*vitro* biochemical studies have shown that R227Q reduces the maximum velocity of enzyme reaction ($V_{max}$) to approximately 3.2% of normal activity (12), indicating that R227Q is a hypomorphic mutation retaining a low residual enzymatic activity; 2) missense mutations with less than 0.4% of normal activity usually cause ambiguous or female external genitalia, whereas those with 3–15% of enzymatic activity usually permit masculinization of the external genitalia (3, 24, 25); 3) R227Q mutation has previously been identified in a homozygous status in two Vietnamese brothers only (7, 11), one with micropenis alone and the other with micropenis and hypospadias, and has not been found in patients with ambiguous or female genitalia; 4) TE therapy resulted in subnormal responses in cases 1–3; and 5) a heterozygous R227Q mutation has been identified in two of 100 Japanese control males (this study) and in five Chinese males after examining 543 normal males of various ethnic origins (12). These findings imply that R227Q may be relatively frequent in Asian populations and that the low residual enzyme activity, although it is insufficient for normal male genital development, has permitted a considerable degree of masculinization including micropenis phenotype as well as subnormal response to TE therapy.

Genital findings of cases 1–3 with SRD5A2 mutations are noteworthy in two points. First, case 3 had mild micropenis of −2.4 SD. This implies that even patients with mild micropenis above −2.5 SD may have 5α-reductase-2 deficiency. In this regard, because case 3 was homozygous for both R227Q with residual enzyme activity and V allele with high enzyme activity, this may have served to prevent the development of severe micropenis. Second, micropenis was more severe in case 2 than in case 1, and cryptorchidism was observed in case 3. Because R227Q and G34R retain approximately 3.2% and approximately 1.2% of enzymatic activity, respectively (8, 12), and Y26X is predicted to lose both the ligand (T) and the cofactor (nicotinamide adenine dinucleotide phosphate) binding domains (4, 9), the results of mutations, together with those of the V89L polymorphism, suggest that the degree of genital development is not simply dependent on the 5α-reductase-2 activity. Indeed, genital development is considered to be subject to multiple genetic and environmental factors such as the AR-mediated signal transduction activity and intrauterine T concentration. In this regard, it is unlikely that cryptorchidism in case 3 is due to his young age, because spontaneous descent is rare after 3 yr of age (26), and his testicular descent occurred after treatment.

Furthermore, several matters appear to be worth pointing out for the diagnostic and therapeutic findings of cases 1–3. First, the hCG stimulated T/5αDHT ratio was unequivocally elevated in cases 1–3. This suggests that 5α-reductase-2 deficiency can be diagnosed by standard endocrine studies, even in boys with micropenis only phenotype. Second, case 3 showed low T response in the hCG test and slightly high FSH response in the GnRH test, as has occasionally been described in 5α-reductase-2 deficiency (11, 27). Although this would be ascribed to secondary testicular dysfunction resulting from cryptorchidism (11, 27), such endocrine data may lead to misdiagnosis of defective testicular steroidogenesis unless the hCG stimulated T/5αDHT ratio is examined. Third, urinary steroid hormone profile analysis revealed markedly elevated ratios of 5β to 5α metabolites, as has been reported previously (28, 29). Although this would primarily be due to defective 5α-reductase-2 activity in the liver, which would mainly catalyze adrenal rather than testicular steroid hormones (6, 10, 30), urinary steroid hormone profile analysis is a highly sensitive and noninvasive test and, therefore, appears to be more advantageous than serum androgen measurement for the diagnosis of 5α-reductase-2 deficiency. In addition, it can often identify heterozygotes (this study and Ref. 31). Lastly, TE therapy with a standard dosage showed a subnormal effect, and 5αDHT gel treatment caused sufficient penile growth. This is consistent with impaired activity of 5α-reductase-2 that converts exogenous as well as endogenous T into 5αDHT, and it implies that early diagnosis of 5α-reductase-2 deficiency enables application of appropriate therapy with 5αDHT gel and prevention of adverse effects such as skeletal maturation of TE therapy.

The V89L polymorphism was similar in both allele and genotype frequencies between patients with micropenis and control males. This suggests that V89L polymorphism, although it has been shown to reduce the enzyme activity by approximately 30% (12, 13), had no discernible effect on the development of micropenis in the patients examined here. It remains possible, however, that V89L polymorphism constitutes one of susceptibility factors for the development of androgen-related disorders, so that it may be detected as a positive modifier for micropenis in other patient populations. Indeed, on the basis of an inverse relationship between CAG repeat length at exon 1 and transactivation function or expression level of the AR gene (32, 33), a large number of studies have been performed to examine CAG repeat lengths in patients with androgen-related disorders such as undermasculinization and azoospermia, showing both positive and negative results for the association between expansion of CAG repeat lengths and predisposition to such disorders (15, 34–36).

In summary, the present study suggests that hypomorphic mutations of the SRD5A2 gene can cause micropenis phenotype in Japanese patients, with R227Q mutation being most prevalent, and that V89L polymorphism is unlikely to raise the susceptibility to the development of micropenis. Further studies will permit better clarification of the relevance of the SRD5A2 gene to the development of micropenis.

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# TESTOSTERONE ENANTHATE THERAPY IS EFFECTIVE AND INDEPENDENT OF SRD5A2 AND AR GENE POLYMORPHISMS IN BOYS WITH MICROPENIS

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ABSTRACT

Purpose: We report penile length (PL) responses to testosterone enanthate (TE) therapy for micropenis, and the relevance of the V89L polymorphism of SRD5A2 encoding the 5α-reductase type 2 and CAG repeat length polymorphism of AR encoding the androgen receptor.

Materials and Methods: A total of 53 Japanese boys with micropenis (less than −2.0 SD) 0 to 13 years old who had no SRD5A2 or AR mutation were examined. TE was given at a dosage of 25 mg intramuscularly, and PL was measured at least 4 weeks after the injection. The 2 polymorphisms were determined by direct sequencing.

Results: PLs became −2.0 SD or greater in all the boys after TE therapy (1 injection in 4 boys, 2 in 28, 3 in 19 and 4 in 2), with a significant increase in the medians of PLs (from 2.5 to 3.5 cm, $p < 0.0001$) and SD score, (from −2.6 to −0.7, $p < 0.0001$). The increment in actual PL at the first injection ranged from 0.2 to 1.5 cm (median 0.6) and was independent of age ($r = 0.22$, $p = 0.12$) and body surface area ($r = 0.11$, $p = 0.43$), while that in PL SD score at the first injection ranged from 0.3 to 2.5 (1.0) and was inversely correlated with age ($r = -0.33$, $p = 0.02$) and body surface area ($r = -0.37$, $p = 0.008$). The actual PL increment at the first injection was also unrelated to initial PL ($r = -0.03$, $p = 0.81$). The median of actual PL increments at the first injection was similar among boys with V/V, V/L and L/L genotypes of SRD5A2 (0.6 cm in 18, 0.7 cm in 30 and 0.5 cm in 5, respectively, $p = 0.77$), and between boys with and without long CAG repeats (26 or greater) of AR (0.65 cm in 6 and 0.6 cm in 47, respectively, $p = 0.77$). In addition, there was no significant correlation between actual PL increment at the first injection and CAG repeat length ($r = 0.06$, $p = 0.67$).

Conclusions: Our results suggest that administration of 25 mg TE is effective for micropenis in prepubertal boys with no SRD5A2 or AR mutation, with variable but significant PL increments, and that the penile responsiveness to TE therapy is independent of the V89L and the CAG repeat length polymorphisms.

KEY WORDS: penis/abnormalities, testosterone 5-alpha-reductase, polymorphism (genetics)

Testosterone (T) therapy has been advocated for micropenis in infancy to childhood.[1,2] T is usually given intramuscularly in doses of 25 to 50 mg, although it can be given by daily topical application.[1–5] Injection therapy may be repeated a few times at roughly 4-week intervals until sufficient penile length (PL) has been achieved.

Exogenous as well as endogenous T is converted into 5α-dihydrotestosterone by 5α-reductase type 2 in the external genitalia, and subsequently 5αDHT binds to androgen receptor (AR) much more effectively than T, leading to penile growth.[6] Thus, the 5α-reductase type 2 activity and the AR function have a crucial role in the PL response to T therapy. In this context the 5α-reductase type 2 gene (SRD5A2) on 2p23 carries a V89L polymorphism (Val to Leu substitution at the 89th codon) at exon 1, which has been shown to decrease 5α-reductase type 2 activity by approximately 30%, and previous studies have suggested that this polymorphism may decrease the occurrence of androgen dependent prostate cancer.[7,8] Similarly, the AR gene on Xq11 to 12 harbors a CAG repeat length polymorphism at exon 1, which has been found to be inversely correlated with transactivation function of the AR gene, and previous studies have revealed that expanded CAG repeat lengths are frequently associated with infertility and under-masculinization as well as decreased prostate growth after T therapy in hypogonadal men.[9–12] Therefore, it is possible that the 2 polymorphisms are also relevant to the effect of T therapy for micropenis.

To our knowledge there has been no report documenting the effects of T therapy in a large number of boys with micropenis, nor has the relationship been analyzed between the PL increment and the V89L or the CAG repeat length polymorphism. Thus, we systemically studied the PL responses and the relevance of the 2 polymorphisms to T therapy.

