Cosmetics & Toiletries **magazine's 2005**
Advanced Technology Conference

HAIR GROWTH EFFECTS OF ADENOSINE

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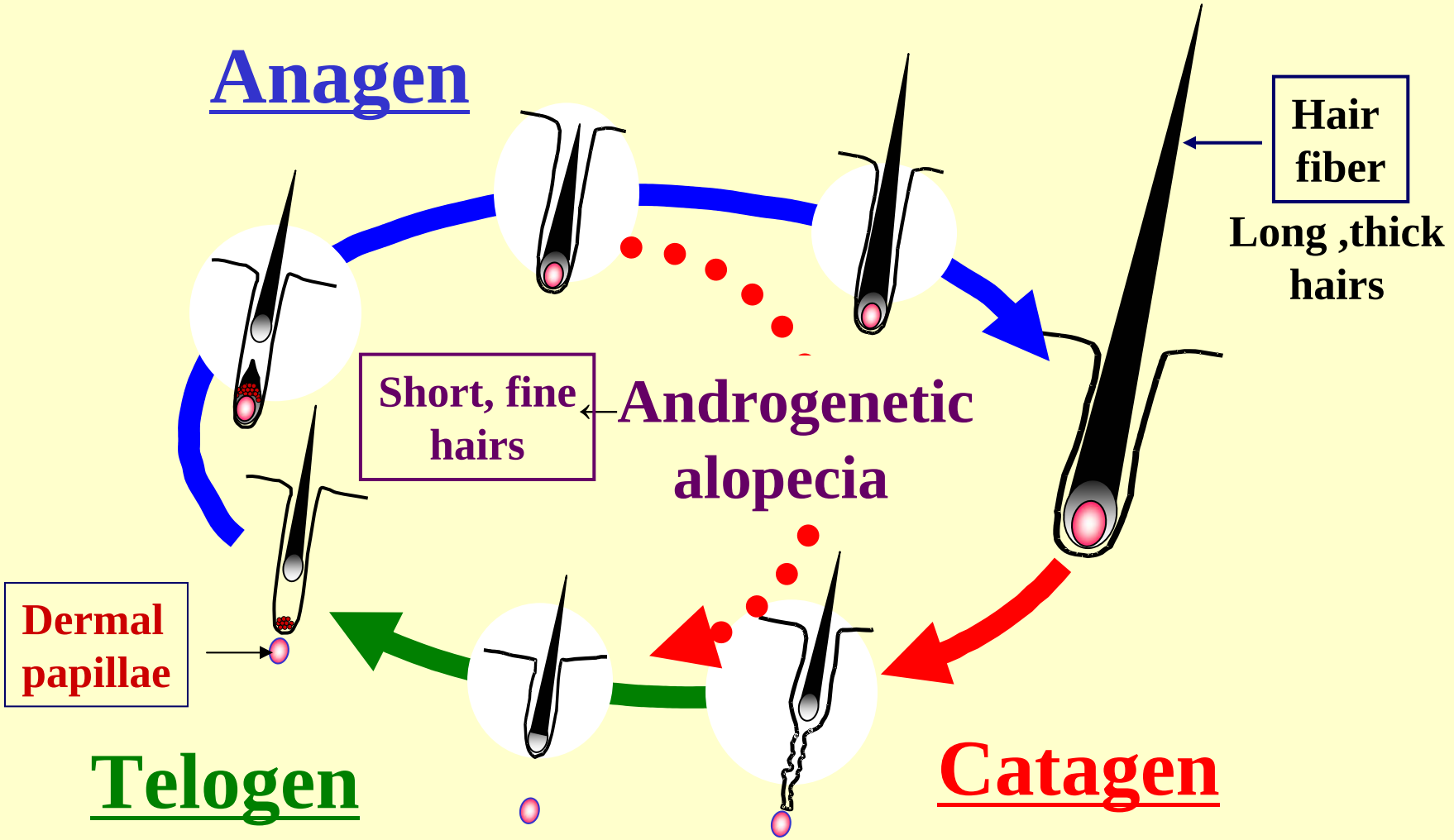
Shiseido Research Center

Tuesday, April 12, 2005
9:15-9:45 am:

the Palais am Funkturm located
in Building 19 at the Messe Fairgrounds
in Berlin.

Hair cycle and androgenetic alopecia

Short, fine hair of androgenetic alopecia results from short duration of anagen and in turn miniaturization of hair cycle.



Minoxidil: hair growth promoter

Effects of topical minoxidil in human:

Messenger and Rundegren
Br J Dermatol 2004; 150: 186-194.

1. Prolongation of anagen
2. Increase of hair follicle size

Effects of orally administration:

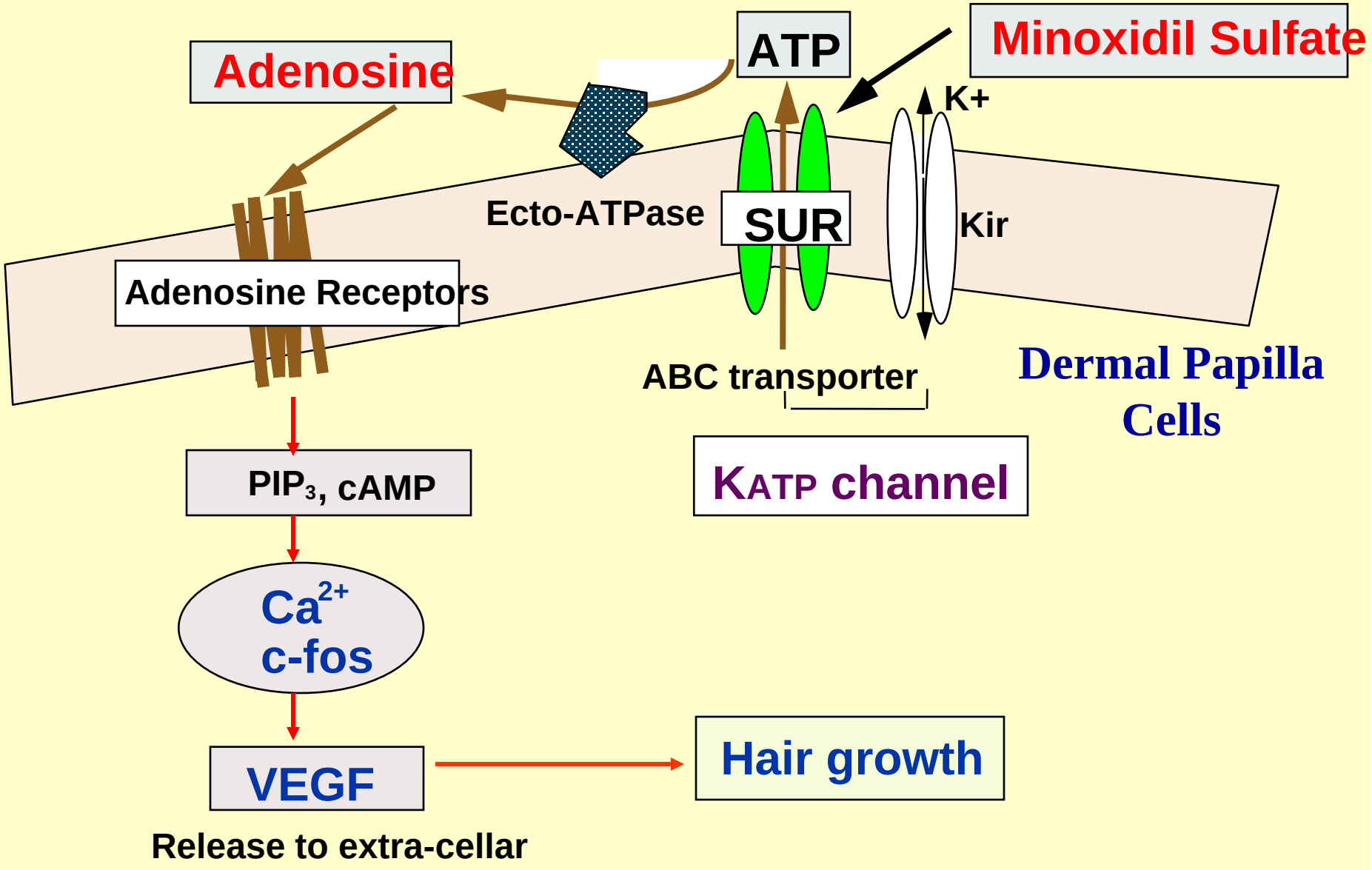
1. Decrease of blood pressure via minoxidil sulfate, as K_{ATP} channels opener

In hair, no clear determination of K_{ATP} channels .

