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Incidence of low bioavailability of leuprolide acetate after percutaneous administration to rats by dissolving microneedles

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ABSTRACT

Two-layered dissolving microneedles of which acral portion contained leuprolide acetate (LA) as solid dispersion were prepared with sodium chondroitin sulfate as the base and the systemic absorption efficiency of LA was studied in rats after administration to their abdominal skin. A patch contained 100 dissolving microneedles of which length and basement diameter were 469.8 ± 4.7 μm and 284.5 ± 9.8 μm, where LA content was 14.3 ± 1.6 μg. In vitro dissolution experiment showed that LA was released from dissolving microneedle patch within 3 min. LA was stable in the patch, % recoveries for 3 months were 102.2 ± 1.9–95.3 ± 1.9%. One and half-patch of LA dissolving microneedles were administered to the rat skin and plasma LA concentrations were measured by LC–MS/MS. Plasma LA concentrations increased immediately after administration, and reached to the maximum level at 15 min, where C_{max} were 6.0 ± 0.7 and 16.4 ± 0.9 μg/ml, respectively. The AUC were 5.8 ± 0.8 and 14.5 ± 0.4 μg h/ml and BA were 33.8 ± 4.3% and 31.7 ± 0.8%. When LA solution was subcutaneously (s.c.) injected to rats, 50 μg/kg, the BA was 32.0 ± 2.1%. Relative BA of LA from dissolving microneedles against s.c. solution was 105.6 ± 13.5%. The degradation rate of LA in the rat skin tissue homogenate was very fast where the half-life was 16.3 ± 5.7 min. The degradation of LA in the skin tissue was the cause of the low BA of LA after percutaneous administration to rats.

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1. Introduction

Leuprolein acetate (LA) is a synthetic agonist analogue of gonadotropin-releasing hormone. By the continuous administrain of leuprolein, the suppression of gonadal steroid sysnthesis is induced and as a result pharmacological castration is obtained. Clinically, it is used for the therapy of prostatic cancer and endometriosis (Tombal et al., 2010; Nanda et al., 2009; Laimou et al., 2010). Due to the pharmacological mechanism of the action of leuprolein, chronic administration of leuprolein is needed. Therefore, studies on the long-term sustained-release preparation based on microencapsulation technology and liposomes have been performed (Toguchi et al., 1991; Guo et al., 2003; Woo et al., 2001). As leuprolein is a peptide drug, its membrane permeability through the small intestinal tract is low and it receives the hydrolysis by the digestive enzymes, the bioavailability (BA) of leuprolide is low (Degim and Celebi, 2007). Therefore, patients receive subcutaneous (s.c.) injection therapy and as a result the quality of life (QOL) of them is low. To improve the QOL of the patients, several drug delivery systems (DDSs) including nasal spray, inhalation (Adjei et al., 1992), intravaginal suppository (Okada et al., 1984), have been challenged. However, most of them are failed to develop as pharmaceutical preparation because of low BA and wide inter- and intra-subject variations of BA.

We have focused on the transdermal drug delivery systems (TDDS). TDDS has the following advantages over the traditional pharmaceutical preparation like tablets and capsules (De Jager et al., 2007), (1) no first-pass effects of the liver associated with oral delivery; (2) less or no degradation by hydrolytic enzymes compared to that in the GI tract; (3) less or no pain compared to subcutaneous injection; (4) better convenience of administration than intravenous injection; (5) better and more continuously controlled delivery rate than that of oral or subcutaneous sustained-release preparations; and (6) easy removal if side-effects appear.

Recent advances in microfabrication technology have made it possible to prepare microneedles that have a possible application as novel TDDS. Since the first publication by Henry et al. in 1998 (Henry et al., 1998), microfabrication techniques for the production of silicon, metal, glass and polymer microneedle arrays with micrometer dimensions have been reported (Teo et al., 2005; Park et al., 2006; Davis et al., 2005; McAllister et al., 2003). The microneedles are either solid or hollow and possess a geometrical shape. Microneedle TDDS are roughly defined by a micron size needle

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preparation through and by which drug is percutaneously administered. Microneedle TDDS are classified as follows; (1) extremely small needles through which drug solution can be injected into the skin, (2) metallic and/or silastic microneedles on whose surface drug is coated, and (3) metallic and/or silastic microneedles with which conduits (micropores) are made on the skin and drug solution is applied after removing the microneedles.

