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Review

REVIEW

Epidermal growth factor receptor inhibitor-induced diarrhea: clinical incidence, toxicological mechanism, and management

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Abstract

The epidermal growth factor receptor (EGFR) family is a class of receptor tyrosine kinase playing a central role in carcinogenesis and cancer progression. The members of this family, particularly EGFR and human epidermal growth factor receptor 2 (HER2), are the most extensively studied drug targets for malignancy. Today, numerous tyrosine kinase inhibitors targeting EGFR family have been developed to combat non-small-cell lung cancer and breast cancer. However, severe gastrointestinal (GI) toxicity leading to dose reduction and treatment discontinuation hampers the therapeutic outcome of EGFR inhibitors. Diarrhea is one of the most frequent GI side effects, especially when it comes to second-generation EGFR inhibitors. Enterocytes apoptosis and increased inflammation accompany with many oral EGFR inhibitors. Loperamide and budesonide are the first-line treatment to manage such adverse effects. However, current prophylaxis and management are all empirical interventions to relieve the symptom. They do not specifically target the toxicological mechanism of EGFR inhibitors. Hereby, those anti-diarrhea agents do not work well when used in cancer patients experiencing EGFR inhibitor-induced diarrhea. On the other hand, the toxicological mechanism of EGFR inhibitor-induced diarrhea is poorly understood. Thus, determining the mechanism behind such diarrhea is urgently in need for developing genuinely effective anti-diarrhea agents. This review aims to call attention to EGFR inhibitor-induced diarrhea, a highly occurring and devastating cancer drug toxicity.

Key words: epidermal growth factor receptor, tyrosine kinase inhibitors, gastrointestinal toxicity, diarrhea

Introduction

Epidermal growth factor receptor (EGFR) is fundamentally important in cell proliferation and differentiation and is one of the most extensively studied drug targets for many cancers. In 2004 and 2009, FDA approved first-generation EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, for patients having EGFR mutation-positive non-small-cell lung cancer (NSCLC). However, many responsive patients develop T790M-driven gatekeeper drug resistance after 12 months of treatment [1]. To address this resistance, second-generation EGFR TKIs were developed, like afatinib and dacomtinib, to treat NSCLC patients harboring T790M. Meanwhile, lapatinib and neratinib were approved for human epidermal growth factor receptor 2 (HER2)-positive breast cancer [2]. However, gastrointestinal (GI) toxicities are associated with second-generation EGFR TKIs [3]

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because of wide-spectrum inhibitory effect on both mutant- and wild-type EGFR. Currently, third-generation EGFR TKIs are emerging to selectively target T790M mutants while sparing wild-type EGFR. Nevertheless, a new mechanism of resistance toward third-generation TKIs was recently discovered, that is, the EGFR C797S mutation [1, 4]. Aiming at targeting C797S mutant, fourth-generation EGFR TKIs are now under development.

To date, many clinical trials have revealed that EGFR TKIs, especially second-generation inhibitors, cause remarkable GI toxicities such as diarrhea, vomiting, and nausea. In a pooled analysis, diarrhea occurred to over half of patients receiving EGFR TKIs-based treatment. Diarrhea happened to 51% of patients having lapatinib monotherapy and to 65% of patients having lapatinib plus capecitabine [5]. Cancer drug-induced diarrhea also causes considerable economic loss. Hogsett *et al*. [6] suggested that additional costs up to $25 000 (USD) per therapy cycle might happen. Such costs are due to increased risk of mucositis, prolonged hospital stays, and additional supportive care. Thus, the mechanism of EGFR TKIs-induced diarrhea needs to be better understood. Effective assessment, prevention, and management of such GI toxicity should be established.

In this review, we summarize the first three generations of EGFR TKIs. We focus on the differences in the mechanism of action between each generation and its relevance to GI toxicity. We review the primary toxicological mechanisms underlying EGFR TKIs-induced diarrhea. Finally, we discuss contemporary non-medical supportive care and medication for EGFR TKIs-induced diarrhea.

EGFR inhibitors causing diarrhea

A large portion of human carcinoma is caused by the excessive activation of human EGFR family. While staying inactive, all EGFR members remain as monomers. Once one member is activated by ligand, it will couple with another EGFR member to trigger downstream signaling. Without forming dimer with another EGFR member, single EGFR member cannot initiate downstream transduction (Fig. 1). Such dimerization stimulates several oncogenes, including mitogen-activated protein kinase (MAPK), phosphoinositol 3-kinase/protein kinase B (PI3K/PKB), and janus kinase/signal transducer and activator of transcription (JAK/STAT). Eventually, those events lead to increased cellular motility, proliferation, and invasion [7]. Based on this conceptual framework, EGFR family proteins are identified as the first and foremost target in treating many types of carcinoma from NSCLC, breast cancer, head and neck cancers, and pancreatic cancer to renal cancer. Up to date, three generations of EGFR inhibitors have been posted to market for malignant carcinoma (Fig. 2).

First-generation EGFR inhibitors: reversible inhibition

The most frequent mutations in EGFR encoding gene are L858R and Del19 (exon 19 deletions between amino acids 746 and 750), resulting in most of the EGFR-driven carcinogenesis [8]. Patients with these somatic mutants are treated with first-generation EGFR inhibitors, such as gefitinib and erlotinib. First-generation EGFR inhibitors usually have promising response rates for the first 11–14 months [9]. First-generation EGFR inhibitors display potency by blocking the adenosine triphosphate (ATP) binding site of EGFR kinases. Generally, the EGFR inhibitors of this generation are anilinoquinazoline derivatives, a class of ATP homologous [10]. This similarity allows them to compete for the ATP-binding domain of protein kinases to prevent the activation of EGFR downstream signaling, which ultimately suppresses cancer.

