Pharmacokinetic and Pharmacodynamics of Self-Assembled Cubic Liquid Crystalline Nanoparticle Gel After Transdermal Administration

**Background:**
The aim of this study was to assess the pharmacokinetics after transdermal administration by a novel skin microdialysis technology in rats. The guinea pig model was established by investigating the pharmacodynamics.

**Material/Methods:**
Three different agents were given after hair removal, and the samples were extracted by microdialysis and detected by HPLC. Subcutaneous/plasma concentration-time curves of the 3 different agents were analyzed and the pharmacokinetic parameters were calculated. The SS-04B UV light therapy instrument was used in the modeling. Changes in melanin index and histopathology were observed with HE staining.

**Results:**
The increment and decrement results showed that the concentration had no significant effect on drug recovery both in vivo and in vitro. After the paeonol cubic liquid crystalline nanoparticles gel (PAE-LCNPs) was administered, the maximum peak time ($t_{\text{max}}$) of paeonol skin concentration appeared at 2.42±0.20 h, the maximum skin concentration $C_{\text{max}}$ was (926±105) ng/ml, and the area under the curve $AUC_{0–8}$ was (8056±954) ng/h/ml. The $t_{\text{max}}$ was shortened much more than in the other groups, and the performance of PAE-LCNPs targeting was good. Pharmacodynamic results showed that PAE-LCNPs can reduce melanocytes and reduce the melanin index, proving its utility in the treatment of melanin deposition.

**Conclusions:**
The skin microdialysis study indicated PAE-LCNPs have good transdermal permeability and efficacy. Pharmacological experiments based on the study found that the topical pigmentation model of guinea pigs showed a better therapeutic effect.

**MeSH Keywords:** Microdialysis • Nanoparticles • Pharmacokinetics

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Background

Paeonol is the main active ingredient of the plant *Cynanchum paniculatum* (Bge.) Kitag. Xu Changqing, which has many pharmacological activities such as antipyretic, analgesic, anti-inflammatory, and anti-allergic reactions. It is effective in treatment of eczema, urticaria, contact dermatitis, skin itching, and other dermatological diseases and various types of pain [1].

In the transdermal drug delivery system (TDDS), also known as the percutaneous treatment system, a drug passes through the skin and, through capillary absorption, into the systemic circulation at a certain rate, causing local or integral effects on the body. TDDS has become an important research topic in China and abroad in recent years [2,3]. Monoglyceride monoollein is a lipid widely used to construct bicontinuous cubic phases, which spontaneously forms bicontinuous cubic phases upon the addition of water [4]. The cubic liquid crystal nanoparticle drug delivery system has the advantages of controlled release in the body, as well as external advantages that also captured domestic and foreign researchers’ attention. Due to the characteristics of cubic liquid crystalline nanoparticles gel (PAE-LCNPs), it can be used as a drug carrier. Estracanholli [5] once again proved that the cubic liquid crystal has good percutaneous permeability.

According to the material concentration gradient diffusion and semipermeable membrane on the permeability of small molecules with the principle of design, the microdialysis sampling technology used in the active ingredient of paeonol research does not require pre-treatment, and can be applied directly to sample analysis. This saves time and cost, and is a major breakthrough in drug analysis [6–9]. In recent years, microdialysis technology has been used to achieve online analysis with the chromatographic analysis, and also made commercially available a set of equipment based on micro-penetration analysis of the technology, especially the study of pharmacokinetics [10–13]. This test uses microdialysis technology to determine the pharmacokinetics of PAE-LCNPs in rats after transdermal administration.

In the present study, paeonol was used as the model drug to construct the transdermal drug delivery system, with paeonol lipid cubic nanoparticles as carrier. Microdialysis sampling technique and HPLC combined method [14] were used to monitor the paeonol in vivo. The concentration of PAE-LCNPs, paeonol ordinary gel (PAE-GEL), and commercially available paeonol ointment (PAE-OIT) were measured in the subcutaneous tissue fluid after administration on abdominal skin to further evaluate the absorption and metabolism of paeonol lipid cubic nano-gel in vivo. Paeonol has antioxidant properties and inhibits the pharmacological effects of skin pigmentation. In this study, the cubic liquid crystal nanoparticles were prepared and applied in a UV-induced guinea pig pigmentation model.

