THE EXPRESSION LEVEL OF GLUT-1 RECEPTOR IN THE BRAIN OF NICOTINE - DEPENDENT RAT

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A. INTRODUCTION

- Nicotine is an addictive substance with devastating effect on health. Smoking habit can lead to nicotine addiction.

- High risk diseases:
  - Respiration, Cardiovascular, Cancer \(\rightarrow\) ACTIVE/ PASSIVE smokers

- Difficult to withdraw: 70% smokers intended to quit smoking \(\rightarrow\) only 3% succeeded

- Some medications have been studied to be used for the treatment of nicotine addiction, and the methods widely used currently are nicotine replacement therapies (Berretini, 2005).

- Previous research:
  - The role of arachidonic acid pathway in drug dependent: Diclofenac as COX inhibitor decrease reward in rat nicotine addiction (Sjah, 2007)
  - Low dosage of diclofenac (1 mg/kg bw and 3,2 mg/kg bw) decreased EP receptor (Faridah, 2010; Anggraeney et al, 2012)
Nicotine and neurotransmitters

- Dopamine: Pleasure, Appetite Suppression
- Norepinephrine: Arousal, Appetite Suppression
- Acetylcholine: Arousal, Cognitive Enhancement
- Serotonin: Mood Modulation, Appetite Suppression
- Beta-Endorphin: Reduction of Anxiety and Tension
- GABA: Reduction of Anxiety and Tension

Benowitz, 2008
Neuro-signaling in nicotine addiction

Nestler, 2001
Arachidonic acid pathway: drug addiction and inflammation pathway

Stimulus

Activation of PLC, PLD, PLA₂

Phospholipids

Glycerol

Arachidonic acid

Cyclooxygenase reaction

O₂

2e⁻

COX

PGG₂

Peroxidase reaction

PGH₂

12-HPETE

5-HPETE

5,12-HETE

5,12,15-Lipoxygenase

O₂

NADPH

Cytochrome p450 epoxygenase

Dehydrase

Leukotriene A₄

EET Dihydroxyacids

Isomerases/synthases

TXA₂

PGD₂

PGE₂

PGF₂

PGI₂

12-HPETE

5-HPETE

5,12-HETE

GST

Hydrolase

Lipoxygenases

LTC₄

LTD₄

LTE₄

LTB₄

Lipoxins

The three major pathways involved in arachidonic acid metabolism

Expert Reviews in Molecular Medicine ©2003 Cambridge University Press
Background

- GLUT-1 is one of glucose transporters that plays important role in brain glucose transport (Gerhart et al., 1989; Maher et al, 1994; Kumagai et al., 1994)
- Chronic use of addictive substance influences the rate of metabolism in the brain, especially glucose metabolism (Volkow et al., 1997)
- Drug dependence and withdrawal changed brain glucose metabolism (Volkow et al., 1991)

GLUT-1 has important role in drug addiction
Aim of the research

• To study the expression of GLUT-1 receptor in nicotine dependent rat brain

• To study the effect of diclofenac as *Non Steroidal Anti Inflammatory Drugs* (NSAID) in GLUT-1 receptor expression
Research methodology

CPP test: nicotine dependent rats

- mRNA isolation
  - RT-PCR
    - Electrophoresis
      - Data Analysis

- Protein Isolation
  - Electrophoresis
    - Western Blotting & ECL
      - Data Analysis
Outline for CPP test

- Nicotine 0.5 mg/kg 1x/hari i.p
- Preference test (CPP)

- Diclofenac 3.2 mg/kg i.p
  Sebelum induksi nikotin

- Nicotine 0.5 mg/kg 1x/hari i.p
- Preference test (CPP)

Extinction 7 days

- Diclofenac 3,2 mg/kg i.p
  Before nicotine induction
Research design

Rat habituation

Pre conditioning

Dependence group

Post Conditioning

Conditioning with nicotine

Conditioning with nicotine & Diclofenac

Organ isolation (mRNA & Protein)

Spectrophotometry

PCR

WB

Dependence group

Conditioning with nicotine

Organ isolation (mRNA & Protein)

Spectrophotometry

PCR

WB

Relapse group

Conditioning with nicotine

Post Conditioning

Extinction

Relapse with nicotine

Relapse with nicotine & Diclofenac

Organ isolation (mRNA & Protein)

Spectrophotometry

PCR

WB
Based on CPP test, nicotine induced dependency in rats, and diclofenac reduced nicotine dependency.
Expression level of GLUT-1 mRNA in Hipocampus

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GLUT-1

GAPDH

Dependence

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Relapse

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Expression level of GLUT-1 mRNA in Bulbus

GLUT-1

GAPDH

Dependence

Relapse

Expression level of GLUT-1 mRNA in Bulbus

GLUT-1

GAPDH

Dependence

Relapse
Expression level of GLUT-1 mRNA
Expression level of GLUT-1 receptor in Hipocampus

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Dependence

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Expression level GLUT-1 receptor in bulbus

GLUT-1

Aktin

Relapse

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Dependence

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<td>N+D (R)</td>
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Expression level of GLUT-1 receptor

- Kontrol: 10.57586936
- Nikotin (D): 13.37536532
- Diklofenak (D): 12.90639586
- Nikotin (R): 14.97378323
- Diklofenak (R): 3.913674037
Expression level of GLUT-1 receptor mRNA

Expression level of Protein Receptor GLUT-1

Post-transcriptional modification: nicotine dependent (D/R) GLUT-1 receptor increased, diclofenac (D/R) decreased GLUT-1 receptor
POSSIBLE MECHANISM OF NICOTINE-DEPENDENT AND GLUT-1 RECEPTOR EXPRESSION, AND DICLOFENAC IN AA PATHWAY AS ANTI-ADDICTION DRUG
POSSIBLE MECHANISM OF NICOTINE-DEPENDENT AND GLUT-1 RECEPTOR EXPRESSION, AND DICLOFENAC IN AA PATHWAY AS ANTI-ADDICTION DRUG
Conclusion

- Nicotine reduced GLUT-1 receptor in mRNA level
- Diclofenac (3,2 mg) treatment increased GLUT-1 receptor in mRNA level
- There is post-transcriptional modification → in nicotine dependent rat brain GLUT-1 receptor in protein level increased
- Diclofenac (3,2 mg) treatment decreased GLUT-1 receptor in protein level