However, a big portion of patients develop resistance to such competitive EGFR inhibitors due to a secondary EGFR kinase domain mutation, T790M. T790M restores EGFR-dependent signaling [11]. The mechanism of T790M-driven resistance has not been fully illustrated but is believed to consist of three actions. T790 mutant exerts increased ATP binding affinity. Then, it generates steric clash between Met790 gatekeeper side chain and the aniline moiety of first-generation EGFR inhibitors, hindering the drugs from inserting into kinase back pocket. Besides, such mutation changes the conformational dynamics of EGFR catalytic domain [12, 13].

Second-generation EGFR inhibitors: irreversible inhibition and multiple targeting

The second-generation EGFR inhibitors, irreversible inhibitors, are developed to resolve the resistance to first-generation inhibitors. Irreversible inhibitors have advantages over reversible compounds since they achieve complete and sustained target engagement even with a high concentration of the endogenous ligand, ATP. It requires the physical turnover of targeted protein to restore suppressed signaling [14]. Indeed, irreversible inhibitors of EGFR family protein, like dacomitinib and afatinib, demonstrate increased potency against EGFR oncogenic variants such as EGFR L858R/T790M [13]. The main drawback of second-generation EGFR inhibitors is that they potently block wild-type EGFR too and cause epithelium-based toxicity such as diarrhea [15].

In addition to irreversible inhibition, another signature feature of second-generation EGFR inhibitor is multi-targeting, like lapatinib and neratinib. This class of compound blocks more than one EGFR family member, so it is named as pan-HER inhibitor. It is known that cross-activation between EGFR family members generates complementary cascades. This system is one of the main factors jeopardizing the clinical benefit of first-generation EGFR inhibitors and causing cancer recurring [16, 17]. The strength of multi-targeting is disrupting such crosstalk signaling, but it makes normal epithelium more vulnerable to toxicity as well. One example is that Pifzer suspended the development of canertinib (CI-1033), a pan-HER inhibitor, due to its uncontrollable GI toxicities in Phase I trial [18].

Third-generation EGFR inhibitors: targeting T790M mutant

The third-generation EGFR inhibitors selectively target T790M mutants to tackle T790M-driven resistance. Third-generation inhibitors bind covalently to Cys797 but spare wild-type EGFR. Increasing third-generation EGFR inhibitors have advanced into clinical trials or got approval, such as osimertinib (AZD9291), rociletinib (CO1686), olmutinib (HM61713), zazartinib (EGF816), and naquotinib (ASP8273) [19]. The Phase II trial of osmertinib in NSCLC patients having T790M showed that the incidence of diarrhea significantly decreased. The Grade 2 diarrhea in this trial is 5%, while Grade 3 diarrhea is less than 1%, which is impressively better than the second-generation inhibitors [20].

Clinical incidence of EGFR inhibitors-induced diarrhea

In cancer patients treated with EGFR inhibitors, diarrhea is the second most common adverse events, affecting up to 95% of

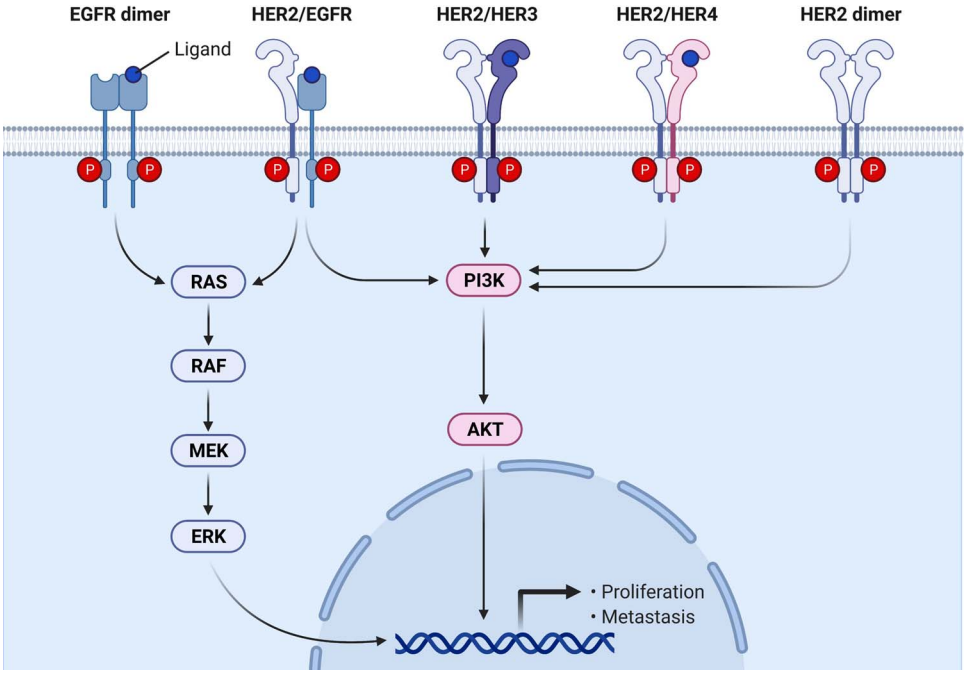

Figure 1: EGFR signaling in cancer proliferation.

them. The occurrence of diarrhea has been suggested to predict tumor response. Diarrhea of Grade 3 or higher occurs in around 30% of patients taking EGFR inhibitors. Diarrhea typically starts as early as 2–3 days after doing EGFR inhibitors. As for most EGFR inhibitors, the severity of diarrhea is dose dependent. Nevertheless, high risk of diarrhea compromises the therapeutic outcome of EGFR inhibitors (Table 1). The current supportive cares are all empirical practices. In the field of cancer supportive care, empirical intervention is distinct from target-oriented intervention. Loperamide and budesonide are used because they alleviate diarrhea in other scenarios, like inflammatory bowel disease. They are not specifically developed to target EGFR-induced diarrhea. Thus, their effectiveness is not always satisfactory when tested in cancer patients taking EFGR inhibitors.

