In Vitro and in Vivo Characterization of a Newly Developed Clonidine Transdermal Patch for Treatment of Attention Deficit Hyperactivity Disorder in Children

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Received May 14, 2004; accepted October 14, 2004

The aim of this study was to characterize a newly developed clonidine transdermal patch, KBD-transdermal therapeutic system (TTS), for the treatment of attention deficit hyperactivity disorder in children. In vitro release, penetration, and in vivo pharmacokinetics in rabbits were investigated. The smaller size of KBD-TTS (2.5 mg/2.5 cm²) showed a similar in vitro penetration to those of Catapres-TTS (2.5 mg/3.5 cm²), a clonidine transdermal patch used for the treatment of hypertension, Alza Corporation, U.S.A.). The transdermal penetration rate of clonidine was mainly controlled by the ethylene vinylacetate membrane used in the patch. The skin layer may be only a minor rate-limiting barrier after the topical skin layer at the dosing site is saturated with penetrating clonidine in the initial phase (0 to 12 h). A sensitive liquid chromatography-mass spectrometry method for the quantification of clonidine in rabbit plasma was developed using solid-phase extraction and gradient elution on LC combined with the selected-ion monitoring (SIM) mode. A single dose of clonidine transdermal patch (KBD-TTS) or Catapres-TTS was transdermally administered to rabbits (n = 6 each) and removed after 168 h. The average half-life, T_max, C_max, and C_t values of clonidine in rabbits following administration of KBD-TTS were 19.27 ± 4.68 h, 52.56 ± 25.77 h, 27.39 ± 9.03 ng/ml, and 25.82 ± 9.34 ng/ml, similar to those of Catapres-TTS, respectively. The clonidine plasma concentration of KBD-TTS reached a steady state at 24 h through 168 h. The in vitro release rate of the clonidine from KBD-TTS significantly correlated with the in vivo absorption rate (p < 0.001).

Key words: clonidine; KBD-transdermal therapeutic system (TTS); LC-MS; transdermal penetration; pharmacokinetics; rabbit

Clonidine, also known as Catapres, was first synthesized in the early 1960’s. Clonidine is an imidazoline derivative and acts as an agonist on alpha-2 receptors peripherally as well as in the central nervous system. It has been used in the treatment of arterial hypertension and as an analgesic. Attention deficit hyperactivity disorder (ADHD) is characterized by the three primary symptoms of hyperactivity, impulsivity, and inattention, often resulting in poor academic performance and impaired social functioning. An estimated 3—6% of school-aged children have ADHD, which is more prevalent in boys. Clonidine has been used for the treatment of ADHD.

The transdermal drug delivery route is believed to have greater therapeutic benefits compared with the oral and parenteral routes, including decreased drug degradation due to gastrointestinal metabolism and first-pass metabolism in the liver, maintaining the therapeutic drug concentration over a long period of time, avoiding fluctuations in drug concentration and thus decreasing adverse reactions. Clonidine is available in several different dosage forms including oral, parenteral, and transdermal. The Catapres-transdermal therapeutic system (TTS), a clonidine transdermal patch, was developed by the Alza Corporation (Palo Alto, U.S.A.) in the mid-1980’s and has been used for treatment of hypertension, but is still unavailable for Chinese patients. A new clonidine transdermal patch (KBD-TTS) with a smaller size was developed by Beijing Kangbeide Pharmaceuticals, Ltd. in 2001 and has used to treat ADHD in children.

With oral or intravenous administration of a dose 50 to 200 μg/d, the clonidine concentration is in the range of nanograms to picograms per milliliter of plasma. Several quantification methods were reported for the measurement of the clonidine plasma concentration, including combined gas chromatography/electron-capture negative-ion chemical ionization mass spectrometry and radioimmunoassay (RIA). The liquid chromatography/UV-detection procedures are not suitable for concentration measurements in vivo because of the lack of sensitivity. Gas chromatography is sensitive, but sample preparation is time-consuming due to the numerous steps. However, solid-phase extraction on octadeyl-cartridges appears to be easier to separate the analyte from the complex matrix. Liquid chromatography coupled with mass spectrometry has recently been applied to analysis of drugs and used for the measurement of clonidine in human plasma.

Pharmacokinetic studies on Catapres-TTS are unavailable, although those of M-5041T, another clonidine transdermal patch that is different from Catapres-TTS in patch structure and in dose (2.5/5.0/7.5 mg for Catapres-TTS versus 6 mg for M-5041T) were performed in healthy human subjects.