Effects of *in vitro* experiments:

1. Stimulation of cell proliferation: but reported controversial results.
2. Stimulation of prostaglandin E_2 synthesis by dermal papilla cells (DPC)
3. Stimulation of VEGF synthesis by DPC.

Minoxidil-induced hair growth mediated by adenosine



Li *et al.*, *J Invest Dermatol*, **117**, 1594-, 2001

Working hypothesis and research strategy

Working hypothesis:

If minoxidil-induced hair growth is mediated by adenosine receptor on DPC, adenosine also should improve baldness.

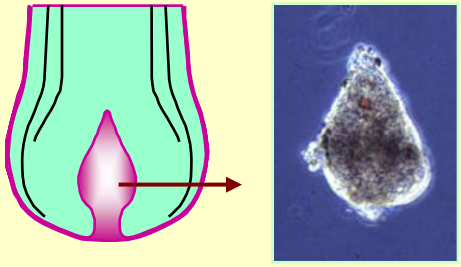
Research objects:

1. Growth factors other than VEGF released by adenosine stimulation on DPC.

2. Intracellular signaling by adenosine stimulation on DPC.

3. Efficacy and safety of topical adenosine application for androgenetic alopecia.

Microarray analysis of adenosine stimulation on DP



DP from
34y, female

Confluent (P4)

Adenosine (10 μM) vs **Control (DMSO)**

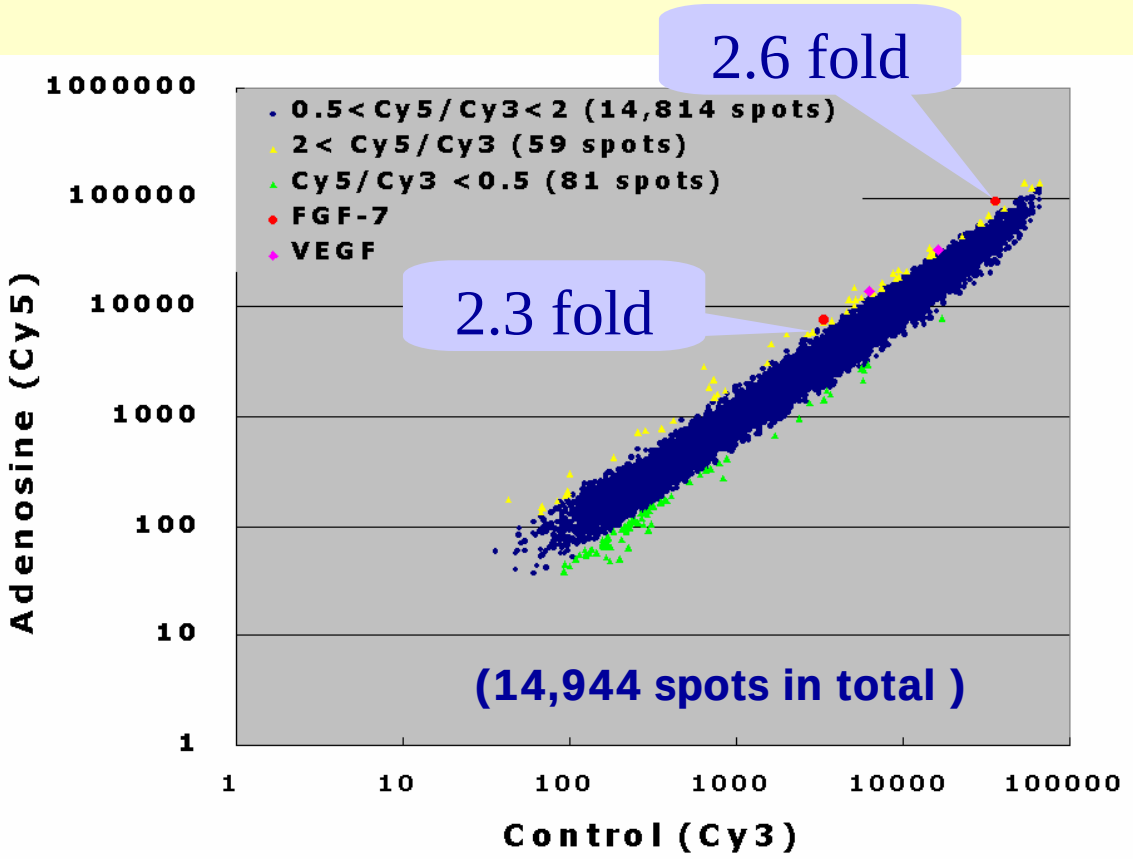
4h

Total RNA

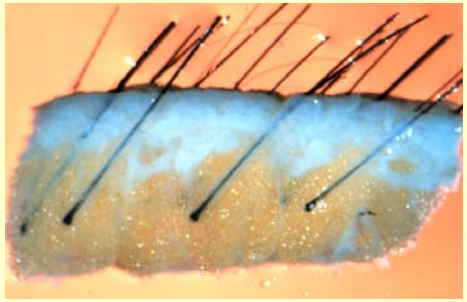
Cy3 / Cy5 label

Human 1 cDNA Microarray (Agilent)

Up-regulation of Fibroblast Growth Factor 7 (FGF-7)

