Rational application of fructose-1,6-diphosphate: From the perspective of pharmacokinetics

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Fructose-1,6-diphosphate (FDP), a glycolytic metabolite, has been reported to protect susceptible organs during hypoxia or ischemia. However, there is paucity of human data on its pharmacokinetics after being exogenously administered. In the current study, the preliminary pharmacokinetics of FDP given orally to humans was investigated, and no typical peak was observed in the serum drug-time curve. Then, the pharmacokinetic studies were performed following multiple doses of FDP in rats, and the Caco-2 monolayer model was used to study the absorption of FDP *in vitro*. The results suggested that plasma FDP concentration was significantly increased after oral multiple doses of 180 mg kg^{-1} but not 90 mg kg^{-1} of FDP, and FDP was partly depleted during the absorption, which was supposed to be consumed by the intestinal epithelium cells. Thus, we conclude that a high dose of FDP should be orally administered in order to get an effective plasma level.

Keywords: fructose-1,6-diphosphate, ischemia, hypoxia, pharmacokinetics

Fructose-1,6-diphosphate (FDP) is an endogenous intermediate metabolite of glucose. Most of glucose and fructose is converted to FDP when entering a cell. It has been increasingly recognized that FDP can protect ischemic or hypoxic tissue after acute ischemia, although the mechanisms have not been fully clarified yet.

Previous studies suggested that exogenous FDP could augment the anaerobic carbohydrate utilization and improve the cellular energy metabolism in ischemic and hypoperfused tissues. Hypoxia forces ischemic tissue to anaerobic glycolysis for energy, which yields two molecules of ATP per glucose in contrast to 36 molecules of ATP generated during oxidative phosphorylation (1). Addition of exogenous FDP can produce two more molecules of ATP in an uncompensated anaerobic environment and hence facilitate the recovery of ischemia tissue. FDP breaks down into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, which will further break down into two molecules of pyruvate and finally produce two molecules of ATP (Fig. 1).

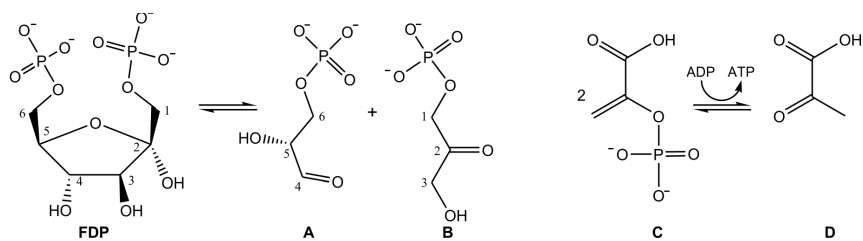* Correspondence; e-mail: jxh1013@vip.163.com

Fig. 1. The role of fructose-1,6-diphosphate (FDP) in energy metabolism. A – glyceraldehyde-3-phosphate, B – dihydroxyacetone phosphate, C – phosphoenolpyruvate, D – pyruvate.

Other mechanisms include inhibition of the generation of oxygen free radicals by neutrophils, stabilization of cell membranes, and maintainance of the correct xanthine dehydrogenase/oxidase ratio by preventing the depletion of phosphorylated compounds in ischemic tissues (2, 3).

In myocardial infarction patients, FDP can improve the hemodynamic parameters, attenuate ECG proven ischemic injury and arrhythmia, prevent ATP and creatine phosphate depletion from ischemic myocardium, reduce infarct size, and increase survival rate (4). Exogenously administered FDP has also been proven beneficial for a variety of other ischemic organs, such as liver, kidney, bowel and even brain as a consequence of its ability to penetrate to the blood brain barrier (5–7). In China, FDP has been approved and marketed as a commercial drug.

The pharmacokinetic study of FDP is demanding due to endogenous interferences. A very high dose is needed to achieve a substantial increase of FDP plasma concentration and generate a comprehensive plasma concentration-time course. Doses used in the animal study varied from 350 to 700 mg kg^{-1} when given by gavage or intraperitoneally (8, 9).