HE staining was used to observe the melanin index and tissue disease changes to evaluate pharmacological aspects of initial treatment and possible mechanisms of action, and to provide a reference for new drug approval and clinical medication.

Material and Methods

Material and regent

Paeonol (>99% pure) was purchased from Dahua Weiye Co. Ltd. ( Wuhan, China). GMO was ordered from BaoMan Co. Ltd. (Shanghai, China) and Poloxamer 407 was provided by YuanYe Co. Ltd. (Shanghai, China). Commercial paeonol ointment was purchased from LiFang Co. Ltd. (Hefei, China), and the Milli-Q water was prepared by Millipore purifying system (Molsheim, France) throughout this study. HPLC-grade methanol was obtained from Merck (Darmstadt, Germany).

Animals

Male Sprague-Dawley (SD) rats weighing 200 g to 220 g were supplied by the Laboratory Animal Service Center of Shanghai, animal qualified number: SYKK (shanghai) 2012-0006, and male guinea pigs weighing 200 g to 260 g were supplied by the Animal Breeding Farm of Nanjing, Animal qualified number: SCXK (Nanjing) 2002-0004. They were all kept in animal facility rooms under standard controlled environment.

Chromatographic system

The HPLC system (Shimazu, Kyoto, Japan) was used in the following condition: column 250× 4.6 mm with particle size of 5 μm (Kromasil C18; Biomics™ Co. Ltd., Nantong, China); mobile phase consisting of water-methanol (25: 75; v/v), the mobile phase was subjected to ultrasound before filtering through a 0.45 μm Millipore filter and degassed prior to use; injection volume of 20 μl; and UV detection at 274 nm.

Preparation of paeonol cubosomes

The paeonol cubosomes were established according to our previous study [15]. In brief, GMO and Poloxamer 407 (P407) were melted at a ratio of 9: 1 in a 60°C water bath. When the sample was almost completely melted, 60°C deionized water was added gradually to the mixture and mixed for 1 min to achieve a homogenous state. Then, the sample was allowed to sit at room temperature for more than 1 week. Fragmentation of the gel was performed in water by intermittent probe sonication for 10 min using pulse mode with 400 W energy input. The resultant milky, coarse, fragmented gel was homogenized thoroughly using a high-pressure homogenizer (JN-02HC; JUNENG Co. Ltd., Guangzhou, China) at 60°C to obtain an opalescent dispersion of cubosomes.
**Microdialysis experiments**

**Microdialysis system**

The MD system consisted of a MD-1001 syringe pump and a MD-1201 microfraction collector. The MD probes have a 20 KDa cut-off with a polycarbonate membrane (length 10 mm).

**Recovery rate of probe in vitro**

The relative recovery of probe refers to the percentage that was the ratio of the component under test and the standard concentration [16]. The perfusion rate of perfusion fluid was 1.5 μl/min. We used the increment and decrement method to investigate the influence of different concentrations of paeonol on the recovery of probe in vitro.

The influence of incremental method to the recovery of probe was as follows: Firstly, we put linear probe in 3 different mass concentrations (0.186, 0.372, and 0.744 μg/ml) of paeonol physiological saline (PAE-NS) solution, and the temperature of the water bath was set to 37±0.5°C. Then, we used blank NS as the perfusate, keeping the perfusion rate at 1.5 μl/min, and we collected the samples after 1 h and then collected samples once every 30 min. All samples were analyzed in triplicate. We calculated the recovery rate of PAE as:

\[ R_c = \frac{C_b - C_d}{C_b} \times 100\% \]

where: \( R_c \) expresses the recovery rate of PAE; \( C_b \) and \( C_d \) express the initial concentration and the concentration after dialysis, respectively.

