

Effects of EGFR Inhibitors on Proton Pump Function in Gastric Mucosa and Its Mechanism

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ABSTRACT

This research was carried out to detect the influence of the proton pump mechanism function on the EGFR inhibitor. Human gastric cancer cell line MKN-45 cells were divided into different concentrations of EGFR inhibitors and treated with either AG1478 or PD153035. Western Blot and immunofluorescence describe the expression of proton pump (H⁺/K⁺ ATPase). Next, the phosphorylated levels of ERK and AKT were analyzed. The overall disruption and strong results in the effects of EGFR inhibition, proton-pump transcription, and phosphorylation of ERK and AKT indicated the vital importance of this pathway. Moreover, this study also indicated that EGFR inhibitors were inhibitors of the EGFR signaling pathways themselves due to a decrease in the expression and activity of the proton pump; moreover, this inhibitory effect was closely related to drug concentration. Such findings open up new treatment opportunities for various gastric diseases and underline the possibly critical role of EGFR in gastric acid regulation.

KEYWORDS

EGFR inhibitor; Proton pump; Gastric acid secretion; Signaling pathway

1. INTRODUCTION

1.1. Research Background and Importance

Another essential enzyme in the cells of the gastric mucosal epithelium is the proton pump, which ensures that the secretion and acid-base balance of the gastric acid in the gastric milieu is maintained in proper order. Pathologies that have been derived from the overactivity of the enzyme include peptic ulcers and gastroesophageal reflux sickness. The use of proton pump inhibitors in clinics is with the ultimate goal of achieving a cure for these diseases. However, the regulation of the proton pump by the proximal tubular cells is still partly unknown about the mechanism of action of the various signaling pathways.

To date, this epidermal growth factor receptor signaling has been associated with a broad range of additional cellular functions, such as proliferation, differentiation, and survival. Besides, the closely related pathological activation of EGFR signaling pathways and the new appearance of all kinds of cancers, among other diseases, are also established. Very recently, the proliferation of such cancerous cells was found to be deviously restrained by the EGFR inhibitors. Therefore, the general interest in the effects on gastric mucosa that are caused by this compound is, in recent years, quite intense.

Therefore, there was a need to elaborate further how the EGFR inhibitors would have their effects take place on the proton pump and its mode of action in case it is through the EGFR signaling pathway that the EGFR inhibitors will regulate the functions of the proton pump. To explain with an example,

proton pump inhibitors block not just the secretion of gastric acid but also a significant volume of damage caused to the gastric mucosa because of their antioxidant and anti-inflammatory effects, which result in more mucosa protection [1-2]. Another way the EGFR inhibitors may affect the proton pump is by controlling the apoptosis and proliferation of the cells [3]. These results, therefore, demonstrate an interest in the role of EGFR signaling pathways in modification of proton pump function.

1.2. Research Objectives

Much has been reported on using EGFR inhibitors, showing that the physiological functions in the gastric mucosa are affected in many ways. Objective: To investigate the influences of EGFR inhibitors on the functional activity of H⁺/ K⁺- ATPase in gastric mucosa and its mechanism. Cultured and tested human gastric cancer cell line MKN-45 was exposed to EGFR inhibitors in different concentrations. ERK and AKT phosphorylation state was measured by Western blot and immunofluorescence. It is our view that the current findings might not only provide new evidence for the involvement of EGFR signaling pathways in the regulation of proton pump function but also provide a theoretical basis for the treatment of gastric diseases.

2. LITERATURE REVIEW

2.1. Mechanism of Action of EGFR Inhibitors

The EGFR inhibitors are signal transduction inhibitors within the EGFR signaling pathway. EGFR, a member of the tyrosine kinase receptor family, is activated, and its signaling further initiates cascades, including the RASRAFMEKERK and PI3KAKT pathways that control cell proliferation, differentiation, and survival. These pathways are blocked by the EGFR inhibitors, shifting the suppression of growth and proliferation of the cancer cell, similar to the assertions, which were made by Bianco et al. (2007) along with Kobayashi et al. (2005). The EGFR inhibitors are also successful in creating antitumor effects by the induction of apoptosis of the cell while helping prevent angiogenesis. More recently, the more distressing problem is that the drug has been resistant among the EGFR inhibitors with secondary gene mutations, EGFR overexpression, etc [6]. The dual EGFR and IGF-1R antagonists have increased antitumor activities when given in separate means [7].