According to Xu and Stringer (10), a complete time course of the levels in blood and brain was observed after intraperitoneal administration of a single 500 mg kg^{-1} dose of FDP to adult male Sprague-Dawley rats. The recommended dose for patients is 1 g, tid, *i.e.*, 42.8 mg kg^{-1} per day. In this study, we first investigated whether the currently recommended total daily dose of FDP (3 g, 42.8 mg kg^{-1}) was adequate to increase the plasma level of FDP in human volunteers. Then, a study was conducted to investigate the pharmacokinetic profile of FDP in rats.

EXPERIMENTAL

Materials

Fructose diphosphate sodium tablets (1 g of FDP per tablet) and the corresponding placebo tablets were manufactured by Ling Kang Pharmaceutical Co., Ltd. (China). The FDP standard ($C_6H_{11}Na_3O_{12}P_2 \cdot 8H_2O$, M_r 550.17, purity 73.4 %) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China). Blank plasma was supplied by the West China Hospital, Sichuan University (Chengdu, Sichuan, China) and was covered by institutional approval. The special solid complex enzyme reagent, a

mixture of aldolase (ALD), triosephosphate isomerase (TIM) and glycerol-3-phosphate dehydrogenase (GDH) in a certain proportion, was purchased from Chung Yi Long Run Co., Ltd. (China), and nicotinamide adenine dinucleotide (NADH) from Sinopharm Chemical Reagent Co., Ltd. (China). The deionized water used in this study was purified using a Millipore AFS-10 water purification system (Millipore, USA). All other chemicals were of analytical grade and were purchased from local chemical suppliers.

Caco-2 cells were supplied by the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified eagle's medium (DMEM) was purchased from Gibco Laboratories (Life Technologies Inc., USA), HyClone™ fetal calf serum from Thermo Fisher Scientific (USA), and trypsin-EDTA solution (0.25 %) from Amresco (USA). Hank's balanced salts solution (HBSS) was purchased from Solarbio (Beijing Solarbio Science &Technology Co., Ltd., China). Polycarbonate filter membranes (Transwell inserts) were obtained from Corning Costar Corp. (Corning, USA).

Assay development

Analytical methods and principles. – FDP concentration was determined by monitoring the absorbance of NADH reduced by a special solid complex enzyme (ALD, TIM and GDH) reagent. Aldolase (ALD) cleaves FDP into dihydroxyacetone phosphate (DAP) and *D*-glyceraldehyde 3-phosphate (GAP). Then DAP and GAP are interconverted by the enzyme triosephosphate isomerase (TIM). In the presence of the above reaction products, glycerol-3-phosphate dehydrogenase (GDH) catalyzes the reduction of DAP by NADH (10).

Briefly, 30 mg of the special solid complex enzyme and 3 mg of NADH were dissolved in 10 mL of tetraethylammonium buffer (TEA, 0.4 mol L^{-1}, pH 7.6 with 40 mmol L^{-1} of EDTA) before analysis, which was called »mingle enzyme«. For FDP measurements, 2 mL of TEA buffer and 1 mL of the »mingle enzyme« were added into a 4-mL cuvette, followed by mixing for 5 min at room temperature. The absorbance was then repeatedly measured each minute for 10 min at 340 nm. The concentration of FDP in the sample was proportional to the change of absorbance intensity.

FDP standard (54.51 mg) was accurately weighed and dissolved in 10 mL of deionized water to prepare the stock solution. Working solutions of FDP with concentrations of 12.5, 25, 50, 100, 200, 400, 500 µg mL^{-1} were prepared by appropriately diluting the stock solution using deionized water (for FDP analysis in plasma) or Hank's Balanced Salt Solution (HBSS) (for FDP analysis in the transport medium). The stock solution and working solutions were stored at 4 °C and tested to be stable for at least 1 month. Calibration standards for FDP analysis in plasma were prepared by spiking 50 µL of working solutions into 500 µL of blank plasma (for FDP analysis in plasma) or HBSS (for FDP analysis in the transport medium). Three levels of QC samples were chosen as low, medium and high calibration standard concentrations, *i.e.*, 25, 100 and 400 µg mL^{-1}. The QC samples were stored at –20 °C with the sample to be analyzed. Blank sample and FDP standard samples were run in parallel with every assay. The environmental temperature for detecting FDP was set at 25 °C.