The influence of decremental method to the recovery of probe was as follows: We used 3 different mass concentrations (0.186, 0.372, and 0.744 μg/ml) of PAE-NS as infusion solution, keeping the perfusion rate at 1.5 μl/min, then we collected the samples after 1 h and then every 30 min. All samples were analyzed in triplicate. We calculated the recovery rate of PAE as:

\[ R_c = \frac{C_b - C_d}{C_b} \times 100\% \]

where: \( R_c \) expresses the recovery rate of PAE; \( C_b \) and \( C_d \) express the initial concentration and the concentration after dialysis, respectively.

**Skin pharmacokinetic and retention test**

The rats were anesthetized during in vivo microdialysis before administration of model drug. Nine rats were randomly divided into 3 groups; they were administered 3 types of formulations (PAE-OIT, PAE-GEL, and PAE-LCNPs, with 0.5 g in each) in the abdomens of mice, with samples collected every 30 min for a total of 8 h. Cuticle and epidermis/dermis were examined for drug retention in this test. After the skin pharmacokinetic study, the skins were reused and washed quickly with normal saline to remove the remaining drug. The skins were then dried with filter paper, and 3M tapes were used to collect the cuticle samples by tapping and stripping away the cuticle layer repeatedly for a total of 20 times. The remaining skin was epidermis/dermis layer. The 3M tapes with cuticle samples and epidermis/dermis samples were cut into pieces and tissue-homogenized. After that, the samples were mixed with 5 ml methanol ultrasonicated for 30 min. Then, the extracts were centrifuged at 4000 rpm for 30 min and the supernatants of 20 μl supernatants were injected for HPLC analysis after filtration by a 0.45-μm microporous membrane.

**Pharmacodynamics experiments**

**Grouping and dose**

After the animal model was successfully established, the skin of the dorsal region in the rat was divided into 4 parts and smeared with different types of formulations: Vaseline (a commercially available ointment), 3% hydroquinone cream (3% HDC), and cubic crystal nanoparticle gel. On alternate days, for a total of 3 times with 60 min every time, and each medication area being 4×3 cm², the interval between different parts was 1 cm. We removed the back fur every 3 days in the process of drug administration.
Model in skin chromatosis

Using the SS-04B type ultraviolet phototherapy device, the lamp spectrum value was 280~320 nm. Before irradiation, we used an electric knife to remove hair, baring 4×3 cm² of the skin, and the distance between the lamp and the back of the guinea pig back was 15 cm. We administered a one-time dose of 0.5 J/cm².

Results

Specificity and linearity

The experiment used HPLC methods to detect the blank skin microdialysis fluids of PAE-LCNPs, the skin microdialysis fluids of PAE-LCNPs, and the PAE. The retention time of PAE solution and the skin microdialysis fluids of PAE-LCNPs were the same, at about 5.76 min, and there was no interference peak at this time compared with the blank skin microdialysis fluids of PAE-LCNPs (Figure 1).

The linearity and calibration curves were produced using 7 standards within the range of 0.047, 0.093, 0.372, 1.488, 2.976, 5.952, and 11.904 μg/ml for the PAE reference substance. The linearity of the relationship between peak area ratio or peak area and concentration was demonstrated by the determination coefficient (r²) obtained for the paeonol regression lines. The stand curve equation was: A=34491C−971.65, r=0.9996; the drug concentration was within 0.047~11.904 μg/ml and had a good linear relationship. The quantitative limit was 0.047 μg/ml.

Precision

Precision was calculated for 3 different concentrations of PAE perfusion fluid. This procedure was repeated 6 times over 3 days to determine the inter-day precision. The intra- and inter-day precision for PAE perfusion fluid during the experiments are shown in Table 1. The intra- and inter-day precision of different groups were less than 3%; therefore, the method was considered to be accurate and precise for quantifying paeonol in biological samples.

Stability

The concentration of 1.488 μg/ml PAE was prepared at room temperature for 0 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h, and the peak area was recorded according to the “chromatographic system”. Table 2 shows that the PAE perfusate had good stability within 24 h.