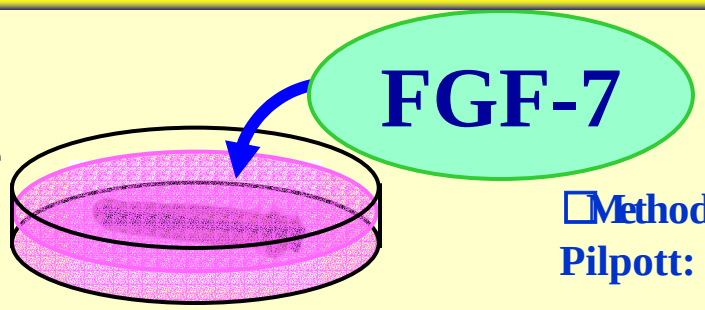


FGF-7 elongates hair fiber *ex vivo*



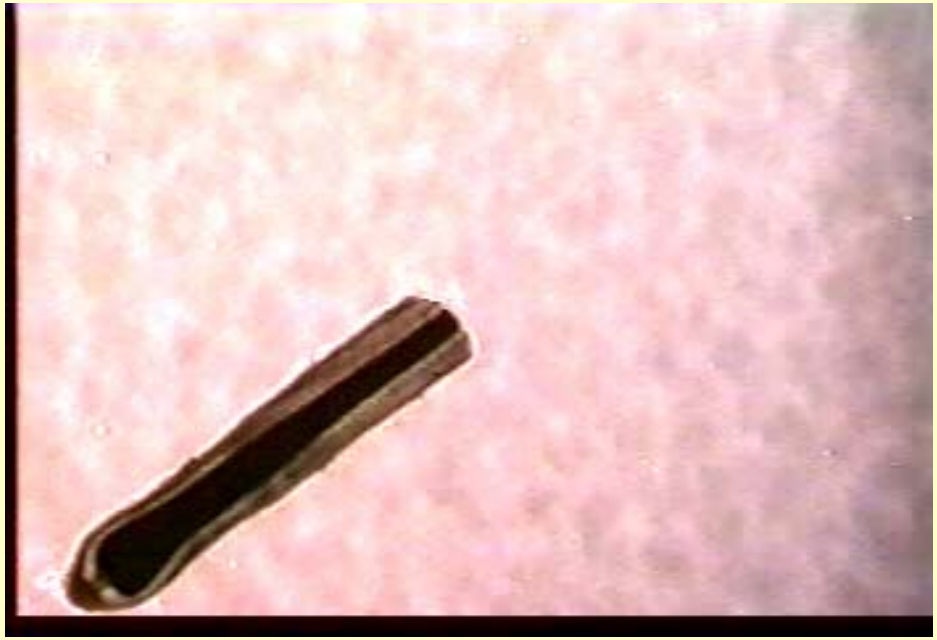
Scalp tissue of a male 42y

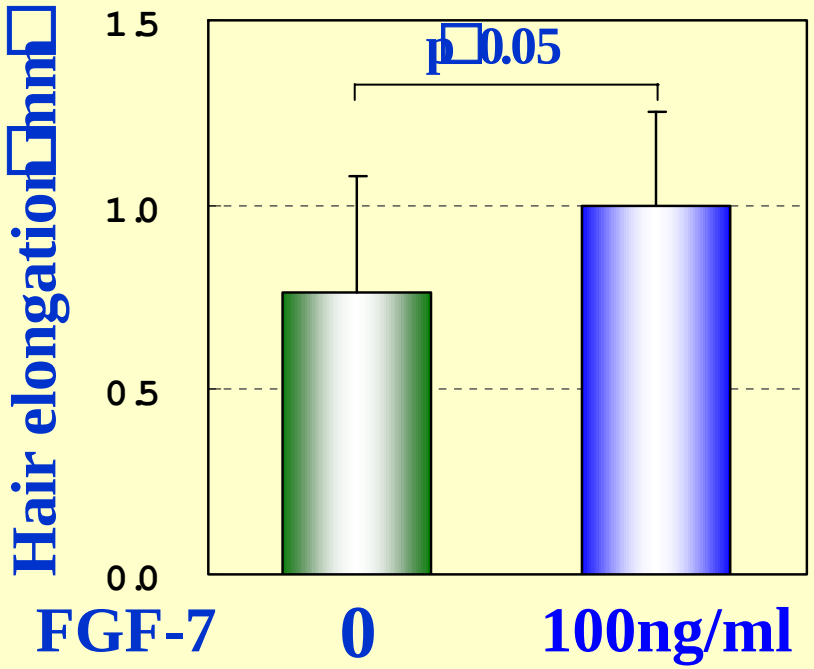
Isolation of hair follicle



Organ culture for 11days

Method;
Pilpott: *J Cell Sci*, 97:463, 1997





FGF-7 stimulates hair growth.

Working hypothesis and research strategy

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If minoxidil-induced hair growth is mediated by adenosine receptor on DPC, adenosine also should improve baldness.

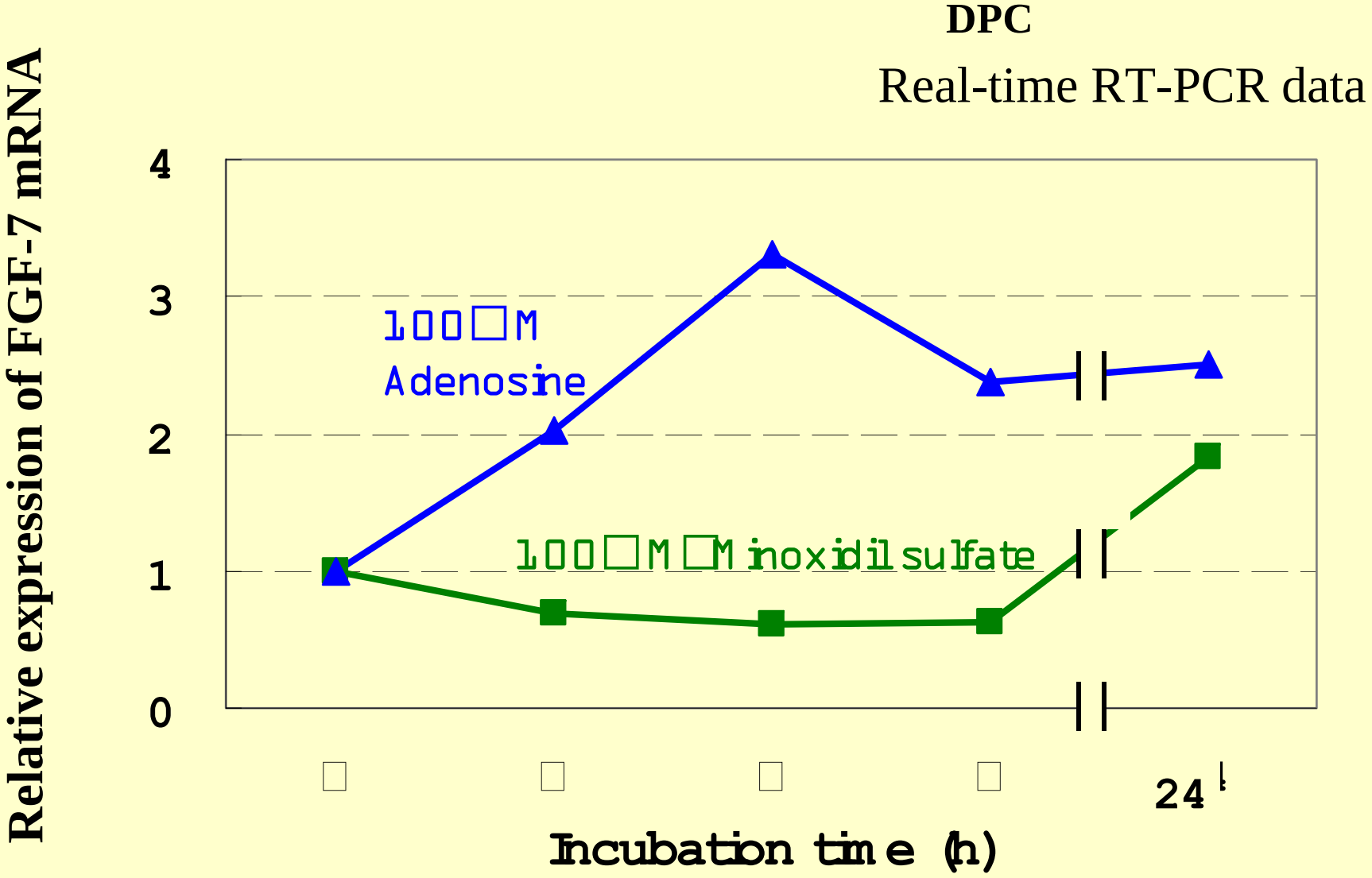
Research objects:

1. Growth factors other than VEGF released by adenosine stimulation on DPC. (FGF-7)

2. Intracellular signaling by adenosine stimulation on DPC.

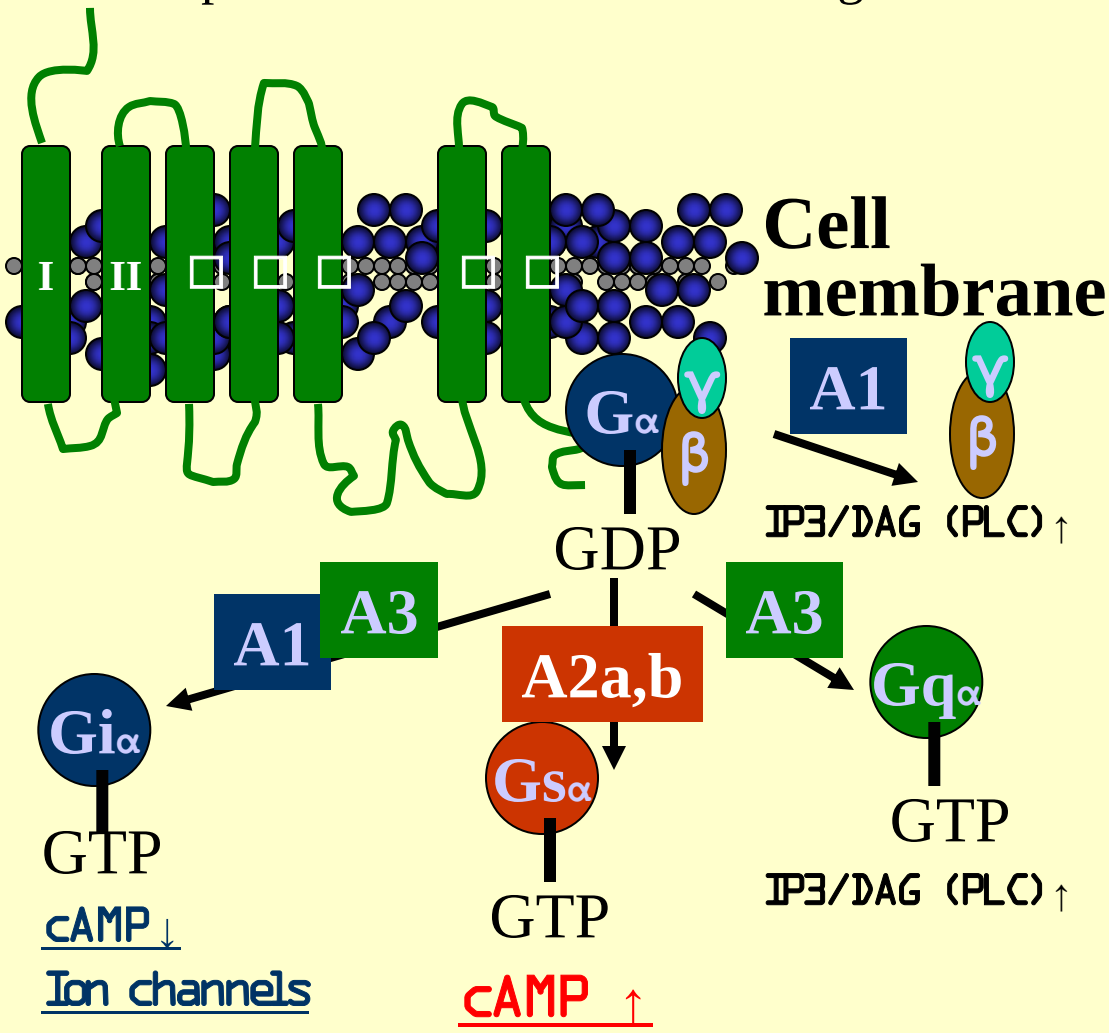
3. Efficacy and safety of topical adenosine application for androgenetic alopecia.