## SUBJECTS AND METHODS

*Subjects.* This study consisted of 53 Japanese boys 0 to 13 years old (median 7) who received T therapy because of micropenis. All of the boys satisfied the selection criteria of stretched PL less than −2.0 SD as compared with the age matched Japanese PL standards, lack of hypospadias, absence of recognizable extragenital anomalies, 46,XY karyo-

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TABLE 1. *Effects of intramuscular testosterone enanthate therapy in boys with micropenis*

| | Age (yrs) | BSA ($m^2$) | TE Dosage (mg) | Penile Length (cm) | | | Penile Length (SD score) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Before | After | p Value* | Before | After | p Value* |
| Total (53 pts): | | | | | | | | | |
| Mean ± SE | 6.5 ± 0.5 | 0.942 ± 0.045 | 59.0 ± 2.3 | 2.50 ± 0.06 | 3.70 ± 0.08 | | −2.74 ± 0.09 | −0.72 ± 0.09 | |
| Median | 7 | 0.96 | 50 | 2.5 | 3.5 | <0.0001 | −2.6 | −0.7 | <0.0001 |
| Range | 0–13 | 0.37–1.59 | 25–100 | 1.5–3.0 | 2.7–5.0 | | −4.6–−2.1 | −2.0–0.5 | |
| Group 1 (24 pts): | | | | | | | | | |
| Mean ± SE | 7.3 ± 0.8 | 1.001 ± 0.069 | 53.1 ± 3.5 | 2.77 ± 0.06 | 3.97 ± 0.12 | | −2.23 ± 0.02 | −0.56 ± 0.09 | |
| Median | 9 | 1.07 | 50 | 3.0 | 4.0 | <0.0001 | −2.2 | −0.6 | <0.0001 |
| Range | 0–13 | 0.37–1.52 | 25–75 | 2.2–3.0 | 2.8–5.0 | | −2.5–−2.1 | −1.3–0.5 | |
| Group 2 (29 pts): | | | | | | | | | |
| Mean ± SE | 5.9 ± 0.6 | 0.893 ± 0.060 | 63.8 ± 2.9 | 2.28 ± 0.08 | 3.48 ± 0.07 | | −3.16 ± 0.10 | −0.91 ± 0.13 | |
| Median | 6 | 0.90 | 50 | 2.5 | 3.5 | <0.0001 | −2.9 | −1.0 | <0.0001 |
| Range | 0–11 | 0.38–1.59 | 50–100 | 1.5–3.0 | 2.7–4.3 | | −4.6–2.6 | −2.0–0.5 | |
| p Value† | 0.15 | 0.19 | 0.04 | 0.0001 | 0.001 | | <0.0001 | 0.05 | |

* Before vs after therapy.
† Difference between groups 1 and 2.

type and no demonstrable SRD5A2 or AR gene mutation.[13–15] Since −2.5 SD and −2.0 SD have been used as the lower limit of normal PLs, the boys were divided into 2 groups—group 1 consisted of 24 boys with borderline micropenis from −2.1 to −2.5 SD, and group 2 consisted of 29 boys with definite micropenis less than −2.5 SD.[1,2] Cryptorchidism was present in 3 boys in group 1 (2 bilateral and 1 unilateral) and in 2 boys in group 2 (1 bilateral and 1 unilateral), and the testis was palpable in the inguinal region in all 5. Basal serum gonadotropin and T values were within age and pubertal tempo matched Japanese reference data in most boys, except for low follicle-stimulating hormone levels in a 9-year-old boy in group 1 (0.2 mIU/ml) and in a 7-year-old boy in group 2 (less than 0.2 mIU/ml).[16] However, more detailed endocrine studies were not performed, so that possible underlying endocrine disorders such as gonadotropin deficiency remained unidentified in the 53 boys. In each boy SD score (SDS) for PL and body surface area (BSA) were obtained.

*Testosterone therapy.* T was injected intramuscularly as testosterone enanthate (TE) at a dosage of 25 mg. PL was measured at least 4 weeks after the injection. The administration of TE was repeated up to 4 times, depending on the PL response and the request of the patients and/or parents.

*Polymorphism analysis of the SRD5A2 and AR genes.* After informed consent was obtained the V89L polymorphism of the SRD5A2 gene and the CAG repeat length polymorphism of the AR gene were examined by direct sequencing, using the leukocyte genomic DNA of each boy. The methods have been reported previously.[14,15]

*Statistical analysis.* After the normality of variables was excluded by the chi-square test the statistical significance of the median was examined by the Mann-Whitney U test for 2 independent groups, by the Kruskal-Wallis test for 3 independent groups, by the Wilcoxon rank sum test for 2 paired groups and by the Friedman test for 3 paired groups. The statistical significance of the correlation coefficient was analyzed by the Spearman rank test. A p value of less than 0.05 was considered significant.

## RESULTS

*Testosterone therapy.* The PLs became −2.0 SD or greater in all of the boys (after 1 injection in 4 boys, 2 injections in 13 and 3 injections in 7 of group 1, and after 2 injections in 12 boys and 4 injections in 2 of group 2). In addition, biochemical studies performed roughly 1 month after final TE injection showed no abnormal findings such as increased serum hepatic enzymes, and bone age assessment approximately 1 year after final TE injection revealed no accelerated advancement of skeletal maturation.

The overall results are summarized in table 1. Age and BSA were similar between groups 1 and 2, and TE dosage was significantly higher in group 2 than in group 1. PL and PL SDS medians were significantly increased in groups 1 and 2 combined, and in each group separately, although they were still significantly shorter in group 2 than in group 1 after therapy. The increment in actual PL at the first injection of TE ranged from 0.2 to 1.5 cm, and was independent of age and BSA, whereas that in PL SDS at the first injection of TE ranged from 0.3 to 2.5, and was inversely correlated with age and BSA (fig. 1).

PL responses at the first, second and third injections of TE are summarized in table 2. In groups 1 and 2 combined and in group 2 separately the median of PL increments was significantly decreased at the second injection compared to the first, and was similar between the second and third injections. There was no significant difference in the median of PL increments between the first, second and third injections in group 1. In groups 1 and 2 the median of PL increments was similar at each injection, especially at the first injection. Consistent with this finding, no correlation was observed between actual PL before therapy and actual PL increment at the first injection of TE (fig. 2). Furthermore, the median of PL increments was significantly decreased at the second injection compared to the first in boys treated with 2 or 3 injections, whereas it was similar between the second and third injections in boys treated with 3 injections. The median of PL increments at the first injection was higher in boys receiving 2 injections than in those receiving 3, as was that of PL increments at the second injection.

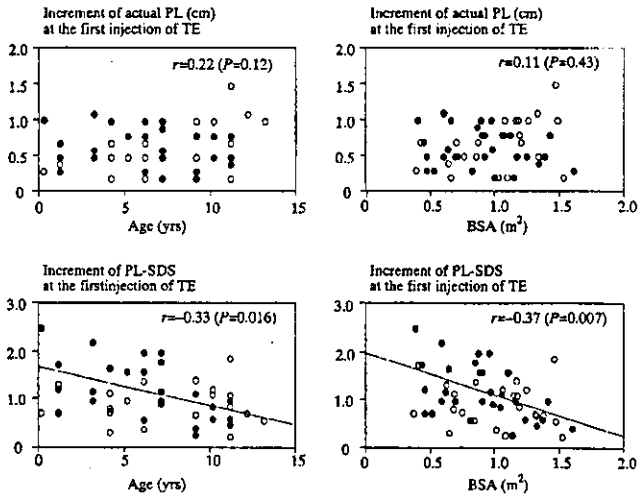


FIG. 1. Relationship of penile length (*PL*) response at first injection of testosterone enanthate (*TE*) to age and body surface area (*BSA*). Closed circles indicate definite micropenis less than −2.5 SD. Open circles represent borderline micropenis −2.1 to −2.5 SD. *SDS*, SD score.

TABLE 2. *Penile length increment at first, second and third injections of testosterone enanthate*

| | Total | Group 1 | Group 2 | p Value* | 2 Injections | 3 Injections | p Value† |
|---|---|---|---|---|---|---|---|
| First injection: | | | | | | | |
| Mean ± SE | 0.64 ± 0.04 | 0.65 ± 0.07 | 0.63 ± 0.05 | | 0.74 ± 0.05 | 0.51 ± 0.06 | |
| Median | 0.6 | 0.6 | 0.6 | 0.96 | 0.7 | 0.5 | 0.009 |
| Range | 0.2–1.5 | 0.2–1.5 | 0.2–1.1 | | — | 0.2–1.0 | |
| No. pts | 53 | 24 | 29 | | 28 | 19 | |
| Second injection: | | | | | | | |
| Mean ± SE | 0.43 ± 0.03 | 0.50 ± 0.07 | 0.39 ± 0.03 | | 0.50 ± 0.05 | 0.33 ± 0.04 | |
| Median | 0.4 | 0.50 | 0.4 | 0.26 | 0.5 | 0.3 | 0.006 |
| Range | 0.1–1.5 | 0.1–1.5 | 0.1–0.7 | | 0.1–1.5 | 0.1–0.6 | |
| No. pts | 49 | 20 | 29 | | 28 | 19 | |
| Third injection: | | | | | | | |
| Mean ± SE | 0.36 ± 0.06 | 0.46 ± 0.12 | 0.31 ± 0.07 | | | 0.38 ± 0.06 | |
| Median | 0.3 | 0.4 | 0.3 | 0.33 | | 0.3 | |
| Range | 0.0–1.1 | 0.1–1.1 | 0.0–0.7 | | | 0.0–1.1 | |
| No. pts | 21 | 7 | 14 | | | 19 | |
| p Value: | | | | | | | |
| Between injections 1 and 2 | 0.0002 | 0.09 | 0.0007 | | 0.0006 | 0.02 | |
| Between injections 2 and 3 | 0.23 | 0.61 | 0.30 | | | 0.52 | |
| Among injections 1, 2 and 3 | <0.0001 | 0.14 | 0.0004 | | | 0.07 | |

* Difference between groups 1 and 2.
† Two vs 3 injections.

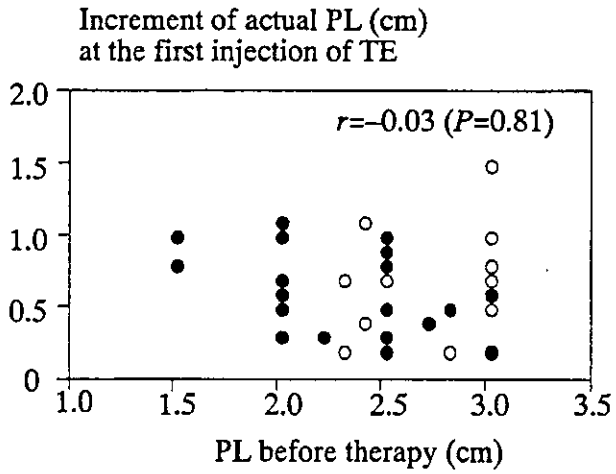


FIG. 2. Relationship between actual penile length (*PL*) increment at first injection of testosterone enanthate (*TE*) and PL before therapy. Closed circles indicate definite micropenis less than −2.5 SD. Open circles represent borderline micropenis −2.1 to −2.5 SD.

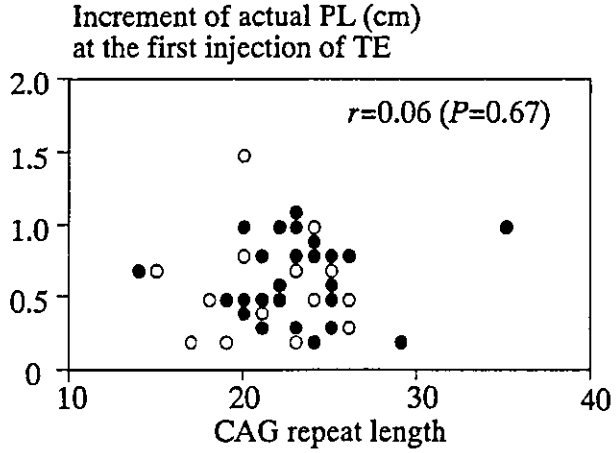


FIG. 3. Relationship between actual penile length (*PL*) increment at first injection of testosterone enanthate (*TE*) and CAG repeat length of AR gene. Closed circles indicate definite micropenis less than −2.5 SD. Open circles represent borderline micropenis −2.1 to −2.5 SD.