Recovery rate of probe in vitro and in vivo

We used the increment and decrement method to investigate the influence of different concentrations on the linear probe. When the perfusion rate was 1.5 μl/min, the average recoveries measured by increment and decrement method were 47.22±1.97% and 49.48±2.25%, respectively. The recovery rates of different groups were similar, which indicated that there was no relationship between concentration and recovery (Table 3). The results of recovery rate in vivo are summarized in Table 4. The in vivo recovery rates of different groups were similar, which indicated the recovery rate in vivo was not affected by the drug concentration under the condition of constant flow rate. Thus, the in vivo recovery rate can be used to correct the data in the microdialysis test.

Skin pharmacokinetic and retention test

The HPLC method was used to determine the amount of PAE in microdialysis samples. After the content of PAE in the subcutaneous tissue fluid was corrected, we obtain the real drug concentration of PAE, which represents the actual quality of PAE concentration in the subcutaneous tissue of rats (C=C_{subtissue}/R). Three group of blood drug concentration after treatment are shown in Figure 2, and the main pharmacokinetic parameters are shown in Table 5. After administering PAE-LCNPs, the concentration of PAE in the skin reached a peak quickly. The AUC was higher than the PAE-OIT (P<0.05) and was significantly higher than the PAE-GEL (P<0.01), and the C_{max} was significantly higher than in the other 2 groups (P<0.01), indicating that the PAE-LCNPs group had a better transdermal effect.

Eight hours after skin pharmacokinetic test, we continued to conduct the retention study. Table 6 shows the results. PAE-LCNPs, PAE-OIT, and PAE-GEL were examined in the retention study. The results show that the retention of PAE-LCNPs was higher than that of PAE-GEL (P<0.05) and was extremely significantly higher than that of PAE-OIT (P<0.01) when analyzed using SPSS 16.0 software, which indicates that the performance of PAE-LCNPs targeting was good; thus, the concept of skin targeting [15] was proposed.

Skin appearance change

After the irradiation, skin erythema appears, along with desquamation and chromatosis after 1 day, 5 days, and 1 week, respectively. The chromatosis reached a peak and tended to be stable after 9 days. In the PAE-LCNPs group, there was no obvious irritation reaction such as erythema and edema in administering the drug. The chromatosis phenomenon decreased obviously after 4 weeks. In the 3% hydroquinone cream group, there was mild erythema after 1 week and the chromatosis phenomenon gradually decreased after 4 weeks. In the PAE-OIT group, there was no irritant reaction such as erythema or edema in the process of drug administration. The chromatosis phenomenon was not obviously decreased after 4
weeks. The Vaseline group had no obvious signs of chromatosis phenomenon.

Melanin index investigate

The melanin index of animal models is shown in Table 7. After ultraviolet lamp irradiation, the melanin index of skin was significantly increased compared with before the modeling

Table 1. Results of precision test (n=6).

| Drug amount (μg/ml) | RSD(%) |
|---------------------|--------|
|                     | Intra-day | Inter-day |
| 0.108               | 1.77      | 2.54      |
| 0.87                | 0.98      | 1.17      |
| 6.95                | 1.06      | 1.35      |

Table 2. Stability test.

| Time (h) | 0   | 2   | 4   | 6   | 8   | 12  | 24  | Mean (%) | RSD (%) |
|----------|-----|-----|-----|-----|-----|-----|-----|----------|--------|
| Drug amount (μg/ml) | 1.486 | 1.485 | 1.484 | 1.485 | 1.483 | 1.485 | 1.484 | 1.485 | 0.976 |
Table 3. Recovery rate in vitro (mean ±SD, n=3).

| Drug amount (μg/ml) | Increment method (%) | Decrement method (%) |
|---------------------|----------------------|----------------------|
| 0.186               | 45.83±1.06           | 48.65±2.37           |
| 0.372               | 47.30±1.58           | 50.66±1.80           |
| 0.744               | 48.50±0.94           | 49.13±1.12           |

Table 4. Recovery rate in vivo (mean ±SD, n=3).

| Drug amount µg/ml | Recovery rate % |
|-------------------|-----------------|
| 0.186             | 40.28±1.70      |
| 0.372             | 44.09±1.33      |
| 0.744             | 45.69±1.97      |

Table 6. The retention of different groups after 8h (n=3).

| Samples          | Cuticle (μg) | Epidermal/dermal (μg) |
|------------------|-------------|------------------------|
| PAE-LCNPs        | 67.15±3.50** | 31.09±3.29**           |
| PAE-OIT          | 36.65±2.12   | 17.97±1.58             |
| PAE-GEL          | 50.56±3.37   | 24.08±2.07             |

Compared with PAE-OIT: ** P<0.01; Compared with PAE-GEL: * P<0.05.