On the other hand, we have been studying the dissolving microneedles that are made of water soluble thread-forming polymer like chondroitin sulfate, dextran and albumin etc. and the usefulness were suggested with insulin (Ito et al., 2006a, 2008a), erythropoietin (EPO) (Ito et al., 2006b, 2007), interferon (Ito et al., 2008b) and recombinant human growth hormone (Ito et al., 2008c). They were well absorbed through rat skin by dissolving microneedle preparations about 2-3 mm in length with an outer diameter of 0.3–0.4 mm. In addition, a dissolving microneedle array comprising 10 lines and 10 columns was formed on a 1.0 cm^2 tip, where water-soluble thread-forming polymers like chondroitin sulfate and dextran were used as the base and insulin was formulated as a model peptide drug (Takada, 2008). By comparing the hypoglycemic effects of insulin from dissolving microneedles and subcutaneous injection preparation, relative pharmacological availability was 30% (Ito et al., 2009). The molecular weight (MW) of insulin is 6 kDa, though the MW of leuprolide is 1.2 kDa. As the MW of the peptide decreases, the membrane permeability is thought to be increased. Therefore, dissolving microneedle technology was applied to leuprolide and the factors affecting the BA of LA from dissolving microneedles was evaluated in the rat experiment.

2. Materials and methods

2.1. Materials

Leuprolide acetate (LA) was obtained from Techno-science (Chiba, Japan) Sodium chondroitin sulfate, dextran (MW = 50–70 kDa), methylene blue (MB) and polyethyleneglycol 400 (PEG) were obtained from Nacalai Tesque Inc. (Kyoto, Japan). All other materials used were of reagent grade and were used as received. Male Wistar Hannover rats used in the study were obtained from Japan SLC Inc. (Hamamatsu, Japan). A standard solid-meal commercial food (LabDiet®; Japan SLC Inc., Hamamatsu, Japan) was used. All other materials used were of reagent grade and were used as received.

2.2. Preparation of dissolving microneedle patches and injection solutions

To produce the dissolving microneedle patches, 0.2 mg of LA and 10 mg of sodium chondroitin sulfate was dissolved with 50 μl of 0.4 w/v% MB solution. The obtained drug glue was mixed well. After the drug glue was degassed under reduced pressure, it was dispensed into a mould containing 100 inverted cone-shaped wells, each with area of 1.0 cm^2. Each well was 500 μm deep, with 300 μm diameter at its top. The mould was covered with a 300 g steel plate; then the drug glue was filled into the wells. After the plate was removed, glue made of either 15 mg of chondroitin sulfate and 25 ml of distilled water was painted over the mould. It was then dried under the pressure of the stainless steel plate for 3 h. Thereafter, the plate was removed and a dissolving microneedle patch was obtained by detaching it with a supporting material.

LA, 1.0 mg, was dissolved with 480 μl of saline and solution for subcutaneous and intramuscular injection experiments was obtained. Injection solution for i.v. infusion experiment was prepared by diluting the 80 μl of the above prepared solution with 0.92 or 4.92 ml of saline.

2.3. LA content in dissolving microneedle patch

The LA was extracted from dissolving microneedle patches with 10.0 ml of 0.1 mM phosphate buffer, pH 7.4; a 100 μl aliquot was used for the LC–MS/MS assay described below.

