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丙戊酸恒速给药在小鼠体内时间药物动力学及进食条件的影响

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目的：研究丙戊酸(VA)药物动力学昼夜节律变化及进食条件对节律的影响. **方法**：对自由进食及限定进食的 ICR 小鼠分别以渗透压微泵技术(1.062 mg·h^{-1})及 iv (50 mg·kg^{-1})给予丙戊酸钠，并测定 VA 动力学的时间依赖性变化. **结果**：血浆 VA 浓度及清除率在稳态时呈昼夜节律性变化(P<0.01)，限定进食时间影响 VA 动力学的节律，使峰值位相移动约12 h. **结论**：用药时间是影响 VA 药动学的重要因素，进食条件是 VA 药动学节律的同步因子之一.

关键词 丙戊酸；药物动力学；昼夜节律；用药计划表

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Absorption of indometacin from nasal cavity in rats

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AIM: To investigate if identical bioavailability, rapid T_{max}, and C_{max} of indometacin (Ind) could be achieved when Ind is administered in rats via intranasal (ina) route. **METHODS**: The pharmacokinetics of Ind solution at a dosage of 3 mg·kg^{-1} was studied after iv, ina, and *po* in rats using HPLC. **RESULTS**: It showed that the time to peak (T_{max}) of ina Ind 3 mg·kg^{-1} solution was 0.08 h, approached that after iv route the peak concentration (C_{max}) following ina was 20.0 mg·L^{-1}, 2.4 times higher than *po* dosing. **CONCLUSION**: It demonstrated that the ina administration of Ind was superior to *po* in rats, and that Ind absorption through nasal mucosa was a reasonable approach at lower doses.

KEY WORDS indomethacin; high pressure liquid chromatography; intranasal administration; pharmacokinetics

Indometacin (indomethacin, Ind) is an anti-inflammatory and analgesic-antipyretic drug in experiments, but produces erosions and ulcers in the gastrointestinal tracts[1,2]. Our laboratory showed that index of Ind-induced ulcer was highest in *po*, then iv, and sc routes. If there is an alternative route of administration through which Ind could be at a lower dose to avoid gastric irritation[3], while at the same time is enough to produce desired pharmacological effects[4]? The present study is to investigate if identical bioavailability, rapid T_{max}, and C_{max} of Ind could be achieved when Ind is administered in rats via intranasal (ina) route.

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MATERIALS AND METHODS

Chemicals Ind was from Sigma. Phenacetin (used as an internal standard), acetonitrile, and sodium acetate trihydrate were from Tsukishima Pharmaceutical Co. Kanto Chemical Co. and Wako Pure Chemical Industries, respectively. All other chemicals were of AR.

Preparation of Ind solution Ind 50 mg in 3.5 mL of 2.5 % Na_2CO_3 solution, was mixed with 1.5 mL of HCl 0.5 mol·L^{-1}, and shaken for about 20 min. The pH was adjusted to 7. Using HPLC, Ind in the solution was shown to be stable for at least 48 h. All the solutions were freshly prepared.

***In vivo* absorption experiments and sample preparation** Sprague-Dawley rats ♂, weighing 300±*s* 10 g (n=12), from Japan Laboratory Animal Institute, Tokyo, were fasted for 16 h (water *ad lib*) and equally divided into 3 groups. The surgical procedures to separate nasal cavity from oropharyngeal cavity for *in vivo* nasal administration were those described by Hirai *et al*[5], after 30 min, Ind 3 mg·kg^{-1} within 0.1 mL of volume was injected into the nasal cavity by a microsyringe attached to a soft and blunt medical-grade tubing (0.12 mm in ID, 0.25 mm in OD, Dow Corning, USA) through one of the nostrils. An iv bolus injection and *po* of the solution were carried out as controls.

Blood samples (0.2 mL each), taken from the jugular vein at 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360 min following each dose, were collected into heparinized polypropylene tubes (Quality Scientific Plastics, USA) and centrifuged at 1000×g at 4 ℃ for 10 min. The plasma was kept at −80 ℃ until assay.

Plasma 50 μL and acetonitrile 100 μL were mixed with phenacetin 50 μL (10 mg·L^{-1}). After 10 min, the precipitated protein was removed by centrifugation (1000×g, 4 ℃, 10 min), and about 100 μL aliquot of the supernatant was transferred to another polypropylene tube for measuring Ind.

