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Role of oral food intake amount in intestinal absorption of morphine in rats

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ABSTRACT
Defects in oral intake cause morphological changes,such as atrophy in the intestine; however, the resulting changes in pharmacokineticsof administered drugs have not been elucidated. The present study focused on the effect of fasting and feed restriction on morphine pharmacokinetics.Morphine was orally administered to fasted and feed restriction rat groups.Plasma morphine concentration and the area under the curve (AUC) were measured and compared among groups.The AUC in fasted and feed restriction groups markedly increased compared with that reported for the control group.This increment was dependent on the feed restriction ratio, and not on the fasting period.The findings of this study will help predict the safety of morphine after oral administration.

Keywords: morphine; pain management; oral absorption; food intake; fasting

INTRODUCTION

Orally administered nutrition and drugs are absorbed in the small intestine,which provides a barrier against antigens, pathogens, and drugs.Total parenteral nutrition (TPN) can cause a decrease in oral intake frequency. The decrease in oral intake frequency leads to defects inintestinal barrier functionthat has been reported to be caused by histological changes in the intestine,such as intestinal atrophy [1-3], deranged epithelial integrity [4, 5], transporter protein expressionsuch as p-glycoprotein,[6] and bacterial translocation [7, 8].The impaired barrier function causes some substances, which are hardly absorbed or not absorbed from the normal intestine,to permeate through the intestinal mucosa and get absorbed into the systemic circulation[9, 10].Because these defects lead to over-absorption, easily absorbable substancessuch as small molecular drugs may cause adverse events. Evaluation of the drug pharmacokinetics is important and useful for predicting such adverse events. However,only a few studies have reported drug pharmacokinetics in patients with intestinal atrophy caused by fasting and food restriction.

Morphine is one of the most commonly used and importantdrugs for pain relief in terminal palliative care [11]. It acts directly on the central nervous system; however, its activity on the peripheral tissues leads to many secondary complications, such as addiction, tolerance, constipation, nausea, vomiting, deliria, somnolentia, and respiratory depression [12]. Considering the intended use i.e. pain relief, determining when to discontinue morphine is difficult. Thus, optimized blood/plasma concentration of morphine would improve patient's quality of life and its effective usage.

Cancer patients, who take morphine for pain relief, often reduce their food intake because of depressed physical and psychological conditionsdue to cancer and chemotherapy[13].This decreasedfood intake could result in an altered morphine plasma concentration-time profile,which can cause adverse effects.

The present study focused on the relationship between amount of food intake and kinetics of morphine. The fasted or food restriction group rats were used as a modelto investigate the pharmacokinetics of morphine and observe villous histology after fasting. Clinical biological parameters were also determined to investigate the influence of fasting on some organ functions.

MATERIALS AND METHODS

Materials

Morphine hydrochloride was obtained from Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). All other chemicals purchased were of analytical grade and were used without any purification.

Animals

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Male Wistar rats weighing 200 to 250 g (aged 6 weeks) were purchased from Sankyo Labo Service Corporation, Inc. (Saitama, Japan). All animal experiments were performed according to the guidelines for the care and use of laboratory animals approved by the Institutional Animal Care and Use Committee of Josai University.

Feed restriction and fasting schedule

Rats were acclimatized to the environment and adapted to the diet consisting of 6 pieces of MF pellet (diameter 12 mm, 3.5 g per pellet; 21 g per day; 75 kcal per day; Oriental Yeast Co., Ltd., Tokyo, Japan) daily until the start of the experiment. No other food intake restriction was imposed on the control group rats; they were provided 6 pellets daily.The 1-day and 3-day fasting groups were fasted for 1 day and 3 days before the experiment (1d fast and 3d fast groups), respectively. Feed restriction groupswere provided1 pellet and 2 pellets daily for 3 days (3d 1/6 fed and 3d 1/3 fed groups), and 2 pellets daily for 7 days (7d 1/3 fed group).

