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# Influence of VKORC1 Polymorphism on the Pharmacokinetics of Vitamin $K_2$ (Menatetrenone) and its Epoxide Metabolite (Menatetrenone Epoxide) in Healthy Volunteers

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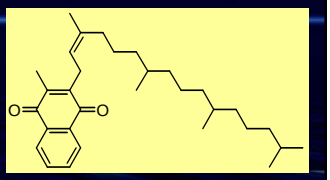
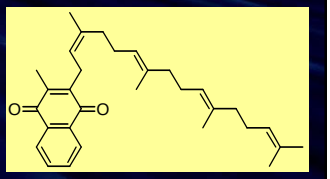
# Introduction

- Vitamin K separate two types
- Vitamin K1 (phylloquinone)
  Be synthesized by plants



- Vitamin K2 (menaquinone-n, MK-n)
  Be synthesized by bacteria
  Menatetrenone
  : used as a treatment agent of osteoporosis



- They are essential for the modulation of glutamic acid residues of substrate proteins into γ-carboxyglutamic acid(Gla) residues

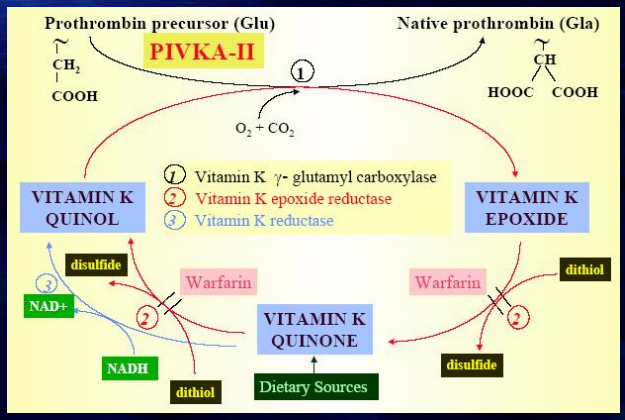
# Introduction

- Gla-proteins
  - Clotting factor II, VII, X : Hemostatic roles
  - Bone Gla-protein : Bone metabolism
  - Matrix Gla-protein : Bone metabolism
- Biological role of vitamin K
  - Cofactor for blood coagulation
  - Cofactor for Bone related re-absorption, metabolism and elimination
  - Increase bone mineral density
  - Reduce fracture rate

# Introduction

- Vitamin K epoxide reductase (VKOR)

  - The enzyme for the recycling of vitamin K form its oxidized form




# Introduction

- Vitamin K epoxide reductase complex subunit 1 (VKORC1)

  - The main target enzyme of anticoagulant warfarin dosage

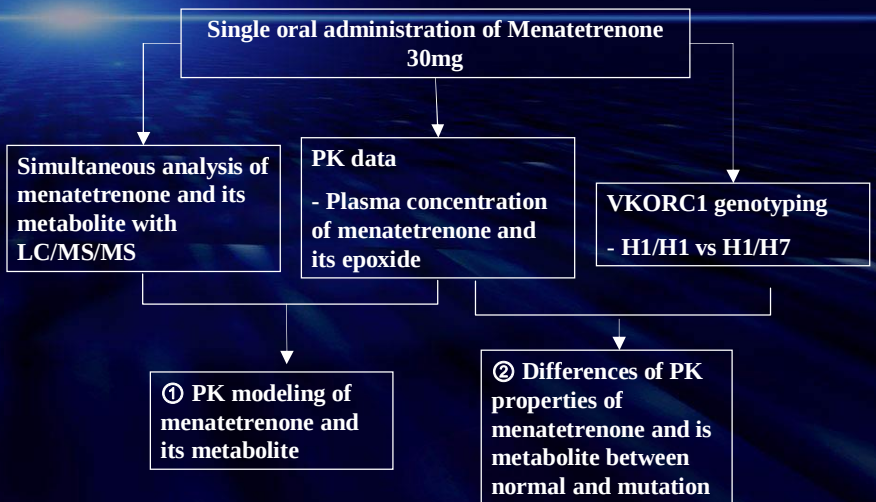
- The relation betwrrn single nucleotide polymorphism (SNP) of VKORC1 and wafarin dose adjustment