2.4. In vitro dissolution experiment

In vitro dissolution experiment was performed with a rotator RT-50 (Taitec, Saitama, Japan) by put the test dissolving microneedle patch into a bottle with 1.0 ml of 10 mM phosphate buffer, pH 7.4, rotated at 13 rpm at 37 °C. To determine the amount of LA released from the patch, 1.0 ml of the dissolution medium was collected for the assay at 0, 0.5, 1, 2, 3, 5 and 10 min and replaced with fresh dissolution medium that was degassed before. The cumulative released amount of LA from dissolving microneedle array patch was defined by the following equation:

$$\text{Cumulative amount released} = \left(\sum_{t=0}^{t} \frac{M_t}{M_{10\,\text{min}}} \right) \times 100\%$$

where M_t is the amount of LA dissolved at time t, and $M_{10\text{min}}$ is the dissolved amount of LA at 10 min after the start of the dissolution experiment.

2.5. Stability study

The dissolving microneedle patches were kept for 1 and 3 months under four different conditions: −80, 4, 23 and 37 °C. Thereafter, LA was extracted from the patches with 10 ml of phosphate buffer, pH 7.4. The LA contents were measured by LC–MS/MS method.

2.6. In vivo absorption experiments in rats

Male Wistar Hannover rats, 298–363 g, were anesthetized with intraperitoneal injection of sodium pentobarbital, 50 mg/kg. One group consisted of 3–4 rats. At 10 min before drug administration, 0.25 ml of blank blood samples was obtained from the left jugular vein with a heparinized syringe. The hair on the abdominal region was removed with a shaver (ES7111; Panasonic Inc., Osaka, Japan). One or half-patch of the dissolving microneedles were inserted to the skin by pressing the base with two fingers for 3 min without any treatment. The dissolving microneedles were administered not to the dermis but to the epidermis by making sure of no bleeding. At 5, 15, 30, 60, 120, 180, 240 and 300 min after administration, 0.25 ml blood samples were collected from the left jugular vein. By centrifuging at 12,000 rpm for 10 min at 4 °C using a centrifuge, Kubota 1700 (Kubota, Tokyo, Japan), 100 μl of plasma samples were obtained. The resultant plasma samples were stored at −80 °C until analysis by LC–MS/MS.

For the s.c. injection experiment, LA solution was injected into the abdominal skin where the hair was removed previously, 50 μg/kg. In another group of rats, LA solution was infused into the left femoral vein for 30 min, 50 μg/kg, after a 0.25 ml blank blood sample was obtained from the left jugular vein. Additional blood samples of 0.25 ml were obtained at 5, 15, 30, 60, 120, 180, 240 and 300 min using a heparinized syringe. After centrifugation, plasma samples were obtained. All these plasma samples were immediately frozen in a deep freezer at −80 °C until analysis. All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation, Kyoto Pharmaceutical University.

2.7. In vitro stability study with skin tissue homogenate

Rat skin specimens were obtained from their abdominal skin under anesthesized condition and tissue homogenate was prepared by homogenizing the isolated skin tissue with five times volume of saline at 4 °C. After the homogenate was centrifuged at 3000 rpm for 15 min, the supernatant was used. To the 180 μl of thus obtained supernatant, 20 μl of LA solution, 100 μg/ml, was added and was incubated at 37 °C for 30, 60 and 120 min. By adding 0.5 ml of acetonitrile, the reaction was stopped and the obtained samples were immediately frozen in a deep freezer at −80 °C until analysis. The elimination ratio was estimated from the amount of LA in spiked samples.

2.8. LC–MS/MS assay methods for LA

The LA samples were measured by modifying mass spectrometric methods (Sofianos et al., 2008) following a solid phase extraction (Singh and Singh, 2007). The LA extraction in sample was performed using an Oasis® HLB 1 cc (30 mg) extraction cartridge (Waters Corp., Massachusetts, USA). In a 1.5 ml microtube, 100 μl of LA sample was mixed with 200 μl of acetonitrile. After centrifugation at 1200 × *g* for 10 min, the supernatant was added to 700 μl of 1% formic acid. The resultant solution was loaded to an extraction cartridge that was pre-equilibrated by washing with acetonitrile (1 ml, once) followed by 1% formic acid (1 ml, three times). The column was washed with 1% formic acid (1 ml, two times), and the eluent was discarded. Then LA was eluted with acetonitrile and 1% formic acid at a ratio of 1:1 (v/v; 1 ml, three times), and eluent was collected in a test tube and evaporated to dryness at 60 °C under the flow of nitrogen gas. The residue was reconstituted with 100 μl of mobile phase, of which 50 μl was injected into the LC/MS/MS system, as described below.