Morphine kinetics in rat after i.v. or p.o. administration

Morphine hydrochloride dissolved in 150 mM NaCl aq. was administered to rats in the control, 1d fast, and 3d fast groupsorally (p.o. administration;70 mg/kg body weight, b.w.) or intravenously(i.v. administration;5 mg/kg b.w.).Blood sampling was carried out at optimized time intervals (0.2 mL per sample) from jugular vein.

Morphine determination

Morphine concentration in the plasma samples was detected and measured using a high-performance liquid chromatography (HPLC) system consisting of the following components: an LC-9A pump, an SIL-6B auto injector, an SPD-6A UV spectrophotometric detector (all from Shimadzu Co. Ltd., Kyoto, Japan), an SSC-2300 column oven (Senshu Scientific Co., Ltd.), and a Mightysil RP-18 GP 150-2.0 column (Kanto Chemical Co., Inc.) packed with C18 silica reversed-phase particles (3 μm). The mobile phase consisted of 38% acetonitrile in 0.02% sodium tetradecyl sulfate solution.The flow rate, injection volume, column temperature, and detection wavelength were set at 0.22 mL/min, 20 μL, 40°C, and 230 nm, respectively.

Histological and morphological examination of the intestine

After blood sampling, the intestine was excised and carefully rinsed with phosphate buffer saline (PBS). Proximal from the cardia, part of the intestine between 10 to 13 cm was cut and weighed, whereas the part between 13-16 cm was fixed in 4% paraformaldehyde[14]. Preparation of paraffin-embedded tissue sections and H&E-staining were performed byBiopathology Institute Co., Ltd. (Oita, Japan). Height of the jejunal villirepresents the mean height of30 villi.

Measurement of biochemistry parameters

VetoScan® (Abaxis Inc., CA, USA) was used to measure the clinical biochemistry parameters such as sodium (Na^+), potassium (K^+), total CO_2(tCO_2), creatine kinase (CK), glucose (GLU), calcium (Ca^{++}), blood urea nitrogen (BUN), creatinine (CRE), aspartate transaminase (AST), total bilirubin (TBIL), gamma GTP (GGTP), albumin (ALB), total protein (TP), and globulin (GLOB) in the blood. Briefly, anEquine Profile Plus® (Abaxis Inc., CA, U.S.A.) filled with 100 μL of blood from 1d or 3d fast groups was set on the VetScan® and the analysis was started.

Statistical analysis

Data are represented as the mean ± the standard deviation (SD) unless otherwise noted. Statistical analyses were performed using Student's t-tests vs control with Microsoft® Excel. A P value of less than 0.05 was regarded significant.

RESULTS

Plasma concentration-time profile after oral administration of morphine to fasted groups

Figure 1 shows the concentration-time profile (A) and area under the curve (AUC) (B) after oral administration of morphine to the control, 1d fast, and 3d fast rats. The concentration of morphine in the control group rats was almost close tothe lower limit of quantification, higher than the concentration reported for control group ratsin the 1d fast group, and the highest in 3d fast group rats. The AUC for 1d and 3d fast groupswas 21-fold and 182-fold higher than that reported for the control group. The $C_{max}$of morphine inthe 3d fast rats was higher than that in the 1d fast rats; however, k_a, k_{el}, and T_{max}were not different (Table 1).
Fig. 1 and Table 1

Evaluation of histological and morphological characteristics and biochemistry parameters

Heightof villi was not significantly different between the control group rats and 1d fast rats, but the heightof villi in 3d fast ratssignificantly decreased

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compared with that reported for the other groups (Fig. 2A). As shown in Fig. 2B, weight of the intestinedecreased with longer fasting periods, decreasing to 59.2% in the 3d fast group. Drug distribution and elimination-related biochemistry parameters including CRE and ALB were not different among the groups, whereas energy level indicatorsincluding TP and GLUdecreased with longer fasting period (Table 2).