*Relevance of the SRD5A2 and AR gene polymorphisms.* The effects of the polymorphisms on the PL responses to TE therapy are summarized in table 3. The median of PL increments at the first injection of TE was independent of the V89L genotype and of the presence or absence of relatively long CAG repeats (26 or greater). Furthermore, there was no significant correlation between CAG repeat length and actual PL increment at the first injection of TE (fig. 3). In addition, actual PL increment at the first injection of TE was 0.8 cm in 1 boy with the association of L/L genotype and CAG repeat length of 26, and ranged from 0.2 to 1.5 cm (median 0.55, mean ± SE 0.62 ± 0.07) in 18 boys with the combination of V/V genotype and CAG repeat length of 25 or less.

## DISCUSSION

This study indicates that systemic T administration is an effective therapeutic method for micropenis in boys with no demonstrable SRD5A2 or AR mutation. The PLs were increased −2.0 SD or greater in all 53 boys after 1 to 4 injections. Although such beneficial effects of parenteral T therapy have been described in the literature, the previous reports are based on the data for a relatively small number of boys with micropenis, ie 4 boys reported on by Guthrie et al,[3] 14 reported on by Burstein et al[4] and 13 reported on by Chalapathi et al.[5] Thus, our results provide compelling evidence for the application of parenteral T therapy for micropenis in infancy to childhood.

TABLE 3. *Effects of SRD5A2 and AR polymorphisms on penile length increment at first injection of testosterone enanthate*

| | SRD5A2 V89L Genotype | | | | No. AR CAG Repeats | | |
|---|---|---|---|---|---|---|---|
| | V/V | V/L | L/L | p Value | 25 or Less | 26 or Greater | p Value |
| Mean ± SE | 0.62 ± 0.07 | 0.66 ± 0.05 | 0.56 ± 0.15 | | 0.64 ± 0.04 | 0.60 ± 0.13 | |
| Median | 0.6 | 0.7 | 0.5 | 0.77 | 0.6 | 0.65 | 0.77 |
| Range | 0.2–1.5 | 0.2–1.1 | 0.2–1.0 | | 0.2–1.5 | 0.2–1.0 | |
| No. pts | 18 | 30 | 5 | | 47 | 6 | |

The same dosage of 25 mg TE was applied in all boys, with no dosage adjustment for age or BSA. This simple and convenient method appears to be clinically acceptable because it virtually resulted in the normalization of PLs in all of the boys. Furthermore, since the relative TE dosage (TE dosage per body size) should be decreased with age and BSA, this may explain why the increment in the actual PL was independent of age and BSA, whereas that in PL SDS was inversely correlated with age and BSA. In this regard the lack of correlation between actual PL increment and age or BSA is noteworthy because it implies that the actual PL increment can serve as an indicator for TE therapy in all boys irrespective of age or BSA (thus, increments in actual PL rather than PL SDS were primarily analyzed in this study).

Although the TE therapy was effective in all boys, the extent of PL increments was variable, with the actual PL increments at the first TE injection ranging from 0.2 to 1.5 cm. This finding would primarily be due to the heterogeneity of micropenis in this study. Although a demonstrable SRD5A2 and AR mutation was excluded in the 53 boys, micropenis can still be caused by multiple factors that can be classified into 2 major categories—defective endogenous T production due to primary or secondary testicular dysfunction, such as impaired testis formation or gonadotropin deficiency, and impaired responses of external genitalia to T, such as defective post-AR signal transduction and impaired anlage formation of external genitalia. Therefore, it is inferred that penile response to TE therapy is good for micropenis primarily resulting from defective endogenous T production and poor for micropenis primarily caused by impaired response of external genitalia to T, although the severity of the underlying factors for micropenis would also be involved in the variation of PL responses. Furthermore, this notion would explain why the PL increments were similar between groups 1 and 2 and were unrelated to the initial PLs before therapy, because the PL response would primarily depend on the major cause of micropenis, ie defective endogenous T production or impaired responses of external genitalia to T.

Several matters are noteworthy with respect to TE therapy. First, the effect of TE therapy was as a whole decreased at the second injection compared to the first injection, and remained similar between the second and the third injections. The decreased PL response at the second injection implies that the responsiveness to TE therapy was attenuated after the stimulation at the first injection, although it is unknown why the responsiveness remained similar between the second and third injections (thus, PL increments at the first injection were primarily examined in this study).

Also noteworthy is the fact that the PL increments at the first and second injections were larger in boys receiving 2 injections than in those receiving 3. This finding suggests that the TE dosage required for sufficient PL increase depends not only on PLs before therapy, which is indicated by the larger amount of TE used in group 2 than in group 1, but also on the penile responsiveness to TE therapy, which is considered to be good for micropenis due to defective endogenous T production and poor for micropenis resulting from impaired responses of external genitalia to T.

In addition, the minimum PL increment was 0.2 cm at the first injection. Since similar PL responses (0.1, 0.2 and 0.25 cm/25 mg TE) have been observed in 3 previously reported Japanese patients with SRD5A2 mutations, this finding may suggest that partial 5α-reductase type 2 deficiency, and probably partial androgen resistance as well, should be examined in boys with poor PL responses.[14] Indeed, the 3 boys responded well to 5α-dihydrotestosterone gel applied after the diagnosis of 5α-reductase type 2 deficiency was established.[14]

Finally, the maximum PL increment was 1.5 cm at the first injection. This finding implies that an injection of 25 mg TE would not cause unfavorably large PL responses exceeding the normal range.

The PL increment was irrelevant to the V89L polymorphism in the SRD5A2 gene and to the CAG repeat length polymorphism in the AR gene. This finding suggests that the 2 polymorphisms, although associated with functional alteration, are unlikely to influence penile responsiveness to TE therapy.[7,9] This outcome is consistent with our previous finding that the development of micropenis is independent of the 2 polymorphisms in Japanese boys, including the 53 boys examined in this study, because the effect of endogenous and exogenous T is mediated by 5α-reductase type 2 activity and AR function.[14,15] However, previous studies have shown positive and negative results for the association between the 2 polymorphisms and predisposition to various androgen related disorders such as under-masculinization, azoospermia and prostate cancer.[8,10,11,14,15,17–20] Thus, it is possible that the 2 polymorphisms may actually constitute minor modifying factors for responsiveness to TE therapy, and that they may be detected as positive modifying factors in other patient populations with micropenis.

## CONCLUSIONS

This study indicates that administration of 25 mg TE is an effective therapeutic method for micropenis in prepubertal boys with no SRD5A2 or AR mutation, and can be applied irrespective of age, BSA or initial PL. Furthermore, the results imply that the PL response to TE therapy is independent of the V89L and CAG repeat length polymorphisms. However, it is noteworthy that the underlying causes for micropenis have not been elucidated in these 53 patients, although SRD5A2 and AR mutations have been excluded. Thus, further studies are necessary to determine the PL responses in specific disorders such as gonadotropin deficiency.

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Drug Metab. Pharmacokin. 18 (3): 203-211 (2003).

## Regular Article

# *Population Pharmacokinetics of an Angiotensin II Receptor Antagonist, Telmisartan, in Healthy Volunteers and Hypertensive Patients*

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**Summary:** Objective: To describe the factors affecting pharmacokinetics of telmisartan, an angiotensin II receptor antagonist, a population pharmacokinetic (PPK) model has been developed based upon the data collected from healthy volunteers and hypertensive patients.

Methods: A total of 1566 plasma samples were collected from 20 healthy volunteers and 129 hypertensive patients, together with the demographic background. The data were analyzed by the NONMEM program using two-compartment model with first-order absorption. The robustness of the obtained PPK model was validated by the bootstrapping resampling method.

Results: The oral clearance (CL/F) was found to be associated with age, dose and alcohol consumption, but neither related to serum creatinine nor smoking history. The volume of distribution for the central compartment was related to age and dose, and the volume of distribution for the peripheral compartment was related to body weight and gender. The absorption rate constant (Ka) and the absorption lag time were described as function of dose. The CL/F decreased with advanced age. The CL/F decreased and Ka increased with higher dose, reflecting the super-proportional increase in the plasma levels of telmisartan. The AUC and $C_{max}$ values predicted by the present PPK model were well consistent with the observed values. The means of parameter estimates obtained with 200 bootstrap replicates were within 95–111% of the final parameter estimates from the original data set.

Conclusion: A PPK model for telmisartan developed here well described the individual variability and exposure, and robustness of the model has been validated by the bootstrapping method.

**Key words: telmisartan; population pharmacokinetics; modeling; covariate; NONMEM; model validation**

## Introduction

Telmisartan is a nonpeptide angiotensin II receptor antagonist,[1,2] and is used for the treatment of hypertension.[3,4] Because of its long elimination half-life, telmisartan shows sustained anti-hypertensive effect over the 24-hour dosing interval by once-daily dosing.[5] Telmisartan is a lipophilic compound and extensively distributes to tissues. Telmisartan is highly bound to plasma proteins (99.5%)[5] mainly albumin, and also bound to α1-acid glycoprotein, γ-globulin and lipoproteins.[5] Telmisartan is metabolized to an inactive acylglucuronide conjugate, which is account for approximately 10% of the circulating drugs following a single 40 mg dose.[6] Biliary-faecal excretion is the primary elimination route of telmisartan and its metabolite. Following a single oral or intravenous dose of 40 mg [$^{14}$C] telmisartan to normal volunteers, more than 98% of total radioactivity was recovered in the faeces and $<1\%$ of radioactivity was recovered in urine.[6]

Telmisartan shows a large interindividual variability in plasma concentration profile.[5] The absolute bioavailability at 40 mg dose was 42% in fasting volunteers[5] and it was reduced when administered after food intake. The absolute bioavailability was increased to 97.2% in patients with hepatic impairment, and the $C_{max}$ and $AUC_{0-\infty}$ in hepatic impairment patients were approximately 3-fold greater than those in healthy subjects.[7] The $C_{max}$ and AUC showed dose-proportionality after intravenous administration of 10 to 120 mg

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**Table 1.** Sources of plasma telmisartan concentration data used in the present population analysis

| Study Number | Study Objective | Dose (mg) | Number of subjects | Number of measurements | Average number of measurements | Ref. No. |
|---|---|---|---|---|---|---|
| 1 | Pharmacokinetic study in hypertensive patients | 20, 40, 80 | 90 | 784 | 8.7/subject | 10 |
| 2 | Pharmacokinetics and pharmacodynamics in hypertensive patients | 40, 80 | 20 | 298 | 14.9 | 11 |
| 3 | Pharmacokinetics and pharmacodynamics in hypertensive patients with renal dysfunction | 40 | 19 | 263 | 13.8 | 12 |
| 4 | Effect of food on bioavailability in healthy male volunteers | 40 | 20 | 221 | 11.1 | 13 |
| | Total | | 149 | 1566 | | |

dose,[5] whereas $C_{max}$ increased disproportionally with dose after oral administration for both single and multiple dosing,[3,5] which indicates the saturable first-pass metabolism.