Adenosine, but not minoxidil, directly regulates FGF-7 expression



Adenosine Receptors

Four adenosine receptors, A1, A2a, A2b and A3, have distinct in tissues. Adenylate cyclase is a common effector , which is negatively coupled to A1 and A3 receptors and positively coupled to A2 receptors. They may contribute fine tuning of signaling pathway. A1 and A2 receptors also controls ion exchange.



A1

αi1/2/3 cAMP↓

□βγ□ IP3/DAG (PLC)↑

A2a

αs cAMP ↑

A2b (low affinity)

αs cAMP ↑

A3

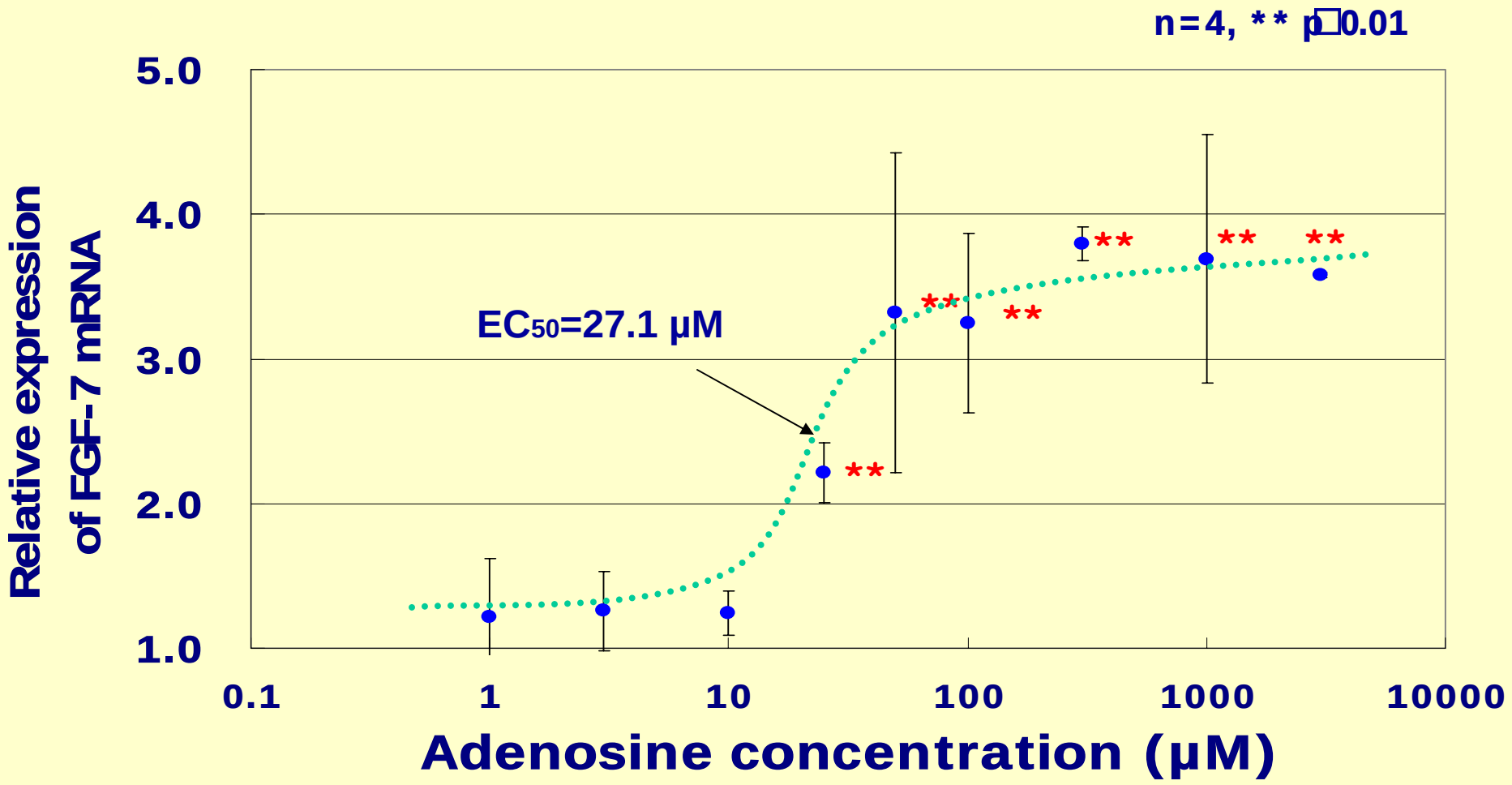
αi2/3 cAMP↓

αq/11 IP3/DAG (PLC)↑

Dose dependency of FGF-7 mRNA up-regulation

DPC

Real-time RT-PCR data

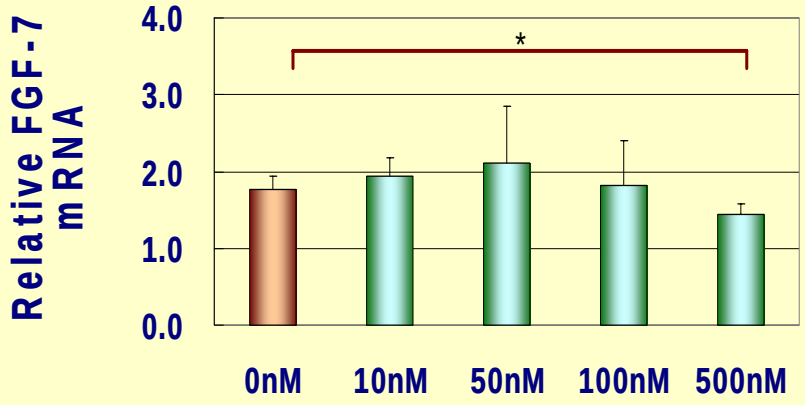


Incubation: 2h

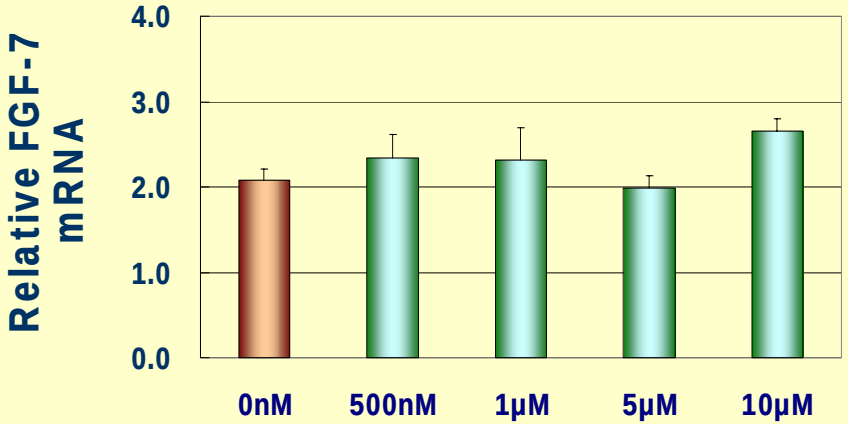
A2b antagonist suppressed FGF-7 up-regulation