Fig. 2 and Table 2

Elimination profiles of morphine in control and fasted group

To evaluate the elimination kinetics, morphine was intravenouslyadministered to both control and fasting group rats. As shown in the plasma concentration-time profiles(Fig. 3),no differences in elimination kinetics were observed between the control and fasted group rats. The pharmacokinetic parameters such as volume of distribution (V_d) and eliminationrate constant (k_{el}) were also not different between both groups (Table 3).

Fig. 3 and Table 3

Effect of feed restriction on plasma concentration-time profiles

To evaluate the effect of feed amount on morphine pharmacokinetics, plasma concentration and AUCwere measured after feed restriction (Fig. 4).Feed restriction increased the plasmaconcentration of morphine compared with that reported for thecontrolgroup.The 3d1/6 group showed higher morphine plasma concentration than 3d 1/3 and 7d 1/3 groups did (Fig. 4A). Although notsignificantly different, AUC in the 3d 1/6 group was higher than that in the other groups (Fig. 4B).

Fig. 4

Figure 5 shows the relationship between feed reduction ratio at 3 days and logarithm of AUC; a good correlation was observed (r = 0.996). Feed reduction ratio was calculated from the feed amount (fasted rat means 100% reduction). Reduction of the feed amount resulted in a logarithmic increase in the AUC of morphine. Rat body weight decreased by 0.92-, 0.87-, and 0.86-fold in 3d 1/3, 3d 1/6, and 7d 1/3 fed groups, respectively. Short-term feed restriction ratioinfluenced the rate of weight loss; however, it was not correlated with AUC.
Fig. 5

DISCUSSION

Meal is the most efficient way of energy intake.A reduction in food amount due to any reason, such as feed restriction, fasting, TPN, physiological and pathological conditions, and adverse drug reactions in patients, can cause physical problems. This studywas performed to investigate the influence of feed amount on morphine pharmacokinetics. The results indicated that plasma morphine concentration increased upon feed restriction.

Although T_{max} was almost similar among the control, 1d, and 3d fast groups, C_{max} in the fasted groupswas significantly higher than that in the control group. Plasma concentration after administration of a drug depends on absorption rate or bioavailability and elimination rate. In the present study, increased plasma concentration was observed after p.o. administration to 3d fast rats, but not after i.v. administration to control and 3d fast rats, indicating that absorption or bioavailability was the main factor of increased plasma concentration in this case. The elimination profiles of the control and 3d fast groups were similar. The difference in C_{max}between fasted and control groupscan therefore be explained by increased bioavailability and absorption rate of morphine. This can lead to transient adverse effects after p.o.morphine administration tothe patients who cannot eat adequate amounts. Repeated morphine administration to such patients can trigger a severe adverse event such as somnolentia and respiratory depression[13]. The plasma morphine concentration in the 3d 1/6 group was lower than that in the 3d fast group. Therefore, it can be suggested that oral food intake can prevent acute and substantial increments of morphine concentration caused by fasting, indicating that absorption is related to the use-status of the intestine (i.e. whether the intestine is empty or processing food).

Fasting decreased the heightof villi,and we speculate that it might also have decreased the number of villi. This intestinal atrophy could have resulted in smaller absorption area for morphine. Morphine is a substrateof p-glycoprotein[15].The activity of p-glycoprotein is an important factor in determining the oral morphine bioavailability [16]. We speculated that absorption per unit area was affected more by the increased absorption compared to that by the reduced absorption area. A decrease in the feed amount per day increased the plasma morphine concentration with a good correlation; however, no difference was observed in the morphine AUC between 3d 1/3 and 7d 1/3 feed restriction group rats, suggesting that not calorie intake but intake amount plays a key role in morphine pharmacokinetics. Continuous calorie

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defects caused weight loss. It has been previously reported thatthe height of intestinal villi is decreased by parenteral nutrition[5, 11, 17, 18], and the TPN decreasesthe expression of tumor necrosis factor (TNF)-α, epidermal growth factor receptor (EGFR), and p-Akt/t-Akt [19].Another study reported thatan indigestible polystyrene material partly restored the reduction in mucosal height [20]. Based on these reports and results of the current study, we speculate that not energy but physical stress or stimulation by day-to-day diet is important to maintain the function of intestinal mucosa.