Analytical Apparatus A Model SC-8010 HPLC (Tosoh, Tokyo), equipped with a detector (242 nm), a CCPM pump, and a PP-8010 Chart Recorder, was employed. Separation was performed at 25 ℃ on a stainless-steel column (Nucleosil 120-5C8 4.6 mm×250 mm, GL Sciences Inc.). Samples were automatically introduced onto the column using an AS-8000 Auto Sampler with a 20 μL loop valve.

Mobile phase Acetonitrile : NaAc 0.1 mol·L^{-1} buffer (55/45 v/v) was used as mobile phase, pH adjusted to 4.6 by dropwise addition of HAc. The flow rate was 1.0 mL·min^{-1}.

Internal standard Phenacetin 1 mg was dissolved in 100 mL of acetonitrile to obtain a 10 mg·L^{-1} internal standard.

Recovery study For the determination of recovery 5 replicate samples at levels of 1 and 4 mg·L^{-1} for Ind, and 1 mg·L^{-1} for phenacetin, were spiked in fresh blank heparinized plasma and run through the procedures as decribed in extraction of samples. The absolute peak heights obtained for extracted samples were compared with those of fresh standard for Ind and phenacetin in mobile phase.

Calibration curve Plasma calibration curves were prepared from a solution of Ind (10 mg·L^{-1}, prepared daily from a stock solution of 500 mg·L^{-1} in acetonitrile and stored at 4 ℃ in darkness up to 1 wk) by serial dilution with plasma (0.05—50 mg·L^{-1}).

Calculations Ind was determined from the calibration curve of concentration *vs* the peak height ratio of Ind to phenacetin. Linear regression analysis and interpolation were performed with a Macintosh classic microcomputer, Tokyo. Pharmacokinetic study and statistical analysis were performed with NEC microcomputer. Peak plasma concentration of Ind (C_{max}) and time to peak (T_{max}) were determined by inspection. Plasma elimination half-time ($T_{1/2}$) of Ind was estimated from the terminal portion of the log plasma concentration-time curves by least square regression analysis on semilogarithmic plots. AUC was calculated by the trapezoidal rule. The AUC from last datum point to t=infinity was estimated by C/beta, where C represented the last measurable plasma concentration and beta ($0.693/T_{1/2}$), the elimination rate constant.

The t test was used for comparison of the data.

RESULTS AND DISCUSSION

Ind and the internal standard yielded sharp symmetrical peaks under the conditions given in the experiment with retention times of 6.33 and 4.47 min, resectively (peak a and b in Fig 1, B and C). Fig 1A showed a chro-

matogram of the extract of fresh blank plasma. Fig 1B showed a chromatogram, obtained when the method was applied to spiked plasma containing Ind 4 mg · L^{-1} and the internal standard phenacetin 10 mg · L^{-1}, where no endogenous peaks interfered. Fig 1C was a chromatogram of a 50 μL plasma sample 6 h post-dosing from a rat which received Ind 3 mg · kg^{-1} iv. The sample was estimated to contain Ind 5.8 mg·L^{-1}.

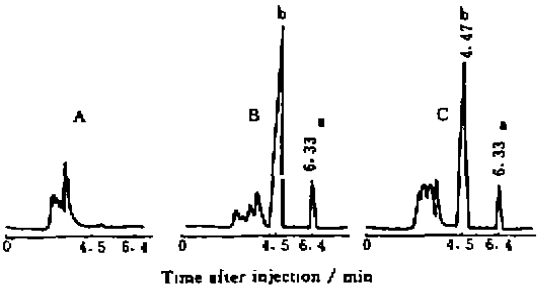

Fig 1. Chromatogram of extracts from rat plasma. A) Blank plasma; B) Plasma with indometacin 4 mg · L^{-1} (peak, 6.33 min) and phenacetin 10 mg · L^{-1} (peak, 4.47 min); C) Plasma from a rat 6 h after indometacin 3 mg · kg^{-1} iv containing indometacin 5.8 mg · L^{-1}.

The linearity was evaluated in plasma in concentrations of 0.05—50 mg·L^{-1}. The calibration curve was best described by a linear equation: $\hat{Y} = 22.11X + 0.17$, where $\hat{Y}$ was Ind concentration in mg·L^{-1}, and X was peak height ratio of Ind to phenacetin. A correlation coeffecient of 0.999±0.003 ($n=8$, $P<0.01$) was obtained, indicating a high degree of linearty.

