【S-15】

Safety Issues of Growth Factors including EASYEF

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Numerous active growth factors such as PDGF-BB, EGF, bFGF and KGF are emerging in the world market and playing an increasing role in the therapy of human disease. In the nonclinical safety studies of growth factors, specific considerations should be focused on manufacturing process, immunogenecity, and carcinogenecity to ensure their safety, quality, efficacy and economic advantages in humans. EASYEF (DWP401) was developed as the first rhEGF for diabetic foot ulcer and was approved as Orphan Drug in 2001 by KFDA. This agent has subsequently been marketed and Phase III clinical trials are ongoing now in Korea. In terms of toxicity studies of DWP401, 13-week repeated subcutaneous injection studies reported NOAEL of 40 g/kg in mice and 1 g/kg in rats. Reproductive toxicity tests showed no significant abnormalities. Genotoxicity tests gave results that the DWP401 is non-genotoxic. Immunogenicity tests were performed to verify that the subject animals did not develop antibodies to the DWP401. Local irritation tests to skin and eye showed that DWP401 is non-irritant. Based on the result of toxicity studies, the possibility of DWP401 to elicit adverse reactions upon topical application to open wounds appears to be insignificant in clinical practice. In the Phase II clinical study for 12 weeks treatment of DWP401 in diabetic foot ulcer patients, significant difference was not observed among the groups in the incidence of adverse events ($p>0.05$) and there was no adverse event that seems to be related to the DWP401. Due to the physiological function of growth factors especially cell proliferation activity, the issue of their carcinogenic potentials has been raised. However, there is no evidence that EGF is related to carcinogenesis. DWP401 was identical to human EGF and gave negative responses to all genotoxicity tests. In phase II clinical study for 12 weeks treatment of DWP401 on diabetic foot ulcer, there were no adverse events such as metaplasia and neoplasm. In the overexpression of EGF did not induce any carcinoma in transgenic mice. Based on these facts, rhEGF is not considered to have carcinogenic effects in the therapeutic dose range.

Safety Issues of Growth Factors including EASYEF®

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Numerous active Growth Factor are emerging

- Regranex (PDGF-BB) : Chiron/J&J (Dec. 1997, US)
  - Diabetic foot ulcers : $98m in 2001
  - Speed-up healing times for patients with diabetic foot ulcers
- EASYEF (rhEGF) : Daewoong (May 2001, Korea)
  - Diabetic foot ulcers : $1m in 2002
  - Improving complete healing ratio
- Fiblast (bFGF) : Scios/Kaken (Oct. 2001, Japan)
  - Healing a recalcitrant dermal wounds $6m in 2002
  - Phase III in US
- Palifermin (rhKGF) : Amgen (Feb.2005)
  - Radiation and chemotherapy-induced oral mucositis

Growth factors sales forecasts (2003-2010)

($m)

Brand	2003	2005	2007	2010
Regranex	117	125	122	109
KGF	0	75	199	457
Somatokine	0	12	66	156
ART123	0	0	60	93
Total	161	273	534	934

Datamonotor (2004. Apr)

Promise of Growth Factors

1. Specific Activity on Biological Process
2. Huge potential market for growth factor therapies
3. Stimulate the repair & regeneration of tissue and organs

Challenges of Growth Factors

1. **Efficacy & Safety proving**
   - from initial research through late-stage clinical trials
2. **Economic advantages**
   - extreme competition from other therapies
   - ex) KGF: 2,000-3,000$/procedure
3. **Body natural growth factor balancing**
   - some G.F may cause unwanted toxicities
   - High conc. of IGF-1 : more risk of prostate cancer

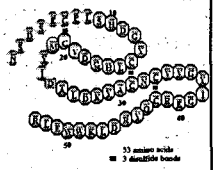
EASYEF®: The First Approved rhEGF in the World

Spray type (EGF 50 μg/ml)
Orphan drug

What is EGF ?