Table 7. The change of melanin index before and after molding (mean ±SD).

| Group            | n   | Melanin index |
|------------------|-----|---------------|
| Before molding   | 10  | 140.10±51.83  |
| After molding    | 10  | 347.60±76.52* |

Compared with Before molding: * P<0.01.

Table 8. The change of melanin index before and after administering (mean ±SD).

| Group          | n   | Before administration | After administration |
|----------------|-----|-----------------------|-----------------------|
| Vaseline       | 5   | 346.70±74.15          | 315.90±86.55          |
| PAE-OIT        | 5   | 343.50±93.90          | 289.70±96.03^         |
| 3% HDC         | 5   | 351.20±87.41          | 192.10±67.58**        |
| PAE-LCNPs      | 5   | 349.10±68.03          | 187.90±63.41**^       |

Compared with the Vaseline: ^ P>0.05; Compared with the 3% HDC: ^ P>0.05; Compared with the Vaseline and PAE-OIT: ** P<0.01; Compared with the Vaseline and PAE-OIT: ** P<0.01.
Figure 3. Before and after modeling of guinea pigs skin histopathological images (HE×100). (A) Before modeling; (B) After the model was not the medication group; (C) Vaseline drug group; (D) PAE-OIT drug group; (E) 3% hydroquinone cream application group; (F): PAE-LCNPs medication group.
(P<0.01). In the Vaseline and PAE-OIT group, there was no obvious change in the melanin index after 4 weeks (P>0.05). In the 3% hydroquinone cream and PAE-LCNPs group, the melanin index significantly decreased after 4 weeks, there was no significant difference between the 2 groups (P>0.05). Compared with the Vaseline and PAE-OIT groups, the 3% hydroquinone cream group had a significantly lower melanin index (P<0.01). Compared with the Vaseline and PAE-OIT groups, the PAE-LCNPs group had a significantly decreased melanin index (P<0.01) (Table 8).

Histopathological observation

Before the model was established, the melanin particles were not found in the guinea pig epidermis. With ultraviolet light therapy device irradiation, the area of melanin particles increased significantly. Four weeks after the application of Vaseline cream, the basal layer of melanin particles showed almost no signs of reduction. In the PAE-OIT group at 4 weeks after treatment, melanin granules decreased significantly, and the basal layer still had more melanin particles (Figure 3D). Hydroquinone cream was applied for 4 weeks after the use of PAE-OIT was significantly reduced. The PAE-LCNPs topical smear was significantly lower than the PAE-OIT (Figure 3).

Discussion

Tissue microdialysis is a minimally invasive technique used to assess local energy metabolism through measurement of energy metabolites such as glucose, pyruvate, and lactate, and has been used in lamellar tissue in normal horses for this purpose [17]. This experiment, through the comprehensive application of increment and decrement method to investigate the factors influencing recovery rate, showed that the change of the concentration had no effect on recovery when the flow rate of perfusion fluid was constant, providing a reference for determination of the recovery rate of a drug in vivo. During the in vivo microdialysis study, since the concentration of the drug outside the probe is unknown, the reverse recovery method is usually used in the microdialysis test, and the recovery of the probe is determined by calculating the amount of the drug passing through a semipermeable membrane [18].