Fasting did not affect the biochemical parameters such as BUN and AST, suggesting that it hardly influencedthe hepatic and kidney function until 3 days. Furthermore, fasting did not alter the elimination profiles of morphine after i.v. administration,indicating that it had little or no influence on distribution and elimination-related factors of morphine.

Feed restriction leads to defective intestinal barrier function that can result in altered morphine pharmacokinetics, as shown by the histological observations. A strong correlation between feed restriction ratio and log AUC indicates that the patient's meal amount can help in predicting the beneficial and adverse effects of morphine.

CONCLUSION

In summary, plasma morphine concentration was increased by the reduction inper day food intake. Thecorrelation between reduction ratio of food and AUC can beused to predict the beneficial and adverse effects of morphine. A thorough understanding of the involved mechanism—from physical, biochemical, and genetic point of view—canhelp improve morphine administration by decreasing adverse reactions and customizingpain therapy for patients.It can be concluded that morphological atrophic changes and over-permeation in the intestine are related to food intake amount in day-to-day diet.

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Table 1

Pharmacokinetic parameters of morphine afterp.o. (70 mg/kg b.w.) administration to rats fasted for 1day and 3days.

	Fasted for 1day	Fasted for 3days	p value
k_{el} (min^{-1})	0.00207±0.00234	0.00246±0.00127	0.815
k_a (min^{-1})	0.0299±0.0267	0.0842±0.0392	0.118
T_{max} (min)	90.0 ±30.0	75.0 ±39.6	0.629
C_{max} (μM)	8.36±7.52	93.1±16.2	0.00120
AUC_{0-360} (μM・min)	2340 ±974	22300 ±2040	0.000106

Table 2

Clinical biological parameters of the control and fasted groups (fasted for 1day or 3days).

Parameters	**Control**		**fasted for 1day**		**fasted for 3days**	
Na^+	134	±1.53	138	±3.46	131	±4.36
K^+	5.9	±1.82	4.9	±0.173	4.77	±1.78
tCO_2	29	±1.73	27.3	±0.577	94.6	±123
CK	288	±78.5	458	±57.2	178	±50.8
GLU	209	±20.7	179	±21	163	±19.0
Ca^{++}	8.8	±0.681	9.1	±0.493	6.8	±0.513
BUN	10	±2.52	13	±2.00	14	±0.577
CRE	<0.3		0.3	±0.100	<0.3	

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AST	64.3	±9.61	77.7	±11.7	92	±60.6
TBIL	0.3	±0	0.3	±0	0.33	±0.0577
GGT	<5		<5		<5	
ALB	3.6	±0.252	4.4	±0.173	3.5	±0.173
TP	4.1	±0.321	4.8	±0.100	3.6	±0.116
GLOB	0.53	±0.289	0.367	±0.0577	0.133	±0.153

Table 3

Pharmacokinetic parameters of morphine afteri.v. (5mg/kg b.w.) administration to the rats fasting of 1day and 3days.

	Control	Fasted for 3days	P value
$k_{el}(min^{-1})$	0.0119±0.00415	0.0130±0.00331	0.743
V (mL)	191±33.9	184±13.3	0.745
AUC_{0-360} (μM・min)	13.4±7.69	11.8±1.84	0.679
C_0 (μM)	26.7±4.40	27.3±1.98	0.835

Figure caption

Fig. 1 Effect of fasting period on the plasma concentration (A) and AUC (B) of morphine after p.o. administration. Each value represents the mean±S.D. (n=3)

Fig. 2 Histological evaluation of the jejunum in the HE stained tissue section (A), villus height (B), and tissue weight (C).

Fig. 3 Elimination profiles of morphine after i.v. administration to control (○) and 3-day fasted (●) groups. Each point represents the mean ± S.D. (n=3).

Fig. 4 Effect of feed amount on the plasma concentration (A) and AUC (B) of morphine after p.o. administration. Each point represents the mean ± S.D. (n=6).