The LC–MS/MS system consisted of an API 3200 triple quadrupole mass spectrometer equipped with turbo ion spray sample inlet as an interface for electrospray ionization (ESI), an analyst workstation (Applied Biosystems, CA, U.S.A.), a micropump (LC-10AD; Shimadzu Corp., Kyoto, Japan) and an automatic sample injector (AS8020; Tosoh Corp., Tokyo, Japan). The mobile phase, 1% formic acid/acetonitrile (70:30, v/v), was degassed and pumped through an ODS column (2.1 mm i.d. × 100 mm, 3 (m size, Quicksorb; Chemco Scientific, Co. Ltd., Osaka, Japan) at a flow rate of 0.2 ml/min. The column temperature was maintained at 25 °C. For LA (MW: 1209) detection, the transitions of m/z 605.5 → 249.1 were optimized for the following conditions. Ionization occurred *via* the turbo ion spray inlet in the positive ion mode. The flow rates of nebulizer gas, curtain gas, and collision gas were set, respectively, at 8.0, 8.0, and 2.0 l/min. The ion spray voltage and temperature were set, respectively, at 5 kV and 500 °C. The declustering potential, the entrance potential, the collision energy and the collision cell exit potential were set, respectively, at 40.0, 7.0, 90.0, and 2.5 V.

2.9. Pharmacokinetic analysis

Pharmacokinetic parameter values were calculated by a non-compartment analysis method (Yoshikawa et al., 1998). The maximum drug concentration, C_{max}, and the time to reach maximum concentration, T_{max}, were determined from the authentic plasma drug concentration *vs.* time data. The area under the plasma drug concentration *vs.* time curve, AUC, after percutaneous administration or intravenous injection was calculated using the linear trapezoidal rule up to the last measured drug concentration, $C_{p(last)}$, and was extrapolated to infinity by addition of the correction term $C_{p(last)}/k$, where k was the terminal elimination rate constant. The absolute bioavailability (BA) and relative bioavailability (RBA) of LA from dissolving microneedles against s.c. injection solution were calculated using the following equation.

$$BA(\%) = \left(\frac{AUC_{patch}}{AUC_{i.v.}}\right)\left(\frac{Dose_{i.v.}}{Dose_{patch}}\right) \times 100$$

$$RBA(\%) = \left(\frac{AUC_{patch}}{AUC_{s.c.}}\right)\left(\frac{Dose_{s.c.}}{Dose_{patch}}\right) \times 100$$

where AUC_{patch}, $AUC_{i.v.}$, $AUC_{s.c.}$, are the AUC obtained after percutaneous, intravenous (i.v.) and s.c. administration of LA and $Dose_{i.v.}$, $Dose_{patch}$ and $Dose_{s.c.}$ are the administered dose of LA by percutaneous, intravenous (i.v.) and s.c. administration.

Table 1
Physical characteristics of dissolving microneedles containing LA.

Length (μm)	Diameter* (μm)	Length of the drug loaded space (μm)	LA content (μg)
469.8 ± 4.7	284.5 ± 9.8	224.2 ± 12.8	14.3 ± 1.6

Each value represents the mean ± S.E. (n = 6).
* The diameter of the basement.

2.10. Statistics

All values are expressed as their mean ± S.E. Statistical differences were assumed to be significant when p 0.05 (Student's unpaired t–test).

3. Results

3.1. Physicochemical properties of dissolving microneedles

The physical properties of the prepared dissolving microneedles are shown in Table 1. The mean lengths of the dissolving microneedles were 469.8 ± 4.7 μm and the basement diameters were 284.5 ± 9.8 μm. The mean length of the drug-loaded space was 224.2 ± 12.8 μm from the top of the dissolving microneedles. The drug content was 14.3 ± 1.6 μg.