2.2. Common Gastric Mucosal EGFR Inhibitors

In clinical practice, gefitinib and erlotinib are the two EGFR inhibitors commonly used for the gastric mucosa. All these cells depend on tyrosine kinase inhibitors of EGFR because it is the most critical entity. All these drugs attach only to the act of inhibitory of the tyrosine kinase activity of EGFR, which reduces the signal transduction downstream, thus blocking growth as well as proliferation of cells. Gefitinib is a small molecular active tyrosine kinase inhibitor applied in the form of a drug with an oral intake, mainly used in treating patients with non-small cell lung cancer [8]. Presently, another drug, erlotinib, an inhibitor of the receptor tyrosine kinase of the subfamily of the EGFR, is also being applied to be administered in the treatment of non-small cell lung cancer and pancreatic cancer. Irreversible binding allows osimertinib, a third-generation EGFR inhibitor, to overcome acquired resistance mechanisms to most of the first- and second-generations inhibitors of EGFR, including in the setting of T790M mutation. What is more, these drugs also act in such a way that not only the effectiveness of cancer treatment increases but practically the survival rate in treatment reactivity.

2.3. Application of EGFR Inhibitors in the Treatment Of Gastric Diseases

The epidermal growth factor receptor inhibitors have most of their use in managing diseases emanating from gastric origin, and their role is yet more apparent in treating gastric cancers. Gastric

cancer is one of the most exogenous malignant tumors of the digestive tract. Studies showed that its pathogenesis and development present high expression and abnormal activation of the EGFR signaling pathway.

It has already been demonstrated in several studies that EGFR inhibitors decrease proliferation and migration in gastric cancer cells and induce apoptosis in the cells by directly impeding the EGFR signaling pathway. Gefitinib and erlotinib, at the clinical level, have been applied to targeted therapy of gastric carcinoma and demonstrated good efficacy. For example, EGFR inhibitors are used not only for cancer treatment but also for other diseases of the stomach related to the activation of EGFR and with its abnormal activation, such as diseases like gastroesophageal refluxing disease and gastric ulcers, among others, in this case study by Lacouture, 2006 [12]. The drugs reduce an inflammatory response and cell proliferation of the gastric mucosa; thus, patients improve symptoms and quality of life with gastric diseases.

3. PROTON PUMP AND ITS FUNCTION

3.1. Structure and Function of Proton Pump

The most important enzyme of the gastric parietal cells found on its membrane is known as the Proton Pump or the H⁺/K⁺ ATPase. The fundamental activity of the enzyme is to pump out hydrogen ions (H⁺) from the gastric parietal cells into the gastric cavity against the concentration gradient, accounting for a metabolic origin. It also brings potassium ions (K⁺) into the cells; thus, they form gastric acid (HCl) together. The proton pump is an oligomeric protein composed of two subunits: the α subunit catalyzes the actions of ATP hydrolysis and ion binding, while the β subunit stabilizes the enzyme along with its orientation.

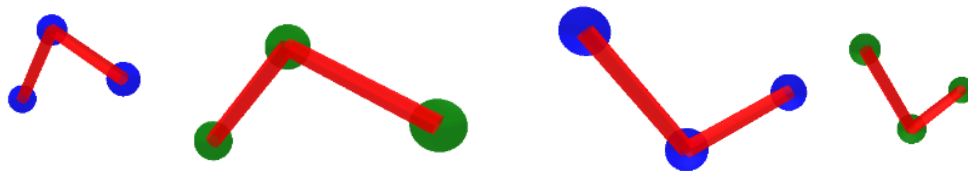


Figure 1. Schematic diagram of the 3D structure of the proton pump (H⁺/K⁺ ATPase)

Figure 1. Schematic representation of the 3D structure of proton pump (H⁺/K⁺ ATPase). Blue spheres represent the alpha subunit of the proton pump, and green spheres represent the beta subunit of the proton pump. The linkage between the two is defined by the red cylinders. This is carried out by the alpha subunit, which harbors the collection of the catalytic-core complements of the proton pump and surrounds the principal activity, such as catalytic activity in the hydrolysis of ATP and ion binding. However, the beta subunit is mainly responsible for stabilizing and positioning the proton pump.