Fig. 5 Relationship between feed restriction ratio per day (for 3days) and log AUC value. Control and completely fasted groups are shown as 0% and 100% in the restriction ratio, respectively. Each point represents the mean ± S.D. (n=3-6).

Fig. 1

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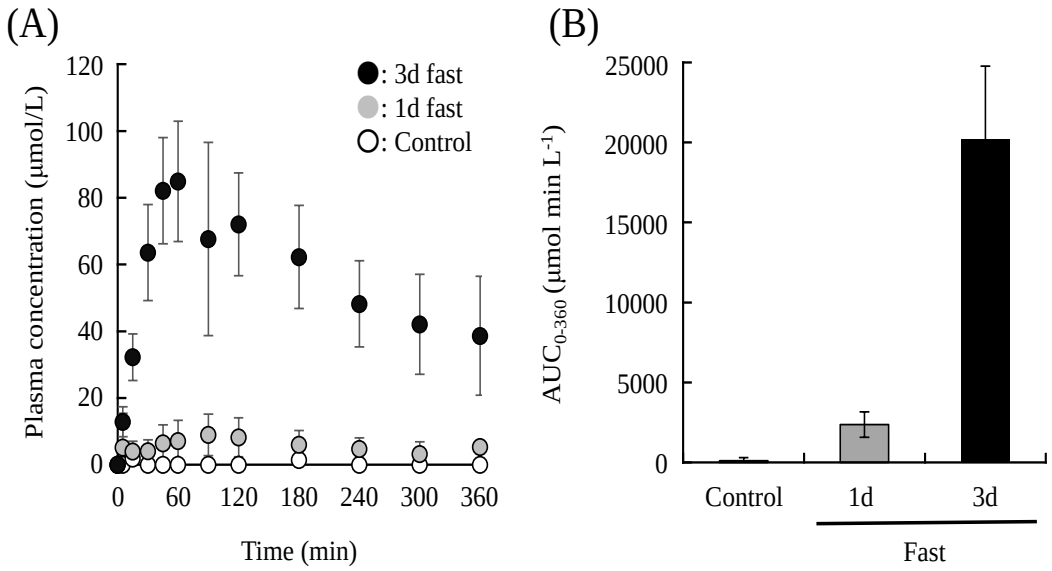