3.2. In vitro dissolution study of LA from dissolving microneedles

Dissolution experiment was performed with LA dissolving microneedle patch and the result is shown in Fig. 1 under perfect sink condition. The dissolution rate of LA was rapid and approximately 100% of LA was released from the dissolving microneedles within 5 min after the start of the dissolution experiment, where

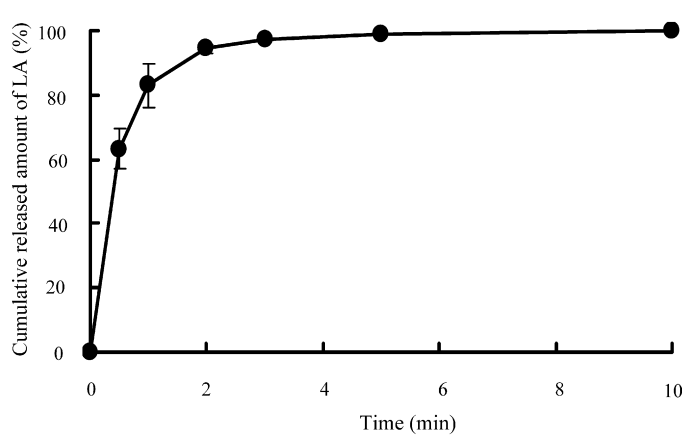

Fig. 1. Release profile of LA from dissolving microneedle patch. Each point represents the mean ± S.E. of 4 experiments

Table 2
Stability of LA in dissolving microneedles at 1 and 3 months after the start of the experiment.

Temperature (°C)	% Remaining of LA	
	1 month	3 month
−80 °C	97.7 ± 2.9	97.3 ± 2.2
4 °C	98.1 ± 1.0	102.2 ± 1.9
23 °C	95.4 ± 3.1	95.1 ± 1.4
40 °C	96.1 ± 1.5	95.3 ± 1.9

Each value represents the mean ± S.E. (n = 5).

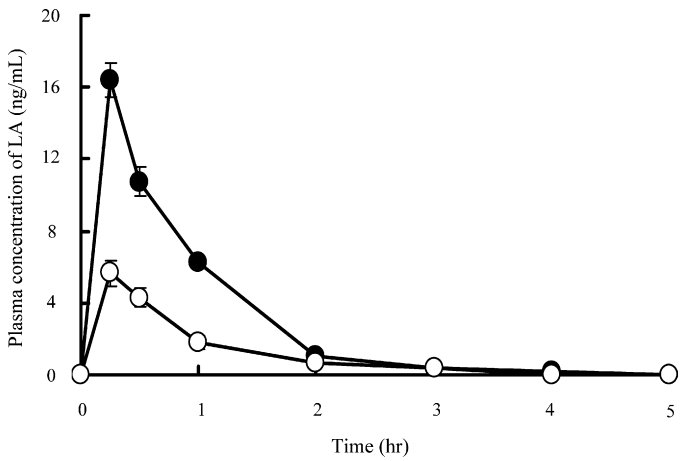

Fig. 2. Plasma LA concentration *vs.* time profiles after percutaneous administration of LA dissolving microneedle patch to the abdominal rat skin. Plasma LA concentrations were measured by LC–MS/MS method. The open and closed circles denotes that obtained after the administration of half patch and one patch of dissolving microneedles. Each point represents the mean ± S.E. of 3–4 experiments.

the time when half amount of the formulated LA was released, T50%, was 0.40 ± 0.04 min.