The aims of this study were to evaluate the in vitro and in vivo pharmacokinetics in animals of the newly developed clonidine transdermal patch with a smaller in size (2.5 cm² of KBD-TTS versus 3.5 cm² of Catapres-TTS), KBD-TTS, for the treatment of ADHD in children.

MATERIALS AND METHODS

Materials Clonidine base, the clonidine transdermal patch (KBD-TTS) and the ethylene vinylacetate (EVA) were provided by Beijing Kangbeide Pharmaceuticals, Ltd. (Beijing, China). Catapres-TTS was commercially obtained from Alza Corporation. Acetonitrile was obtained from Fisher (HPLC grade) and ammonium hydrogen carbonate and am-
monia solution (analytical grade) from Beijing Chemical Plant (Beijing, China). The solid extraction columns (Oasis HLB) were purchased from Waters (Beijing, China). The Millipore filter membranes (0.2 µm) were obtained from Millipore (Beijing, China). Franz-type glass single-diffusion cells were purchased commercially.

The high-performance liquid chromatography (HPLC) system (Waters, U.S.A.) was used for in vitro studies including a Waters alliance 2690 autosampler, a Waters 2487 dual-wavelength absorbance UV-detector, and a Waters Xterra® C₁₈ column (5 µm, 150 mm×3.9 mm i.d.). Liquid chromatography coupled with mass spectrometry (LC-MS) was used for in vivo studies [Waters Alliance 2790 linked to a Micromass ZQ-4000 MS and the MS was equipped with an electrospray ion-spray (ESI) interface] The LC column used in vivo assay was Xterra C₁₈ (5 µm, 150 mm×2.1 mm i.d. Waters).

Guinea pigs (200—250 g) and rabbits (2.5—3.0 kg) were provided by the Animal Center of Peking University (Beijing, China).

**In Vitro Assay Using HPLC**  
HPLC ultraviolet detection was used for the in vitro clonidine assay. The mobile phase consisted of acetonitrile–0.05 mol/l ammonium hydrogen carbonate solution (20 : 80, v/v) adjusted to pH 9.50 with ammonia solution. The flow rate was 0.8 ml/min and detection wavelength 254 nm. The retention time of clonidine was about 8.5 min. The peak areas versus clonidine concentrations (r²=0.9999) were linearly correlated in the range of 2.0—60.0 µg/ml and the limit of quantification was 0.5 µg/ml. The coefficient of variation was less than 2%.

**Apparent Partition Coefficient of Clonidine**  
Supersaturated clonidine was placed in a screw-capped test tube and allowed to partition between the 2.5-cm² skin of guinea pigs (n=5) and water or between the 2.5-cm² EVA membranes (n=5) and water. The volume of dorsal or abdominal skin of guinea pigs was calculated based on the measured thickness and area (2.5 cm²) of the skin. The test tube was equilibrated in a constant-temperature shaker maintained at 32±0.5°C for 24 h. The saturated solution of clonidine was filtered through a 0.2-µm filter membrane. The residual solution of skin and EVA membrane were wiped off using filter paper. The extraction of clonidine from the skin and EVA membrane was performed using 50 ml of methanol and then methanol was filtered through a 0.2-µm filter membrane. The filtrates were assayed by HPLC as described below. The apparent partition coefficients (K_{app}) were the ratio of clonidine concentration in skin to the concentration in water or the ratio of clonidine concentration in EVA membrane to the concentration in water, respectively.

**In Vitro Release and Penetration**  
The EVA membrane and excised skin from male guinea pigs were separately used as a barrier membrane of in vitro release or transdermal penetration. The release and transdermal penetration of KBD-TTS (2.5 mg/2.5 cm²) were carried out in Franz-type glass single-diffusion cells with a’ 2.5-cm² penetration area and 45.0-ml receptor volume. The receiving fluid (degassed water) in Franz-type glass single-diffusion cells was magnetically stirred at 32.±±0.5°C. Sampling time-points were set at 3, 6, 12, 24, 48, 72, 96, 120, 144, and 168 h. The same volume of fresh degassed purified water was supplemented to the receiver after each sampling. The samples were filtered through a 0.2-µm filter membrane and analyzed by HPLC. Catapres-TTS (2.5 mg/3.5 cm²) was used as a control for transdermal penetration.

