can cause hospitalisation and interfere with cancer treatment, thus compromising the quality of life of patients with cancer. ErbB1 is widely expressed in the intestine which functions to maintain homeostasis. Therefore, it is hypothesised that LAP inhibits ErbB1 normal physiological in the intestine, leading to diarrhoea. This study aimed to investigate possible changes in Caco-2 intestinal monolayer following LAP treatment due to ErbB1 inhibition. Several parameters such as intestinal permeability changes, alteration of tight junctions and intestinal inflammation due to ErbB1 inhibition were studied using Caco-2 cells. Caco-2 is a colon cancer cell line, but it can differentiate into enterocytes and mimic normal intestinal cultures. Prior to Caco-2 differentiation, cytotoxic effect of LAP and LAP+recombinant epidermal growth factor (LAP+rEGF) on Caco-2 were evaluated at 24, 48, 72 and 96 hours using WST-1 assay. Caco-2 cells were seeded in a transwell insert for 21 days to form an intestinal epithelial monolayer prior to treatment. Monolayer integrity and permeability were assessed via transepithelial electrical resistance (TEER) and Lucifer yellow (LY) assay. Expression of tight junction proteins (TJPs) claudin-1, occludin and ZO-1 and inflammatory cytokines TNF-α, IL-1β and IL-6 were analysed using qPCR and immunofluorescence staining. In WST-1 assay, LAP exhibited different IC_{50} values at different time points. No IC_{50} value was observed after 24 hours. Recorded IC_{50} values were 28μM±7.39, 29μM±1.45 and 14μM±0.95 at 48, 72 and 96 hours while the median IC_{50} over 48 to 96 hours was 28μM±2.51. This concentration was used to treat Caco-2 cells in combination with rEGF. rEGF promoted 35.5% cell proliferation in LAP-treated cells at 325nM±5.18. Caco-2 cells grown on a transwell insert showed optimum TEER reading on day-19 at 810.74±243.16 Ωcm^{-2}. LAP-treated Caco-2 monolayer showed significantly lower TEER at 96 hours ($p<0.05$) while LAP+rEGF increased TEER reading at 24 hours when compared to control untreated monolayer ($p<0.05$). Increased LY permeability was observed but was not statistically different from the control. Meanwhile, LAP+rEGF reduced LY permeability, but the difference was not statistically significant. LAP significantly suppressed TJPs mRNA expression (*claudin-1*: $p<0.05$, *occludin*: $p<0.05$ and *ZO-1*: $p<0.001$) and altered their respective protein expression, except for ZO-1. rEGF counteracted LAP inhibition (claudin-1: $p<0.05$; occludin: $p<0.05$; ZO-1: $p<0.001$) at the transcriptional level. ZO-1 protein level, however, was not significant in any group. LAP increased *IL-6* expression ($p<0.01$). Interestingly, rEGF significantly increased *IL-6* expression ($p<0.001$) compared to other groups. *TNF-α* expression was also increased in LAP+rEGF group, but the difference was not significant. LAP significantly inhibited *ErbB1* expression ($p<0.01$) compared to LAP+rEGF group. *ErbB2* expression decreased in the LAP group, with no significant differences. Meanwhile, LAP+rEGF significantly upregulated *ErbB1* expression ($p<0.01$) compared to control. *ErbB2* and *ErbB3* levels were increased in LAP+rEGF group but were not statistically significant. Protein analyses showed ErbB1 protein was significantly higher in LAP+rEGF group than in the LAP group; however, Erbb2 protein showed no significant intensity any in of the groups. In conclusion, the mechanism involved in LID is through ErbB1 inhibition, which leads to increased monolayer permeability and altered tight junctions.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Molecular targeted therapy is one of the recent advances in molecular biology, especially in cancer treatment. Molecular targeted therapy refers to targeted molecules such as macromolecules (monoclonal antibodies (mAbs), polypeptides, antibody-drug conjugates, nucleic acid) (Zhong et al., 2021), and small molecule tyrosine kinase inhibitors (SM-TKIs). Specifically, mAbs modulate the immune system of tumour cells by targeting T-cell receptors (Scott et al., 2012), whereas SM-TKIs and non-TKIs regulate the function of target proteins by interacting with surface receptors as well as receptors inside the cell (Pathak et al., 2018). Examples of mAbs are trastuzumab, bevacizumab and cetuximab (Coulson et al., 2014), whereas SM-TKIs include lapatinib, erlotinib and gefitinib (Pathak et al., 2018). Although chemotherapy is an important cancer treatment, its success is severely limited due to lack of selectivity, which results in insufficient tumour cell elimination and systemic toxicity. Molecular targeted therapy is gaining popularity because of its specificity to cancer cells, while sparing normal cells. Hence, molecular targeted therapy has become a successful alternative treatment for patients who cannot receive suitable conventional chemotherapy.

Yet, the popularity of these medications especially SM-TKIs, are notorious for causing adverse effects due to their non-targeted mechanisms of action, which have a detrimental influence on patients' quality of life (QOL). Skin toxicity (Brian et al., 2018; Klastersky, 2014), cardiotoxicity (Chaar et al., 2018; Orphanos et al., 2009) and gastrointestinal (GI) toxicity (Bowen et al., 2014; Klastersky, 2014; Demirci et al., 2012) are several undesirable adverse effects and GI toxicity appears to be one of the most common toxicities associated with SM-TKIs (Collins et al., 2019; Bowen et al., 2014).

Diarrhoea induced by SM-TKIs is a frequent, debilitating and dose-limiting toxicity that affects patients with cancer and must be carefully differentiated from other causes of diarrhoea. Tyrosine kinase inhibitors (TKIs) that are directed against the