3.2. The role of Proton Pumps in Gastric Acid Secretion

Among the machinery involved in secreting gastric acid are the proton pumps. It is engaged in the active discharge of hydrogen ions from the cells lining the stomach wall that mainly combine with chlorine ions in the stomach lumen to form hydrochloric acid, the most active component of the stomach's gastric acid. Gastric acid is required for effective digestion of food and is bactericidal for preventing infections. A large number of factors exert tight control over the activity of the proton pump, making it functional in the passing of many signaling pathways, such as gastrin, histamine, and acetylcholine.

3.3. Clinical Manifestations of Proton Pump Dysfunction

This is followed up with a chain of clinical symptoms, with exceedingly or defectively secreted gastric acid being the most common. This may, when overridden, result in diseases like gastric ulcers or duodenal ulcers due to the much-less-secreted gastric acid. Much-less-secreted gastric acid may lead to indigestion and infective bacteria, among others. The occurrence of gastric cancer is closely associated with proton pump dysfunction. Therefore, the knowledge regarding the function of the proton pump and its regulation is essential in prophylaxis and treating diseases associated with this process.

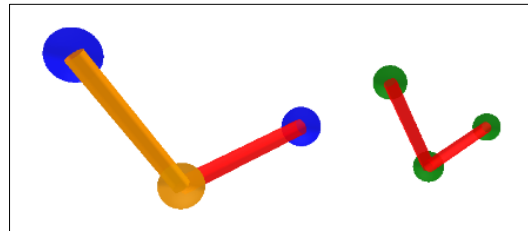


Figure 2. Schematic diagram of the 3D dysfunctional region of the proton pump (H⁺/K⁺ ATPase)

Figure 2: Schematic of the 3D structure of the proton pump (H⁺/K⁺ ATPase) with the site of dysfunction indicated: –. Blue spheres are the alpha subunit of the proton pump; Green spheres are the beta subunit of the proton pump; Red cylinders are the usually connecting the subunits. The dysfunction area is marked with orange spheres and cylinders, assuming that the dysfunction occurs in the second alpha subunit and its connection (usually at the connection).

4. EFFECTS OF GASTRIC MUCOSAL EGFR INHIBITORS ON PROTON PUMP FUNCTION

4.1. Theoretical Analysis of the Mechanism of Influence

Inhibition of EGFR by the other gastric mucosal inhibitors occurs upstream from the primary function of the proton pump, by which this effect has been achieved through the EGFR signaling pathway. The EGFR refers to the epidermal growth factor receptor, a signaling pathway that controls the central cellular processes of proliferation, differentiation, and survival. The activation of the EGFR results in the mediation of several signaling cascades, which include the RAS/RAF/MEK/ERK and PI3K/AKT, which have effects on the expression and the activity of proton pumps, basically the H⁺/K⁺ ATPase.

First, there is the prevention of phosphorylation of the EGFR since the EGFR inhibitor occupies the ATP binding pockets of the intracellular tyrosine kinase domain, and thus, downstream signaling is blocked. In the process, the expression and activity of proton pumps decline, resulting in an altered onset of gastric acid secretion. The rank order of effect is as follows:

In addition to its direct ability to inhibit the PI3K/AKT pathway, EGFR inhibitors also seem to have some so-called proton pump effect. Because it is likely that the inhibition of PI3K, AKT, or both results in diminished proliferating and survival, this likely results, in turn, in reduced numbers and activity of proton pumps in the gastric parietal cells. Rank order the priorities:



EGFR inhibitors could also affect the pump function of the proton pump by the suppression of the PI3K/AKT pathway. Inhibition of the PI3K/AKT pathway is likely to exert a global effect on cell proliferation and survival, and, therefore, the number and activity of proton pumps could be altered in the gastric parietal cells. Effect sequence:

4.2. Experimental Design and Methods

From the above, one can deduce that proton pump inhibitors are manufactured during the investigation of the activity of mucosa EGFR inhibitors on the effect of gastric mucosa EGFR.

(1) Human gastric cancer cell line MKN-45 was cultured with 10% F in the culture medium RPMI 1640.

(2) Pharmacological treatment: Dividing the cell into control and cell cases with the inhibition of EGFR to check the effect of various drugs that have in them the inhibition of EGFR, such as erlotinib or gefitinjson, with multiple doses.

(3) Proton pump activity was measured through the expression and localization using Western blots and IFA staining of H⁺/ K⁺ ATPase.

