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Effects of alga polysaccharide capsule shells on in-vivo bioavailability and disintegration*

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Abstract Gelatin has been used in hard capsule shells for more than a century, and some shortcomings have appeared, such as high moisture content and risk of transmitting diseases of animal origin to people. Based on available studies regarding gelatin and vegetable shells, we developed a new type of algal polysaccharide capsule (APPC) shells. To test whether our products can replace commercial gelatin shells, we measured in-vivo plasma concentration of 12 selected volunteers with a model drug, ibuprofen, using high performance liquid chromatography (HPLC), by calculating the relative bioavailability of APPC and Qualicaps® referenced to gelatin capsules and assessing bioequivalence of the three types of shells, and calculated pharmacokinetic parameters with the software DAS 2.0 (China). The results show that APPC shells possess bioequivalence with Qualicaps® and gelatin shells. Moreover, the disintegration behavior of four types of shells (APPC, Vegcaps®, Qualicaps® and gelatin shells) with the content of lactose and radioactive element (^{99m}Tc) was observed via gamma-scintigraphic images. The bioavailability and gamma-scintigraphic studies showed that APPC was not statistically different from other vegetable and gelatin capsule shells with respect to in-vivo behavior. Hence, it can be concluded that APPCs are exchangeable with other vegetable and gelatin shells.

Keyword: bioavailability; bioequivalence; Gamma-scintigraphy; vegetable capsules; gelatin capsules

1 INTRODUCTION

Two-piece hard capsules have been used as a drug delivery system for more than a century (Chiwele et al., 2000). Most commercially available ones are produced from gelatin. Gelatin is a collagen protein, originating from the bones and skin of cattle and pigs, as it has properties that make it suitable for capsule manufacturing, such as thermo-reversibility, film-forming ability, and a triple-helix structure. However, there are some drawbacks, such as the cross-linking reaction of gelatin (Digenis et al., 1994; Brown et al., 1998; Ofner et al., 2001).

Firstly, the moisture of gelatin capsule shells is about 13%–15% (Nagata, 2001), which decreases the stability of the contents and promotes gelatin cross-linking. In addition, there is the risk of transmitting animal-original diseases, such as bovine spongiform encephalopathy (BSE) (Honkanen et al., 2002). Furthermore, the application of gelatin capsules in Islamic areas and amongst vegetarians is limited.

For the above reasons, vegetable capsules were introduced to the market. Many plant-derived materials, such as cellulose, polysaccharide, starch and derivatives (Vilivalam et al., 2000; Nagata, 2001; El-Malah et al., 2007; Misale et al., 2008; Sakata and Otsuka, 2009), were studied to produce vegetable capsule shells. These shells overcome the shortcomings of gelatin capsules, and possess additional advantages, such as the lower oesophageal sticking tendency of hydroxypropyl methylcellulose

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(HPMC) capsules (Honkanen et al., 2002), and their temperature-independent dissolution (Chiwele et al., 2000).

Although a lot of literature on this topic has been published, there is little about the in-vivo studies of the effect of the shells on drug absorption and disintegration behavior. Our laboratory developed a new type of capsule shell composed of a cellulose derivative and algal polysaccharide. Studies on in-vitro dissolution and disintegration of shells have been conducted, and the results showed that there was no significant difference between the algal polysaccharide shells and gelatin shells.

With the permission of the ethical committee of the Affiliated Hospital of Medical College Qingdao University, we conducted present studies on relative bioavailability and assessed the bioequivalence of four types of capsules (APPCs, Qualicaps®, Vegcaps® and Gelatin capsules) using ibuprofen as a model drug. Bioavailability investigations can provide in-vivo pharmacokinetic parameters of the drug. However, all of the data are estimates because they are based on blood sample collection and measurement post-dose. In this case, a radionuclide imaging technique was developed. Gamma-scintigraphy is one of the techniques widely used to study local and time-specific formulation, because it can trace the drug delivery process non-invasively in real time (Newman et al., 2003; Marvola et al., 2004, 2008). In present studies, we combined the above two in-vivo research techniques to obtain more accurate data.