Recently, population pharmacokinetic (PPK) analysis has been applied to new drug development for a variety of drugs.[8] The PPK analysis is helpful to identify factors that affect the pharmacokinetics of a drug, or to explain variability in a target population.[9] To date, however, there is no report on the PPK of telmisartan although this drug is widely used as an antihypertensive agent. In the present article, we have developed a PPK model for telmisartan by analyzing the pooled data obtained in the course of clinical trials in Japan. Since telmisartan shows a large individual variability in pharmacokinetics, it is useful to develop a PPK model by integrating the currently available information for this agent. The obtained PPK model explains several factors that can cause the inter-individual variability in pharmacokinetics, and the model is capable to describe and predict the plasma concentration-time profiles for patients with various backgrounds.

## Methods

**Subjects and studies:** A total of 1566 plasma concentration data were collected from 149 subjects (20 healthy subjects, 129 hypertensive patients) participated in four clinical trials conducted in Japan (**Table 1**).[10-13] **Figure 1** shows the plasma concentration-time data of telmisartan used for the present population pharmacokinetic analysis.

The study 1 was performed to collect the pharmacokinetic data for the population analysis in hypertensive patients.[10] Ninety hypertensive patients were administered a single oral dose of 20, 40 or 80 mg of telmisartan after breakfast. Ten blood samples were drawn from the vein of each patient, immediately before dosing and 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours after administration. The study 2 obtained the pharmacokinetic data after multiple oral dosing in hypertensive patients.[12] Twenty essential hypertensive patients were given 40 or 80 mg telmisartan once daily for 14 days. The study 3 was a pharmacokinetic study performed in 19 hypertensive patients with renal dysfunction (Serum creatinine: 1.5 to 4.0 mg/100 mL) to evaluate the influence of renal impairment on the pharmacokinetics of telmisartan.[11] A dose of 40 mg was orally given once daily for 7 days. The study 4 investigated the effect of food.[13] Twenty healthy male subjects received 40 mg of telmisartan orally as a single dose. The 221 plasma concentrations obtained in the fed condition were included in the data set, because our purpose was to obtain the PPK model in a standard clinical situation and because the other three studies were all conducted in the fed condition.

**Assay of telmisartan concentrations:** Plasma concentrations of telmisartan were determined by a validated reverse-phase high-performance liquid chromatographic method using a column-switching technique. All plasma samples collected in the four clinical studies were analyzed by the same procedure at the Department of Drug Metabolism and Pharmacokinetics, Kawanishi Pharma Research Institute, Nippon Boehringer Ingelheim Co., Ltd. In brief, telmisartan was extracted and concentrated on an enrichment column (LiChroprep RP-8, 20×4.0 mm, 25～40 μm) using water containing 400 μL/L piperidine. Then, the extract was chromatographically separated on a reversed-phase column (BDS-Hypersil, 125×4.0 mm, 5 μm) using a mixture of acetonitrile–water–piperidine (235:800:0.16 [vol/vol/vol]) as the mobile phase. Quantification was made based on the peak height ratio of telmisartam to the internal standard, 4′-[[4-methyl-6-(1-methyl-2-benzimidazolyl)-2-butyl-1-benzimidazolyl]methyl]-2-biphenyl carboxylic acid, detected by a fluorescence detector (excitation 300 nm, emission 385 nm). The lower limit of quantitation of the present assay was 0.5 ng/mL. Intra- and inter-day variation of assay precision was less than 5% and the average bias was within 8%.

**Demographic background of the subject population:** Demographic background for the population participating in the present PPK analysis is summarized in **Table 2**. A total of 149 subjects (20 healthy volunteers

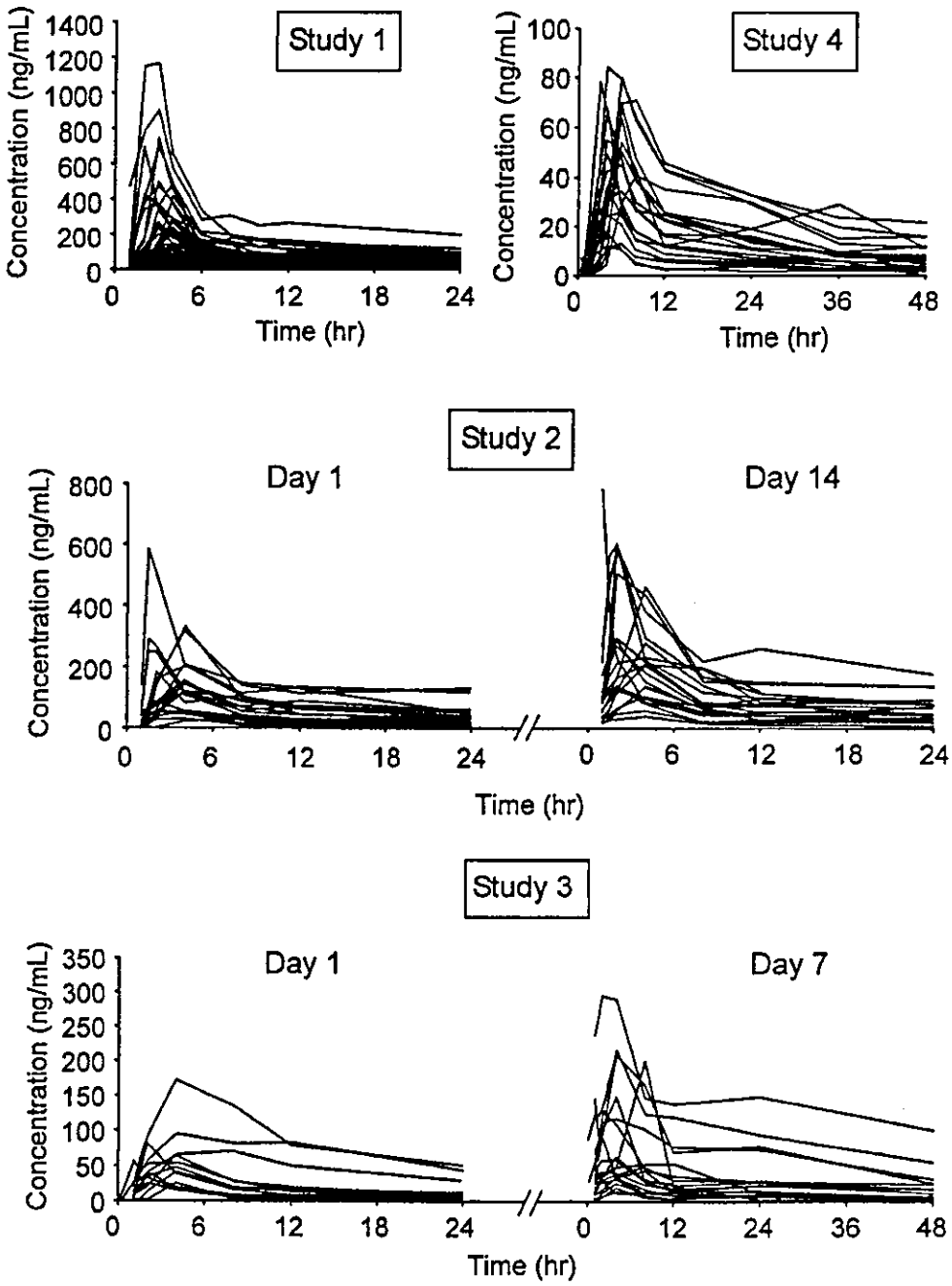


**Fig. 1.** Plasma concentration-time profiles of telmisartan following a single or multiple doses. The detail of each study is described in **Table 1.**

and 129 hypertensive patients) were enrolled in the study. A total of 1566 drug concentration measurements were pooled and used for computation. The study population comprised 107 males and 42 females. The age ranged from 20 to 77 years old, body weight from 30.5 to 118.3 kg, and serum creatinine from 0.59 to 4.10 mg/100 mL. The subjects received 20, 40 or 80 mg telmisartan by single or once daily multiple dosing after meal.

**Non-compartmental analysis:** Before developing a PPK model, individual AUC values were calculated by a non-compartmental analysis with linear trapezoid rule (WinNonlin® professional software version 3.1).

**Model development:** The PPK modeling was performed using the NONMEM program (double precision, version V, level 1.0) with its library subroutines ADVAN4 and TRANS4.[14] A two-compartment open

**Table 2.** Description of the population participating in the present study

| | | |
|---|---|---|
| Total number of subjects | 149 | |
| Number of healthy volunteers | 20 | |
| Number of hypertensive patients | 129 | |
| Gender: Male | 107 | |
| Female | 42 | |
| Dose: 20 mg | 31 | |
| 40 mg | 78 | |
| 80 mg | 40 | |
| Alcohol consumption: Non-drinker | 57 | |
| Drinker | 92 | |
| Smoking history: Non-smoker | 63 | |
| Ex-smoker | 19 | |
| Smoker | 67 | |
| Food condition: Fasting condition | 0 | |
| Non-fasting condition | 149 | |
| Renal function: Normal (Scr: ≤1.5 mg/100 mL) | 128 | |
| Moderate impairment (SCr: 1.5–3.0 mg/100 mL) | 10 | |
| Severe impairment (SCr: 3.0–4.0 mg/100 mL) | 11 | |
| Hepatic function: Glutamic-oxaloacetic transaminase ≤ 60U | 145 | |
| Glutamic-oxaloacetic transaminase > 60U | 4 | |
| Age (year) | 50.5 ± 16.0[a] | [20–77][b] |
| Weight (kg) | 63.9 ± 13.0 | [30.5–118.3] |
| Serum creatinine (SCr, mg/100 mL) | 1.22 ± 0.74 | [0.59–4.10] |
| Glutamic-oxaloacetic transaminase (U) | 22.5 ± 12.9 | [5–97] |

a) Mean ± S.D. b) Minimum-maximum values.

model with first-order absorption was used as a structural model. The basic pharmacokinetic parameters were oral clearance (CL/F, L/hr), volume of distribution for the central compartment ($V_1$/F, L), inter-compartmental clearance (Q/F, L/hr), volume of distribution for the peripheral compartment ($V_2$/F, L), first-order absorption rate constant (Ka, $hr^{-1}$) and absorption lag time (ALAG, hr). The first-order estimation (FO) method was used.