Microdialysis is a semi-invasive sampling technique that allows for determining free, pharmacologically active drug concentrations in the interstitial space fluid of virtually any tissue [19]. This experiment uses microdialysis technology to inspect the dynamic changes in drug metabolism in rat skin tissue. The results showed that the Cmax, tmax, and AUC of the PAE-LCNPs group increased the local absorption of drug compared with PAE-OIT and PAE-GEL, and the difference was statistically significant. PAE-LCNPs formed an effective active drug repository in local tissue, could quickly permeate through the skin and reach the peak at about 2.4 h, and control the release of drug in 8 h, which indicated the sustained-release effect and better targeting of the skin. But according to the related literature [20], after long-term administration, the drug can accumulate on the surface of the skin. How deposition affects drug penetration changes of the drug in the blood remains to be determined, and long-term transdermal penetration testing and assessing the drug level in the blood need further investigation.

With the accelerated pace of life and the impact of poor environmental quality, skin pigmentation diseases are commonly encountered in dermatology and they affect physical and mental health. UV radiation can cause the skin to produce many biological effects and adverse reactions, the most common of which is erythema and melanin production. The degree of adverse reactions usually depends on the size of the UV wavelength, exposure, and individual skin differences, particularly in skin melanin content, but changes in melanin particles are difficult for the casual observer to detect [21]. Guinea pigs are widely used as experimental animals due to their gentle temperament, moderate size, easy feeding, and low cost. They are suited to batch research and the animal pigmentation model has been widely used in China and in other countries [22,23]. The present study was conducted to investigate the efficacy of PAE-LCNPs in pharmacodynamics and to provide a valuable reference for clinical treatment by performing pharmacological studies in guinea pig models after skin pigmentation.

This study successfully established the guinea pig skin chromatosis model induced by ultraviolet radiation, and verified the feasibility and effectiveness of the model. The changes in skin pigmentation in guinea pigs were observed by use of a skin physiologic analyzer, and the melanin index was recorded. A higher index is associated with a higher content of melanin in the skin [21]. Pharmacodynamic results show that the PAE-LCNPs reduced melanin cells and melanin index, proved to have an effect for the treatment of melanin deposition, and caused no stimulation in the process of medication on the skin. These results have guiding significance for prevention or treatment of chromatosis. However, drug use in the test was only 30 days, providing only short-term results. Further research is needed to determine if there is similar long-term efficacy of hydroquinone cream. Skin pigmentation disease is complex and the mechanism is unclear, requiring further study of the efficacy of PAE-LCNPs.

Conclusions

Based on the results of skin pharmacokinetic and retention studies, the PAE-LCNPs group had increased local absorption of drug compared with the PAE-OIT and PAE-GEL groups,
indicating that the drug has sustained-release effect and better targeting of the skin. The in vivo skin melanin index investigation showed that the PAE-LCNPs can reduce melanin cells and melanin index, showed an effect on the treatment of melanin deposition, and showed no stimulation in the process of medication on the skin. In conclusion, the paeonol cubosomes effectively promote the transdermal permeability of the drug and might be an excellent natural penetration enhancer.

References:

1. Peng LH, Liu S, Xu SY et al: Inhibitory effects of salidroside and paeonol on tyrosinase activity and melanin synthesis in mouse B16F10 melanoma cells and ultraviolet B-induced pigmentation in guinea pig skin. Phytomedicine, 2013; 20(12): 1082–87
2. Malipeddi VR, Awasthi R, Ghisleni DD et al: Preparation and characterization of metoprolol tartrate containing matrix type transdermal drug delivery system. Drug Deliv Transit Res, 2017; 7(1): 66–76
3. Singla SK, Sachdeva V: Current and emerging lipid-based systems for transdermal drug delivery. Ther Deliv, 2015; 6(9): 1063–70
4. He X, Li Q, Liu X et al: Curcumin-loaded lipid cubic liquid crystalline nanoparticles: Preparation, optimization, physicochemical properties and oral absorption. J Nanosci Nanotechnol, 2015; 15(8): 5559–65
5. Estracanhelli EA, Praça FS, Cintra AB et al: Liquid crystalline systems for transdermal delivery of celecoxib: In vitro drug release and skin permeation studies. AAPS Pharm Sci Tech, 2014; 15(6): 1468–75
6. Wang D, Liu CS, Lv J et al: Automated on-line microdialysis sampling coupled with HPLC for synchronous determination of puerarin in subcutaneous tissue and plasma following topical administration. Clin Lab, 2015; 61(9): 1297–302
7. Pigatto MC, Roman RM, Carrara L et al: Pharmacokinetic/pharmacodynamic modeling of etoposide tumor growth inhibitory effect in Walker-256 tumor-bearing rat model using free intratumoral drug concentrations. Eur J Pharm Sci, 2017; 97: 70–78
8. Demand D, Schack-Kirchner H, Lang F: Assessment of diffusive phosphate supply in soils by microdialysis. Journal of Plant Nutrition & Soil Science, 2017; 180(2): 220–30
9. Ran R, Sun Q, Baby T et al: Multiphasic microfluidic synthesis of micro and nanostructures for pharmaceutical applications. Chemical Engineering Science, 2017; 169: 78–96
10. Bue M, Birke-Sørensen H, Thillemann TM et al: Single-dose pharmacokinetics of vancomycin in porcine cancellous and cortical bone determined by microdialysis. Int J Antimicrob Agents, 2015; 46(4): 434–38
11. Sahuquillo J, Merino MA, Sánchez-Guerrero A et al: Lactate and the lactate-to-pyruvate molar ratio cannot be used as independent biomarkers for monitoring brain energetic metabolism: A microdialysis study in patients with traumatic brain injuries. PLoS One, 2014; 9(7): e102540
12. Ali Suliman H, Kim J, Sani SN: Microdialysis-directed intra-tumor pharmacokinetic modeling of methotrexate in mice and humans. J Pharm Pharm Sci, 2016; 19(2): 239–51
13. Pigatto MC, Mossmann DL, Dalla CT: HPLC-UV method for quantifying etoposide in plasma and tumor interstitial fluid by microdialysis: Application to pharmacokinetic studies. Biomed Chromatogr, 2015; 29(4): 529–36
14. Gotit A, Olstedt EL, Baix F et al: Simultaneous measurement of heroin and its metabolites in brain extracellular fluid by microdialysis and ultra-performance liquid chromatography tandem mass spectrometry. J Pharmacol Toxicol Methods, 2012; 66(1): 14–21
15. Li IC, Zhu N, Zhu JX et al: Self-assembled cubic liquid crystalline nanoparticles for transdermal delivery of paenolin. Med Sci Monit, 2013; 21: 3298–310
16. Liao WC, Wang LS, Fan WT et al: [Study on recovery and its influencing factors of fericul acid and tetramethylpyrazine in cerebral microdialysis probe]. Zhongguo Zhong Yao Za Zhi, 2015; 40(21): 4275–80
17. Olausson P, Gerdle B, Ghafouri N et al: Relative recovery over time – an in vivo microdialysis study of human skeletal muscle. Scand J Clin Lab Invest, 2013; 73(1): 10–16
18. Li SS, Li GF, Liu L et al: Evaluation of paeonol skin-target delivery from its microsponge formulation: In vitro skin permeation and in vivo microdialysis. PLoS One, 2013; 8(11): e79881
19. Guo J, Shang EX, Duan JA et al: Determination of lignastilide in the brains of freely moving rats using microdialysis coupled with ultra-performance liquid chromatography/mass spectrometry. Fitoterapia, 2011; 82(3): 441–45
20. Jiang S, Liang Z, Hao L, Li L: Investigation of signaling molecules and metabolites found in crustacean hemolymph via in vivo microdialysis using a multifaceted mass spectrometric platform. Electrophoresis, 2016; 37(7–8): 1031–38
21. Fuhri JW, Sassning S, Lademann O et al: In vivo skin treatment with tissue-tolerable plasma influences skin physiology and antioxidant profile in human stratum corneum. Exp Dermatol, 2012; 21(2): 130–34
22. Tobishi M, Haratake A, Kaminaga H et al: Pigmentation in ultraviolet B-induced pigmentation in Guinea pig skin. Pigment Cell Res, 2004; 17(6): 651–58
23. Lee TH, Seo JO, Baek SH, Kim SY: Inhibitory effects of resveratrol on melanin synthesis in ultraviolet B-induced pigmentation in Guinea pig skin. Biomol Ther (Seoul), 2014; 22(1): 35–40