Method validation

Method validation was done by evaluating linearity, intra-day and inter-day precision as well as accuracy and recovery. The QC samples were prepared by spiking the three

levels of QC working solutions (25, 100 and 400 μg mL^{-1}) into blank plasma. Accuracy and precision of the method were assessed by determining QC samples using five replicate preparations on 3 separate days. Extraction recoveries at low, medium and high concentrations were evaluated by comparing the change of absorbance intensity obtained from the QC samples with that from the samples prepared by spiking working solutions into deionized water.

Pharmacokinetic studies

Study participants. – Preliminary pharmacokinetic studies in humans were performed in two healthy male Chinese volunteers (21 and 22 years old) by administering the doses of 3 g FDP (~ 43 mg kg^{-1}). Two healthy male Chinese volunteers (body mass 70.3 and 69.5 kg) were selected after clinical assessment of their health status to ensure that they had no history or current evidence of any gastrointestinal, hepatic, renal, or hematologic abnormality, acute infection, chronic disease, or allergy to any drugs. Both of them were nondrinkers and nonsmokers, and did not use drugs for 2 weeks before the study. The protocol was approved by the Ethics Committee of the Xijing Hospital, Fourth Military Medical University of Chinese People's Liberation Army. Both provided written informed consent.

Food intake may modify the pharmacokinetics of a drug by altering the bioavailability of the drug during absorption (11). Caffeinated and alcoholic beverages may also affect the metabolism of certain drugs (12). In this study, volunteers were fasted for at least 12 hours. Food and beverage were prohibited for 4 hours post dosing. Consumption of beverages containing caffeine or alcohol was not allowed during the study.

Study design and drug administration. – The study was conducted with a single blind and parallel design. The first day, two volunteers received a single 3-g oral dose of placebo and 4-mL blood sample was drawn into a heparinized tube before and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6 and 8 h post dose to analyze the baseline levels of FDP in plasma. The next day, they received the same oral dose of FDP and the corresponding blood samples were collected as aforementioned. Blood samples were centrifuged at 4000 rpm for 5 min. The plasma sample was separated and stored at –20 °C until analysis.

Sample preparation. – Fifty μL of deionized water was added to 0.5 mL of plasma sample and mixed. Then, 50 μL perchloric acid (30 %, *m/m*) was added and mixed for 5 min. After centrifugation at 12000 rpm for 5 min at 4 °C, the supernatant was collected in another tube and 20 μL of sodium carbonate solution (2.5 mol L^{-1}) was added in order to adjust the pH to 3.5. Then, the samples underwent another mixing and centrifugation at 12000 rpm for 5 min at 4 °C. The supernatant was collected and used for determination of FDP levels as described above.

Pharmacokinetic studies of FDP

The pharmacokinetic study of FDP following oral administration was carried out using adult male Sprague-Dawley rats (180–230 g) obtained from the Laboratory Animal Center of West China University of Medical Science (Sichuan, China). All the animals were housed individually with free access to food and water under standard conditions (controlled temperature and humidity with 12-h light:dark cycles). Animal experiments in this

study were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Sichuan University.

Ten rats were randomly divided into low dose (90 mg kg^{-1}) or high dose (180 mg kg^{-1}) groups. FDP was dissolved in phosphate buffered saline (pH = 7) to a concentration of 10 mg mL^{-1}. The rats received FDP three times a day for 7 consecutive days. Before dosing, 0.4 mL of blood was drawn after 12 h fasting as a control sample to measure the baseline. On the morning of the eighth day, 0.4-mL blood samples were drawn from the tail vein before and at 10, 20, 30 and 45 min and 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 h after the dose. Samples were centrifuged at 4000 rpm for 5 minutes and plasma was separated and stored at –20 °C until analysis.

Study of biliary excretion of FDP in rats

Ten adult male rats were randomly divided into two groups (test and control). The rats received either FDP (180 mg kg^{-1}, three times a day) or equal amount of phosphate buffered saline by gavage for 7 consecutive days. On the eighth day, after the last dose, bile samples were taken after 2, 4, 6, 8 and 12 h from the bile duct cannulation. All bile samples were stored at –20 °C for later analyses.

Absorption of FDP on monolayers of human intestinal epithelial (Caco-2) cells

Cell culture. – Caco-2 cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum. Cells were cultured in a humidified atmosphere of 5 % CO$_2$ at 37 °C. After reaching 80 % confluence, the Caco-2 cells were harvested using 0.25 % trypsin-EDTA. For transport experiments, the cells were seeded onto polycarbonate filter membranes in 6-well plates at a density of 1.0×10^5 cells cm^{-2} and grown for 21 days before the experiments. The protocols for cell culture in the polycarbonate filter membranes were similar to those described previously (13).