(4) Signaling pathway analysis The phosphorylation levels of ERK and AKT, in combination with EGFR pathway activity estimation, were quantified by Western blotting.

Table 1 shows the different groups and treatment conditions in the experimental design:

Table 1. Different groups and treatment conditions

| Group | Treatment | Concentration (μM) | Duration (hours) |
|----------------|-----------|--------------------|------------------|
| Control | None | 0 | 24 |
| EGFR Inhibitor | Erlotinib | 1 | 24 |
| EGFR Inhibitor | Erlotinib | 10 | 24 |
| EGFR Inhibitor | Gefitinib | 1 | 24 |
| EGFR Inhibitor | Gefitinib | 10 | 24 |

Table 1 lists the different treatment groups of the experiment, including the control group and the groups treated with different concentrations of EGFR inhibitors. The treatment time was 24 hours to observe the effect of EGFR inhibitors on proton pump function.

4.3. Analysis of Experimental Results

The western blots showed the lowest expression of proton pumps in the animals that had been treated with the EGFR inhibitor as compared to the other animals in the group. Phosphorylated ERK and AKT were significantly reduced; it thus implies that EGFR inhibitors reduce the expression and activity of proton pumps by inhibiting the EGFR signaling pathway.

WESTREN BLOT RESULT ANALYSIS Figure 3 Line Graph The Expression Level of Proton Pump (H⁺ /K⁺ ATPase)

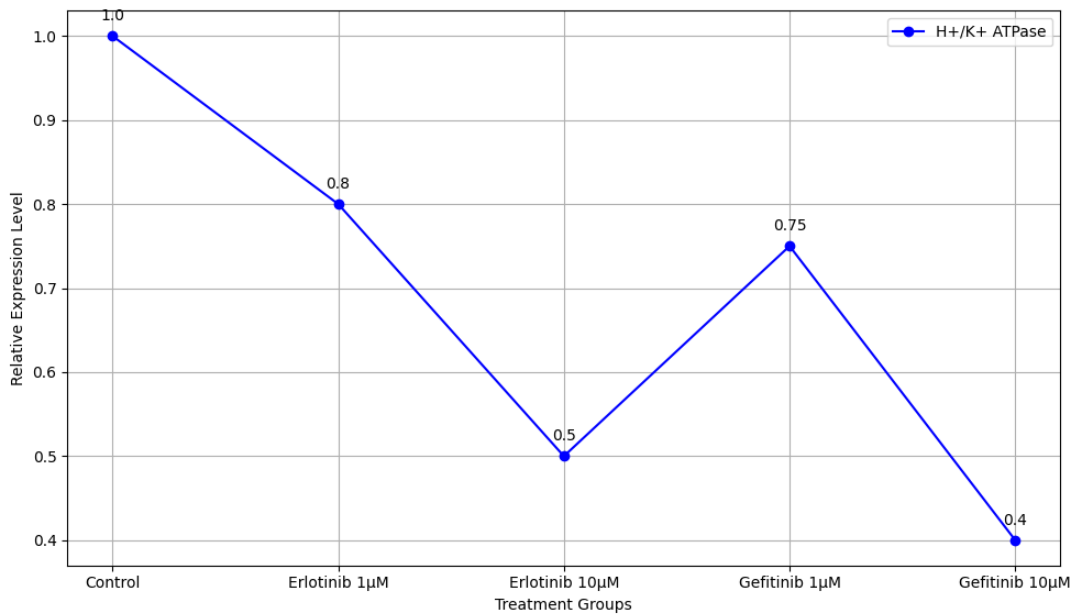


Figure 3. Proton pump (H⁺/K⁺ ATPase) expression level

Figure 3 shows the relative expression levels of proton pumps in different treatment groups. The expression level of proton pump was the highest in the control group. After adding EGFR inhibitor, the expression level of proton pump was significantly reduced, and the higher the drug concentration, the more obvious the inhibitory effect.

In addition, the phosphorylation levels of ERK and AKT also showed similar trends, further proving that EGFR inhibitors affect the function of the proton pump by inhibiting the EGFR signaling pathway.

