EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR AFTER LOW DOSE CAPSAICIN ADMINISTRATION IN RAT DUODENUM

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Abstract: Growth factors are essential for the development, growth and homeostasis of multicellular organisms and play an important role in the development and the maintenance of the gastrointestinal tract. Capsaicin (CAP) with neurotoxic properties has been shown to have a protective effect against experimental gastric mucosal injury in animals and humans. Epidermal growth factor (EGF) and ıt's receptore has been shown to exert gastric hyperemic and gastroprotective effects via capsaicin-sensitive afferent neurons, including the release of calcitonin gene-related peptide (CGRP). The. aim of the present study was to investigate the effects of low dose capsaicin (CAP) on epidermal growth factor receptor (EGFR) expression in the duodenum. In this study, 21-day-old rats were divided into two groups as CAP-treated and vehicle. CAP prepared in a solvent and injected subcutaneously to CAP-treated group (0.5 mg/kg/d) and vehicle group was injected with only solvent for 20 days. At the end of the experiment, tissue samples were collected and paraffin-embedded tissues were processed for standard immunohistochemistry by the labelled streptavidin-biotin technique. The EGFR localizations were identified on the surface epithelium of the villi, Lieberkühn crypts, in the Brunner's glands and smooth muscles layer of the duodenumIn the experimental group, the expression of EGFR in surface epithelial cells was not different compared to the control group, while the expressions in the Lieberukhn crypts, Brunner's glands and smooth muscle layer were stronger than the control group. As a result, the low dose capsaicin increased EGFR expression in the digestive system and probably have positive effects on the digestive system
Key Words: Capsaicin, duodenum, epidermal growth factor, rat.

Introduction

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the pungent ingredient in red peppers and chillies that has been a pharmacological tool for the study of thin afferent fibres for almost half a century (Hwang et. al 2010). Capsaicin at a low dose stimulates release of neuropeptides such as catecholamines, neurokinin A (NKA), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), and substance P (SP) from sensory neurons endings (Holzer 1991, Surh and Lee, 1996). In contrast, high dose capsaicin shows neurotoxic effect and induces an irreversible long-standing inactivation of the capsaicin-sensitive nerve endings with a loss of their sensory-afferent functions and their ability to release sensory neuropeptides (Holzer 1991).

Sensitive primary afferent neurons of capsaicin participate in the regulation of gastrointestinal (GI) motility (Barthó et. al 2002). The effects of CAP are dependent on the concentration of capsaicin and of the mode of application. Also, Capsaicin induces the apoptosis of cancers cells, including myeloid leukemia (Ito et. al 2004), human hepatoma (Lee et. al 2004), and colon cancer (Kim et. al 2007). However, epidemiologic and animal experimental evidence suggests that capsaicin also acts as a carcinogen or cocarcinogen, particularly during the tumor promotion stage (Surh and Lee, 1996).

Growth factors are essential for the development, growth and homeostasis of multicellular organisms (Wieduwilt et. al 2008). The epidermal growth factor (EGF) is a polypeptide of 53 amino acids originally isolated from the rodent submaxillary gland (Cohen, 1962). EGF is continuously secreted from the salivary glands and the duodenal Brunner's glands (Konturek et. al 1995, Konturek, 1990). Intragastric EGF has been shown to enhance the healing of gastric mucosal injury (Konturek et. al 1995, Konturek, 1990, Olsen et. al 1986) and to protect the gastric mucosa against various stimuli such as stress, ethanol, hypertonic saline, and aspirin (Hui et al. 1993, Kang et al. 1998, Konturek, 1988, Konturek et. al 1981a, Konturek et. al 1981b). Therefore, it has therapeutic potential in digestive system disorders. The protective effects of EGF-family members are most likely related to their ability to modulate epithelial cell migration (Dignass et. al 1993), mucosal blood flow (Hui et al 1993) gastrointestinal motility (McLeay et al.1990), mucus production and secretion (Kelly and Hunter 1990), and gastric acid secretion (Rhodes et. al 1986). In addition, EGF and related peptides are potent mitogens for cells of the gastrointestinal tract in vivo (Al-Nafussi and Wright 1982) and in vitro (Carpenter and Cohen 1979)

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that mediates intracellular signaling in response to various extracellular stimuli (Cohen, 2003). The epidermal growth factor family of receptor tyrosine kinases plays essential roles in regulating cell proliferation, survival, differentiation and migration (Wieduwilt and Moasser 2008).