Cytotoxicity of FDP. – The cytotoxicity of FDP to Caco-2 cells was examined using a sulforhodamine B (SRB) approach. Briefly, Caco-2 cells were seeded onto 96-well plates (0.1 mL per well, containing 500 cells per well) and incubated for 48 h at 37 °C. Then, cells were incubated with various concentrations of FDP (0, 10, 40, 80, 100, 120, 140, 160, 200, 300, 400 μg mL^{-1}) for 3 h. Afterwards, the cells were fixed using methanol and cellular proteins were dyed with 0.1 mL 0.4 % (*m/V*) SRB in 1 % acetic acid. Cell-bound dye was dissolved with 0.1 mL 10 mmol L^{-1} Tris buffer (pH = 10.5) and the absorbance of each concentration was measured at 492 nm. No FDP treated group was set as control. Cell viability was proportional to the absorbance.

Transport experiments. – The integrity and transportation ability of the Caco-2 cell monolayer were examined by measuring the transepithelial electrical resistance (TEER) with an epithelial volt-ohm meter and detecting the activity of alkaline phosphates. Only the cell monolayer with a TEER of above 500 ohm cm^{-2} was used for transport assays. Differentiation of Caco-2 cells was checked on the 3rd and 14th day by determining the activity of alkaline phosphates.

After washing the Caco-2 cell monolayer twice with HBSS medium (pH = 7.4) pre-warmed to 37 °C, transport experiments were conducted by adding the FDP solutions to the apical chamber (AP, 1.5 mL) while 2.5 mL of blank HBSS medium was added to the basolateral (BL) chamber. After 0, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min incubation at 37 °C, a 0.1-mL sample was collected from the BL side to study the drug transport of the apical to basolateral (AP to BL) chamber and meanwhile an equal volume of blank HBSS was replenished to BL. Also, 0.1-mL samples from the AP side were collected at the beginning and at the end of the experiment. Three loading concentrations of 100, 200 and 400 µg mL^{-1} were used in triplicate. All samples were preserved at –20 °C until analysis.

FDP released by Caco-2 cells. – Caco-2 cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) for 21 days, followed by replacing it with flesh medium and incubating for another 48 h (14). The medium at the end of the 48-h incubation was collected to determine the FDP concentration compared to that in fresh medium. All samples were preserved at –20 °C until analysis.

Data analysis

The cumulative mass (µg) of FDP in the basolateral (BL) chamber at time *i* was calculated using the following formula:

$$QrBi = 0.1 \times [\gamma r1 + \gamma r2 + ... + \gamma r(i–1)] + 2.5 \times \gamma ri \qquad (1)$$

where QrBi (µg) is the cumulative amount of FDP in BL chamber, γr1 is the FDP level (µg mL^{-1}) of the sample in BL chamber from the first time point, γri is the level of the sample at time *i* in BL chamber, and 2.5 refers to the volume of BL chamber (2.5 mL).

Usually, drugs that are well absorbed in the intestine have permeability coefficients $> 1 \times 10^{-6}$ cm s^{-1}, and those which are hardly absorbed have permeability coefficients $\leq 1 \times 10^{-7}$ cm s^{-1}. The apparent permeability coefficient (P_{app}), the coefficient that reflects the absorption of a drug, was calculated based on the following equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A\gamma_0} \qquad (2)$$

where dQ/dt (µg s^{-1}) is the cumulative transport rate of the compound on the receiving side, A is the surface area of the cell monolayer (cm^2), and γ_0 is the initial concentration in the donor compartment (µg mL^{-1}).

The recovery and absorption ratio of the transport experiment was calculated as:

$$Recovery\ (\%) = \frac{\gamma_{Af} \times V_A - \gamma_{Bf} \times V_B}{\gamma_{A0} \times V_A} \qquad (3)$$

$$Absorption\ ratio\ (\%) = \frac{\gamma_{Bf} \times V_B}{\gamma_{A0} \times V_A} \qquad (4)$$

where γ_{Af}, γ_{Bf} and γ_{A0} refer to the final concentration of FDP in the AP chamber, BL chamber, and initial concentration of FDP in AP chamber respectively, and V_A and V_B are the volume of AP and BL chambers.