**Measurement of Clonidine in Plasma Using LC-MS**  
Gradient elution was used in the LC-MS system consisting of acetonitrile (solvent A) and ammonium hydrogen carbonate buffer 0.05 mol/l (solvent B, pH 10.50 adjusted with ammonia solution). The mobile phase was delivered at a flow rate of 0.2 ml/min using a linear gradient from A:B=10 : 90 (v/v) to A:B=90:10 between 0.00 min and 8.00 min, followed by an isocratic elution up to 12.00 min with A:B=90:10 (v/v), and elution with A:B=10:90 (v/v) at 12.10 min through 15.00 min. The optimized ms parameters were: electrospray ionization (ES+) mode; capillary voltage of 3.0kV, cone voltage of 40 V; source temperature of 105°C; and desolvation temperature of 150°C. The instrument was operated at unit resolution in the selected-ion monitoring mode (SIM) monitoring the molecular ion at m/z 230.

Solid-phase extraction was used for the extraction of clonidine in the plasma samples. Briefly, Oasis HLB cartridges were preconditioned with 1 ml of methanol and 1 ml of water. Plasma sample 0.2 ml was added to the cartridge and washed with 1.0 ml of water followed by 1 ml of 5% methanol. Clonidine was finally eluted with 1.0 ml of methanol. The eluent was evaporated to dryness under a stream of nitrogen at 40°C. The residue was reconstituted with 0.2 ml of acetonitrile and 10 µl of sample was injected into the LC-MS system.

Clonidine concentrations in rabbit plasma were set at 1.0, 4.0, 10.0, 20.0, 40.0, and 80.0 ng/ml, respectively, for preparation of calibration curves and extracted using the above procedures. Three concentrations of clonidine in rabbit plasma, 4.0, 20.0, and 80.0 ng/ml, were prepared for the measurement of accuracy (recovery) and precision (intra- and interday relative standard deviations). Measurement at each concentration was performed in five replicates. For measurement of interday precision, concentration samples were determined on 5 consecutive days.

**Dosing Regimen of KBD-TTS**  
Twelve rabbits were equally divided into two groups, and a single-dose clonidine transdermal patch (2.5 mg/2.5 cm², KBD-TTS) or Catapres-TTS (2.5 mg/3.5 cm²) was transdermally administered to the rabbits. Sampling time-points were set at 0, 3, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h. The patch was removed after 168 h. Blood samples were collected from the ear vein. Plasma samples were separated by centrifugation (3500 rpm) for 10 min, processed using the solid extraction cartridge for extraction of clonidine as described above, and analyzed using the LC-MS method.

**Pharmacokinetics, Correlation and Statistics**  
Pharmacokinetic parameters were calculated using CRF0 software (Chinese Academy of Science Calculating Center, Beijing, China). Correlation was performed between the in vitro release rate of clonidine from KBD-TTS and the in vivo absorption rate after administration of KBD-TTS to rabbits. The two-tailed t-test was used for statistical analysis of the transdermal penetration rate, analysis of variance (ANOVA) for the clonidine plasma concentration of the two patches, and correlation coefficient test for the correlation between in vitro release rate and in vivo absorption rate.
Table 1. Clonidine Apparent Partition Coefficient between Guinea Pig Skin or EVA Membrane and Water after Soaking the Skin or the Membrane in Excess Clonidine Solution at 32 °C for 24 h

<table>
<thead>
<tr>
<th></th>
<th>Amount of clonidine extracted (µg)</th>
<th>Volume of skin or membrane (cm³)</th>
<th>Concentration of clonidine (µg/ml)</th>
<th>$K_{app}^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal skin (n=5)</td>
<td>1538.1 ± 197.8</td>
<td>0.1658 ± 0.0156</td>
<td>9276.8 ± 1192.8</td>
<td>4.733</td>
</tr>
<tr>
<td>Abdominal skin (n=5)</td>
<td>793.6 ± 117.8</td>
<td>0.0877 ± 0.0061</td>
<td>9048.8 ± 1343.3</td>
<td>4.617</td>
</tr>
<tr>
<td>EVA membrane (n=5)</td>
<td>30.2 ± 4.7</td>
<td>0.0124 ± 0.0005</td>
<td>2425.7 ± 377.5</td>
<td>1.238</td>
</tr>
<tr>
<td>Water (n=5)</td>
<td></td>
<td>1960.1 ± 80.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$K_{app}^*$ represents clonidine apparent partition coefficient.