Second-generation inhibitors are reported to cause dose-limiting diarrhea. In NSCLC patients treated with second-generation EGFR inhibitors, the incidence of diarrhea of all grades was 100%, while the incidence of Grade 3 was 23% [21]. In a comparative Phase III study, the incidence of diarrhea in afatinib arm was even higher than the arm of cisplatin plus pemetrexed. About 95.2% of the patients taking afatinib had diarrhea, and diarrhea of Grade 3 was 14%. Meanwhile, the occurrence of diarrhea was 15% in the arm of cisplatin plus pemetrexed and no Grade 3 diarrhea was recorded [3]. Same issue happens to pan-HER inhibitors used for breast cancer. ExteNET, the Phase III trial of neratinib in HER2-positive breast cancer, showed that 96% patients taking neratinib had diarrhea, while the incidence of Grade 3 diarrhea was 41% [22].

Toxicological mechanisms of EGFR inhibitors-induced diarrhea

Although diarrhea is a highly occurring side effect of EGFR inhibitors, the mechanism underlying such toxicity remains unclear. Also, the inter-individual variability in diarrhea incidence is high. It is uncertain whether the enterocytes or enzymes in the GI tract are sensitive to EGFR inhibitors. Typically, drug-associated diarrhea is attributed to three principle reasons: (i) excessive hypertonic substances in lumen, (ii) damage to transporters that control electrolytes flux across enterocyte membrane, and (iii) increased gut motility. Further studies to support the above three hypotheses are expected to be done. Generally, drug-induced diarrhea is driven by multiple mechanisms.

EGFR regulate epithelium regeneration and permeability

It is known that the inhibition of EGFR signaling results in reduced growth and barrier leakage in intestinal epithelium. This leads to mucosal atrophy-associated GI toxicity [23]. GI tract is one of the most rapidly proliferating organs in the body. Berlanga-Acosta *et al.* [24] proved that, in rodent model, EGF promoted enterocytes growth and altered crypt fission. Crypt fission is to maintain the absorption function of gut barrier. Inhibition of crypt fission will reduce the water absorption in GI tract, leading to diarrhea. In rats' colon, EGF is a potent stimulus for crypt fission [24]. Besides, it was found that multiple EGFR ligands were induced in *Drosophila* in response to damage in intestinal epithelium. Increase in those ligands activated the intestinal stem cells. Activation of EGFR signaling promoted epithelium regeneration in *Drosophila*, which eventually maintained intestine homeostasis. Jiang *et al.* [25] showed that EGFR-deficit intestinal stem cells could not support intestinal epithelium regeneration after bowel infection.

In addition to enterocyte proliferation, EGFR regulates tight junction and permeability in gut. Raimondi *et al.* demonstrated that adding cholic acid, deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) decreased transepithelial electrical resistance and increased dextran flux in Caco-2 cell monolayer. Co-incubation of CDCA or DCA with EGF abolished such effect. Those findings proved that EGFR signaling was essential to support the tight junction of Caco-2 monolayer [26]. Basuroy *et al.* [27] reported that EGF suppressed H_2O_2-induced gut leakage