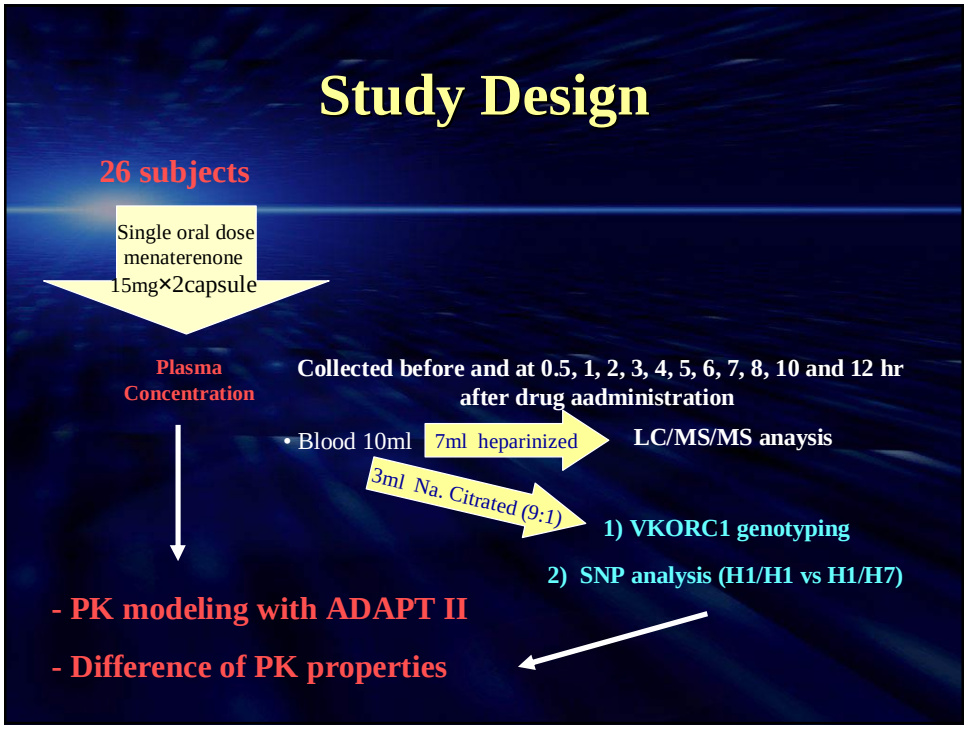
- However there have been no previous studies that how SNP of VKORC1 affect on the metabolism of vitamin $K_2$ (menatetrenone).