Adenosine:25uM

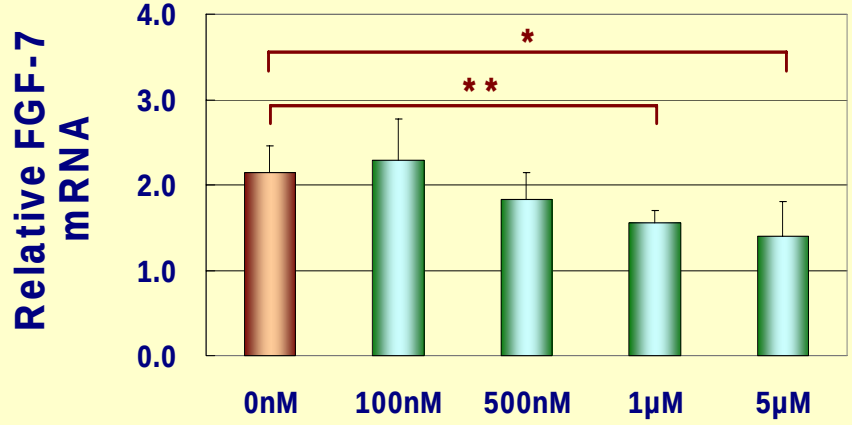
CPX (A1 antagonist)



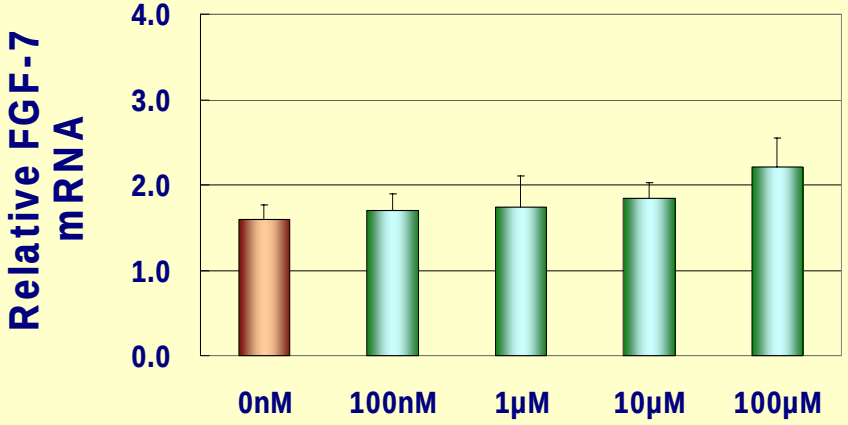
CSC (A2a antagonist)



ALX (A2b antagonist)



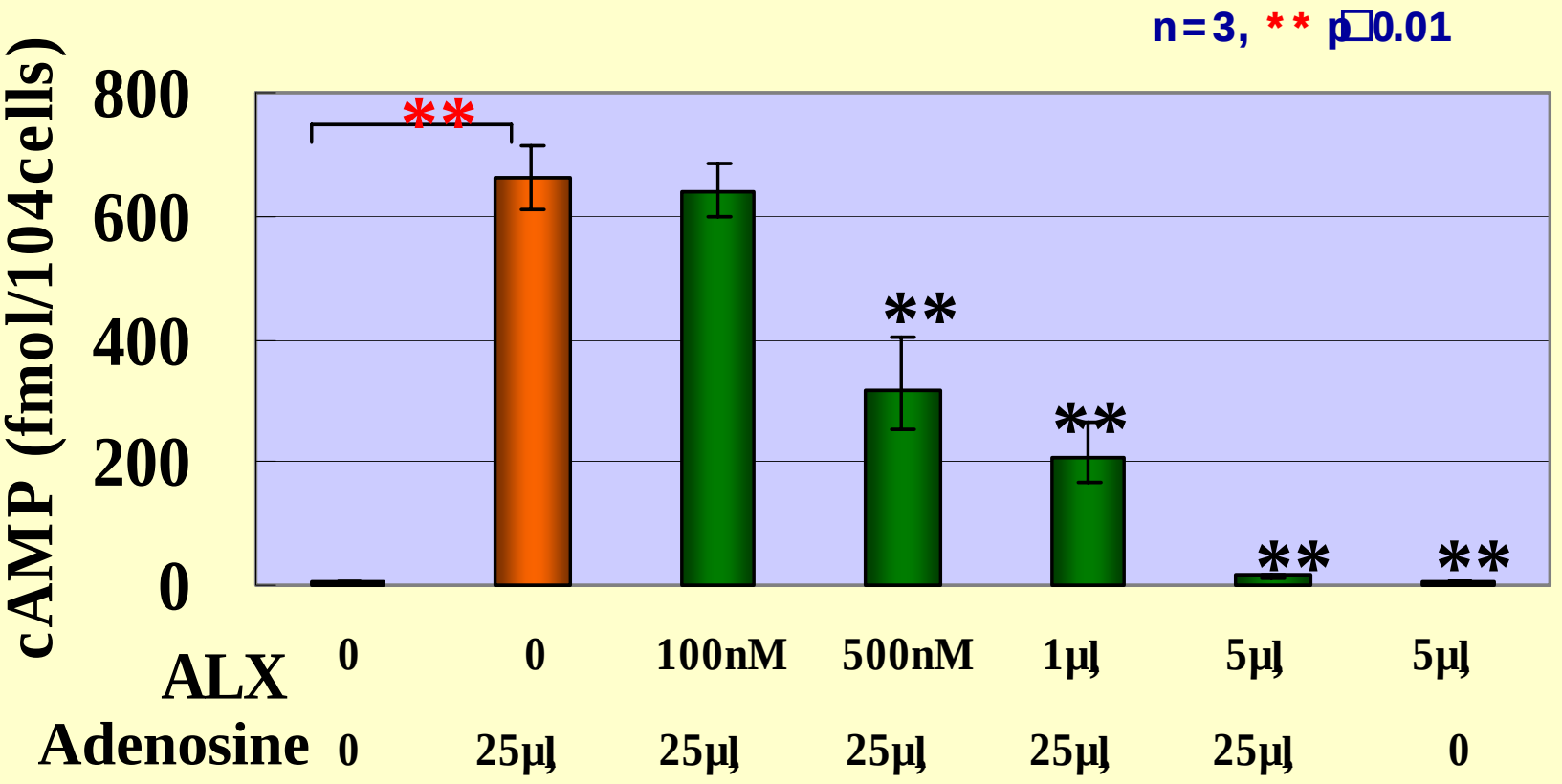
MRS-1191 (A3 antagonist)