3.3. Stability of LA in dissolving microneedles

Table 2 shows the results on the stability study of LA dissolving microneedles. Under four different conditions, the remaining percentage amounts of LA after 1 month were 97.7 ± 2.9% (−80 °C), 98.1 ± 1.0% (4 °C), 95.4 ± 3.1% (23 °C) and 96.1 ± 1.5% (40 °C), respectively. In the case of longer time stability study for 3 months, the remaining % of LA were 97.3 ± 2.2% (−80 °C), 102.2 ± 1.9% (4 °C), 95.1 ± 1.4% (23 °C) and 95.3 ± 1.9% (40 °C), respectively. Consequently, LA was shown to be stable in the dissolving microneedles for 3 months.

3.4. In Vivo absorption of LA from dissolving microneedles in rats

The prepared dissolving microneedles were administered to the rat skin by pressing the patch on the abdominal skin where the hair had been removed. Fig. 2 shows the plasma LA concentration *vs.* time curves after administration to rats with two conditions, one patch and half patch where the LA doses were 29.9 ± 0.8 μg/kg and 13.9 ± 0.3 μg/kg, respectively. The plasma LA concentration increased rapidly and reached its maximum concentrations at 15 min in both cases. The maximum plasma LA concentrations, C_{max}, were 16.4 ± 0.9 ng/ml for 1 patch study and 6.0 ± 0.7 ng/ml for half patch study and are shown in Table 3 with other pharmacokinetic parameters. Thereafter, the plasma LA concentration gradually decreased and disappeared within 5 h after administration. The AUCs were 14.5 ± 0.4 ng h/ml and 5.8 ± 0.8 ng h/ml, respectively. Thus, both C_{max} and AUC were increased in proportion to the dose of LA.

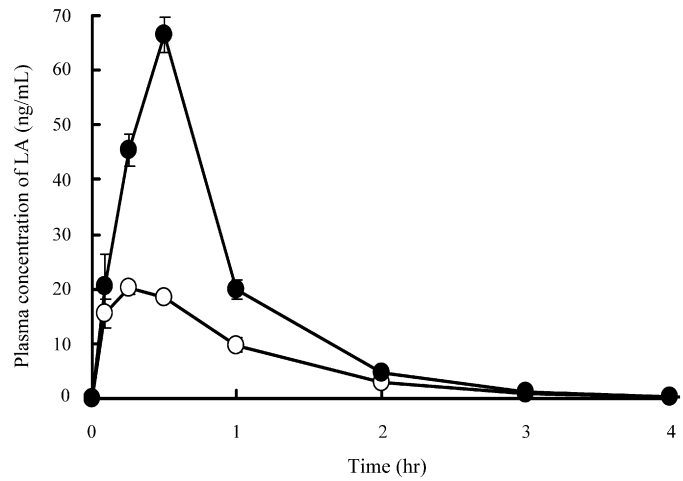

Fig. 3. Plasma LA concentration *vs.* time profiles after subcutaneous (s.c.) and intravenous (i.v.) infusion of LA solution to rats. The open and closed circles denote that obtained after s.c. and i.v. administrations of LA solution, 50.0 μg/kg. Each point represents the mean ± S.E. of 3–4 experiments.

To estimate the BA of LA from dissolving microneedles, LA solution was i.v. administered to another group of rats, 50.0 μg/kg, by infusing for 30 min into the femoral vein. The plasma LA concentration *vs.* time curve is depicted in Fig. 3. Just after the start of the i.v. infusion of LA solution, plasma LA concentration increased and reached to its maximum concentration, 90.0 ± 8.5 ng/ml, and thereafter plasma LA concentration declined. The AUC value obtained after i.v. infusion of LA was 76.9 ± 6.6 ng h/ml. To estimate the absolute BA of LA from dissolving microneedles, the AUC values were compared and absolute BA values were 31.7 ± 0.8% for 1 patch study and 33.8 ± 4.3% for half patch study, respectively.

To elucidate the mechanism why such low absolute BA values were obtained with LA dissolving microneedles, LA solution was s.c. injected to another group of rats. Fig. 3 shows the plasma LA concentration *vs.* time curve after s.c. injection of LA solution to rats, 50.0 μg/kg. The pharmacokinetic parameters were summarized in Table 4. The AUC value was 24.6 ± 2.1 ng h/ml. By comparing the AUC values obtained after administration of LA dissolving microneedles, relative BA values were calculated to be 99.2 ± 2.6% and 105.6 ± 13.5%, respectively.