Statistical analysis

Pharmacokinetic parameters were determined using the DAS version 3.1.6. γ_{max} and t_{max} were determined using visual inspection of the FDP plasma concentration-time flot. All results were expressed as mean ± SEM. The time course for FDP in plasma was analyzed with a one-way ANOVA comparing it to control levels as a function of time. Other comparisons were done using Student's test. A $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION

The intra-day precision of the assay was 8.8, 5.8 and 5.6 % and the inter-day precision are 9.7, 7.3 and 6.5 % for the low, medium and high concentration of FDP, respecting. The accuracies were 93.2, 96.2 and 91.8 % for the low, medium and high concentration of FDP, respectively. A good linear relationship was obtained in the range of 12.5–500 µg mL^{-1} in plasma and HBSS. The lower limit of quantification (*LLOQ*) was 12.5 µg mL^{-1}. The mean extraction recovery was 91.8 ± 7.81, 96.2 ± 7.8 and 93.2 ± 2.5 % for the low, medium and high concentration of FDP, respectively.

Pharmacokinetics of FDP in human plasma

The FDP plasma concentration-time courses of the volunteers following oral administration of 3 g FDP or placebo are shown in Figs. 2a and 2b. Each point represents the concentration of FDP at the corresponding time. The concentration of FDP at baseline level varied from 58.61 to 110.64 µg mL^{-1} and 34.14 to 116.26 µg mL^{-1} for subjects A and B, respectively, which indicated physiological fluctuations. The peak concentration (γ_{max}) and the time to achieve it (t_{max}) in volunteer A were 113.34 µg mL^{-1} and 3 h, and that for volunteer B were 119.83 µg mL^{-1} and 1.5 h.

No significant difference in FDP plasma concentration was observed at 8 hours post dose of FDP tablets and placebo (89.10 ± 3.15 *vs.* 82.58 ± 3.72 µg mL^{-1} for subject A, while 83.47 ± 4.39 *vs.* 84.42 ± 5.39 µg mL^{-1} for subject B). The results demonstrated that FDP failed to rise in plasma of humans at a dose of 3 g compared to an equivalent dose of placebo, indicating that the currently recommended dose of FDP from tablets may be inadequate to achieve clinically effective concentrations.

Plasma pharmacokinetics and biliary excretion of FDP in rats

The FDP plasma concentration in rats following administration of FDP at a dose of 90 mg kg^{-1} (every 8 hours for seven consecutive days) was substantially increased compared to the placebo group, although the increase did not reach a statistically significant level (Fig. 2c). γ_{max} and t_{max} were 118.58 µg mL^{-1} and 45 min, respectively. Average FDP plasma
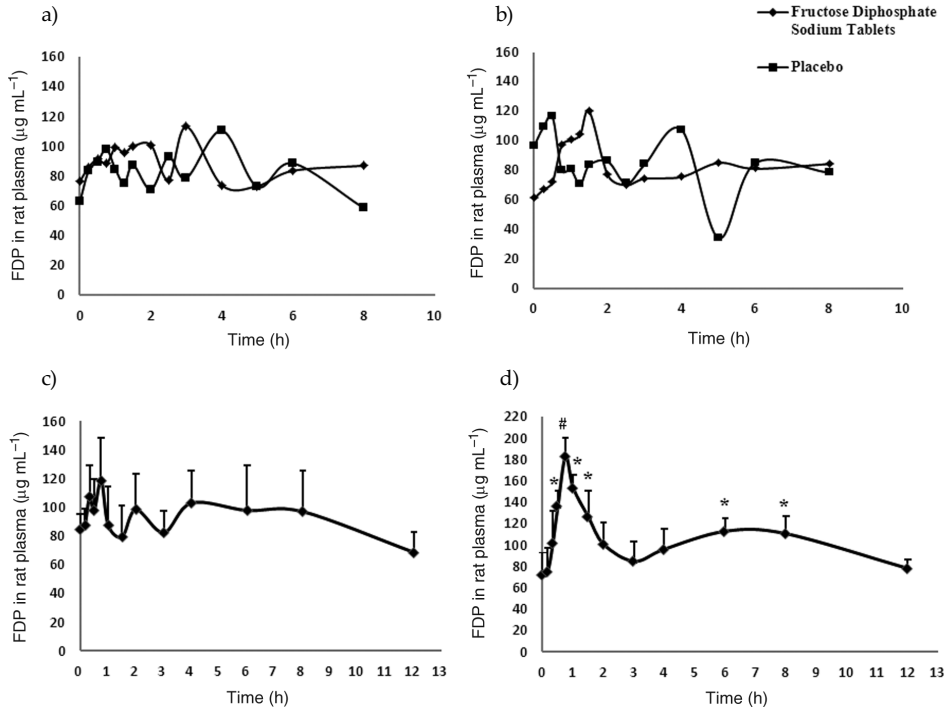
Fig. 2. Level of FDP in plasma of: a) volunteer A and b) volunteer B after administration of 3 g of FDP and placebo. Kinetics of FDP after gavage administration of c) 90 mg kg^{-1} and d) 180 mg kg^{-1} of FDP for consecutive seven days in rats. Each point indicates average and standard deviation, $n = 5$. Significant difference compared to the control value: $^*p < 0.05$, $^{\#}p < 0.01$.