Table 1: GI adverse effect associated with EGFR inhibitors

Drug	Regimen	Reviewed trial	Diarrhea		Stomatitis		Nausea		Vomiting		Ref.
			All grades	≥3	All grades	≥3	All grades	≥3	All grades	≥3	
Erlotinib (OSI-774)	Patients received erlotinib (150 mg/d) or placebo combined with up to six 21-day cycles of chemotherapy (gemcitabine 1250 mg/m^2 on Days 1 and 8 and cisplatin 80 mg/m^2 on Day 1)	Phase III	NA	6%	NA	NA	NA	6%	NA	7%	[62]
Gefitinib	Patients received paclitaxel 225 mg/m^2 and carboplatin area under concentration/time curve of 6 mg/min/ml (Day 1 every 3 weeks) plus gefitinib 500 mg/d or placebo	Phase III	69.3%	25.4%	NA	NA	18.7%	4.1%	12.9%	2.9%	[63]
Icotinib (BPI-2009H)	Patients were randomly assigned (1:1) to receive icotinib (125 mg, three times per day) until disease progression or unacceptable toxicity	Phase III	22%	0%	NA	NA	4%	<1%	5%	0%	[64]
Lapatinib (GW572016)	The starting dose of single agent lapatinib was 750 mg twice daily. Dose delays of up to 2 weeks and two dose reductions, first to 1500 mg once daily and second to 1250 mg, were allowed for toxicities	Phase III	<66%	<14%	NA	NA	27%	3%	24%	4%	[65]
Afatinib (BIBW2992)	Patients randomized to afatinib plus paclitaxel received 40 mg daily and 80 mg/m^2 weekly, respectively	Phase III	53.8%	12.1%	9.8%	1.5%	17.4%	1.5%	15.9%	2.3%	[66]
Neratinib	Oral neratinib 240 mg was administered once per day without breaks, and cycle duration was 28 days	Phase III	41%	26%	NA	NA	13%	5%	13%	5%	[22]
Sapitinib (AZD8931)	Patients received a single oral dose of AZD8931 on Day 1 (D1), followed by a 4-day observation period to allow sufficient time to determine single dose	Phase III	75%	25%	29%	NA	14%	NA	14%	NA	[67]
Dacomitinib	A trial of dacomitinib as initial systemic therapy	Phase III	93%	15%	40%	4%	26%	3%	15%	1%	[30]
Poziotinib	Oral administration	Phase II	92%	10%	59%	18%	8%	0%	5%	3%	[68]
JNJ-26483327 (BGB102)	Oral solution for doses p1200 mg, or capsules of 50, 100, or 300 mg for doses X1500 mg. Medication was taken in combination with food BID with 12-h intervals. A cycle was defined as 28 days of treatment	Phase I	63.2%	0%	NA	NA	68.4%	0%	52.6%	0%	[69]
Osimertinib (AZD9291)	Osimertinib was given 80 mg orally once daily	Phase III	<34%	<1%	11%	0%	NA	NA	NA	NA	[70]
Olmutinib (HM61713)	800 mg once daily	Phase III	59%	0%	NA	NA	39%	0%	NA	NA	[71]
Rociletinib (CO-1686)	1000 mg twice daily	Phase I/II	33%	NA	NA	NA	50%	NA	NA	NA	[72]
Ibrutinib	Patients received 28-day cycles of once-daily ibrutinib 420 mg together with rituximab (375 mg/m^2, intravenously, every week during Cycle 1, then once per cycle until Cycle 6)	Phase II	26%	NA	NA	NA	38%	NA	38%	NA	[73]

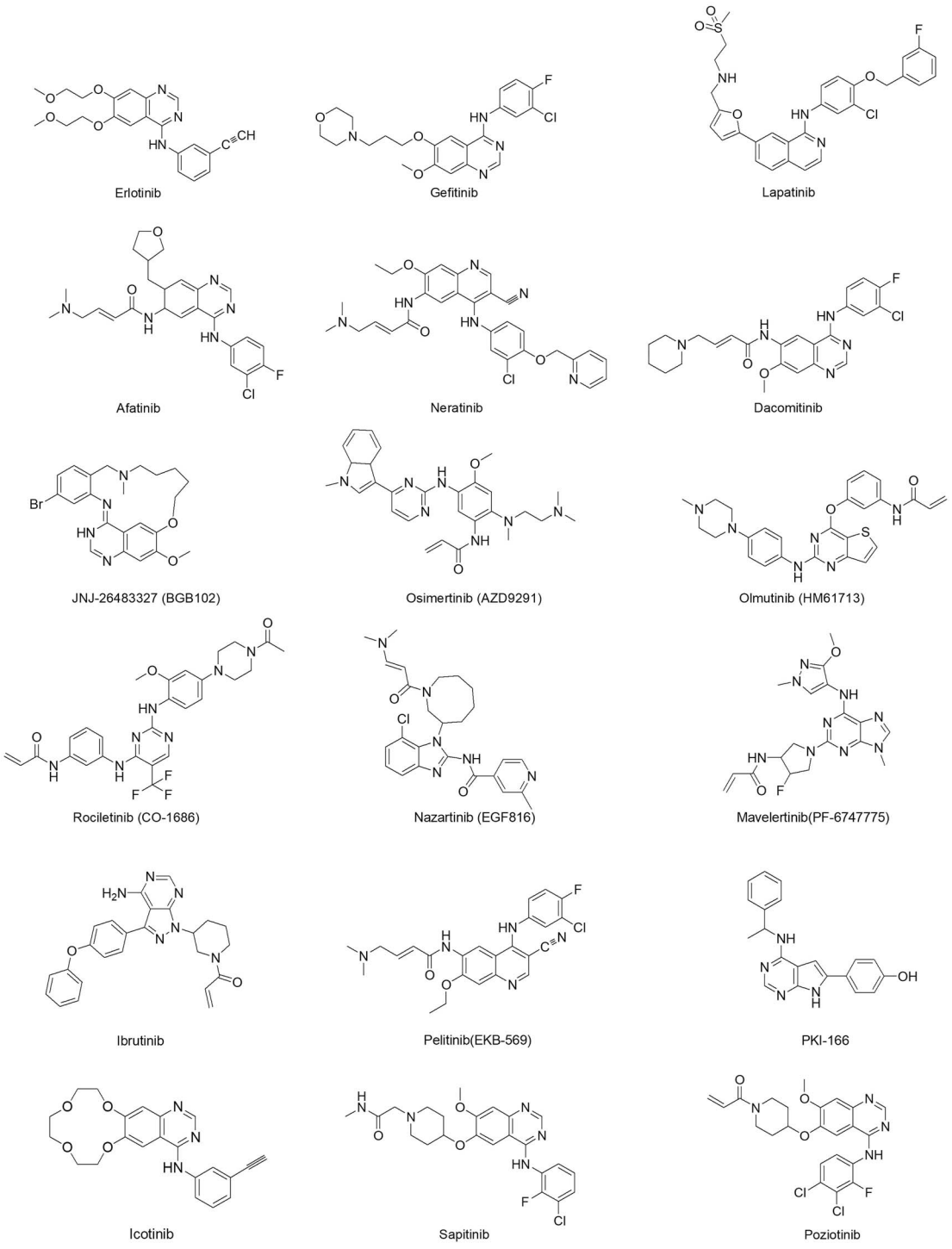

Figure 2: the EGFR TKIs causing severe diarrhea.

by blocking Tyr-phosphorylation, Thr-dephosphorylation and by redistributing zonula occludin-1 (ZO-1). In their study, EGF also protected epithelium from cytoskeleton disruption [27].