* **History**
  - A single-chain polypeptide, 53 amino acids, Mw 6.2 kd
  - First isolated from the Submaxillary glands in mice (Stanley Cohen, 1962)
  - In 1975, Cohen discovered Human EGF

* **Biological Functions**
  - Chemoattractant and mitogen for epithelial cell, fibroblast
  - Collagen syntehsis



Pharmacological Action of EGF on Wound healing

* **Re-epithelialization**
  - Proliferation and migration of epithelial cells
* **Formation of granulation tissue**
  - Proliferation of fibroblasts
  - Synthesis of extracellular matrix
* **Angiogenesis**
  - Induction of angiogenic factors

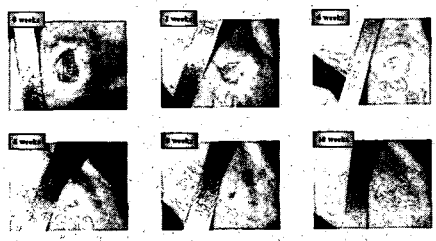
Natural Distribution of EGF in Body Fluids

Handbook of Experimental Pharmacology, Vol. 95/I, 1990

Development Status of Daewoong EASYEF®

* **For diabetic Foot ulcer**
  - Formulation : dermal solution (EASYEF®)
  - Status : phase III in Korea
    Launched as an orphan drug in Korea (2001)
* **For Anti-wrinkle care**
  - Formulation : cream
  - Status : Launched
* **For skin Wound**
  - Formulation : ointment
  - Status : preparing phase II in Korea
* **Potential indication as a wound healing Agent**
  - **Oral Mucositis**
  - **Pressure Ulcers/ Bed Sores**
  - **Burns**

A Case study of EASYEF® for Diabetic Foot Ulcer

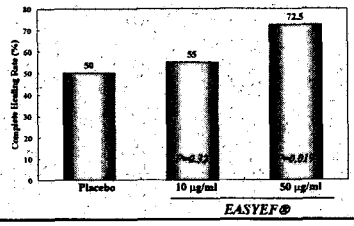


Patient : 53 years old male, Suffering from type-2 DM for 15 years

Ulcer : 4.3cm² on left dorsum, Progressing for 10 month before this study

Efficacy of EASYEF® in PII Clinical study

- Treatment : Twice a day for 12 weeks, Sprayed on the ulcer
- Patients No: 124 (109 patients completed the study), 6 Multi-center
- Foot Ulcer : Grade 2 and 3 by Wagner classification (0.5 - 30cm²)



Safety of EASYEF® in PII Clinical study

There was no statistical difference among the groups (p>0.05)

Category	Placebo (N=41)	EGF 10 ug/ml (N=41)	EGF 50 ug/ml (N=42)
Patients No. With more than one adverse events	10 (24. 4 %)	8 (19.5 %)	11 (26.2 %)
No. of cases	12	11	14
Relationship with study drug	None	1	Unknown (3)
Serious adverse events	None	None	None

Pain & redness in drug application

Preclinical Efficacy Studies of Daewoong rhEGF

Study	Effective dose	Results
Ischemic ears ulcer in rabbit	200 μg/g	Wound area was significantly decreased; - Re-epithelialization and granulation tissue formation were accelerated
Skin graft survival in diabetic rats	10 μg/g	Surviving areas were significantly increased - Thick epidermis, prominent skin appendages and linear organization of collagen fibers were observed
Full-thickness wounds in rats	50 μg/g	Healing time of wounds was decreased; - Re-epithelialization, thickness & maturation of collagen bundles and neovascularization were increased
Split-thickness wounds in pigs	1 ~ 5 μg/g	Healing time of 100% was decreased - 1 μg/g: 5.2 day, 5 μg/g: 5.0 day, vehicle-treated: 8.5 day