CPX : 8-cyclopentyl-1,3-depropylxanthine, CSC : 8-(3-chlorostyryl)caffeine, ALX : alloxazine
MRS-1191 : 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate

n=4, * p□0.05, ** p□0.01

cAMP induction by adenosine and its inhibition by A2b antagonist



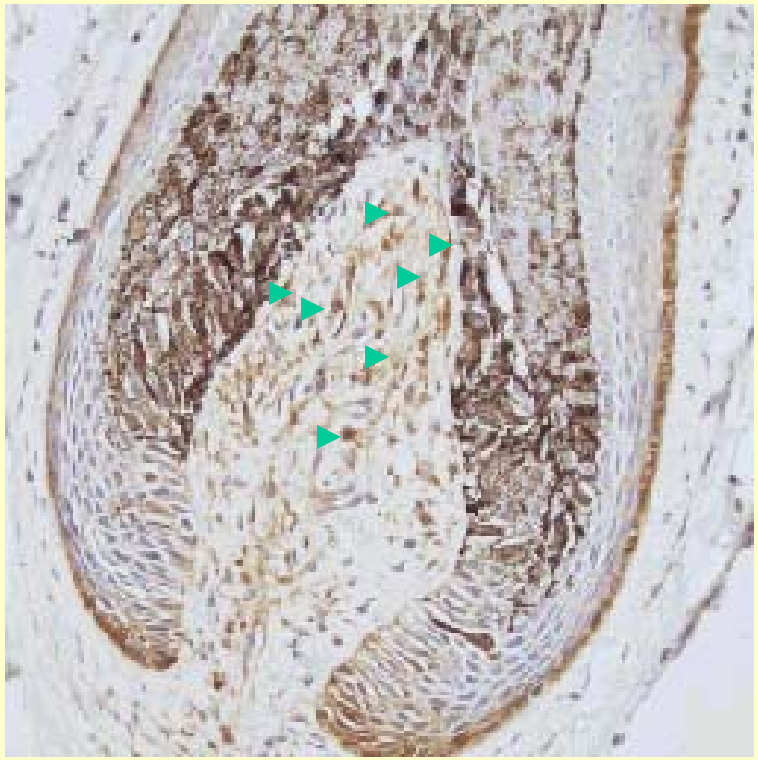
Reaction: 20 min

** p□0.01 ; Compared with adenosine alone

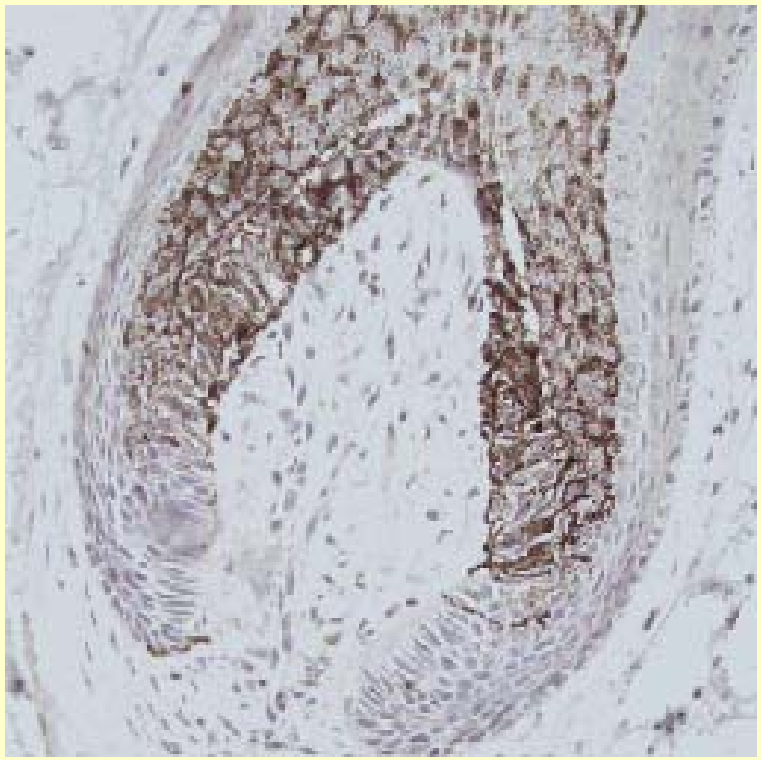
ALX: Alloxazine

Expression of AdoR A2b in human hair DP

Scalp hair from 25y male□DAB



A2b receptor antibody
20μg/ml
Ab1589P□Chemicon□



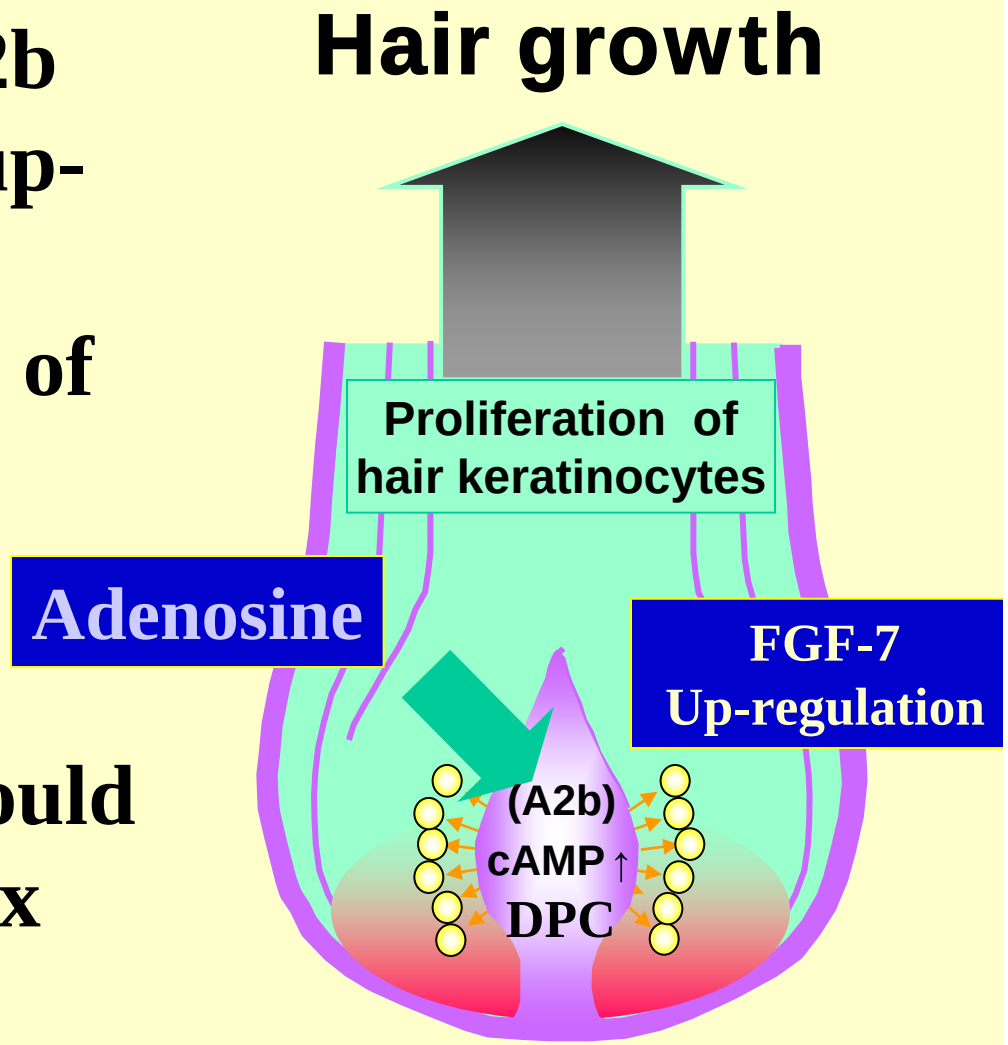
Control
□normal rabbit IgG□
20μg/ml

A2b receptor expression cells indicate with arrow heads.

Hair growth mechanisms by adenosine

Adenosine activates A2b receptor on DPC and up-regulates FGF7 gene expression via increase of intracellular cAMP.

The released FGF-7 would increase the hair matrix cells proliferation and promote hair growth.



Working hypothesis and research strategy

Working hypothesis:

If minoxidil-induced hair growth is mediated by adenosine receptor on DPC, adenosine also should improve baldness.

Research objects:

1. Growth factors other than VEGF released by adenosine stimulation on DPC (FGF-7)

2. Intracellular signaling by adenosine stimulation on DPC. (cAMP via A2b-R)

3. Efficacy and safety of topical adenosine application for androgenetic alopecia.

Clinical Test

Study objective: Evaluation of efficacy for baldness and safety of adenosine lotion versus niacinamide lotion as a positive control.

Study type: Double blind randomized controlled trial.

Modalities of use: Topical applications approximately 3ml of lotions twice daily to vertex area for 6 months.

Efficacy evaluation:

1. Assessment by dermatologists
2. Self-assessment
3. Hair density and diameter in vertex area
4. Global photograph

Safety evaluation: An extensive interview and clinical diagnosis by dermatologists

Volunteers accounting

Randomization in specialized agency Divide by age and type of baldness.