Table 2. Clonidine Residual (%) in EVA Membrane, Skin and KBD/Catapres-TTS after Release and Transdermal Penetration Experiments

<table>
<thead>
<tr>
<th></th>
<th>Clonidine residuals</th>
<th>Residual (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVA membrane after release</td>
<td>1.01 ± 0.11 (n=8)</td>
<td></td>
</tr>
<tr>
<td>KBD-TTS after release</td>
<td>51.21 ± 2.25 (n=8)</td>
<td></td>
</tr>
<tr>
<td>Abdominal skin after penetration using KBD-TTS</td>
<td>5.31 ± 1.04 (n=5)</td>
<td></td>
</tr>
<tr>
<td>KBD-TTS after penetration into abdominal skin</td>
<td>23.72 ± 1.93 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Dorsal skin after penetration using KBD-TTS</td>
<td>7.81 ± 0.28 (n=5)</td>
<td></td>
</tr>
<tr>
<td>KBD-TTS after penetration into dorsal skin</td>
<td>22.78 ± 1.63 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Abdominal skin after penetration using Catapres-TTS</td>
<td>6.97 ± 0.44 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Catapres-TTS after penetration into abdominal skin</td>
<td>16.53 ± 2.34 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Dorsal skin after penetration using Catapres-TTS</td>
<td>10.24 ± 0.53 (n=5)</td>
<td></td>
</tr>
<tr>
<td>Catapres-TTS after penetration into dorsal skin</td>
<td>15.40 ± 2.61 (n=5)</td>
<td></td>
</tr>
</tbody>
</table>

a) $p<0.01$, abdominal or dorsal skin versus EVA membrane.

**Release and Penetration of Clonidine from the Patches**

*In vitro* release % of KDB-TTS from 48 h to 168 h using the EVA membrane were significantly lower ($p<0.01$) than the transdermal penetration % of KBD-TTS or Catapres-TTS at the corresponding time-points using guinea pig abdominal or dorsal skins, as shown in Fig. 1A. No significant difference ($p>0.01$) between the abdominal and dorsal skin was observed in the amount of transdermally penetrating clonidine. No significant difference ($p>0.01$) was found in the transdermally penetrating amount of clonidine between KBD-TTS and Catapres-TTS, as depicted in Fig. 1B. The release rates at steady state were 2.61 µg/cm²·h for KBD-TTS (Fig. 1B) and the release kinetics showed a zero-order process. The steady-state transdermal penetration rates of KBD-TTS were 4.2888 and 4.2173 µg/cm²·h for the abdominal skin and dorsal skin, respectively, and those of Catapres-TTS were 3.1668 and 3.1257 µg/cm²·h for the abdominal skin and dorsal skin, respectively.

Clonidine residuals (%) in the EVA membrane and KBD-TTS after the release experiments were 1.01±0.11 (n=8) and 51.21±2.25 (n=8), respectively. Residuals (%) in the abdominal skin after penetration using KBD-TTS and Catapres-TTS were 5.31±1.10 (n=5) and 6.97±0.44 (n=5), respectively. The residuals (%) in the dorsal skin for the two formulations were slightly higher than those in the abdominal skin, as shown in Table 2. Residuals (%) in KBD-TTS and Catapres-TTS after penetration into abdominal skin were 23.72±1.93 (n=5) and 16.53±2.34 (n=5), while those in KBD-TTS and Catapres-TTS after penetration into dorsal skin were 22.78±1.63 (n=5), 15.40±2.61 (n=5), respectively.

**LC-MS Validation** LC chromatograms of blank plasma and the clonidine in plasma after administering KDB-TTS to

**RESULTS**

**Apparent Partition Coefficient** The saturated concentrations of clonidine in the guinea pig abdominal skin, dorsal skin, EVA membrane and water were 9048.8 ± 1343.3, 9276.8 ± 1192.8, 2425.7 ± 377.5, and 1960.1 ± 80.0 µg/ml, respectively. The $K_{app}^*$ values were 4.617, 4.733, and 1.238, respectively, as shown in Table 1. The results showed that the partition amount per volume of clonidine in the abdominal or dorsal skin was significantly higher than that of the EVA membrane ($p<0.01$) or in water ($p<0.01$), but no significant difference ($p>0.01$) was found between the EVA membrane and water.
rabbits are shown in Figs. 2A and B. The retention time of clonidine was 5.4 min. The molecular ion of clonidine was formed at m/z 230 (M+1). In the MS spectrum, m/z 232 and m/z 233 were the isotope peaks of the molecular ion, as shown in Fig. 2C.