EGF and EGFR have been shown to exert gastric hyperemic and gastroprotective effects via capsaicin-sensitive afferent neurons, including the release of calcitonin gene-related peptide (CGRP) EGF has a protective role in gastric mucosal injury, via capsaicin-sensitive afferent neurons involving calcitonin gene-related peptide (CGRP) mechanisms (Matsumoto et. al 2001**).**

Overexpression of epidermal growth factor receptor (EGFR) is common in many tumors. Specifically in colon and rectal cancer, EGFR is estimated to be overexpressed in 60%-80% of tumors, and is associated with a poor prognosis (Cohen, 2003).

In previous studies, EGFR immunolocalization is in the lung, stomach, duodenum, pituitary gland, thyroid gland, mammary gland, ovary, smooth muscle cells and small intestinal epithelium cells were determined (Carpenter and Cohen 1979, Carpenter 1987, Gómez-Pinilla et al.. 1988, Thompson 1988, Kajikawa et al. 1991, Massagué and Pandiella 1993, Playford et al. 1996, Kelly et al. 1997, Jeffrey et al. 2001, Zeineldin and Hudson 2006)

However, the effects of capsaicin on the small intestine, the role of EGFR is not fully understood. The aim of the present investigation was to determine the localization of EGFR in rat duodenum by immunohistochemistry and to identify different EGFR immunoreactivity after the application of a low dose of capsaicin in the rat's duodenum.

Material and Methods

Thirty immature female Sprague- Dawley rats (21 d old) were used throughout the experiments. The rats were obtained from the Experimental Animals Breeding and Research Centre, Uludag University, Turkey. The animals were housed five per cage, in temperature controlled conditions of (20–24ºC), humidity (60–70%), and lighting (12 h light/dark cycle), and were provided with feed and water *ad libitum*. The experimental protocols were approved by the Animal Care and Use Committee of the Uludag University and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental protocol. The rats were divided at random into 2 groups of 20 animals each. The first group (control) remained without any treatment. The second group (experimental) received subcutaneous injection of CAP (Sigma Chemical Co.) (0.5mg/kg/d), prepared in a solvent consisting of 10% of ethanol, 10% of Tween 80, and 80% of distilled water, for 20 consecutive days, Following 20 d of capsaicin treatment, the animals were euthanasised by the injection of sodium pentobarbital and the abdominal walls were opened. The proximal part of the duodenum was removed and fixed in alcoholic formaldehyde. Tissue samples were embedded in paraffin blocks according to routine histological procedures. Five micrometres thick sections were cut and immunostained for EGFR localisation (Inoue et. al 2002).

Immunohistochemistry analysis

After dewaxing and rehydration, slides were carried out antigen retrieval by boiling sections in microwave oven at 750 W in sodium citrate buffer (1 M, pH 6.1). After cooling, slides were rinsed with PBS and endogenous peroxidase activity was blocked by 10 min incubation at room temperature in 3% H_2O_2 solution in distilled water. After blocking with non-immune serum into kit for 30 minute to reduce nonspecific antibody binding, sections were incubated with primary antibodies, a rabbit polyclonal antibody to EGFR (sc 03, Santa Cruz, CA, USA) diluted to 1:100 for overnight at 4°C. The sections were stained using ImPRESS IgG-peroxidase kits (Vector Labs) (cat. No. MP-7401), according to the supplier's instructions. Finally, 3.3'-diaminobenzidine (DAB) was used for colour development. After counterstaining with haematoxylin, specimens were dehydrated and mounted.