The inter-individual variability for basic pharmacokinetic parameters was modeled by the log normal distribution, as described for CL/F as an example.

$$CL/F_j = TVCL \cdot \exp(\eta_{jCL/F})$$

where $\eta_{jCL/F}$ is a random variable that represents the difference between individual clearance of the $j$-th individual ($CL/F_j$) and the population mean value (TVCL). The random variable $\eta_{jCL/F}$ is normally distributed with an expectation of zero and a variance of $\omega^2_{CL/F}$.

Residual variability was similarly modeled by the log normal distribution as follows:

$$C_{ij} = C_{pred,ij} \cdot \exp(\varepsilon_{ij})$$

where $C_{ij}$ is the $i$-th observed plasma concentration of telmisartan for the $j$-th individual, $C_{pred,ij}$ is the concentration predicted by the population pharmacokinetic model, and $\varepsilon_{ij}$ is a randomly distributed variable with mean of zero and variance of $\sigma^2$.

The minimum value of the NONMEM objective function (MOF) was used as a statistic to choose suitable models during the model-building process. Since the difference in MOF between one model and the other approximates a $\chi^2$ distribution with freedom of the number of parameter difference, a difference in MOF of 3.84 for 1 degree of freedom ($p<0.05$) was considered statistically significant in the model-building process.

**Covariate model:** Starting from a simple two-compartment model, a variety of covariates that could influence the pharmacokinetics of telmisartan were stepwise added to the basic model (forward selection method). Statistical significance for incorporation of each covariate was judged based upon the MOF. Covariates considered for inclusion in the model were subject demographic factors (body weight, age, gender), laboratory tests (renal dysfunction), telmisartan dose, smoking history and alcohol consumption. Once a full model was developed which incorporated all possible covariates, each covariate was in turn examined by removing one by one to confirm the statistical significance (backward selection) using more stringent criterion of MOF with 6.63 ($p<0.01$). The final PPK model was reached by remaining the significant covariates.

**Model validation:** Bootstrap resampling method was used to evaluate the stability and robustness of the final PPK model.[15] The final PPK model was fitted repeatedly to the 200 additional bootstrap datasets. The

means of parameter estimates calculated from the 200 bootstrap replications were compared with the final parameter estimates obtained from the original dataset. Each pharmacokinetic parameter was logarithmically transformed to calculate 95% symmetric confidence intervals.

## Results

**Model development:** A two-compartment open model with first-order absorption was used as a basic structural model, and additional PK parameter such as absorption lag time, random variables for inter-individual variability and covariates were added stepwise to develop the population model for telmisartan pharmacokinetics. The absorption lag time (ALAG) was necessary in the model because its incorporation significantly improved the model fitting ($\Delta$MOF = 184.19, $p < 0.001$). Random variables on the inter-individual variability were needed for the parameters CL/F, $V_1$/F, Q/F and $V_2$/F, but not for Ka and ALAG.

Starting from a simple structural model, a variety of covariates that are likely to influence the pharmacokinetics of telmisartan were added one by one and tested for its statistical significance (forward selection method). The body weight was used as a size factor and incorporated into the parameters CL/F, $V_1$/F and $V_2$/F. For CL/F, further covariates such as age, gender, dose, serum creatinine, alcohol consumption and smoking history were tested.

For continuous variables, the covariate modeling was described according to a power function as described by the following example.

$$CL/F = TVCL \cdot SCr^{\theta_{SCr}}$$

where SCr is the serum creatinine and $\theta_{SCr}$ is the influence factor to be estimated. *TVCL* reveals a typical value of clearance, which describes the *CL/F* value where SCr has no effect. If $\theta_{SCr}$ is significantly different from zero, it is leaded that SCr is associated with CL/F. For categorical variables, covariates were modeled as follows.

$$CL/F = TVCL \cdot GENDER$$

where the variable GENDER takes 1 for female and a parameter $\theta$ for male. If $\theta$ is significantly different from unity, there exists some gender difference in *CL/F*.

Among the examined covariates, age, dose, alcohol consumption and smoking history were found to be associated with the CL/F. The renal function was not picked up as a possible factor affecting CL/F. The influence of hepatic dysfunction was not examined because the number of hepatic impairment subjects was so small (**Table 2**). On the other hand, age showed a significant influence on CL/F ($\Delta$MOF = 23.582). Age was also suggested to be a factor for the $V_1$/F and $V_2$/F. Telmisartan dose was important factor for CL/F, $V_1$/F, Ka and ALAG since this factor significantly improved the model fitting. There observed a gender difference in $V_2$/F. By the above forward selection step, the following full PPK model was suggested, which was described by age, dose (DAMT), alcohol consumption (ETOH), smoking history (SMOK), gender and body weight (WTKG).

$$CL/F = \theta_1 \cdot WTKG^{\theta_8} \cdot AGE^{\theta_{10}} \cdot DAMT^{\theta_{11}} \cdot ETOH \cdot SMOK \cdot \exp(\eta_{CL/F})$$

$$V_1/F = \theta_2 \cdot WTKG \cdot AGE^{\theta_{15}} \cdot DAMT^{\theta_{16}} \cdot \exp(\eta_{V_1/F})$$

$$Q/F = \theta_3 \cdot \exp(\eta_{Q/F})$$

$$V_2/F = \theta_4 \cdot WTKG^{\theta_9} \cdot GENDER \cdot AGE^{\theta_{17}} \cdot \exp(\eta_{V_2/F})$$

$$KA = \theta_5 \cdot DAMT^{\theta_6}$$

$$ALAG = \theta_7 \cdot DAMT^{\theta_{17}}$$

where ETOH = $\theta_{12}$ for drinker, 1 otherwise.
SMOK = $\theta_{13}$ for ex-smoker, $\theta_{14}$ for smoker, 1 otherwise.
GENDER = $\theta_{18}$ for male, 1 for female.

Model refinement was then made by the backward selection, in which statistically insignificant covariates were removed from the above full model (**Table 3**). Remaining only statistically significant factors ($p < 0.01$) leaded to the final PPK model. Through this process, the covariates body weight and smoking history on CL/F, body weight on $V_1$/F, and age on $V_2$/F were removed from the model. Therefore, the refined final PPK model is described as follows.

$$CL/F = \theta_1 \cdot AGE^{\theta_{10}} \cdot DAMT^{\theta_{11}} \cdot ETOH \cdot \exp(\eta_{CL/F})$$

$$V_1/F = \theta_2 \cdot AGE^{\theta_{15}} \cdot DAMT^{\theta_{16}} \cdot \exp(\eta_{V_1/F})$$

$$Q/F = \theta_3 \cdot \exp(\eta_{Q/F})$$

$$V_2/F = \theta_4 \cdot WTKG^{\theta_9} \cdot GENDER \cdot \exp(\eta_{V_2/F})$$

$$KA = \theta_5 \cdot DAMT^{\theta_6}$$

$$ALAG = \theta_7 \cdot DAMT^{\theta_{17}}$$

where ETOH = $\theta_{12}$ for drinker, 1 otherwise.
GENDER = $\theta_{18}$ for male, 1 for female.

The PPK parameter estimates for the final model are summarized in **Table 4**. **Figure 2** represents the comparison of individual $C_{max}$ values observed and calculated by the final PPK model. **Figure 2** also shows the comparison of AUC's computed by non-compartmental trapezoidal rule and calculated by the final PPK model. Both plots show a good agreement, indicating the present PPK model is capable for describing the observed concentrations even though those showed a large individual variability. **Figure 3** and **Table 5** represent how each covariate affects the plasma concentration profile and pharmacokinetic parameters of telmisartan. As described, age and dose could be an important factor for individual variability.

**Table 3.** Hypothesis testing for possible factors affecting pharmacokinetics of telmisartan

| $\theta$ | Factor | Parameter | Estimated value | Hypothesized value | ΔMOF | P value |
|---|---|---|---|---|---|---|
| $\theta_6$ | Dose | Ka | 0.964 | 0 | 62.145 | $p<0.01$ |
| $\theta_8$ | Weight | CL/F | 0.0932 | 0 | 0.614 | N.S. |
| $\theta_9$ | Weight | $V_2/F$ | −0.820 | 0 | 12.111 | $p<0.01$ |
| $\theta_{10}$ | Age | CL/F | −0.243 | 0 | 11.435 | $p<0.01$ |
| $\theta_{11}$ | Dose | CL/F | −0.320 | 0 | 23.222 | $p<0.01$ |
| $\theta_{12}$ | Alcohol consumption | CL/F | 1.18 | 1 | 10.143 | $p<0.01$ |
| $\theta_{13}$ | Smoking history (for ex-smoker) | CL/F | 1.03 | 1 | 0.125 | N.S. |
| $\theta_{14}$ | Smoking history (for smoker) | CL/F | 1.14 | 1 | 5.733 | N.S. |
| $\theta_{15}$ | Age | $V_1/F$ | −1.03 | 0 | 63.861 | $p<0.01$ |
| $\theta_{16}$ | Dose | $V_1/F$ | −0.641 | 0 | 45.852 | $p<0.01$ |
| $\theta_{17}$ | Dose | Absorption lag time | 0.873 | 0 | 42.024 | $p<0.01$ |
| $\theta_{18}$ | Gender (for male) | $V_2/F$ | 1.31 | 1 | 7.968 | $p<0.01$ |
| $\theta_{19}$ | Age | $V_2/F$ | −0.269 | 0 | 4.200 | N.S. |

$CL/F=\theta_1\cdot WTKG^{\theta_8}\cdot AGE^{\theta_{10}}\cdot DAMT^{\theta_{11}}\cdot ETOH\cdot SMOK\cdot\exp(\eta_{CL/F})$, $V_1/F=\theta_2\cdot WTKG\cdot AGE^{\theta_{15}}\cdot DAMT^{\theta_{16}}\cdot\exp(\eta_{V_1/F})$
$Q/F=\theta_3\cdot\exp(\eta_{Q/F})$, $V_2/F=\theta_4\cdot WTKG^{\theta_9}\cdot GENDER\cdot AGE^{\theta_{19}}\cdot\exp(\eta_{V_2/F})$, $Ka=\theta_5\cdot DAMT^{\theta_6}$, $ALAG=\theta_7\cdot DAMT^{\theta_{17}}$
where $ETOH=\theta_{12}$ for drinker, 1 otherwise. $SMOK=\theta_{13}$ for ex-smoker, $\theta_{14}$ for smoker, 1 otherwise. $GENDER=\theta_{18}$ for male, 1 for female.