The calibration concentration ranged from 1.0 to 80.0 ng/ml in plasma. The limit of quantitation of clonidine was 0.25 ng/ml. The detection limit based on the 3:1 peak height ratio of clonidine over noise was 0.05 ng/ml.

The mean recovery of clonidine from the plasma was 92.25 ± 7.21% at 4.0 ng/ml, 94.30 ± 4.43% at 20.0 ng/ml, and 96.20 ± 5.07% at 80.0 ng/ml. Intra- and interday relative standard deviations (R.S.D.) were from 2.42 to 4.48%, as shown in Table 3.

**Pharmacokinetics**  Clonidine plasma concentrations of KBD-TTS were 4.62 ± 2.87 at 3 h, 16.52 ± 8.81 at 12 h, 27.31 ± 9.24 at 24 h, 21.84 ± 8.47 at 192 h, and 5.73 ± 2.35 ng/ml at 240 h. The concentration of clonidine reached a steady state at 24 h through 168 h and the mean concentration was 25.82 ± 9.34 ng/ml. Clonidine plasma concentrations of Catapres-TTS were very similar to those of KBD-TTS at various time-points (p > 0.01), as shown in Fig. 3 and Table 4.

The mean half-life, T_{max}, C_{max}, AU{C}_{0—infty} and CL values of clonidine in rabbits following administration of KBD-TTS were 19.27 ± 4.68 h, 52.56 ± 25.77 h, 27.39 ± 9.03 ng/ml, 5504.17 ± 1995.27 h·ng/ml, 0.51 ± 0.17 l/h, respectively. The pharmacokinetic parameters of clonidine in rabbits following administration of Catapres-TTS were not significantly different from those of KBD-TTS (p > 0.01), as indicated in Table 4.

**Correlation between in Vitro Release and in Vivo Absorption**  The in vitro release rate of clonidine from KBD-TTS was faster than the in vivo absorption rate in the initial phase from 0 h to 12 h after administration to rabbits, and then the release rate showed a strong correlation (p < 0.001) with the absorption rate of clonidine, as shown in Fig. 4.
The clonidine plasma concentrations of KBD-TTS were similar to those of Catapres-TTS. Furthermore, the drug reservoir, control membrane, contact adhesive, and protecting liner. In appearance, it is similar to that of Catapres-TTS but smaller in size because KBD-TTS was developed mainly for the treatment of children with ADHD. Furthermore, the drug reservoir, contact adhesive, and EVA membrane were newly formulated or developed by Beijing Kangbeide Pharmaceuticals, Ltd. and it has applied for a patent for the technique for KBD-TTS in China (application no. 20041000394.7).

The $K_{app}$ values showed that saturated concentrations of clonidine in the skin were significantly higher than those in the EVA membrane, suggesting that the skin is a temporary depot for clonidine after transdermal dosing. Residual measurement further indicated that the clonidine residual in the skin after release or penetration was markedly higher than that in the EVA membrane. The residual clonidine in the controlled release membrane (EVA membrane) was minimal. Results from KBD-TTS penetration using abdominal or dorsal skin showed that the penetration rates for both sites of guinea pig skin were similar in spite of the thicker dorsal skin. In contrast, the release rate of clonidine from the patch, the transdermal penetration rate was markedly higher ($p>0.01$). This suggests that the transdermal penetration resistance is lower than the resistance of release through the EVA membrane. It could further be inferred that the transdermal penetration rate of clonidine was mainly controlled by the EVA membrane and that the release from the patch was the rate-limiting step. The skin layer may be only a minor rate-limiting barrier after the topical skin layer at the patch site was saturated with penetrating clonidine in the initial phase (0 to 12 h). In comparison, in vitro penetration of KBD-TTS was similar to that of Catapres-TTS.

**DISCUSSION**

KBD-TTS is a transdermal patch with ‘a’ five-layered structure consisting of backing, drug reservoir, control membrane, contact adhesive, and protecting liner. In the appearance, it is similar to that of Catapres-TTS but smaller in size because KBD-TTS was developed mainly for the treatment of children with ADHD. Furthermore, the drug reservoir, contact adhesive, and EVA membrane were newly formulated or developed by Beijing Kangbeide Pharmaceuticals, Ltd. and it has applied for a patent for the technique for KBD-TTS in China (application no. 20041000394.7).