Quantitative evaluated according to the staining intensity as follows: no staining (negative, −), slight brown (weak, +), brown-yellow (moderate, ++), and dark brown (strong, +++). The accumulated score of the positive staining represented the relative expression of the protein (Fromowitz et al. 1987, Ergin et al. 2008).

Results and Discussion

The EGFR localizations were identified on the surface epithelium of the villi, Lieberkühn crypts, in the Brunner's glands and smooth muscles layer of the duodenum (Fig. 1a, 2a, 3a). EGFR expression differences between groups are presented in Table 1. In the regions,

In the duodenum of the control group, EGFR expression in the surface epithelium of the tunica mucosa layer showed a strong immunoreactivity. EGFR expression also demonstrated strong immunoreactivity in the surface epithelial cells of the experimental group. (Fig. 1a, 1b) (Table 1.). In the control group, a moderate EGFR immunoreactivity was observed in the Lieberkühn crypts in the Tunica mucosa, while a strong EGFR expression was observed in the experimental group (Fig. 2a, 2b,3b) (Table 1.).

EGFR immunoreaction in Brunner's glands of the submucosa showed intracytoplasmic location. A weak EGFR imunoreactivity was observed in the control group, while a strong immunoreaction was observed in the Brunner's glands of the experimental group (Fig. 2a, 2b) (Table 1.).

Muscle layer. both the circular and longitudinal muscle layers, the dose of CAP administered increased EGFR expression. A weak EGFR expression was determined in the circular muscle layer in the control group, while a moderate EGFR immunoreactivity was observed in the circular muscle layer in the experimental group (Fig 2a 3a, 3b) (Table 1.).

EGFR expression was moderate in the longitudinal muscle layer of the control group, while it showed a strong immunoreactivity in the experimental group. (Fig 2a 3a, 3b) (Table 1.).

Table 1. Semiquantitative observations of the EGFR immunoreactivity in the rat duodenum.

	T. Mucosa		**T.Submucosa**	**T. Muscularis Externa**	
	S. Epithelial Cells	Liβ. Cryps.	Brunner's glands	C.Muscle Layer	L. Muscle Layer
Control	+++	++	+/-	+	++
Experimental	+++	+++	+++	++	+++

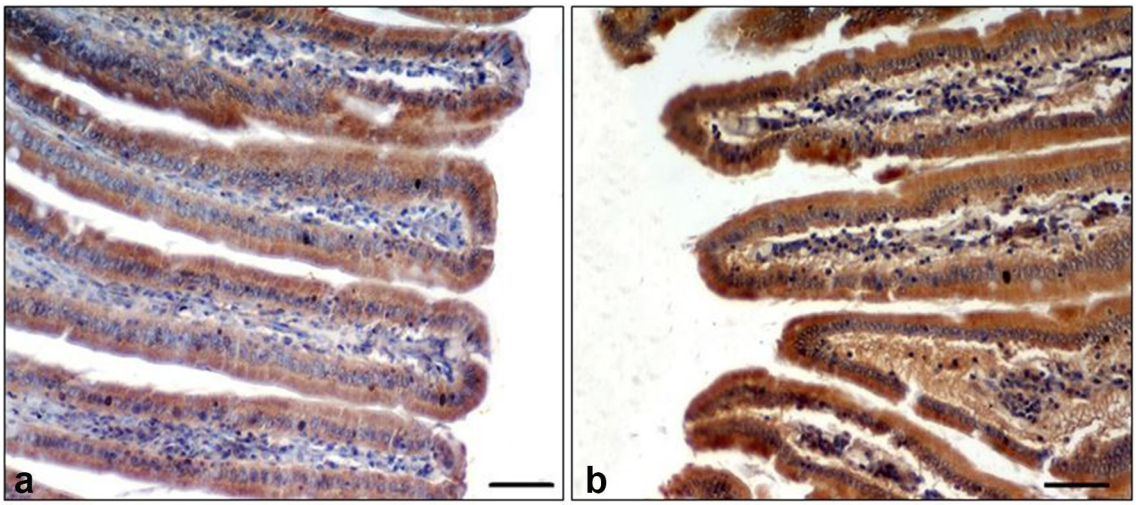

Figure 1. EGFR expression in surface epithelia cells of (a) control group, (b) experimental group. Bar 50 µm.