The recovery of Ind and the internal standard were 93 ± 0.98% and 89 ± 0.82 %, respectively. (Tab 1)

The minimal concentration that could be accurately measured was 20 μg·L^{-1} (signal-to-noise ratio = 5) with 50 μL plasma sample.

Tab 1. Recovery of indometacin and phenacetin from rat plasma. $n=5$, $\bar{x}\pm s$.

Drug	Amount added to 50 μL plasma	Amount recovered	Recovery %
Indometacin	1 μg	0.94 μg	94.00±0.40
	4 μg	3.68 μg	92.00±1.16
Phenacetin	4 μg	3.58 μg	89.50±0.82

The pharmacokinetic parameters of Ind solution 3 g·L^{-1} after *po*, iv and ina administrations were shown in Tab 2.

Tab 2. Pharmacokinetics of indometacin after 3 routes of administration in rats. $n=4$, $\bar{x}\pm s$. $^{a}P>0.05$, $^{c}P<0.01$ *vs po*.

	iv	*po*	Nasal
AUC, mg·h·L^{-1}	84.9±3.6	71.9±3.1	67.4±2.1^{a}
C_{max}, mg·L^{-1}	36.2±1.2	8.3±0.3	20.0±2.4^{c}
T_{max}, h	0	2.3±0.1	0.08^{c}
$T_{1/2}$, h	3.5±0.3	4.0±0.3	3.8±0.3^{a}

The mean T_{max} after ina was shorter than that after *po* and was approaching that after iv, but the T_{max} after *po* was 2.3 h.

The C_{max} after *po* was much lower than those following ina and iv, the ina reached the maximum at a concentration of 20.0 mg·L^{-1}, while *po* route did so at 8.3 mg·L^{-1}.

$T_{1/2}$ of *po* route was 4.0 h, much longer than those of iv and ina dosings.

AUC after ina was close to that after *po* dosing ($P>0.05$). It is suggested that Ind easily passes through both gastrointestinal tract and nasal mucosa to the systemic circulation because of its high lipophylic property.

The plasma concentration after ina reached its maximum within 0.08 h, but the plasma concentration after oral dosing did not rise so quickly or so high. (Fig 2.)

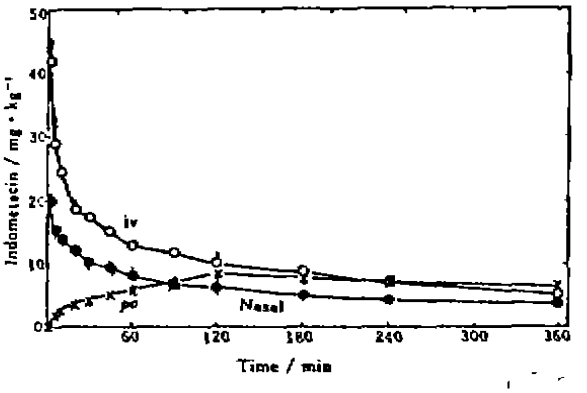

Fig 2. Plasma concentration of indometacin after 3 routes of administration of 3 mg·kg^{-1} in rats.

In conclusion, Ind could easily pass through the nasal mucosa and enter the systemic circulation, nasal absorption was rapid and peak concentration was high, compared with those following *po*. Therefore, the ina administration may be useful for rapid onset of its pharmacological effects. A study is underway on the rapid onset of pharmacological effects both in animals and humans.

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大鼠鼻腔吸收吲哚美辛

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目的：研究吲哚美辛经大鼠鼻腔给药的可行性. **方法**：用高效液相色谱仪测定血浆中吲哚美辛的药浓变化，比较大鼠吲哚美辛3 mg·kg^{-1}水溶液经口服、静脉与鼻腔给药的吸收特点. **结果**：鼻腔给药达峰时间为0.08 h，几乎接近于静脉给药；峰浓度为20.0 mg·L^{-1}，是口服给药的2.4倍. 吲哚美辛水溶液鼻腔给药达峰迅速，峰值浓度高，吸收好. **结论**：低剂量吲哚美辛经鼻腔给药为一合理，可望替代口服的给药方式.

关键词 吲哚美辛；高效液相色谱法；鼻内给药；药物动力学