Table 3
Pharmacokinetic parameters of LA after percutaneous administration of LA microneedle array chip to rats.

Number of microneedles	Dose (μg/kg)	C_{max} (ng/ml)	AUC (ng·hr/ml)	BA (%)	RBA (%)
50	13.9 ± 0.3	6.0 ± 0.7	5.8 ± 0.8	33.8 ± 4.3	105.6 ± 13.5
100	29.9 ± 0.8	16.4 ± 0.9	14.5 ± 0.4	31.7 ± 0.8	99.2 ± 2.6

C: the maximum plasma LA concentration; AUC: area under the plasma LA concentration *vs.* time curve; BA: bioavailability; RBA: relative bioavailability to s.c. injection of LA solution.
Each value represents the mean ± S.E. (n = 3–4).

Table 4
Pharmacokinetic parameters of LA after intravenous and subcutaneous injections rats.

Administration route	Dose (μg/kg)	C_{max} (ng/ml)	AUC(ng hr/ml)	BA (%)
i.v.	50	90.0 ± 8.5	76.9 ± 6.6	100
s.c.	50	20.2 ± 1.2	24.6 ± 2.1	32.0 ± 2.7

Each value represents the mean ± S.E. (n = 3–4).

3.5. Degradation of LA in the skin tissue homogenate

To clarify the incidence of the low absolute BA of LA after the s.c. administration to rats, the degradation of LA in the skin tissue homogenate was estimated from the initial amount of LA in spiked samples and the result was shown in Fig. 4. The % remaining of LA in the homogenate was 36.0 ± 14.5% at 30 min, 17.7 ± 10.0% at 60 min and 5.8 ± 3.6% at 120 min after the start of the incubation experiment. By assuming a first-order degradation process, the degradation half-life of LA, $t_{1/2}$, was 16.3 ± 5.7 min.

4. Discussion

LA is a peptide composed of 9 amino acids and is useful for the therapy of prostate cancer and endometriosis (Fujino et al., 1974). As LA has several pitfalls, short half-life in the systemic circulation and unstable in the body due to the degradation by the hydrolytic enzymes (Degim and Celebi, 2007), those pitfalls prevent the oral administration of LA. As a result, patients must receive the s.c. injection of LA. In this study, we have challenged the new percutaneous administration route of LA by dissolving microneedle patch. Dissolving microneedles physically break the stratum corneum having a strong barrier function and deliver the formulated drug to the epidermis and to the epidermis/dermal junction where there is no neural network system (Monteiro-Riviere et al., 1990; Bauer et al., 2001). Therefore, patients can percutaneously administer LA with no pain. In addition, percutaneously delivered drug do not have a first-pass effect that is usually find out in the case of oral preparation. The used dissolving microneedles differ from microneedles made of metal and/or silicon, because they immediately dissolve after inserted into the skin. FITC-dextran and FITC-insulin were formulated into chondroitin dissolving microneedles and the dissolution kinetics was observed by a fluorescent videomicroscopic analysis. Fluorescent labeled dissolving microneedles dissolved within 3 min after insertion into the rat skin (Fukushima et al., 2010, 2011). As the base polymer of dissolving microneedles, chondroitin sulfate was used.

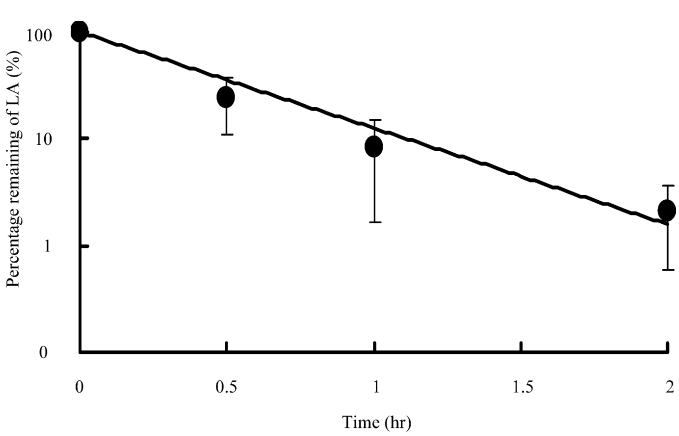

Fig. 4. Stability of LA in the rat skin tissue homogenate. Each point represents the mean ± S.E. of 3–4 experiments.