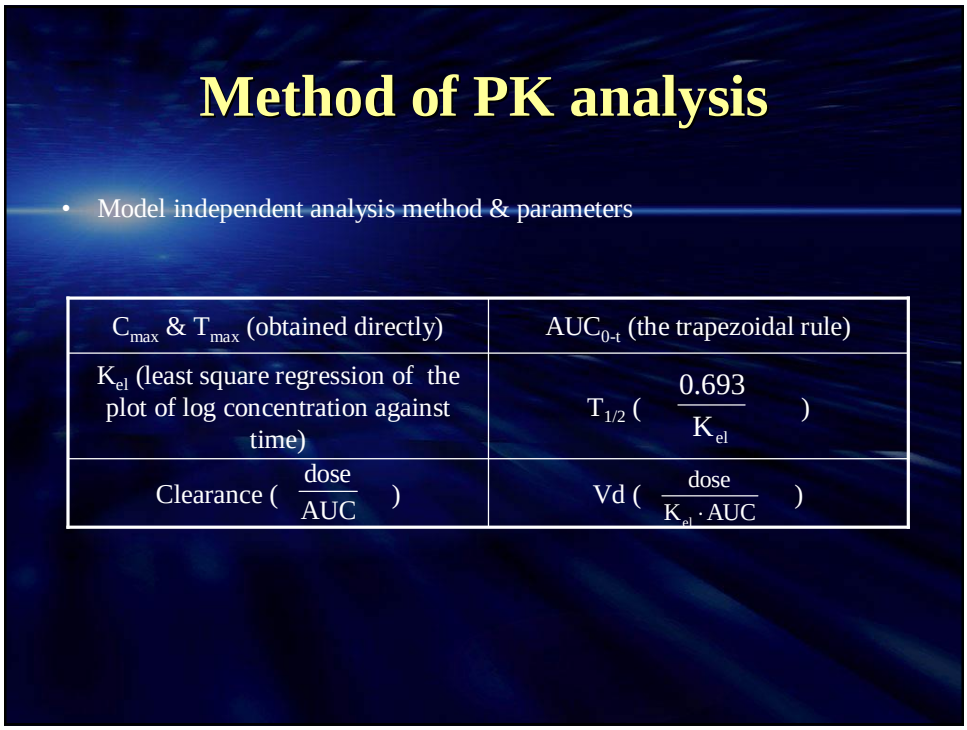
# Objective

- To clarify the relationship between the SNP of VKORC1 and Pharmacokinetic of vitamin $K_2$ (menatetrenone) and vitamin $K_2$ epoxide (menatetrenone epoxide) in healthy male.

# Summary of study

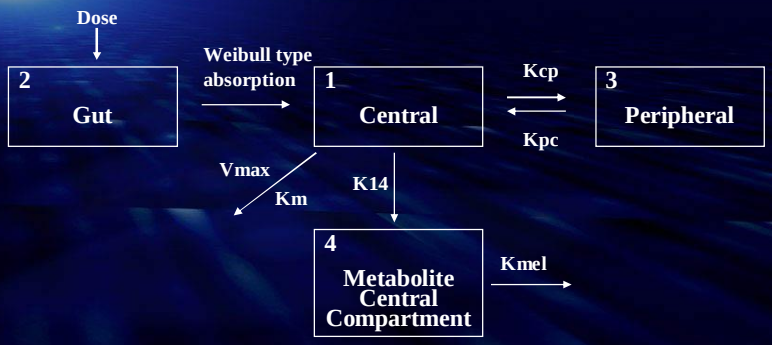



Study Design
26 subjects
Single oral dose menaterenone 15mg×2capsule
Plasma Concentration
Collected before and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hr after drug aadministration
• Blood 10ml
7ml heparinized
LC/MS/MS anaysis
3ml Na. Citrated (9:1)
1) VKORC1 genotyping
2) SNP analysis (H1/H1 vs H1/H7)
- PK modeling with ADAPT II
- Difference of PK properties


Method of PK analysis
• Model independent analysis method & parameters
$C_{max}$ & $T_{max}$ (obtained directly)
$AUC_{0-t}$ (the trapezoidal rule)
$K_{el}$ (least square regression of the plot of log concentration against time)
$T_{1/2}$ ( $\frac{0.693}{K_{el}}$ )
Clearance ( $\frac{dose}{AUC}$ )
Vd ( $\frac{dose}{K_{el} \cdot AUC}$ )

# Method of PK analysis

- Model dependent analysis method & parameters (ADAPT II program)




# Method of PK analysis

- Vitamin K is High oily material and poor aqueous solubility
- Generally, absorption of oily materials has been variously presented, depending on gastro-intestinal tract condition
- In this case, the first-order or zero-order rate constant absorption kinetic was not successful

- Weibull absorption model

  - Described the complex absorption profile more satisfactorily than a first or zero-order absorption and sigmoidal non-linear absorption kinetics

# Method of PK analysis

- Criteria for the fit of the model and estimated parameters

(1) Visual inspection

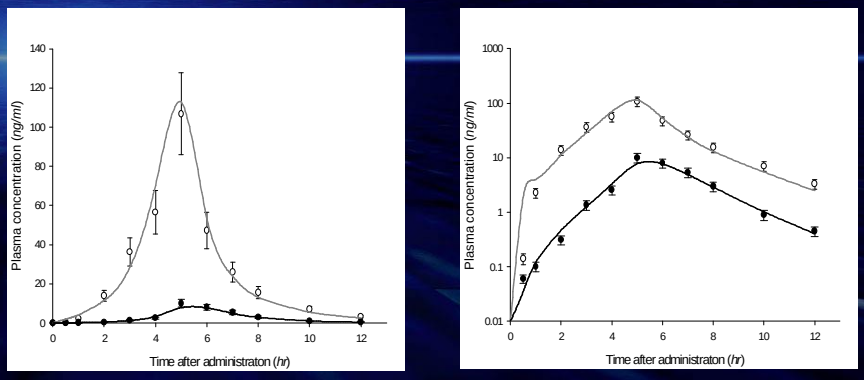
(2) SDRSS (the standardized residual sum-of-squares)
$= \sum(\text{residual}^2/\text{variance})$