**Table 4.** Final estimates for population pharmacokinetic parameters of telmisartan

| |
|---|
| Population mean parameters |
| $CL/F=701\cdot AGE^{-0.359}\cdot DAMT^{-0.345}\cdot(1.22)^{ETOH}$ |
| ETOH: drinker = 1, non-drinker = 0 |
| $V_1/F=281000\cdot AGE^{-1.27}\cdot DAMT^{-0.629}$ |
| $Q/F=79.7$ |
| $V_2/F=13600\cdot WTKG^{-0.724}\cdot(1.42)^{GENDER}$ |
| GENDER: male = 1, female = 0 |
| $Ka=0.00661\cdot DAMT^{1.06}$ |
| $ALAG=0.0166\cdot DAMT^{0.865}$ |
| Interindividual variability (CV%) |
| $\omega_{CL/F}=189\%$ |
| $\omega_{V_1/F}=119\%$ |
| $\omega_{Q/F}=88.3\%$ |
| $\omega_{V_2/F}=86.8\%$ |
| Residual variability (CV%) |
| $\sigma=57.4\%$ |

**Model validation:** The final PPK model was fitted repeatedly to the 200 additional bootstrap re-sampled datasets. All 200 estimation steps were completed successfully and the results are summarized in **Table 6.** The geometric mean of 200 parameter estimates were, in most cases, within ± 10% difference from the final PPK parameters obtained with the original data set.

## Discussion

The PPK model of telmisartan in healthy volunteers and hypertensive patients has been developed based upon the pooled pharmacokinetic data obtained in the four pre-marketing clinical trials conducted in Japan. The CL/F was found to be associated with age, dose and alcohol consumption, but not related to serum creatinine, gender or smoking history. As the total urinary excretion of telmisartan is extremely low,[6] it is reasonable that CL/F was not affected by renal function. This finding was also consistent with the result of a separate clinical study in subjects with mild to moderate renal dysfunction.[16] On the other hand, considering that telmisartan is mainly excreted via bile,[6,17,18] it will be important to examine the effect of hepatic impairment on telmisartan pharmacokinetics. A particular clinical study was performed in Germany in subjects with hepatic impairment. $C_{max}$ and AUC were increased in hepatic impairment subjects compared with healthy volunteers after oral administration of 20 mg or 120 mg of telmisartan. The pharmacokinetic profile of telmisartan in the subjects with hepatic impairment was characterized by rapid absorption and a slow terminal elimination phase.[7] Since the number of patients with hepatic impairment was only four in the present dataset and this number was thought insufficient for the analysis, the effect of hepatic impairment could not be examined in this analysis.

The volume of distribution for the central compartment was related to age and dose, and the volume of distribution for the peripheral compartment was related to body weight and gender. The CL/F decreased with advancing age. A typical AUC value in 70-year-old subject was 1.36-fold greater than that in 30-year-old subject. The AUC in non-drinkers was on average 1.22-fold greater than that in drinkers. The absorption rate constant (Ka) and the absorption lag time were described as a function of dose.

The CL/F and Ka tend to decrease and increase, respectively, with increasing dose. This reflects the super-proportional increase in the plasma levels of

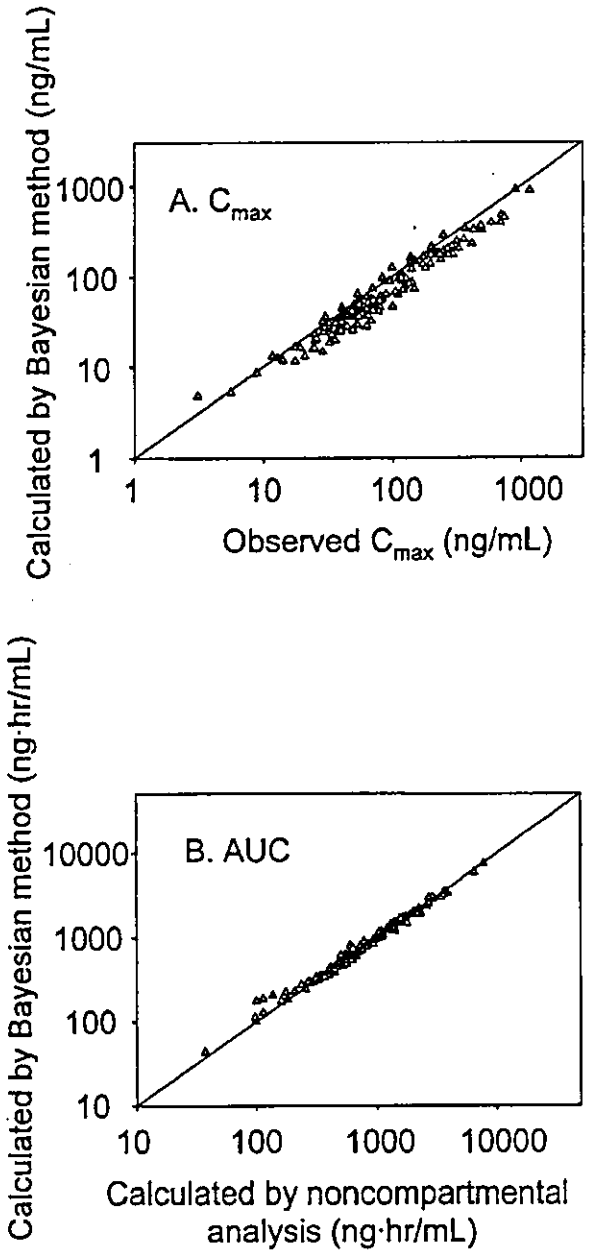


Fig. 2. Comparisons of individual exposure to telmisartan (A: $C_{max}$, B: AUC) observed or calculated by non-compartment method *versus* predicted by the Bayesian estimation based upon the final population pharmacokinetic model.
The solid line represents the unit line.

telmisartan as previously reported.[2,4,5] The AUC for 80 mg dose was 6.45-fold greater than that for 20 mg dose. We also tried a nonlinear pharmacokinetic model, which has a Michaelis-Menten type formula in the absorption phase. But due to the complexity of the model, the computation did not reach the convergence. Although the present PPK model might be a pragmatic expedient from a theoretical viewpoint, this simpler model possesses the advantage of easy clinical applications for describing the factors causing the inter-individual variability or further exposure-response analysis.

As for the estimation step of NONMEM, FO method was used in the present analysis to minimize the calculation time. However, first-order conditional estimation (FOCE) method might be also useful for the estimation.

In the previous clinical studies, a large individual variability in pharmacokinetic profile of telmisartan was observed.[4,17] The large CV(%) values of interindividual variability in the basic model without any covariates reflect this characteristic. After the inclusion of selected covariates as fixed effects in the model, all of interindividual variability and intraindividual variability in the final PPK model were smaller than those in the starting basic model. This indicates that the final PPK model describes the population profile and factors affecting the pharmacokinetics of this agent. However, the CV (%) of interindividual variability especially for CL/F and $V_1$/F were still large, suggesting to require further population PK analysis to find out the causes of the variability. The biotransformation of telmisartan consists exclusively of a phase II reaction, that is, conjugation to glucuronic acid by UDP-glucuronosyltransferases, yielding an acylglucuronide of the compound, but no phase I oxidative metabolites have been identified.[2,19] It was reported that there are polymorphisms in UDP-glucuronosyltransferase genes.[20] At this moment, there is no available information whether the genetic variation of UDP-glucuronosyltransferases is associated with different activity for conjugation reaction of telmisartan, but it is possible that such genetic variation contributes to the large individual variability in the metabolism of telmisartan. In addition, it was reported that the $C_{max}$ and AUC increased more than proportionally with respect to the dose[2,4,5] and it is considered that the super-proportional increase of exposure is a consequence of saturable intestinal first-pass metabolism and high-affinity but limited-capacity uptake of telmisartan by the liver.[2] These complex features might be another factor causing large individual variability in telmisartan pharmacokinetics.

As shown in **Fig. 2A**, the values of $C_{max}$ predicted by the present PPK model were well consistent with the observed values. Furthermore, the values of AUC predicted by this PPK model were in good agreement with the values determined by the non-compartmental analysis, at a range of 2 orders (**Fig. 2B**). These results indicated that the present PPK model is capable of describing and predicting the individual exposure to telmisartan taking into account various patient backgrounds. Thus, this PPK model will be useful to further exposure-response analysis for antihypertensive effect of telmisartan.