EGFR inhibitors induce apoptosis and cytokine secretion

Recently, Hong *et al.* reported that erlotinib caused barrier dysfunction in rat small intestine epithelial cells (IEC-6) by increasing permeability and by down-regulating E-cadherin. Erlotinib induced endoplasmic reticulum stress (ER stress) in both IEC-6 and human colon epithelial cells in a concentration-dependent manner [28]. Hong *et al.* showed that knockdown of C/EBP homologous protein protected IEC-6 cells from erlotinib-induced apoptosis and E-cadherin decrease [28]. Such findings implied that ER stress-mediated injury might contribute to erlotinib-induced diarrhea. Another parallel study indicated that gefitinib and icotinib arrested cell cycle at G_0/G_1 phase

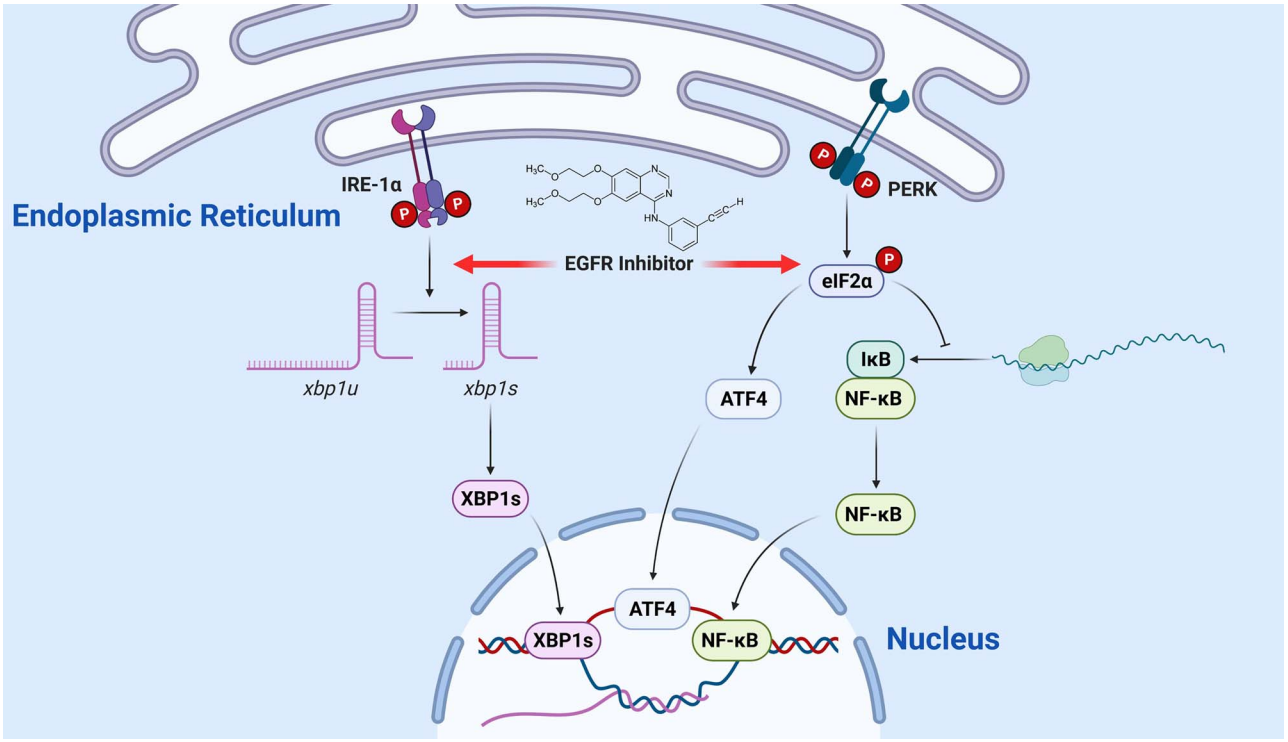

Figure 3: EGFR inhibitor causes inflammation in epithelium by triggering ER stress; following ER stress, PERK activates NF-κB via inhibiting IκB protein translation; this causes the release of NF-κB protein, which then carries out its role as transcription factor to promote inflammation; in addition, ER stress activates IRE-1α to initiate inflammatory response and apoptosis via splicing XBP1 mRNA; the spliced form of XBP1 (XBP1s) translocates into the nucleus and functions as transcription factor; EGFR inhibitors stimulate IRE-1α-mediated XBP1 slicing and PERK-mediated NF-κB activation; abbreviations: eIF2α, eukaryotic initiation factor 2α; IκB protein, inhibitor of kappa B protein; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IRE-1α, serine/threonine-protein kinase/IRE1α; XBP1, X-box binding protein 1.

by increasing cyclin D1 and p27 in IEC-6 cells [29]. In addition, gefitinib and icotinib reduced cell adhesion molecules while increasing IL-6 and IL-25. Those EGFR inhibitors all triggered ER stress response by activating protein kinase R-like ER kinase (PERK) pathway and by increasing serine/threonine-protein kinase/endoribonuclease inositol-requiring enzyme 1α (IRE1α)-mediated XBP1 splicing [28] (Fig. 3).