Fig. 2

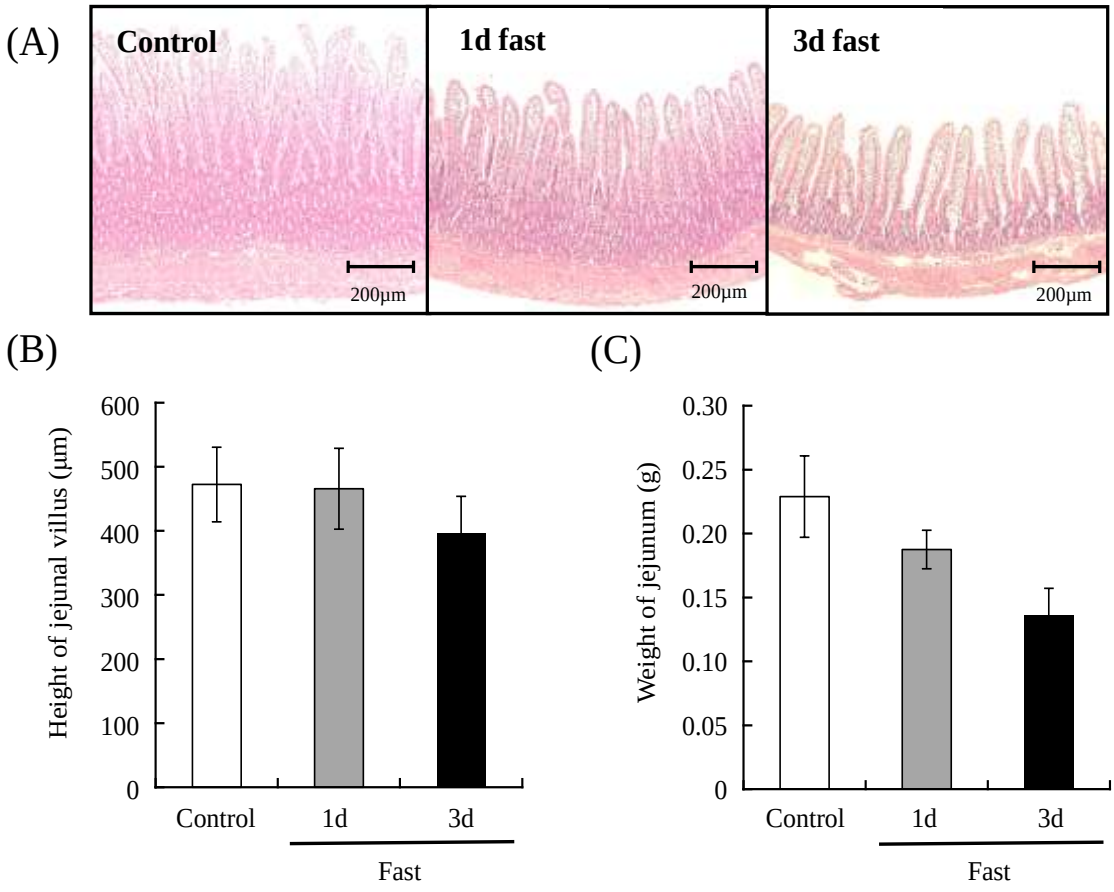

Fig. 3

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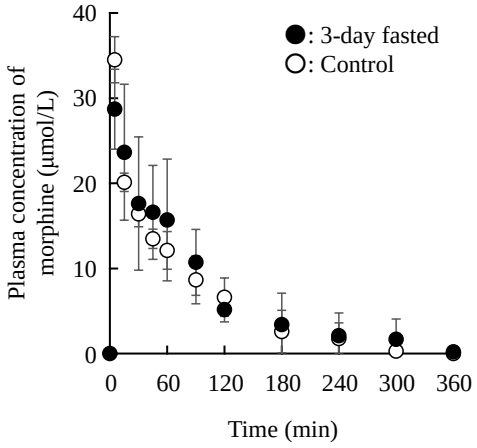

Fig. 4

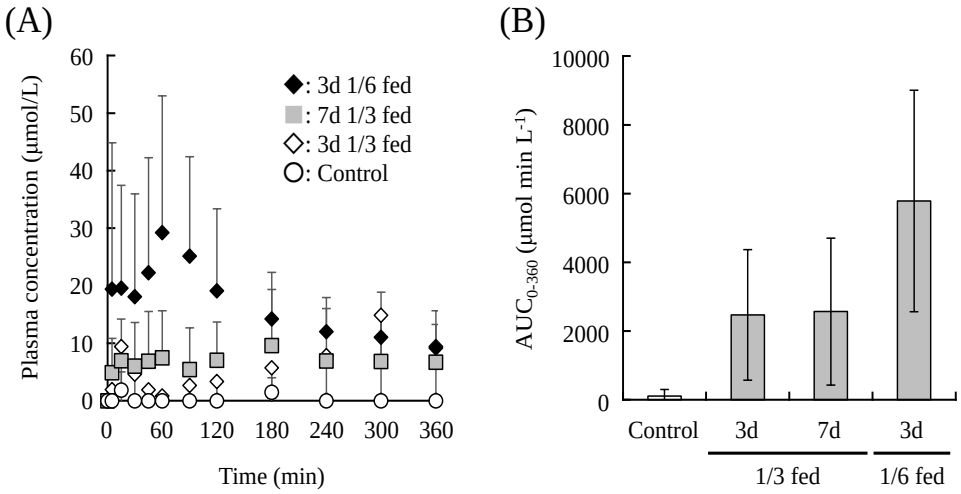

Fig. 5