concentration within 12 h following oral administration of FDP was 93.61 µg mL^{-1}, while the average value in the control group was 84.09 µg mL^{-1}.

The FDP plasma concentration in rats following oral administration of 180 mg kg^{-1} FDP (every 8 hours for seven consecutive days) was increased significantly, especially during the first 0.5–1.5 h post dose (Fig. 2d). The γ_{max} and t_{max} were 182.85 µg mL^{-1} and 45 min, respectively. *AUC (0-t)*, *AUC(0-∞)* and $t_{1/2}$ were 1086.7 µg L h^{-1}, 1975.0 µg L h^{-1} and 8.14 h, respectively. The mean FDP concentration within 12 h post dose was 113.28 µg mL^{-1}, while average for the control group was 71.98 µg mL^{-1}. However, in Xu and Stringer's study (10), the peak plasma concentration was only about 35 µg mL^{-1} after a single dose of 500 mg kg^{-1} FDP by intraperitoneal injection in rats, which was much lower than that in the present study. The multiple-dose in this study may significantly increase the concentration of FDP compared to the single-dose due to drug accumulation in plasma.

In addition, a bimodal phenomenon on the concentration-time curve was observed. The second concentration peak of 115 µg mL^{-1} was observed at 7 h post dose (Fig. 2d), which is consistent with Xu and Stringer's study (10). The results indicated that enterohepatic circulation may exist.
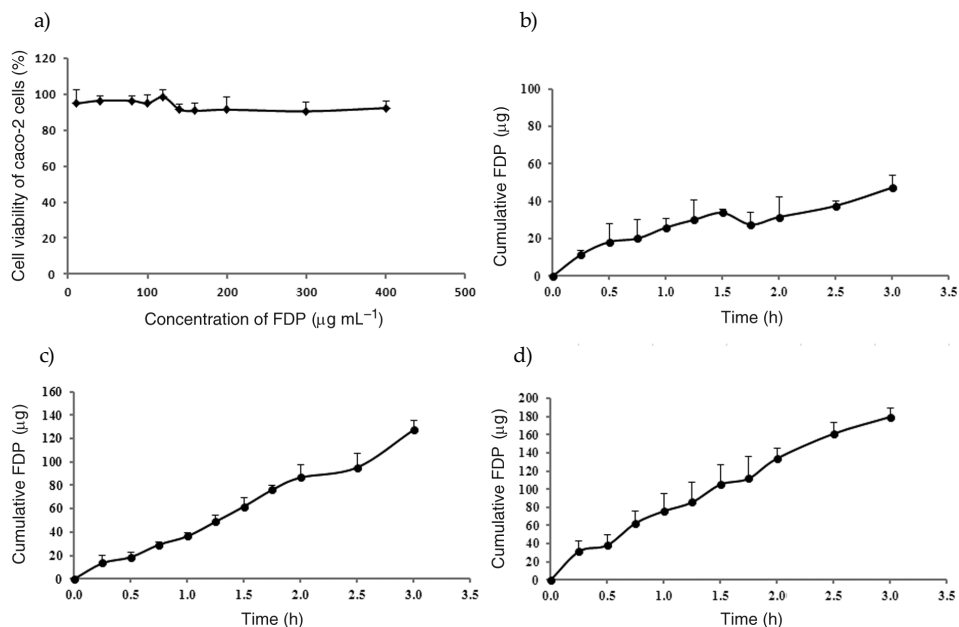
Fig. 3. a) Caco-2 cell viability (%) at different concentrations of FDP (10, 40, 80, 100, 120, 140, 160, 200, 300, and 400 μg mL^{-1}) (mean ± SD, $n = 5$ for 10 μg mL^{-1}, $n = 6$ for others). Time course of FDP transport in Caco-2 cell monolayers at the concentration of: b) 100 μg mL^{-1}, c) 200 μg mL^{-1}, d) 400 μg mL^{-1} (mean ± SD, $n = 3$).