Pharmacokinetics of Daewoong rhEGF

Study	Results
❖ In rats, Subcutaneously injection at dose of 50 μg/kg	
Absorption	Half life: 4 min Cmax: 23.6 ng/ml Tmax: 18 min
Distribution	Mainly distributed in the liver & kidney
Metabolism	Over 93 % of rhEGF was metabolized within 30 min and excreted in urine as amino acid
Accumulation	Multiple dose of 50 μg/kg (s.c): No evidence of accumulation in intact form in any organ
❖ In human skin, Topical administration at dose of 50, 100 μg/ml	
rhEGF Conc. in blood & urine	14 days, 100 cm² of abraded skin / 5ml No significant difference between the placebo

Toxicology Studies of Daewoong rhEGF

Studies	Unit dose	Result
Single dose Tox. (S.C)		
1.Mice		LD_{50} >2 mg/kg
2.Rat		LD_{50} >2 mg/kg
13-week repeated dose Tox.(S.C)		
1.Mice	40, 200, 1,000 μg/kg	NOAEL : 40 μg/kg
2.Rat	0.1, 1, 10 μg/kg	NOAEL : 1 μg/kg
Immunogenicity (Ab response)		
Rats & mice	40, 200,1000 μg/kg	Negative At dose of 1,000 μg/kg, low titer Ab response
Genotoxicity		
1.Ame's test	10 ~ 150 μg/plate	No Mutagenesis
2.Chromosomal aberration	25 ~ 100 μg/ml	Negative
3.Micronucleus (ICR mice)	500~ 2,000 μg/kg	Negative

Toxicology Studies of Daewoong rhEGF

Studies	Unit dose	Result
Reproductive Tox.(S.C.)		
1. Fertility &/General Reproductive Tox.	Rats: 1~1,000 μg/kg	No effect
2. Teratology in rats	0.2, 1, 5 mg/kg	NOAEL= 0.2 mg/kg
3. Teratology in rabbits	0.6, 3, 15 μg/kg	NOAEL= 3 μg/kg
4. Peri-/post-natal	40, 200, 1,000 μg/kg	Dams & F2: NOAEL= 1,000 μg/kg F2: NOAEL= 40 μg/kg
Skin Sensitization	5,000 μg/kg	Negative
Skin & Eye Irritation	(EASYEF)	Non-irritant

Safety Pharmacology of Daewoong rhEGF

Core battery studies	Unit Dose	Result
Central Nervous system		
1. Hexobarbital-induced sleeping time		No effect
2. Locomotor activity	80 μg/kg	No effect
3. Body temperature	160 μg/kg	No effect
4. Rota-rod test	s.c	No effect
5. Antiepileptic		No effect
6. Analgesic		No effect
Cardiovascular system (rabbit)	2.7 μg/kg	No effect
1. Blood pressure	8 μg/kg,	Temporary
2. Hart rate	i.v	decrease BP
Respiratory system		
1. Respiration rate	80 μg/kg	No effect
2. Tidal volume	160 μg/kg	No effect
3. Minute volume	320 μg/kg	No effect
	s.c	

Carcinogenicity study of Marketed G.F

Product (G.F)	Dosage	Carcinogenicity study	Contraindication
Regranex hPDGF	Topical gel (100 μg/g)	No study	Skin cancer or tumors at the site of the ulcer
FIBLAST hbFGF	Topical	Initiation-promotion model	Skin cancer or tumors at the site of the ulcer
Kepivance hKGF	Single IV 20-250 μg/kg	In vivo xenograft in Nude mouse	This medicine may cause some other tumors (not bone marrow tumors) to grow in animal models
Increlex IGF-1	S.C inj. 40-80 μg/kg	2 years study in SD rats	Presence of active or suspected neoplasia

Specific Consideration in Carcinogenicity on Biopharmaceuticals

✱ Carcinogenicity (ICH S6)

- Standard carcinogenicity bioassays are inappropriate
- Carcinogenicity studies needed depending on

1) Patient population and/or biological activity of the product (e.g., growth factors, immunosuppressive agents, etc)

2) When there is a concern about carcinogenic potential
   - induce or support proliferation of cell
   - repeated dose toxicity studies are useful information

Specific Consideration in Carcinogenicity on Biopharmaceuticals

✱ Check Point before the carcinogenicity

- Was long-term human therapy indicated?
- Will human tissues be exposed to supra-physiological levels?
- Is the compound mutagenic or clastogenic?
- Was Antibody formed in animals?
- Was tissue proliferation observed in subchronic or chronic studies?