(3) ECV (the estimator criterion value)

(4) AIC (Akaike information criterion)
$= m \cdot ln(\text{OWLS}) + 2 \cdot p$ *or* $m \cdot ln(\text{ONLL}) + 2 \cdot (p+q)$

(5) SC (Schwartz criterion)

# Result




- Time course of the plasma concentration in healthy subjects after a oral 30mg of menatetrenone. Each point represents the mean±standard error (n=26). (○) observed plasma concentration of menatetrenone and (●) observed plasma concentration of menatetrenone epoxide. The solid line is the result of the maximum likelihood fitting using the ADAPT II program.

# Result

- Pharmacokinetic parameters of menatetrenone and menatetrenone epoxide after a oral 30mg dose (n=26)

| Model independent parameters (mean ± SEM) | | |
|---|---|---|
| Parameter | Menatetrenone | Menatetrenon-epoxide |
| $C_{max}$ (ng/ml) | 112.5 ± 19.2 | 13.9 ± 3.1 |
| $T_{max}$ (h) | 4.5 ± 0.2 | 5.1 ± 0.2 |
| $AUC_t$ (ng h/ml) | 312.4 ± 42.1 | 33.0 ± 8.5 |
| $t_{1/2}$ (h) | 1.7 ± 0.1 | 2.7 ± 0.4 |

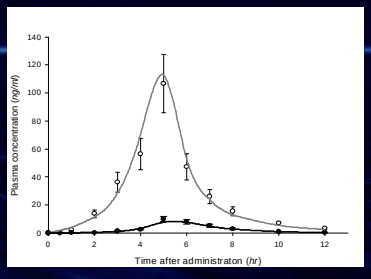
| Model dependent parameters (mean) | |
|---|---|
| $K_m$ (ng) | 0.023 |
| $V_{max}$ (ng/h) | 120400 |
| $V_c$ (L) | 245 |
| $K_{cp}$ ($h^{-1}$) | 34 |
| $K_{pc}$ ($h^{-1}$) | 92 |
| $f$ | 0.035 |
| $A$ ($h^{-B}$) | 0.026 |
| $B$ | 1.798 |
| $K_{14}$ ($h^{-1}$) | 0.845 |
| $K_{mel}$ ($h^{-1}$) | 0.977 |
| $V_4$ (L) | 9.088 |

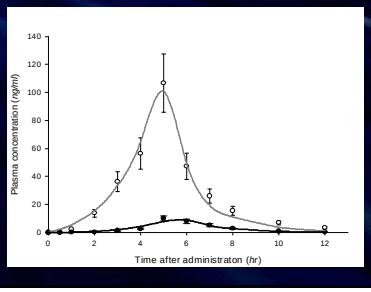
# Result

1. Visual inspection
2. ECV (the estimator criterion value)
3. AIC (Akaike information criterion)
4. SC (Schwartz criterion)

* Estimator :
- WLS (weited least square)
- ML (Maximum likely hood)

| Estimator | WLS | ML |
|---|---|---|
| ECV | 219 | 29 |
| AIC | 142 | 91 |
| SC | 155 | 108 |



# Result

- VKORC1 genotyping

| Haplotype identification code | Haplotype sequence* |
|---|---|
| H1 | CCGATCTCTG |
| H2 | CCGAGCTCTG |
| H3 | CCGGTCCCCG |
| H4 | CCGGTCCGTG |
| H5 | TCGAGCTCTG |
| H6 | TCGGTCCGCG |
| H7 | TCGGTCCGCA |
| H8 | TAGGTCCGCA |
| H9 | TACGTTCGCG |

# Result

- VKORC1 genotyping result (subjects 26)

  - H1/H1 genotype (n=22), H1/H7 genotype (n=4)