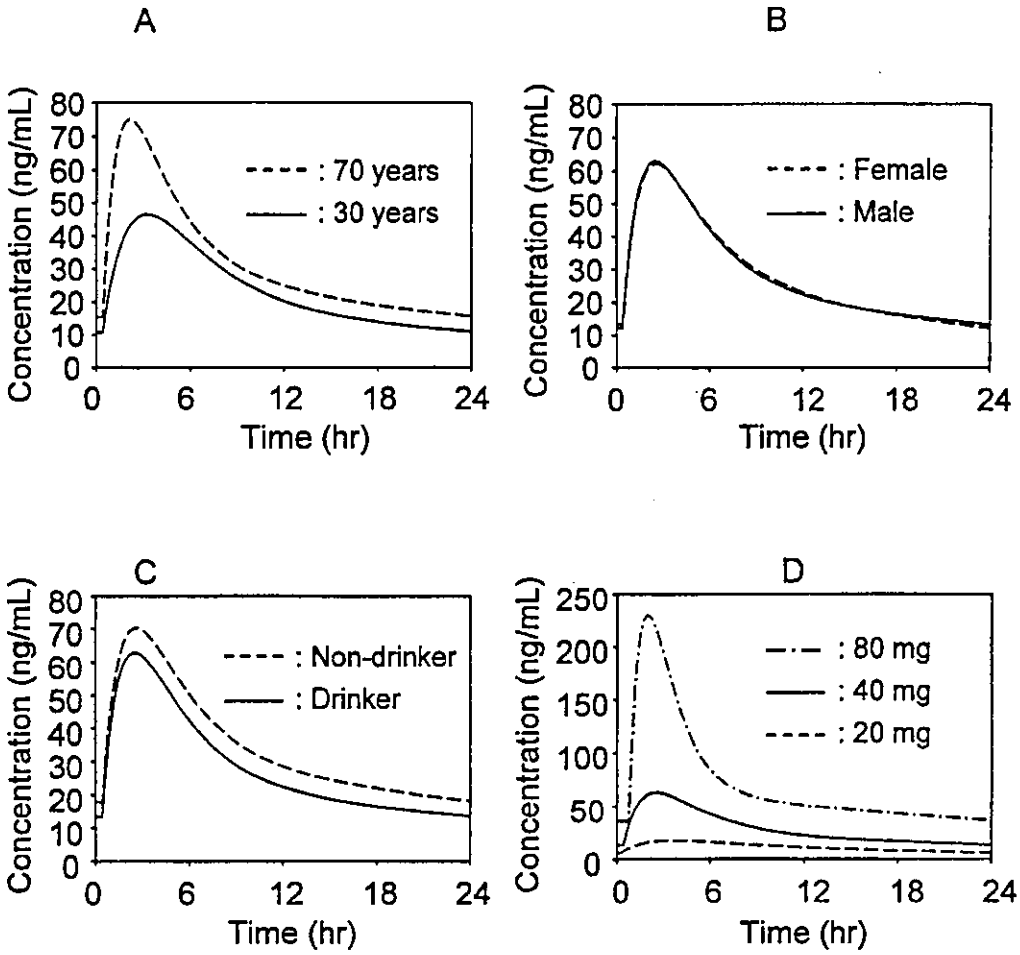


**Fig. 3.** Typical plasma concentration—time profiles of telmisartan at steady state, simulated for various patient subgroups. (A) 30 *versus* 70 years old, (B) male *versus* female, (C) drinker *versus* non-drinker, (D) 20, 40 or 80 mg dose.

**Table 5.** Influences of covariates on pharmacokinetic parameters of telmisartan

| Covariates | | | | | Estimated parameters | | | | | | Calculated exposures | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Model No. | Gender | Weight (kg) | Age (year) | Drinker | Dose (mg) | CL/F (L/hr) | $V_1/F$ (L) | Q/F (L/hr) | $V_2/F$ (L) | Ka ($hr^{-1}$) | Absorption lag time (hr) | $C_{max}$ (ng/mL) | $C_{trough}$ (ng/mL) | $AUC_{0-24}$ (ng·hr/mL) |
| 1 | Male | 60 | 50 | Yes | 40 | 59 | 192 | 80 | 996 | 0.33 | 0.40 | 63 | 13 | 680 |
| 2 | Female | 60 | 50 | Yes | 40 | 59 | 192 | 80 | 702 | 0.33 | 0.40 | 62 | 12 | 680 |
| 3 | Male | 80 | 50 | Yes | 40 | 59 | 192 | 80 | 809 | 0.33 | 0.40 | 62 | 13 | 680 |
| 4 | Male | 60 | 30 | Yes | 40 | 71 | 367 | 80 | 996 | 0.33 | 0.40 | 47 | 11 | 566 |
| 5 | Male | 60 | 70 | Yes | 40 | 52 | 125 | 80 | 996 | 0.33 | 0.40 | 75 | 15 | 768 |
| 6 | Male | 60 | 50 | No | 40 | 48 | 192 | 80 | 996 | 0.33 | 0.40 | 70 | 18 | 830 |
| 7 | Male | 60 | 50 | Yes | 20 | 75 | 297 | 80 | 996 | 0.16 | 0.22 | 18 | 6 | 268 |
| 8 | Male | 60 | 50 | Yes | 80 | 46 | 124 | 80 | 996 | 0.69 | 0.73 | 230 | 35 | 1728 |

* The underline represents the covariate changed from model No. 1.

The reliability and robustness of the final PPK model was validated by the bootstrap resampling method (**Table 6**). The means of parameter estimates for 200 bootstrap replicates of datasets were almost within 10% difference from the final PPK parameters obtained from the original dataset. Furthermore, all 200 trials of computation completed successfully. These results indicate the stability of the final model and the reliability of the parameter estimates.

In conclusion, a PPK model for telmisartan has been

**Table 6.** Summary of the bootstrap validation on the present population pharmacokinetic model and parameters for telmisartan

| | Final estimates of the model parameters | Results of 200 bootstrap simulation | | Bootstrap mean/ final estimate ratio (%) |
|---|---|---|---|---|
| | | Geometric mean | 95% CI (lower, upper) | |
| CL/F | 58.8 | 59.3 | (36.3, 96.9) | 100.8 |
| $V_1/F$ | 192.0 | 202.8 | (129.0, 318.8) | 105.6 |
| Q/F | 79.7 | 82.8 | (60.1, 114.2) | 103.9 |
| $V_2/F$ | 996 | 1060 | (703, 1597) | 106.4 |
| Ka | 0.330 | 0.337 | (0.256, 0.444) | 102.2 |
| Absorption lag time | 0.404 | 0.408 | (0.345, 0.484) | 101.2 |
| $\omega^2_{CL/F}$ | 3.56 | 3.75 | (1.08, 13.0) | 105.2 |
| $\omega^2_{V_1/F}$ | 1.42 | 1.35 | (0.798, 2.28) | 95.1 |
| $\omega^2_{Q/F}$ | 0.779 | 0.75 | (0.302, 1.85) | 96.1 |
| $\omega^2_{V_2/F}$ | 0.753 | 0.84 | (0.200, 3.51) | 111.4 |
| $\sigma^2$ | 0.329 | 0.322 | (0.140, 0.742) | 97.9 |

PK parameters were simulated under following condition: 60 kg, 50 years old, male drinker, 40 mg dose.

developed based upon the data obtained in the pre-marketing clinical trials. Several covariates such as age, gender, body weight, dose and alcohol consumption have been found to be factors that affect the individual variability in pharmacokinetics of telmisartan. The present PPK model well described the individual exposure to telmisartan, and the model has been validated for reliability and robustness.

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# *Relationship between Pharmacokinetic Parameters and Occurrence of Adverse Events in Clinical Trials Performed in Europe and United States for an Angiotensin II Receptor Antagonist, Telmisartan*

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**Summary:** The objective of this study was to clarify the relationship between the pharmacokinetic parameters of telmisartan and the occurrence of adverse events. In order to perform this study, a total of 1500 adverse events was collected from the eight clinical trials performed in Europe and the United States and the pharmacokinetic parameters ($C_{max}$ and AUC) were calculated with the parameters obtained from the population pharmacokinetic model which we have built. Using these data, the pharmacokinetic parameters ($C_{max}$ and AUC) were compared between subjects with or without the occurrence of adverse events. The Mann-Whitney test was performed to analyze ten adverse events selected based on the order of frequency. For eight of these ten adverse events, no significant between-group difference was observed in any pharmacokinetic parameter. For two adverse events, pain and sinusitis, the pharmacokinetic parameters, $C_{max}$ and AUC, were greater in subjects with adverse events as compared with those without adverse events, but the intersubject variability of pharmacokinetic parameters was large and there were many subjects in whom $C_{max}$ and AUC were high without any adverse event. These results suggest that there is no clear relationship between pharmacokinetic parameters of telmisartan and the occurrence of adverse events.

**Key words: pharmacokinetic parameter; adverse event; angiotensin II receptor antagonist; telmisartan; relationship**

## Introduction

Telmisartan is a nonpeptide angiotensin II receptor antagonist,[1,2] which is used for the treatment of hypertension.[3,4] In order to clarify the pharmacokinetic feature of telmisartan, we have already built and reported a population pharmacokinetic (PPK) model for telmisartan in healthy volunteers and hypertensive patients.[5,6] The obtained PPK model revealed several factors that could cause the large inter-individual variability in the pharmacokinetics of telmisartan. The PPK analysis also revealed that plasma concentrations of telmisartan obtained from Japanese clinical trials were lower than those obtained from the clinical trials performed in Europe and the United States.[6] The main cause of the difference was concluded to be the difference of food condition, that is, telmisartan was administered orally after food intake in the Japanese studies and before food intake in the other countries' studies. It has already been reported that angiotensin receptor blockers including telmisartan, are well tolerated and have a low incidence of adverse events.[7,8] In order to evaluate whether the safety of telmisartan would be affected by the above-mentioned variability of pharmacokinetics, we investigated the relationship between pharmacokinetic parameters of telmisartan and occurrence of adverse events.

## Methods

Data were collected from 1194 subjects enrolled in 8 clinical trials performed in Europe and the United States. The details of these trials are summarized in **Table 1**. Trial No. 4 was performed in elderly hypertensive patients at the dose of 20, 40 or 80 mg for 26

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**Table 1.** Summary of trial design

| Trial No. | Subjects | Dosage and administration | Period | Number of analyzed patients |
|---|---|---|---|---|
| 1 | Mild to moderate hypertensive Patients | 40, 80, 120 mg Orally once a day | 4 weeks | 117 |
| 2 | Mild to moderate hypertensive Patients | 20, 40, 80, 120, 160 mg Orally once a day | 4 weeks | 220 |
| 3 | Mild to moderate hypertensive Patients | 40, 80, 120, 160 mg Orally once a day | 12 weeks | 278 |
| 4 | Elderly patients with mild to moderate hypertension | 20, 40, 80 mg Orally once a day | 26 weeks | 94 |
| 5 | Mild to moderate hypertensive patients with moderate renal failure | 40, 80 mg Orally once a day | 12 weeks | 9 |
| 6 | Mild to moderate hypertensive Patients | 40, 80, 160 mg Orally once a day | 52–60 weeks | 358 |
| 7 | Patients with stable chronic symptomatic congestive heart failure | 10, 20, 40, 80 mg Orally once a day | 12 weeks | 106 |
| 8 | Healthy volunteers | 160 mg Orally once a day | 1 week | 12 |

weeks.[9] The trial in hypertensive patients with renal failure (creatinine clearance: 20 to 70 mL/min) was performed at the dose of 40 or 80 mg for 12 weeks (Trial No. 5).[10] Stable chronic symptomatic congestive heart failure patients were given the dose of 10, 20, 40 or 80 mg for 12 weeks (Trial No. 7).[11] A drug-drug interaction study between telmisartan and hydrochlorothiazide (HCTZ), Trial No. 8,[12] was included in this analysis. Healthy subjects were administered 160 mg of telmisartan for a week in this trial. Four other studies (Trial No. 1,[4] 2,[3] 3[13] and 6[14]) were performed in hypertensive patients. Hypertensive patients received 20, 40, 80, 120 or 160 mg of telmisartan for 4～60 weeks. Administrations of telmisartan in these 8 trials were performed before food intake. Placebo control group was included in Trial No. 1, 2 and 3. Trial No. 4, 5, 6 and 7 were performed with active control group (enalapril or lisinopril). The adverse events that occurred in the 8 trials were collected and listed in the order of frequency of their occurrence. Adverse events that occurred in the same subject at different dosages were counted separately. Though there were two distinct periods for each subject, with or without co-administration of HCTZ, in the drug-drug interaction study (Trial No. 8), the same adverse event occurring in the different periods in one subject was counted only once.