Specific Consideration in Carcinogenicity on Biopharmaceuticals

✱ Bioassay Methods for Carcinogenicity

1) *In vitro* proliferation study in normal and malignant tumor cells with product-specific receptors
2) *In vivo* studies in relevant animal models (*in vitro* data, carcinogenic potential)
3) Additional in vivo tests (ICH S1B)
   - Initiation-promotion model
   - Transgenic mouse assays
   - Neonatal rodents

Additional in vivo test for carcinogenicity in Growth Factors

❖ Initiation-promotion model

FIBLAST (bFGF) :
NO evidence of carcinogenic

❖ in vivo xenograft in nude mouse

Kepivance (hKGF):
KGF receptor expressing cancer cell line:
tumor growth in only I cell line out of 7 cell lines

EGF & Tumorigenesis

❖ Transgenic mouse model

➢ EGF overexpression (Chan et al., 2000)
- hEGF expressed at high levels in various organs
- Growth retardation, hypospermatogenesis
- Gross & Histological Exam. at 10 weeks,
- No sign of tumor formation

➢ TGF-α overexpression
(Jhappan et al., 1990; Sandgren et al., 1990)
- Liver neoplasia and breast carcinoma formation

EGF & Tumor Growth

❖ In vivo Xenograft in nude mouse

(World J Gastroenterol, 2002, 8:455-458)

- Gastric adenocarcinoma cell lines: MKN-28, MKN-45, SGC-7901
- Cancer tissue were implanted in BALB/cA nude mouse
- EGF : 15, 30, 60 μg/kg, i.p for 3 weeks
- EGF had no effect on the growth of implanted tumor

[redacted]

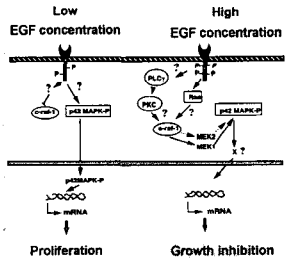
Effects of EGF on the patients for long term treatment

❖ Case study (Brown et al, 1989)

- 12 patients with two skin graft site (5-15 cm^2)
- EGF (10 μg/ml) : the recovery of epidermis was significantly faster than in placebo
- In the follow up study for one year, No metaplasia and Neoplasm were observed in the EGF group.

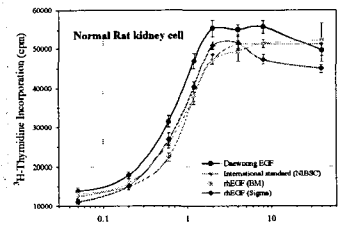
[redacted]

Ambivalent effects of EGF on tumor cells



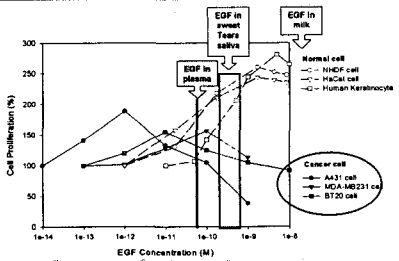
Endocrinology 139 (5):2382, 1998

Mitogenic Activity of Daewoong rhEGF in normal cell



(Daewoong report)

Effects of Daewoong rhEGF on the cell proliferation in normal and cancer cells



Summary

❖ Growth Factors
- Cell proliferation activity
- Carcinogenic potential is controversial

❖ rhEGF & Tumorigenesis
- No tumorigenic effect in Transgenic mouse model
- No stimulating effect on human gastric cancer cell growth in Xenografted nude mouse
- Topical treatment in open wound in patients
  - No neoplasm or hyperplasia
  - No serious adverse effect

❖ rhEGF is not considered to have the carcinogenic effects in the therapeutic dose range