Lately, Van Sebille *et al.* [30] reported a case of dacomitinib GI toxicity. Based on their results, dacomitinib did not affect transepithelial electrical resistance or cell viability *in vitro* but caused diarrhea and weight loss *in vivo*. Dacomitinib left serious injury in distal ileum and elevated the level of monocyte chemoattractant protein 1 (MCP1) [30] in rats. In all, those studies demonstrated that EGFR inhibitors-associated diarrhea is owning to multiple mechanisms beyond inhibition of epithelium regeneration.

EGFR activation reduces chloride secretion

Today, the idea that EGFR inhibitors-associated diarrhea is due to excessive chloride secretion is drawing attention. The chloride in lumen builds up the osmotic gradient for water flowing into lumen. Chloride secretion is instrumental for keeping GI tract moist, but excessive secretion will lead to diarrhea. In intestinal epithelial cells, there are two key pathways governing chloride secretion: cAMP-dependent pathway stimulating delayed and prolonged response; and Ca^{2+}-dependent pathway eliciting rapid and transient response [31]. It is known that EGF inhibits Ca^{2+}-dependent chloride secretion via binding with the EGFR on basolateral membrane [32].

Barrett *et al.* [33] reported several EGFR agonists exerting inhibitory effect on Ca^{2+}-dependent chloride secretion and sodium absorption in intestinal epithelia cells. Another study found that both EGF and carbachol, ligands of M3 muscarinic receptor, reduced chloride secretion *in vitro* [34]. Follow-up study proved that the activation of M3 muscarinic receptor suppressed chloride secretion via an EGFR-dependent manner [35] (Fig. 4). Furthermore, Barrett *et al.* [36] demonstrated that activation of EGFR stimulated Ras–Raf–MEK–ERK as well as PI3K–AKT–mTOR pathways to decrease Ca^{2+}-dependent chloride secretion. According to those findings, EGFR inhibitors disrupt the inhibitory mechanism of EGFR toward chloride secretion. This results in excessive chloride resident in lumen and diarrhea. Latest studies by Duan *et al.* and Kim *et al.* [37, 38] verified this hypothesis, respectively.

Bowen *et al.* suggested that dacomitinib-induced diarrhea was mainly due to increased chloride secretion. Bowen *et al.* showed that crofelemer, a natural product used for diarrhea, suppressed dacomitinib-induced chloride secretion *in vitro*. However, when tested *ex vivo*, crofelemer did not inhibit dacomitinib-induced chloride secretion in ileum and colon tissues. Pharmacokinetics study revealed that crofelemer did not change dacomitinib bioavailability. Bowen *et al.* [39] conducted a large-scale animal experiment to investigate the mechanism of lapatinib-induced diarrhea. Their study showed that lapatinib caused diarrhea symptom but gave no histological damage in the gut. However, the serum level of chloride was decreased in lapatinib group. It suggested that lapatinib-induced diarrhea might be driven chloride loss in GI tract [40].

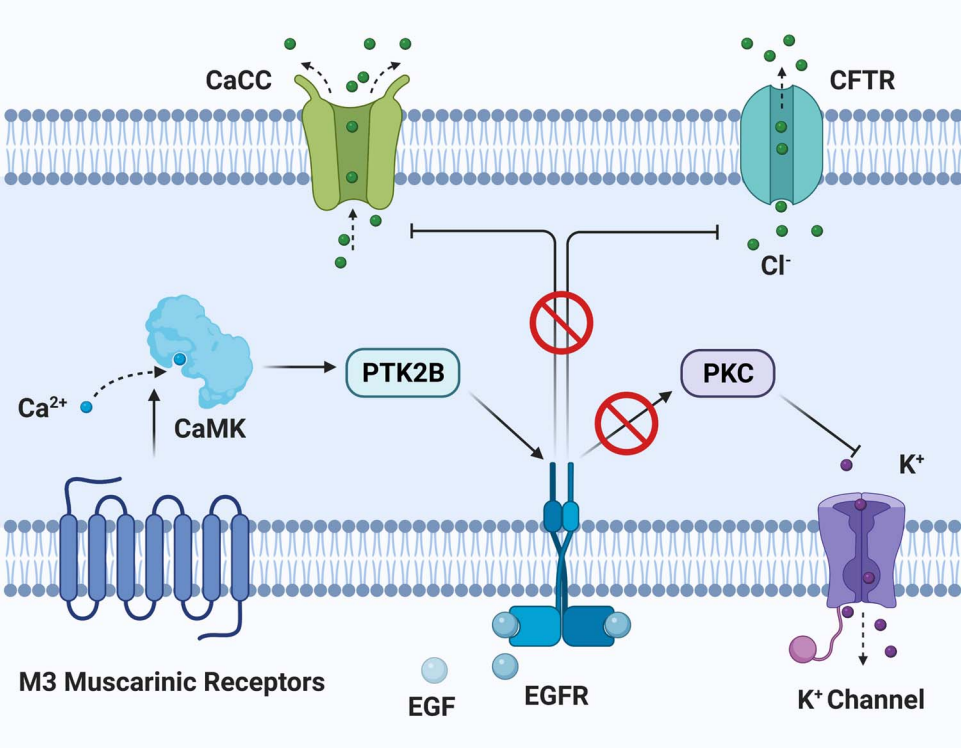

Figure 4: activation of EGFR suppresses Ca^{2+}-dependent chloride secretion in epithelial cells; activation of M3 muscarinic receptor transactivates EGFR and eventually targets CaCC on apical side; the whole signal transduction involves CaMK and PTK2B; direct activation of EGFR by EGF inhibits chloride secretion on lumen side and simultaneously stimulates potassium channel on basal side; EGFR inhibitors are shown to block the inhibitory pathway from EGFR to chloride channel; abbreviations: CaMK, Ca^{2+}/calmodulin-dependent protein kinase; PTK2B, protein tyrosine kinase 2 beta; CaCC, Ca^{2+}-activated chloride channel; CFTR, cystic fibrosis transmembrane conductance regulator; PKC, protein kinase C.