We have already built and reported a PPK model for telmisartan using plasma concentration data from trials in Japan, Europe and the United States.[6] Here, the European and United States plasma concentration data from 8 trials (Trial No. 1 to 8) were used to build the PPK model. From this PPK model, individual Bayesian estimates of pharmacokinetic parameters for each patient were obtained using the POSTHOC option in the NONMEM program (ver V, double precision, level 1.0).[15] With these parameters, the individual pharmacokinetic parameters ($C_{max}$ and AUC) at the steady state were calculated. The relationship between these calculated pharmacokinetic parameters and the occurrence of ten adverse events based on the order of their frequency was investigated. The relationship between these calculated pharmacokinetic parameters and the occurrence of serious adverse events was also investigated. A "serious" adverse event was defined as any fatal or immediately life-threatening clinical experience, any permanently or severely disabling event, an event that required or prolonged inpatient hospitalization, a congenital anomaly, cancer, or overdose. In addition, the relationship between the $C_{max}$ values obtained from the observed plasma concentration profiles (observed $C_{max}$) and the occurrence of the most frequent event, headache, was investigated.

A non-parametric test (Mann-Whitney test) was used to evaluate the significance of differences between groups. Data analysis was performed using SYSTAT® (version 7.0; SPSS Science, Chicago, IL) computer software. The criterion of statistical significance for all analyses was $\alpha = 0.05$.

## Results

The adverse events were collected from a total of 1500 cases, 1194 subjects, in 8 clinical trials.

A total of 1515 adverse events (233 kinds) were observed. **Table 2** shows the ranking of the top 10 adverse events. The most frequent adverse event was headache and it occurred in 146 subjects. The results of

Mann-Whitney tests are summarized in **Table 3** and the box plots of pharmacokinetic parameters ($C_{max}$ and AUC) of the subjects with or without the occurrence of adverse events are shown in **Fig. 1**. No significant between-group differences were observed in eight out of ten adverse events. Concerning two events, pain and sinusitis, significant differences were observed in $C_{max}$ and AUC. For these two adverse events, although the $C_{max}$ and AUC were greater in the subjects with the occurrence of adverse events compared with those without such occurrence, the intersubject variability was large and more than 10% subjects without event occurrence showed higher exposure than the upper quartile for the respective event-occurred groups.

**Table 2.** Ranking of adverse events

| Ranking | Event | Number of subjects |
|---|---|---|
| 1 | Headache | 146 |
| 2 | Upper respiratory tract infection | 136 |
| 3 | Pain | 87 |
| 4 | Dizziness | 69 |
| 5 | Back pain | 51 |
| 6 | Sinusitis | 47 |
| 7 | Coughing | 45 |
| 8 | Fatigue | 41 |
| 9 | Influenza-like symptoms | 37 |
| 10 | Diarrhoea | 35 |

In six of the clinical trials included in this study, only a few sparse plasma samplings were performed, so the observed $C_{max}$ values were obtained from the results of two trials (Trial No. 1 and 8) in which continuous plasma samplings were performed. The result of Mann-Whitney tests of the analysis using the observed $C_{max}$ value and box plot are shown in **Table 4** and **Fig. 2**. No significant between-group difference was observed.

In addition, comparison between the groups with serious adverse events and without serious adverse events was performed. 「All reported Serious adverse events in the 8 trials are listed in **Table 5**.」 As shown in **Table 6** and **Fig. 3**, no significant between-group difference was observed in any pharmacokinetic parameter.

## Discussion

The adverse events were collected from a total of 1500 cases from 8 clinical trials in which all subjects received telmisartan orally over 1 week. It was reported that steady-state plasma concentrations were achieved after approximately 7 days of administration,[4,16] so plasma

**Table 3.** Occurrence of adverse events and estimated PK parameters in the steady state ($C_{max}$ and AUC)

| Adverse event | $C_{max}$ (ng/mL) | | | AUC (ng·hr/mL) | | |
|---|---|---|---|---|---|---|
| | no | yes | p value[a] | no | yes | p value[a] |
| headache | 744±909 (1354) | 732±959 (146) | 0.515 | 4287±4585 (1354) | 3939±3781 (146) | 0.493 |
| upper respiratory tract infection | 728±887 (1364) | 895±1140 (136) | 0.207 | 4181±4377 (1364) | 4981±5670 (136) | 0.135 |
| pain | 730±913 (1413) | 951*±905 (87) | 0.011 | 4230±4563 (1413) | 4629*±3618 (87) | 0.033 |
| dizziness | 728±895 (1431) | 1050±1206 (69) | 0.111 | 4236±4523 (1431) | 4621±4331 (69) | 0.556 |
| back pain | 742±912 (1449) | 749±964 (51) | 0.865 | 4255±4538 (1449) | 4218±3795 (51) | 0.616 |
| sinusitis | 729±900 (1453) | 1171*±1217 (47) | 0.010 | 4204±4492 (1453) | 5783*±4957 (47) | 0.005 |
| coughing | 742±917 (1455) | 754±829 (45) | 0.778 | 4248±4530 (1455) | 4434±3995 (45) | 0.788 |
| fatigue | 735±900 (1459) | 1008±1304 (41) | 0.505 | 4228±4475 (1459) | 5145±5714 (41) | 0.416 |
| influenza-like symptoms | 742±917 (1463) | 770±814 (37) | 0.569 | 4226±4461 (1463) | 5343±6234 (37) | 0.258 |
| diarrhoea | 744±918 (1465) | 708±752 (35) | 0.930 | 4262±4548 (1465) | 3896±2701 (35) | 0.716 |

a) Mann-Whitney test
* $p<0.05$
The numbers represent mean±S.D.
The numbers in parentheses represent numbers of subjects.

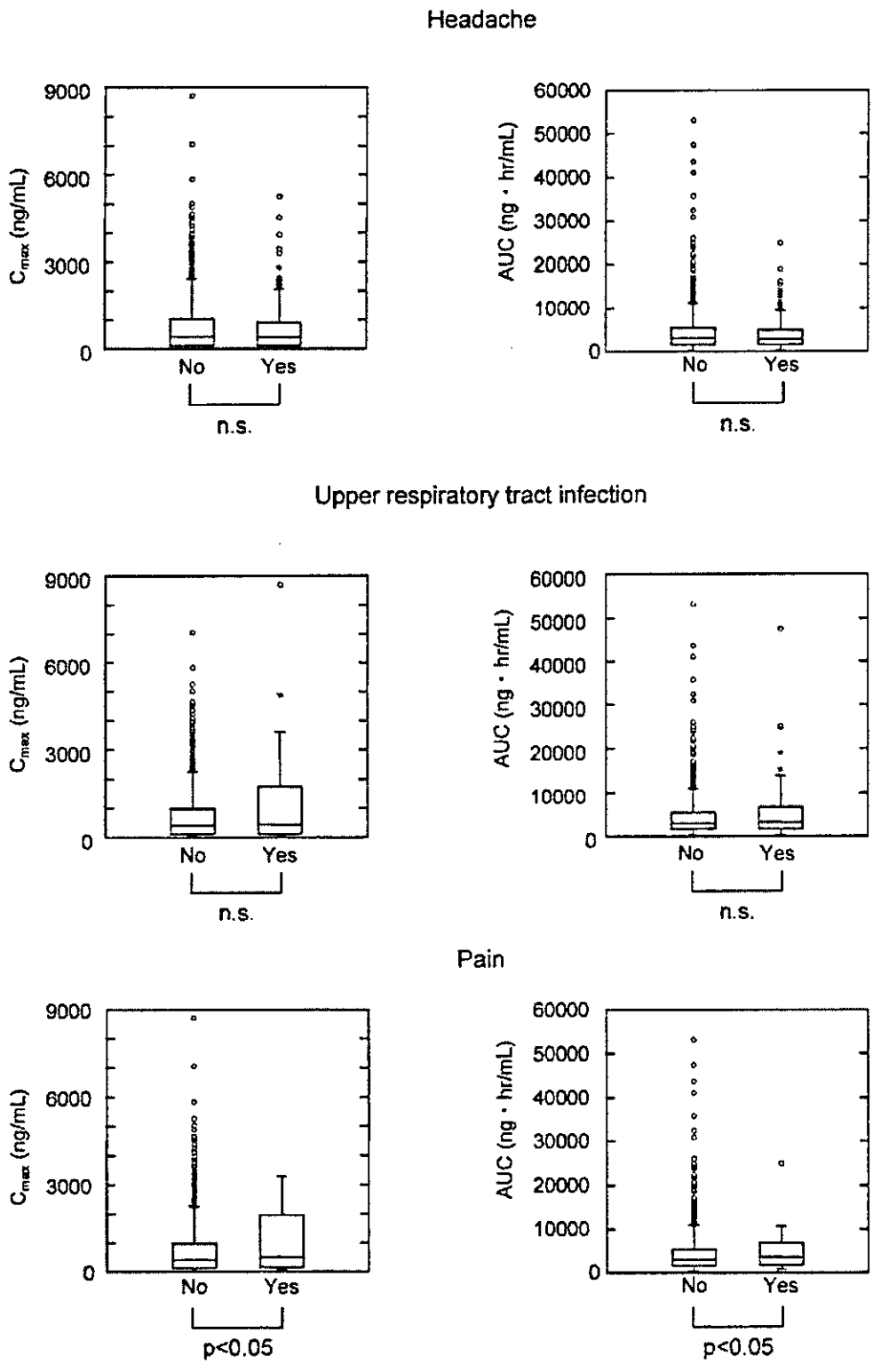
Headache
$C_{max}$ (ng/mL)
9000
6000
3000
0
No
Yes
n.s.
AUC (ng · hr/mL)
60000
50000
40000
30000
20000
10000
0
No
Yes
n.s.
Upper respiratory tract infection
$C_{max}$ (ng/mL)
9000
6000
3000
0
No
Yes
n.s.
AUC (ng · hr/mL)
60000
50000
40000
30000
20000
10000
0
No
Yes
n.s.
Pain
$C_{max}$ (ng/mL)
9000
6000
3000
0
No
Yes
p<0.05
AUC (ng · hr/mL)
60000
50000
40000
30000
20000
10000
0
No
Yes
p<0.05

Fig. 1(1)