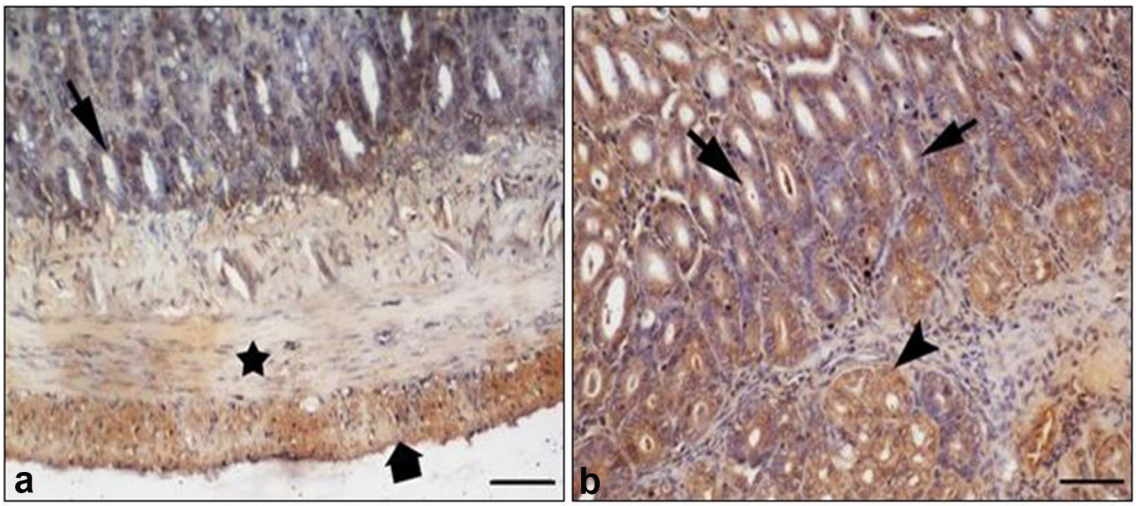

Figure 2. . EGFR expression in Liβ. Cryps, circular and longitudinal muscle layers of (a) control group, (b) experimental group. Bar 50 µm.

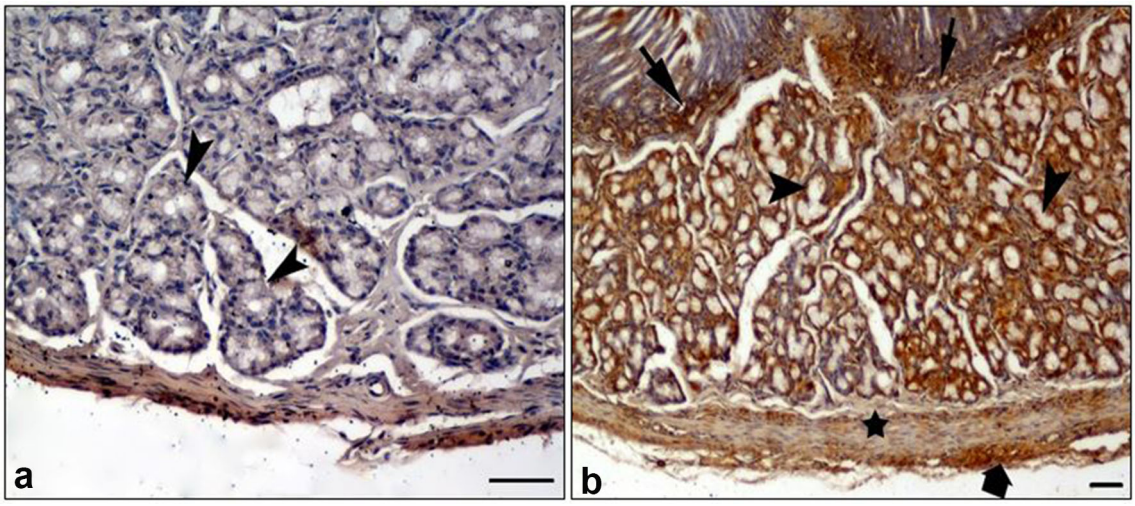

Figure 3. EGFR expression in Brunner's gland, circular and longitudinal muscle layers of (a) control group, (b) experimental group. Bar 50 µm.