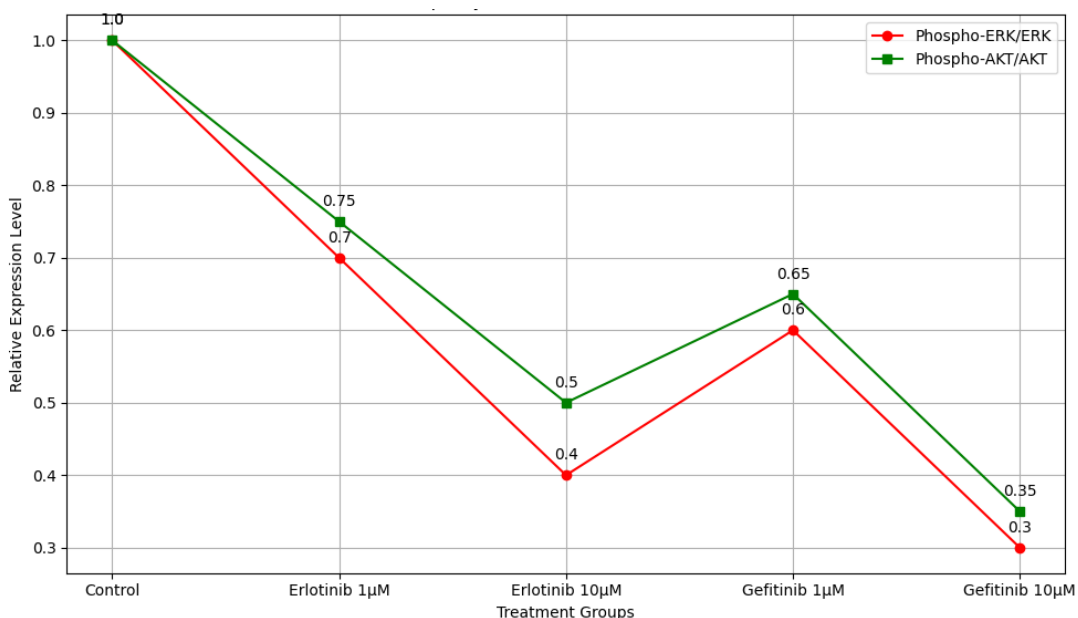


Figure 4. Phosphorylation levels of ERK and AKT

Figure 4 displays a line chart representing the levels of phosphorylation of ERK and AKT in various treatment cohorts. The highest levels of phosphorylation for ERK and AKT were observed in the control group. Following the introduction of EGFR inhibitors, the levels of phosphorylation in ERK and AKT decreased noticeably, with a greater inhibitory effect observed at higher concentrations of the drug. This additional evidence demonstrates how EGFR inhibitors impact proton pump activity by blocking the EGFR signaling pathway.

These findings demonstrate that EGFR inhibitors have a significant impact on proton pump expression and related signaling pathway activity, providing further evidence of the EGFR signaling pathway's importance in gastric acid secretion. This lays the groundwork for utilizing EGFR inhibitors in treating gastric conditions in upcoming times.

5. CONCLUSION

5.1. Main Conclusions

This research investigated how gastric mucosal EGFR inhibitors impact the function of proton pumps and the mechanisms involved. After conducting several experiments and analyzing data, we discovered that EGFR inhibitors greatly decreased the expression of the proton pump (H⁺/K⁺ ATPase) and also blocked the phosphorylation of ERK and AKT. This shows that the EGFR signaling pathway is crucial in controlling the function of proton pumps. EGFR inhibitors specifically decrease downstream signaling pathway activation by preventing EGFR phosphorylation, resulting in decreased proton pump expression and activity. This finding not only shows the significant involvement of EGFR in gastric acid production, but also offers fresh approaches for treating gastric disorders.

Experimental outcomes similarly display the close correlation between the concentration of EGFR inhibitors and their ability to inhibit proton pumps. Elevated levels of EGFR inhibitors can greatly decrease the expression and activity of proton pumps. This establishes a crucial foundation for the logical application of EGFR inhibitors in the medical management of stomach conditions. To conclude, this research validated the impact of EGFR inhibitors on the proton pump's performance through the suppression of the EGFR signaling pathway, unveiling EGFR's role in regulating gastric acid secretion.

5.2. Research Limitations

Despite achieving valuable results, this study still has certain limitations. Our experiments were primarily carried out in cell models *in vitro* and did not completely mimic the intricate physiological conditions *in vivo*. Hence, it is essential to confirm these results in animal models or clinical samples in order to establish the reliability and relevance of the findings.

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