Chondroitin sulfate is a peptideglycan that exists in biological tissues (Morawski et al., 2009; Faissner et al., 1994; Umehara et al., 2004; Wilson and Snow, 2000; Sintov et al., 1995; Tsai et al., 2005). Chondroitin sulfate is a biopolymer and is used for the therapy of arthritis. By dissolving with water, it forms gel and easily mixed with LA. Thereafter, after dry, it forms solid structure again. By considering the safety and the above physicochemical property of chondroitin sulfate, it was used as the base of dissolving microneedles.

Ddissolving microneedle can physically remove the barrier function of the stratum corneum. Therefore, we thought that high absolute BA would be obtained after the percutaneous administration of LA dissolving microneedles. However, the absolute BA of LA from dissolving microneedles was 31.7–33.8%. Generally, the low absolute BA of drugs administered through the skin route is ascribed to the following incidences, (1) low permeability through the stratum corneum, (2) degradation in the skin tissue and (3) low permeability through the microcapillary wall in the dermis. The rate-limiting step of drug penetration into the skin is generally recognized to be the crossing step into the stratum corneum, the hydrophobic barrier of the skin (De Jager et al., 2007). Because the barrier function of the stratum corneum is strong, not only hydrophilic low-molecular-weight organic compounds but also higher molecular weight peptide/proteins are difficult to be permeated into the dermis where microcapillaries exist. However, the dissolving microneedles physically removes the barrier of the stratum corneum and delivers drugs into the epidermis and epidermis/dermal junction. Therefore, the diffusion rate of drugs in the epidermal and dermal regions of the skin and permeation through the microcapillary wall is thought to be the rate-limiting step for the absorption of drugs into the systemic circulation. To make clear the function of dermal diffusion and microcapillary permeability, LA solution was s.c. injected to rats. However, high BA, 96%, reported by (Sennello et al., 1986) was not obtained. Instead, low BA, 31.7–33.8%, was obtained. In their experiment, human volunteers were used and plasma LA concentrations were measured by an ELISA method. On the other hand, rats were used and the assay method was LC–MS/MS method that was more specific to unchanged LA than ELISA. Therefore, the stability of LA in the skin tissue was studied with skin tissue homogenate. LA was unstable in the skin tissue homogenate and over 90% of the spiked LA was degraded within 120 min. On the other hand, LA is stable in water as reported by (Hall et al., 1999), where more than 90% of LA was remained unchanged after being incubated for 1 year at 37 °C. The main metabolic pathway of LA is hydrolysis. In the epidermal region of the skin, there are many enzymes such as peptidases and P-450 (Hayashi et al., 1997; Bando et al., 1996, 1997). Therefore, the degradation of LA in the skin tissue homogenate was ascribed to the hydrolysis by the enzymes there. To increase the BA of LA from dissolving microneedles, we must study the technology to protect LA from the attack of hydrolytic enzymes and/or to decrease the hydrolytic rate of LA in the skin tissue.

5. Conclusions

In conclusion, dissolving microneedles containing LA were prepared with chondroitin sulfate as the base polymer and the absolute BA was measured in rats where low BA value, 31.7–33.8%, was obtained. To clarify the incidence that caused the low BA of LA from dissolving microneedle, stability study of LA in the rat skin tissue homogenate was performed and it was shown that LA was unstable in the skin. As over 90% of LA received the degradation within 120 min, the degradation of LA in the skin tissue caused the low BA after percutaneous administration by dissolving microneedles.

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