

# Topical Vitamin K3 (Menadione) Prevents Erlotinib and Cetuximab-Induced EGFR Inhibition in the Skin

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ASCO 2006, Abstract#3036

Methods and Results

**Background:** The EGFR inhibitors erlotinib and cetuximab cause skin toxicity in about 75% of patients. About half the patients with skin toxicity report significant discomfort. The occurrence of skin toxicity correlates with clinical benefit. There are currently no scientifically-based or proven methods for preventing or treating effectively the skin toxicity secondary to EGFR inhibitors.

**Methods:** We screened a number of EGFR activators and phosphatase inhibitors for their ability to abrogate EGFR inhibition secondary to erlotinib or cetuximab in A431 cells. Phosphorylated EGFR (p-EGFR) expression was assessed by Western blot analysis. Vitamin K3 (VK3) was selected for further *in vivo* studies. The skin toxicity secondary to the topical application of VK3 was evaluated in nude mice. The highest non-toxic concentration was used to determine the ability of topical VK3 to prevent EGFR inhibition in the skin of nude mice treated with oral EGFR inhibitor erlotinib.

**Results:** VK3 was the most potent EGFR activator identified, the maximum effect being observed at concentrations of 0.1-0.5 mM. In the presence of erlotinib or cetuximab, 0.1-0.5 mM VK3 completely prevented EGFR inhibition in A431 cells. The maximal non-toxic concentration of VK3 in nude mice was 15 mM, applied topically BID x 10 days. At this concentration, topical VK3 caused p-EGFR upregulation in the skin. In mice treated with erlotinib (100 mg/kg daily x 5 days), there was no detectable p-EGFR expression in the epidermis of the skin; whereas p-EGFR expression was completely restored in the epidermis of the skin in mice treated with topical VK3 BID x 5 days.

**Conclusions:** VK3 is one of the first reported agents to prevent/reverse EGFR inhibition secondary to anti-EGFR agents. The results strongly justify the development of a topical formulation of VK3 to treat and prevent the cutaneous toxicity secondary to EGFR inhibitors.

## Exogenous ligand EGF fails to rescue the inhibitory effect of erlotinib on pEGFR.

EGF rescues EGFR TK function in A431 cells treated with cetuximab but not small molecule EGFR TKI erlotinib

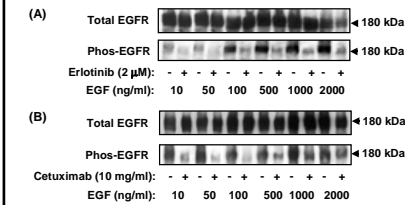
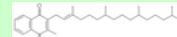


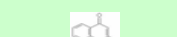
Figure 1. Effect of exogenous EGF on erlotinib and cetuximab-induced EGFR inhibition in A431 cells. Cells were exposed to increasing concentrations of EGF (10 to 2000 ng/ml) in the presence of a fixed concentration of erlotinib 2 μM or cetuximab 10 μg/ml. The expression of total EGFR and p-EGFR in cell lysates was detected by immunoblots.

## Chemical Structure:

Vitamin K1 (WM: 450)



Vitamin K3 (MW: 172)



## VK3 reversibly inhibits the phosphatase activity in A431 Cells.

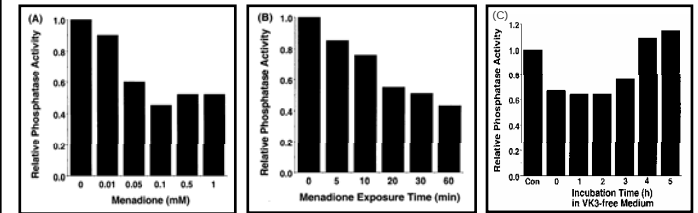


Figure 2. A431 cells were exposed to various concentrations of VK3 for 60 min (A), or 0.1 mM VK3 for the indicated time periods (B). Following exposure, cell lysates were prepared. Phosphatase activity was determined by a colorimetric assay using pNPP (4-nitrophenyl phosphate disodium) as substrate. (C) A431 cells were exposed to 0.1 mM VK3 for 1 hour. After exposure, cells were washed and reincubated in VK3-free medium for the indicated times. Phosphatase activity was measured at different time points. The phosphatase activity in cells without exposure to VK3 was used as a control. The relative phosphatase activity was expressed relative to the expression at concentration 0 or time 0.

## Introduction

### Clinical Significance of Skin Rash



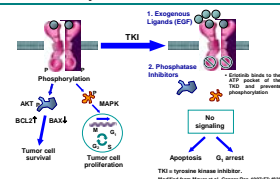
- The EGFR inhibitors erlotinib and cetuximab cause skin toxicity in about 75% of patients.
- About 30-40% of patients treated with anti-EGFR agents experience significant discomfort due to the skin rash (grade 2).
- About 10% of patients treated with anti-EGFR agents need dose reduction or interruption due to the skin rash (grade 3).
- The presence and severity of skin rash have been found to be associated with an improved survival in all studies with EGFR inhibitors in which this relationship has been analyzed.