Subjects n=102

Japanese male :
Age:30□50y□ave.41.5y□
Type□or□
□Ogata's classification□
Ogata, Clinic All-Round 2: 101- 1953


□ □
22 29
Subject numbers

0.75□Adenosine lotion□n=51

0.1□Niacinamide lotion□n=51

□ □
23 28
Subject numbers

2 times daily topical use for 6 month

Withdrew n=□
Job transfer at M4

Completed n=51

Completed n=50

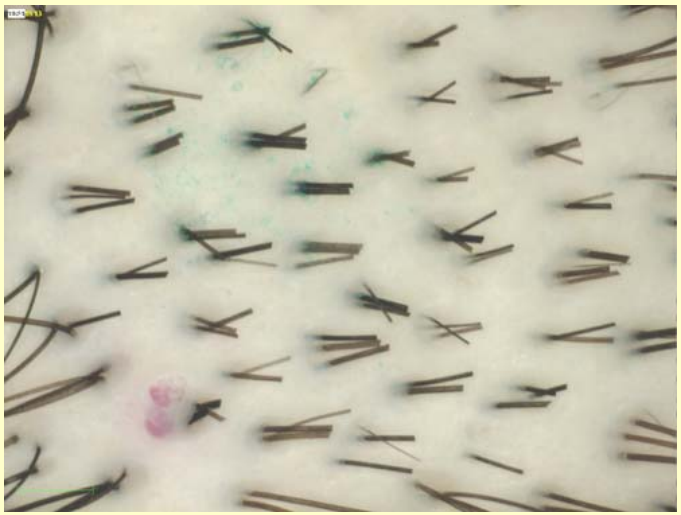
Measurements of hair diameter and density

- Cutting vertex hair with scissors (5×5mm)
- Digital images using video microscope (x40)
- Measurement of hair diameter and number

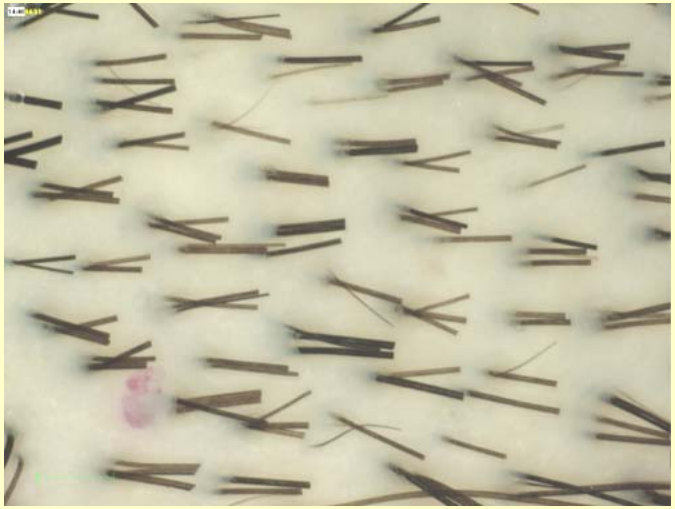
Definition:
Thick hair: 60μm and over in diameter