Table I. Permeability of FDP through Caco-2 cells

γ_0 (μg mL^{-1})	γ_B (μg mL^{-1})	γ_A (μg mL^{-1})	QrB3h (μg)	Absorption ratio (%)	Recovery (%)	Papp × 10^{-5} (cm s^{-1})
100	14.89 ± 1.318	26.66 ± 3.292	31.51 ± 10.78	21.01 ± 7.19	63.27 ± 7.214	2.397 ± 0.5928
200	44.18 ± 3.491	55.60 ± 6.700	127.31 ± 7.83	42.44 ± 2.61	72.58 ± 5.048	4.473 ± 0.7643
400	60.36 ± 6.262	154.51 ± 14.259	179.73 ± 16.01	29.96 ± 2.67	72.17 ± 4.318	2.137 ± 0.2991

γ_0 – Concentration of FDP in chamber AP at the beginning of transport experiments;
γ_B and γ_A – Concentration of FDP in chamber BL and chamber AP at the end of transport experiments.
QrB3h – The cumulative amount of FDP in BL chamber after transporting for 3 h.
P$_{app}$ – The apparent permeability coefficient.
Mean ± SD of 3 independent transport experiments.

FDP was not detected in biliary samples from the both test group and control group. Other mechanisms may exist. Following the oral dose of 180 mg kg^{-1} FDP (every 8 hours for seven days).

Permeability of FDP through Caco-2 cells

Cell viabilities of Caco-2 cells in the presence of FDP at concentrations from 10 to 400 µg mL^{-1} were all above 90 %, which suggested and released FDP by rather low cytotoxicity of FDP on Caco-2 cells within 3 h (Fig. 3a).

The amount of FDP in BL chamber increased with time at loading concentrations of 100, 200 and 400 µg mL^{-1} in AP chamber (Figs. 3b, 3c, 3d). At the end of the experiment, the cumulative FDP in BL chamber was 45.53, 127.3, and 179.61 µg, respectively.

The mean P_{app} value of FDP at three concentrations is listed in Table I; FDP was found to be high permeable ($P_{app} > 1 \times 10^{-6}$ cm s^{-1}). However, the recoveries of FDP were poor (63, 68, and 78 % for 100, 200 and 400 µg mL^{-1} group respectively). The intracellular FDP concentration was determined at the end of the transport experiment and was found to be very low. The results suggested that FDP could be consumed by Caco-2 cells during the transport experiment. Such a possibility would partially explain low recovery.

Caco-2 cells were cultured for 21 days on polycarbonate filter membranes (surface area 4.52 cm^2) and were continuously incubated for another 48 h to investigate the FDP release. The results showed that the level of FDP in the final medium in AP (apical chamber) and BL (basolateral chamber) were 175.35 ± 2.37 and 174.51 ± 5.79 µg mL^{-1}, respectively. No FDP was detected in fresh medium, which implied that FDP may be released from Caco-2 cells. The release of endogenous FDP could explain the second peak in the concentration-time curve (Fig. 2d), although further investigation is necessary to verify the hypothesis.

CONCLUSIONS

The results indicate that the currently recommended dose of FDP may be inadequate to significantly increase the plasma level of FDP and that a higher dose is needed. The consumption of FDP by the intestinal epithelium cells could be a major barrier for FDP absorption. In order to achieve the desired clinical therapeutic effect, a higher dosage may be utilized in clinical settings for mitigating the ischemic injury of organs apart from the intestine itself.

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