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**Table 4. Pharmacokinetic Parameters of Clonidine in Rabbits (n=6 Each) Following Administration at a Single Dose of 2.5 mg (Patch Area 2.5 cm²) of KBD-TTS or 2.5 mg of Catapres-TTS (Patch Area 3.5 cm²)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KBD-TTS</th>
<th>Catapres-TTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{12}$ (h)</td>
<td>0.0380±0.010</td>
<td>0.0297±0.0036</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>19.27±4.68</td>
<td>23.70±3.00</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>52.56±25.77</td>
<td>68.57±54.34</td>
</tr>
<tr>
<td>$C_{e}$ (ng/ml, 24 h to 168 h)</td>
<td>25.82±9.34</td>
<td>26.92±8.83</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (h·ng/ml)</td>
<td>5504.17±1995.27</td>
<td>5935.17±1429.95</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>0.51±0.17</td>
<td>0.45±0.11</td>
</tr>
<tr>
<td>$V$ (l)</td>
<td>13.6±4.1</td>
<td>15.5±4.8</td>
</tr>
</tbody>
</table>

In vivo analysis of clonidine in rabbit plasma, the endogenous ingredients did not interfere with the measurement of clonidine, and high assay specificity and shorter run time were achieved with use of solid-phase extraction and gradient elution on LC combined with SIM mode. Recovery and precision results demonstrated that the determination method met the requirements for the determination of biological samples. The measurement of clonidine using LC-MS, as shown by Naidong et al.,$^{(9)}$ showed that the peak of clonidine appeared at 1.23 min. The present LC-MS system is more suitable for the determination of biological samples because peaks of endogenous ingredients commonly appear at 3 min or earlier in the chromatogram.

The pharmacokinetic results demonstrated that the clonidine plasma concentration of KBD-TTS reached a steady state from 24 h through 168 h. After the patch was removed at 168 h, the concentration still remained at a higher level for about 48 h. This may be due to the reservoir effect of clonidine inside the skin layer and the longer elimination half-time (mean 19 h). Clonidine plasma concentrations within the adult clinically effective range for the treatment of hypertension is 0.2—1.0 ng/ml, as described by Lonnqvist et al.$^{(14)}$ but the effective concentration range for the treatment of ADHD is unknown. A multicenter clinical assessment of KBD-TTS is currently underway in China with the approval of the State Food and Drug Administration. The clonidine plasma concentration versus time profile of KBD-TTS and pharmacokinetic parameters were very similar to those of Catapres-TTS as a control. The plasma half-life was unavailable in the rabbits and reported to be from 12 to 16 h in humans following oral administration, and the half-life increased up to 41 h in patients with severe impairment of renal function.$^{(5)}$ The results of clonidine transdermal patch (M-5041T, Japan) use in healthy human subjects showed that the average plasma half-life was from 32.3 to 40.3 h for different
The in vitro release rate of clonidine from KBD-TTS correlated significantly with the in vivo absorption rate. This indicates that the in vitro release rate of clonidine from the patch could be an indicator to assess the in vivo absorption rate.

In conclusion, the smaller size of KBD-TTS (2.5 mg/2.5 cm²) designed for children shows similar in vitro penetration effects to those of Catapres-TTS (2.5 mg/3.5 cm²). The transdermal penetration resistance was lower than the release through the EVA membrane based on estimates of the release and penetration rates. The transdermal penetration rate of clonidine was mainly controlled by the EVA membrane, and the release from the patch was the rate-limiting step. The skin layer may be only a minor rate-limiting barrier after the topical skin layer at the administration site was saturated with penetrating clonidine in the initial phase (0 to 12 h). The average half-life, $T_{\text{max}}$, $C_{\text{max}}$, and $C_{\text{ss}}$ values of clonidine in rabbits following administration of KBD-TTS were 19.27 ± 4.68 h, 52.56 ± 25.77 h, 27.39 ± 9.03 ng/ml, and 25.82 ± 9.34 ng/ml respectively. The clonidine plasma concentration of KBD-TTS reached the steady state from 24 h through 168 h. After the patch was removed at 168 h, a high concentration remained at about 48 h. The in vitro release rate of the clonidine from KBD-TTS significantly correlated with the in vivo absorption rate. In addition, a sensitive LC-MS method for the quantification of clonidine in rabbit plasma was developed using solid-phase extraction and gradient elution on LC combined with the SIM mode.

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