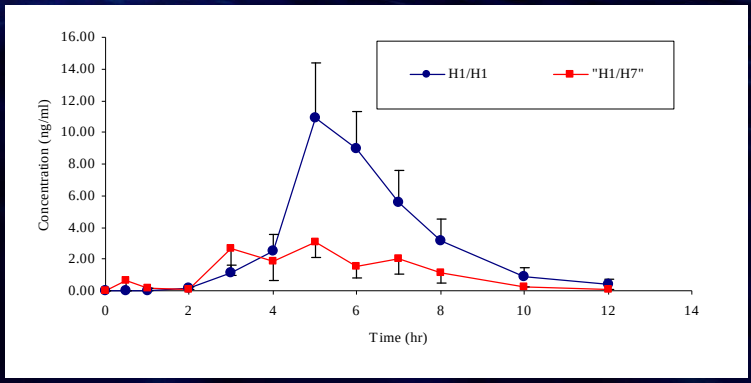
| ID | 1173C>T | 1181T>G | 3730G>A | Genotype |
|---|---|---|---|---|
| 1 | TT | TT | GG | H1/H1 |
| 2 | TT | TT | GG | H1/H1 |
| 3 | TT | TT | GG | H1/H1 |
| 4 | TT | TT | GG | H1/H1 |
| 5 | TT | TT | GG | H1/H1 |
| 6 | TT | TT | GG | H1/H1 |
| 7 | TT | TT | GG | H1/H1 |
| 8 | TT | TT | GG | H1/H1 |
| 9 | TT | TT | GG | H1/H1 |
| 10 | TT | TT | GG | H1/H1 |
| 11 | TT | TT | GG | H1/H1 |
| 12 | TT | TT | GG | H1/H1 |
| 13 | TT | TT | GG | H1/H1 |

| ID | 1173C>T | 1181T>G | 3730G>A | Genotype |
|---|---|---|---|---|
| 14 | TT | TT | GG | H1/H1 |
| 15 | TT | TT | GG | H1/H1 |
| 16 | TT | TT | GG | H1/H1 |
| 17 | TT | TT | GG | H1/H1 |
| 18 | TT | TT | GG | H1/H1 |
| 19 | TT | TT | GG | H1/H1 |
| 20 | TT | TT | GG | H1/H1 |
| 21 | TC | TT | GA | H1/H7 |
| 22 | TT | TT | GG | H1/H1 |
| 23 | TC | TT | GA | H1/H7 |
| 24 | TT | TT | GG | H1/H1 |
| 25 | TC | TT | GA | H1/H7 |
| 26 | TC | TT | GA | H1/H7 |

# Result

- The significant difference of PK properties on VKORC1 SNP

  - Menatetrenone epoxide plasma concentration-time profiles of H1/H1 genotype(n=22, blue) and H1/H7 genotype (n=4, red). The vertical bars represent the standard errors.




# Result

- The significant difference of PK properties on VKORC1 SNP

  - Pharmacokinetic parameters of menatetrenone epoxide carrying genotype H1/H1 and H1/H7

| Parameter | Genotype | | p value* |
|---|---|---|---|
| | H1/H1 (n=22) | H1/H7 (n=4) | |
| $C_{max}$ (ng/ml) | 15.3±3.6 | 5.0±1.0 | < 0.01 |
| $T_{max}$ (h) | 5.4±0.2 | 3.7±0.6 | NS |
| $AUC_t$ (ng h/ml) | 36.6±9.8 | 10.3±1.4 | < 0.05 |
| $t_{1/2}$ (h) | 2.8±0.4 | 3.2±0.7 | NS |

* Significance by t-test, NS ; Not Significantly different

# Conclusion

- The PK analysis of menatetrenone & menatetrenone epoxide
  - Two-compartment with metabolite compartment
  - Absrotion: Weibull absorption model
  - Elimination: Michaelis-Menten elimination process
- The effects of the single nucleotide polymorphism (SNP) in VKORC1 gene on the metabolism of menatetrenone.
  - No significant difference of PK properties of menatetrenone was observed between the normal population and individuals showing H1/H7 haplotype.
  - But, significant differences on $C_{max}$ and AUC of menatetrenone epoxide, and concentration of menatetrenone and menatetrenone epoxide carrying genotype H1/H1 and H1/H7 by time course after administration, were observed.

# Thank you