### EGFR Tyrosine Kinase Activity as a Target to Prevent Skin Toxicity

#### Pathophysiology of Skin Rash:

- EGFR is important in maintaining the physiological function and integrity of epidermis in normal skin.
- The severity of skin toxicities correlates with the dose of erlotinib.

#### Is it possible to topically rescue the EGFR TK activity in the skin? -Possibilities



## Hypotheses

- Inhibition of phosphatase will result in sustained EGFR activation and will prevent the reversible inhibitory effect of EGFR inhibitors on the TK.
- Topical application of a phosphatase inhibitor to the skin should reverse or prevent the skin toxicity secondary to EGFR inhibitors and result in minimal systemic absorption.

## Vitamin K3 (menadione) is a reversible phosphatase inhibitor that activates EGFR tyrosine kinase.

### VK3 is a strong activator of the EGFR pathway in A431 cells

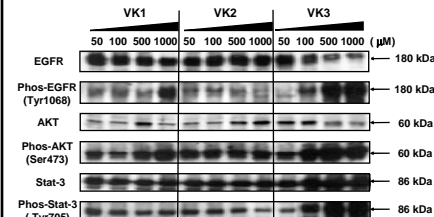


Figure 3. Effect of VK1, VK2, and VK3 (menadione) on the EGFR signaling proteins in A431 cells. After exposure to VKs, cells were collected and cell lysates were prepared. An equal amount of cell lysate (30 μg of protein) was resolved on 7.5% SDS-PAGE. The expression of different signaling proteins was determined by immunoblots.

### VK3 prevents EGFR inhibition by erlotinib in A431 cells

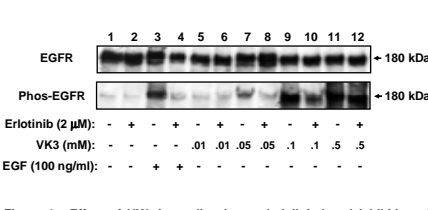


Figure 4. Effect of VK3 (menadione) on erlotinib-induced inhibition of EGFR activation in A431 cells. Cells were incubated in the presence of 2 μM erlotinib alone or with increasing concentrations of VK3 at 37°C for 2 hours. For EGF stimulation, cells were exposed to 100 ng/ml of EGF for 5 min. After treatment, cells were harvested and cell lysates were prepared. Total EGFR and phosphorylated EGFR expression were detected by immunoblots.

### VK3-induced EGFR activation is reversible in A431 cells

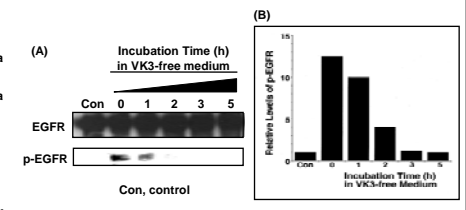


Figure 5. Reversible effect of VK3 on the EGFR activation in A431 cells. Cells were exposed to 0.1 mM VK3 for 1 hour. Following exposure, cells were washed and reincubated in VK3-free fresh medium for the indicated times. Control cells were not exposed to VK3. p-EGFR expression was quantitated as the relative intensity of the p-EGFR band at each time point measured relative to the control (value assigned = 1).

### Topical VK3 rescues skin EGFR TK activity in mice treated with erlotinib.

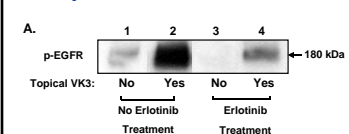


Figure 6. VK3 rescues erlotinib-induced inhibition of p-EGFR expression in mouse skin tissue. Mice were treated with oral 100 mg/kg erlotinib daily x 5 days and areas of mouse skin were exposed to an ethanol solution of 15 mM VK3 every 12 hours or ethanol alone (solvent). Two hours after the last topical application of VK3, mice were sacrificed and skin tissues taken. The expression of p-EGFR was detected by immunoblot (A) or immunohistochemistry (B) using anti-p-EGFR antibody.

## Conclusions

- Vitamin K3 (menadione) is a phosphatase inhibitor and an EGFR TK activator
- VK3 can rescue EGFR TK function in A431 cells treated with cetuximab and the small molecule EGFR TK inhibitor erlotinib
- EGF can rescue EGFR TK function in cells treated with cetuximab but not in cells treated with small molecule EGFR TK inhibitors
- Topical VK3 is an EGFR activator in the skin of nude mice and rescues EGFR TK function in mice treated with oral erlotinib

## Future Directions

Development of a topical Vitamin K3 formulation and clinical testing for the treatment of EGFR Inhibitor-induced skin rash.