Saturation of drug transporters

When EGFR inhibitor and other therapeutic agents share the same efflux transporters or enzymes, there will be drug accumulation in the gut, leading to GI toxicity [41]. First-generation EGFR inhibitors, both gefitinib and erlotinib, show affinity with the ATP-binding cassette (ABC) transporter. *In vitro* experiments showed that gefitinib reversed ABCG2-mediated resistance at high concentration [42]. In clinical setting, patients carrying ABCG2 421C/A polymorphism had higher gefitinib exposure and more diarrhea episodes compared to those carrying wild-type ABCG2 [43]. In mice studies, the absence of ABCB1 and ABCG2 increased the oral bioavailability of erlotinib [44]. It is reported that ABCG2-15622C/T and 1143C/T polymorphisms increased the AUC and C_{max} of erlotinib pharmacokinetics profile [45].

Likewise, second-generation EGFR inhibitors, lapatinib and neratinib, exert an inhibitory effect upon ABC transporters. Lapatinib was reported to be both the substrate and inhibitor of ABC transporters. It reversed ABCB1- and ABCG2-driven resistance in cancer cells [46]. Perry *et al.* [47] reported that lapatinib increased SN-38 intracellular accumulation due to the inhibition of ABCG2. Zhao *et al.* found that neratinib reversed ABCB1-mediated resistance *in vitro* and *in vivo*. Besides, neratinib increased the accumulation of doxorubicin and rhodamine in ABCB1-overexpressing cell lines. It is also known that neratinib suppressed the ATPase activity of ABCB1 [48]. More studies in humans are expected to clarify the role of ABC transporters in the disposition, toxicity of EGFR inhibitors.

Current management

A series of studies have established the causality between EGFR inhibitors-induced diarrhea and excess chloride secretion. In fact, such diarrhea is facilitated by multiple factors from increase in gut motility, damage in epithelium to altered gut microbiome [49]. The complexity of toxicological mechanism is the obstacle for personalized management. Very few clinical studies have been conducted to explore molecular mechanism. Today, very limited options of prophylaxis and management are available in clinical setting. Current guidelines for anti-diarrhea management mainly aim to control dehydration [50], but those managements are not effective in many cancer patients.

Non-medical management

Non-medical supportive care is a critical part to attenuate chemotherapy-induced diarrhea, such as changes in diet and nutrition supplements. Patients should drink three or more liters of clear fluid per day. This quantity should be electrolyte-containing fluids, such as sport drinks, broth, gelatin, decaffeinated tea, and decarbonated soft drinks [51]. After each bowel movement, a barrier cream or ointment, like petroleum jelly, can be applied to the anal area to prevent irritation [51]. The anal area should also be examined for red or broken skin [51]. Grades 3 and 4 diarrhea in some patients require dose reduction or treatment cycle interruption. The dose reduction varies

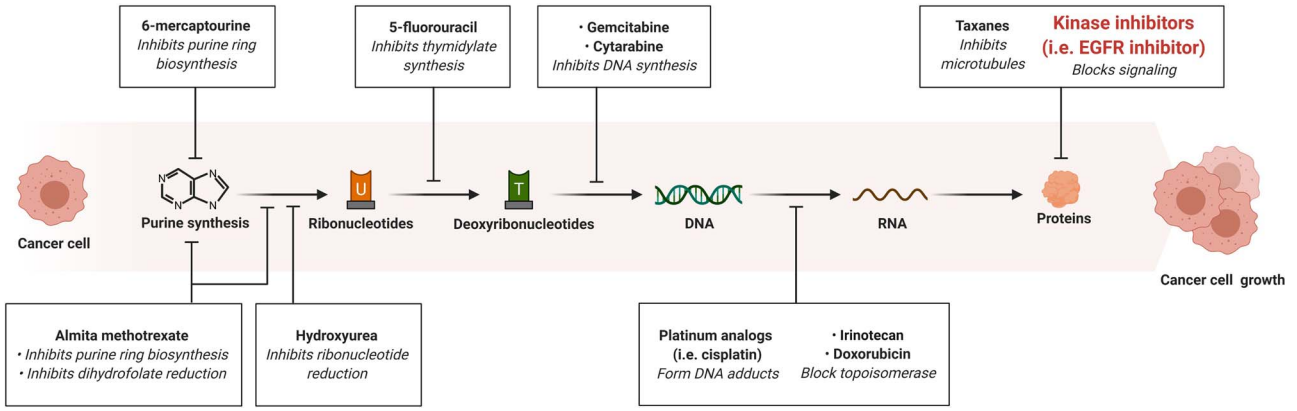

Figure 5: basic methods of chemotherapy.

depending on the grade of diarrhea. But it may compromise the drug efficacy. When non-medical intervention cannot alleviate the side effect, anti-diarrhea medication must chime in.