Vertex scalp

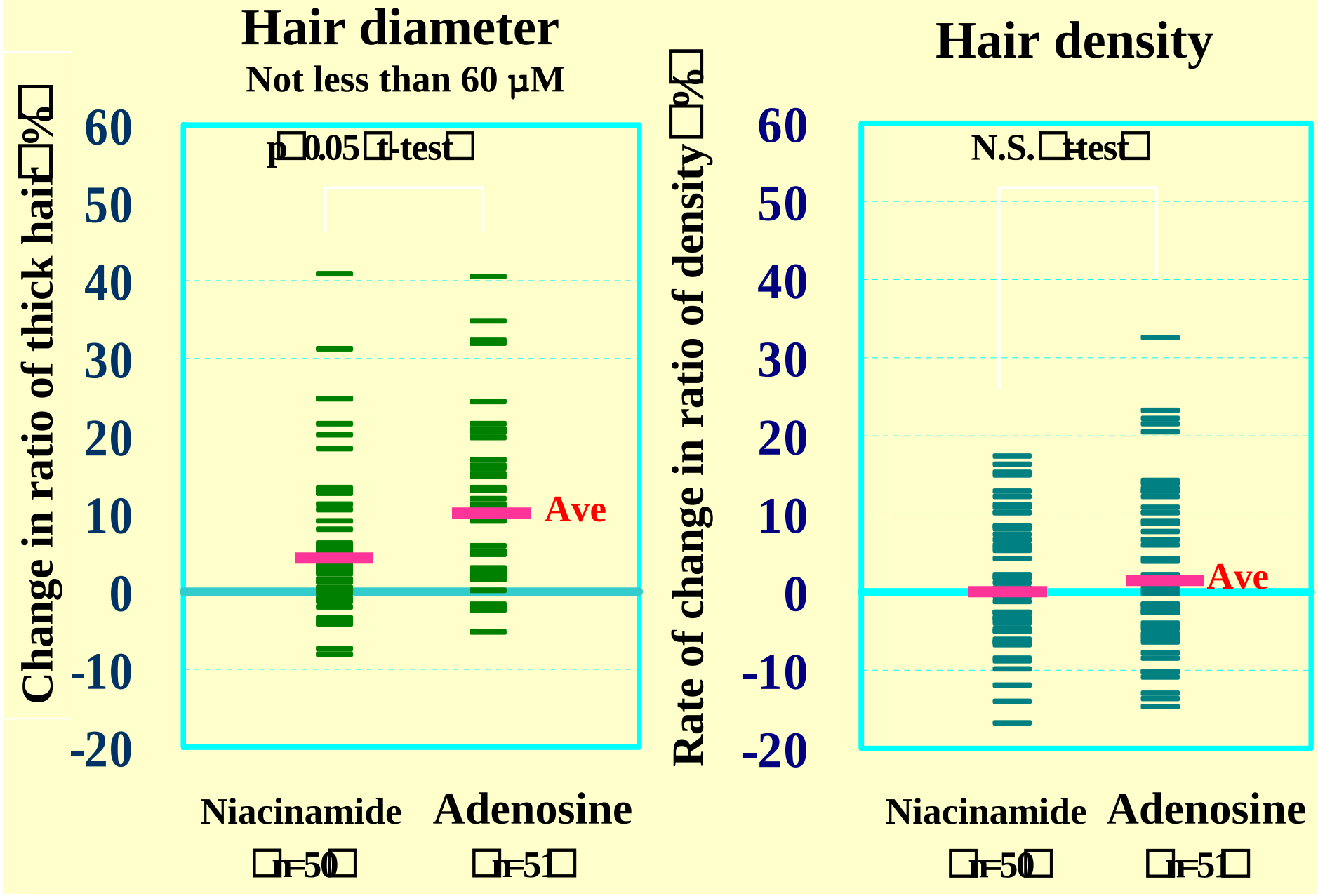
Baseline



6M

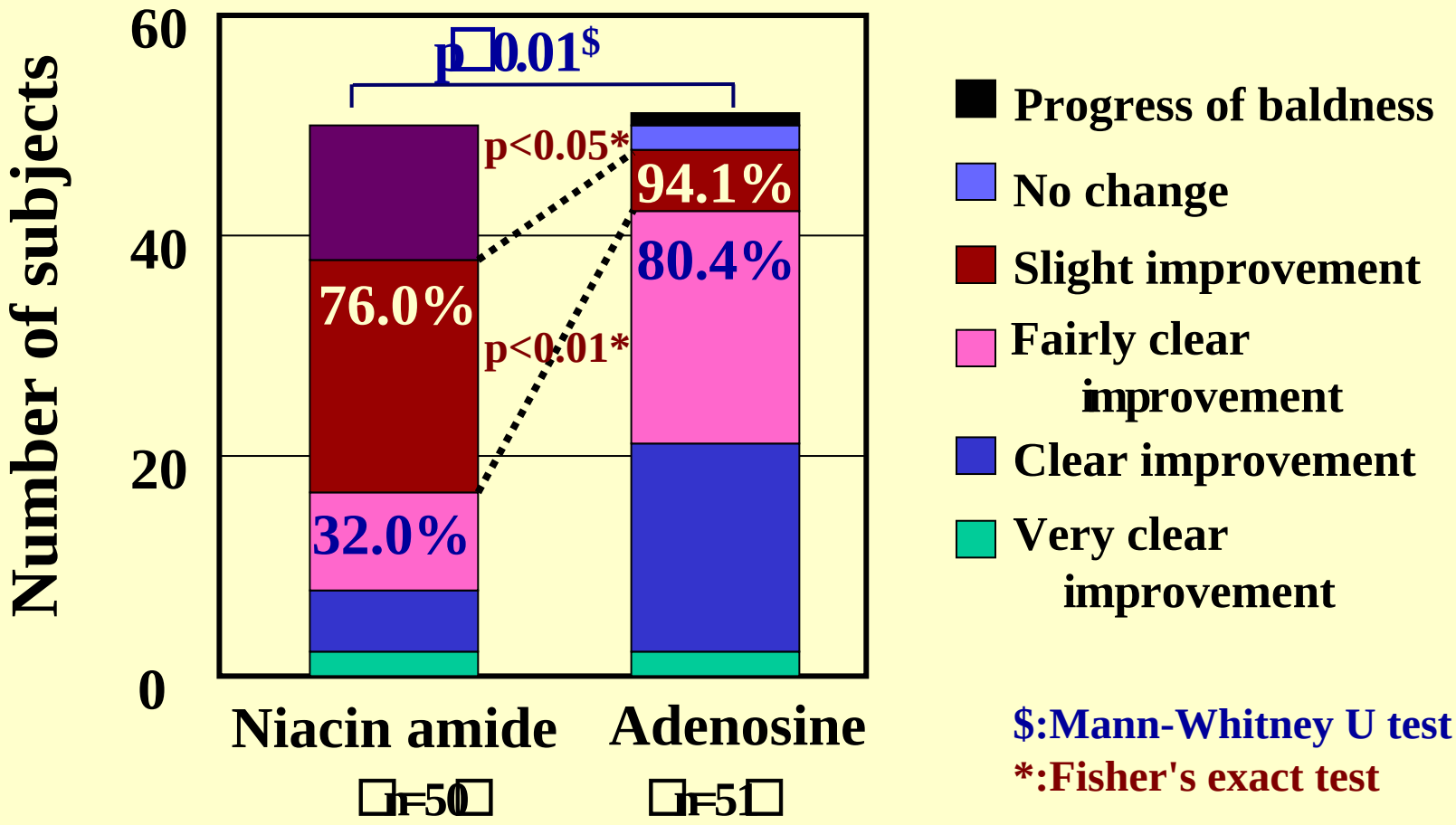


Efficacy at 6M compared with baseline



Global improvement

Two dermatologists evaluate global improvement based on visual evaluation on grounds of hair diameter and density in vertex.



$:Mann-Whitney U test

*:Fisher's exact test

Global photographs

Example of “Clear improvement”

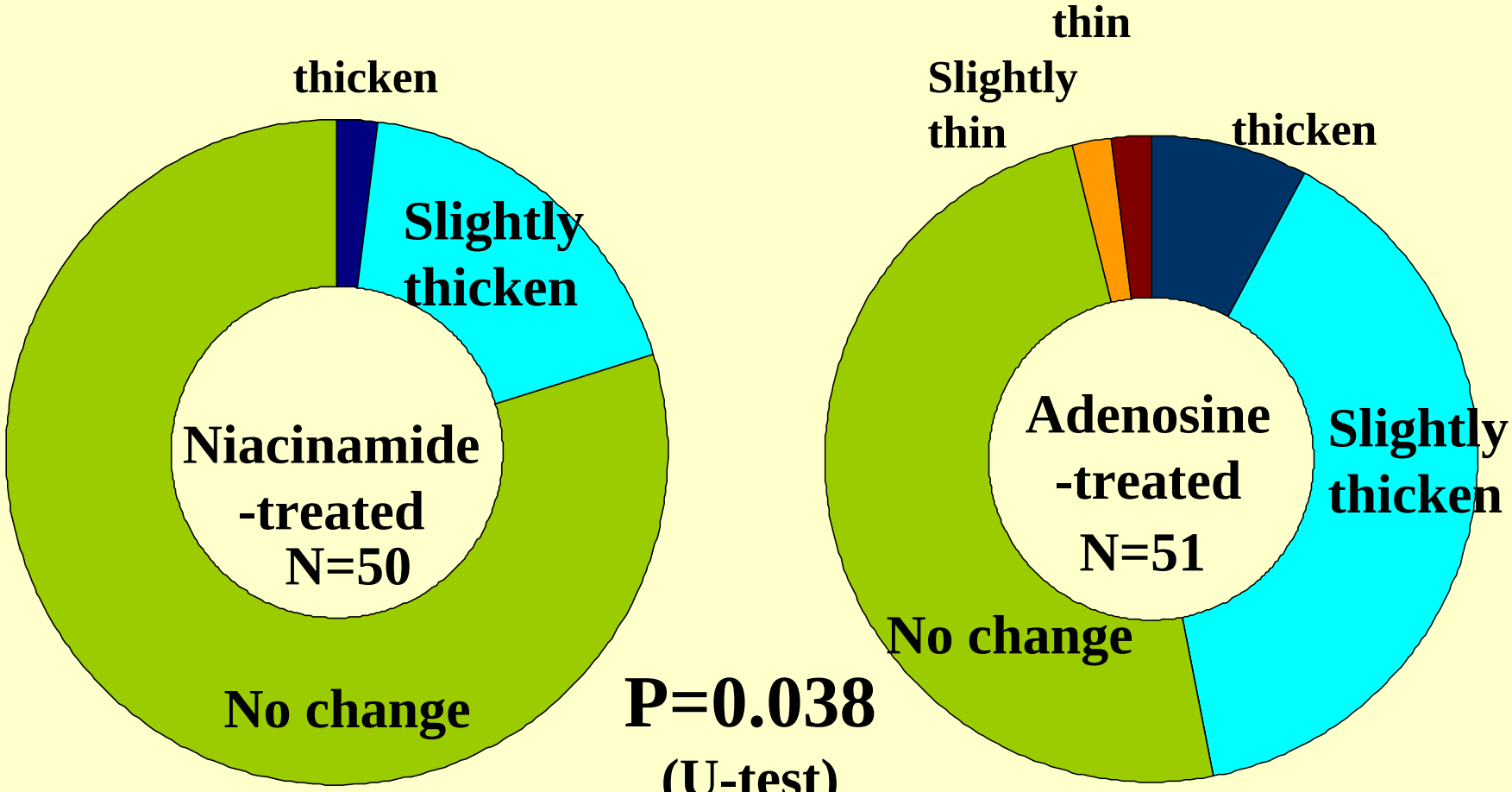
Baseline ☐ 6M

Adenosine-treated Subject

Subjects' self-assessment

Questionnaire about hair thickness

Do you feel that hair thickness change compared with base line?



P=0.038

(U-test)

Safety

Recapitulative table of adverse events

Coetaneous reactions and distemper noted by dermatologists	Adenosine 3M n=51	Adenosine 6M n=51	Niacinamide 3M n=50	Niacinamide 6M n=50	Causal association
Erythema or swelling	☐	☐	☐	☐	no
Eczema	☐	☐	☐	☐	no
Seborrhea	☐	☐	☐	☐	no
Scaling	☐	☐	☐	☐	no
Disorder on Blood Pressure or pulse rate	☐	☐	☐	☐	

☐ disappeared within 1M

Conclusions

1. **Adenosine upregulated FGF-7 gene expression through intracellular cAMP signaling pathway via AdoR A2b in dermal papilla.**

2. **Topical application of adenosine induced hair growth due to thickness of vellus-like hair in men with androgenetic alopecia.**

3. **It is considered that adenosine directly acts on AdoR A2b in dermal papilla and possesses hair growth action similar to minoxidil.**

Collaborators

Shiseido Research Center:
Masashi Ogo, MS
Yosuke Nakazawa, PhD

Hair growth mechanisms

The University of Tokushima:
Prof. Seiji Arase, MD, PhD

Shiseido Research Center:
Masato Iino, MS
Ritsuko Ehama, MS
Tokuro Iwabuchi, PhD

A randomized trial

Watanabe Dermatological Clinic:
Yasushi Watanabe, MD, PhD

Shinjuku Biru Clinic:
Takashi Nagashima, MD, PhD

Shiseido Research Center:
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Akihiro Ishino, PhD
Masaaki Uemura, MS