The EGFR family members have roles in a broad spectrum of human diseases and are a paradig m for the translation of fundamental biological discoveries into therapeutics for human disease (Wieduwilt and Moasser 2008), and numerous studies have shown the existence of capsaicin sensitivity neurons in the gastrointestinal tract (Holzer, 1991, Nozawa et al. 2001, Patterson et. al 2003). In the present study, we observed that the given low-dose CAP increased EGFR expression in the duodenum's Lieberkühn cryptes, Brunner's glands and smooth muscle layer. Capsaicin has been reported to be effective on growth and development by increasing the release of growth factors. On the other hand EGFR plays essential roles in regulating cell proliferation, survival, differentiation and migration (Szalasi and Blumberg 1999, Yıldız et. al 2013, Bakır and Sarı 2015).

Aberrant regulation of EGFR activates downstream signals including ERKs and Akt resulting in increased tumor cell proliferation, survival, and invasiveness. Thus, modulation of EGFR signalingis key in preventing cancers. Capsaicin enhanced the metastasis of murine breast cancer cells by reducing the expressionof apoptosis-related genes (Erin et al. 2006) and induced LNCaP prostatecancer cell proliferation by increasing androgen receptor expression through the activation of ERKs and Akt (Malagarie-Cazenave et al. 2009). Also, another studies have shown that capsaicin-sensitive afferent neurons do not have a possible role in induction of EGFR in the duodenum (Bulut et al. 2008).

The biological effects of capsaicinoids are dependent on the dose of these compounds administered and the time of exposure (Bley et al. 2012).

Studies have shown that high doses of capsaicin (over 100 mg capsaicin per kg body weight) over a long period of time cause peptic ulcers, accelerate the development of prostate, stomach, duodenal and liver cancers and improve breast cancer metastasis (Mózsik et al. 2009, Bley et al. 2012).

Several convergent studies indicate that low-doses of capsaicin display a cancer-chemopreventive, anti-neoplastic activity (Lau et al. 2012, Aggarwal et al. 2008). Capsaicin induces robust apoptosis in multiple types of human cancer cells both in vitro and in mice models. Recent studies have focused on the potential of capsaicin as a viable anti-cancer drug applicable to the management and treatment of human breast cancer, and colon cancer (Lau et al. 2012, Lau et al 2014). The intestinal absorption of low capsaicin in vitro was done using everted intestinal sacs isolated from rats (Monsereenusorn, 1980). It was observed that capsaicin was robustly absorbed both into intestinal tissues, jejunum and serosal fluid. Kawada et al. (1984) studied the in situ metabolism of capsaicin using ligated loops of stomach, jejunum and ileum (Kawada et al. 1984). The application of 1 mM capsaicin led to rapid absorbance of the compound in the lumen within one hour; the absorbance rate was 50% in the stomach, 80% in the jejunum and 70% in the ileum. This indicated that capsaicin was absorbed better in the jejunum and ileum as compared to the stomach. The authors repeated these studies with dihydrocapsaicin and obtained similar results (Kawada et al. 1984).

Intragastric EGF has been shown to enhance the healing of gastric mucosal injury (Konturek et al. 1990, Konturek et al. 1995, Olsen et al. 1986) and to protect the gastric mucosa against various stimuli such as stress, ethanol, hypertonic saline, and aspirin.

Conclusion

Considering all these data, the fact that low dose CAP application increased the expression of EGFR which plays an important role in cell proliferation, differentiation and migration on dudodenum, suggests that it affects the intestinal activities positively and has a more facilitating effect on digestion.

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