Medication prolonging gut transient time

High-dose loperamide is the first-line treatment for many types of diarrhea. Typically, an initial dose is 4 mg. Follow-up dose of 2 mg every 2 h is given until no loose motions last for 12 h [52]. As an agonist acting on the μ-opioid receptors in colon, loperamide prolongs the bowel transit time of food, decreases feces volume, and diminishes the loss of fluid. Budesonide or derivatives of codeine are suggested for patients who are refractory to loperamide [53]. Budesonide is orally administered, topically active steroid. Its potency to restore mucosa function is because of the inhibition of mucosal prostaglandins [53]. Oral budesonide at a dose of 9 mg once daily for 3–5 days may be effective for the treatment of loperamide-resistant diarrhea [54]. Budesonide at a dose of 3 mg twice daily was found to decrease the number of episodes of diarrhea [54]. Diophenoxylate and atropine are other alternatives to loperamide. Although loperamide and budesonide are the most widely used anti-diarrhea drugs, their effectiveness is not satisfying when it comes to EGFR-induced diarrhea.

Medication reducing intestinal secretion

Octreotide is used to control bowel motility and water flow. Octreotide suppresses the response of gut to gonadotropin-releasing hormone [55]. It decreases splanchnic blood flow as well [55]. It inhibits the release of serotonin, gastrin, vasoactive intestinal peptide, secretin, motilin, and pancreatic polypeptide [55]. Octreotide can be given subcutaneously starting at 100–150 μg. Doses of octreotide ranges from 50 μg twice daily to 2500 μg three times daily based on the diarrhea severity [55]. The currently recommended dose is 100–150 μg three times per day [55]. The dose may be escalated to 500 μg three times daily when diarrhea is persistent [55]. As plenty of evidences suggest that chloride secretion plays an important role in EGFR inhibitors-induced diarrhea, octreotide should be considered as an option to contain this toxicity.

Probiotics

Probiotics, such as *Lactobacillus acidophilus* and *Bifidobacterium spp.*, competitively block the invasion of pathogens into intestinal mucosa [56]. Probiotics activate immunity by stimulating the release of cytokines [56]. Although they have been used in infectious diarrhea [57], there is very limited study exploring the effect of *Lactobacillus plantarum* on drug-induced diarrhea [58]. How EGFR inhibitors change the structure of microbiome and how imbalance of microbiome leads to diarrhea need to be answered. It should be noted that cancer patients with weakened immune systems may confront uncontrollable infection when receiving probiotics. Thus, evidence validating the safety of probiotics in cancer patients is needed before we set up the clinical trial to test its anti-diarrhea efficacy.

Case studies of EGFR inhibitors-induced diarrhea

As for afatinib-induced diarrhea, Yang *et al.* suggested that loperamide treatment should start right after the diarrhea episode until there was no bowel movement. If Grade 2 diarrhea lasts for more than 48 h despite anti-diarrhea treatment, afatinib interruption is recommended. In the event of Grades 3 and 4 diarrhea, patients should be admitted to hospitals and should receive intravenous fluid. In this case, loperamide should continue and antibiotics can be considered if neutropenia is diagnosed [59]. According to previous report, after lapatinib was administered, the onset of diarrhea would occur within 2–5 days [39]. When lapatinib-induced diarrhea persists for more than 24 h, increasing the dose of loperamide is suggested. Reducing lapatinib dose and withholding treatment should be considered for patients experiencing Grade 3 diarrhea [60]. If mild diarrhea does not stop after 24 h of high-dose loperamide, second-line agents like octreotide, budesonide, and laudanum should be taken. If the dehydration is severe, fluoroquinolone needs to be administrated. In Phase III trial of neratinib, Grade 3 diarrhea causing dose reduction was recorded [22]. Latest trial showed that adding colestipol to loperamide prophylaxis reduced diarrhea episodes in cancer patients having neratinib [61]. It is suggested that colestipol may also improve neratinib tolerability by decreasing the rate of other adverse events, including fatigue, headache, and abdominal pain [61]. But further follow-up is still necessary to validate the anti-diarrhea function of colestipol in the neratinib case [61].

Conclusion

Diarrhea is one of the most frequent adverse effects hampering the therapeutic outcome of EGFR inhibitors. The third-generation

EGFR inhibitors selectively targeting T790M have been developed to spare wild-type EGFR, in hope to reduce the incidence of diarrhea. However, first- and second-generation inhibitors are still widely prescribed for cancer patients without T790M. The toxicological mechanism of EGFR inhibitors-induced diarrhea is poorly illustrated. Very limited preclinical studies have been done, not to mention the clinical trials. Irinotecan-associated diarrhea is the most extensively studied chemotherapy GI toxicity [41]. Nevertheless, irinotecan is a topoisomerase blocker. The pharmacological mechanism of topoisomerase blocker is distinct from EGFR inhibitors, so what we learn from irinotecan is hardly applicable to EGFR inhibitors (Fig. 5). Diarrhea that resulted from EGFR inhibitors is progressive and requires a prompt and effective management. However, the current guideline for this side effect is not truly effective. The knowledge gap in toxicology is the major barrier for prophylaxis development. Leveraging gut-on-a-chip, humanized mouse model and physiologically based pharmacokinetic (PBPK) modeling, more details behind EGFR-induced diarrhea can be revealed. It will help us to predict the risk of GI toxicity more accurately during new drug development. In terms of reported mechanisms, they mainly fall into three categories: inhibition of epithelium regeneration, excessive chloride secretion, and inhibition of drug transporters. But which one is the main driving force of the EGFR inhibitors-associated diarrhea remains as an open question. We suggest that excessive chloride secretion probably accounts for acute toxicity response, while suppression of epithelium regeneration is responsible for long-term damage. Inhibition of drug transporters partially answers why combinational therapy has a higher diarrhea incidence than the EGFR inhibitor monotherapy.

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Conflict of interest statement

None declared.

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