2 MATERIAL AND METHOD

2.1 Materials

Hard, size 1 algal polysaccharide capsules (APPC; supplied by Qinhuangdao Pharmaceutical Capsule Co. Ltd., China), Qualicaps® (QC; obtained from Shionogi Qualicaps, S. A., Japan), Vegcaps® (VC; gifted by Jiangsu Zodiac Marine Biotechnology Co., Ltd., China) and gelatin capsules (GC; provided by Harbin Yaoyuan Corporate, China) were investigated in this study. The model drug used in the bioavailability study was ibuprofen from Liaoning Fuyuan Pharmaceutics Co. Ltd. (China) and the internal standard, indometacin, was purchased from Huzhou Kangquan Pharmaceutics Co. Ltd. (China). Gamma-scintigraphic images were obtained through a gamma-camera (Millennium VG ECT, General Electric Company, USA). The content of the capsules was lactose from Wuhan Galaxy Co. Ltd., China, and the radioactive marker was technetium-99m (^{99m}Tc, $t_{1/2}$=6.03 h) from Beijing Atom High Tech Co., Ltd. (China).

2.2 Bioavailability and bioequivalence study

2.2.1 Preparation of formulations

An amount of 250±1 mg of drug powder (80% ibuprofen) per capsule was weighed by a precision electronic balance (Mettler AE240). Fifteen capsules of each type were filled manually.

2.2.2 Selection of subjects

A group of 12 healthy male volunteers participated in this study. The ages of the volunteers ranged from 22–32-year-old. The volunteers' weights varied from 58–68 kg, and heights from 167–176 cm. The subjects had no history of drug irritability and diseases of the liver or kidney. Each volunteer passed the physical testing, including electrocardiogram, heart rate, blood pressure, AST (serum glutamic-oxaloacetic transaminase), renal function, routine haematological testing and urine analysis. The volunteers were not administered any drugs within the two weeks leading up to the study, and they abstained from smoking and drinking alcohol during the study. All volunteers were informed of the potential risks, and written informed consent to participate was obtained.

2.2.3 Bioavailability studies

This study was designed as three-cycle, cross-over and double-blinded. Each volunteer received three capsules randomly, one at a time, with an interval of one week. The subjects fasted for at least for 12 h before the studies, and the capsules were administered orally with 180 mL of water. The volunteers were not allowed to drink or eat until 2 h after administration and a standard meal was served at 4 h post-dose. The overall study was supervised by nurses and clinicians.

Blood samples of 3.0 mL from the forearm venous canula were collected into heparinised tubes prior to drug administration (0 h) and at 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 7.0, 9.0, 11.0 h thereafter. Plasma was separated by centrifugation (3 000 r/min for 10 min) and stored at -20°C for analysis within 14 days.

2.2.4 Assay of plasma

The concentration of ibuprofen in the plasma was determined by high performance liquid chromatography (HPLC, Waters 2690). The plasma

samples were separated by Venusil MP-C18 (5 μm, 250 mm×4.6 mm) and measured by a UV detector at 220 nm. The mobile phase was methanol (chromatographic pure, Honeywell International Inc., USA)-0.02 mol/L KH_2PO_4 (80:20) at pH 3.08 adjusted with H_3PO_4. The flow rate was 1 mL/min and the column temperature was 25°C.

The standard curve was established using a series of concentrations of 6.4, 16.0, 32, 80, 200, 500 μg/mL. The ibuprofen standard (purity of 99.9%) was supplied by Dr. Ehrenstorfer GmbH (Germany). Indometacin at a concentration of 10 mg/L in methanol was used as the internal standard. The linear coefficient of the standard curve was 0.999 or higher.

Precisely measured solutions of 200 μL plasma and 600 μL indometacin were placed in 1.5 mL centrifugation tubes and mixed in a swirling shaker (MSI minishaker, IKA works, Guangzhou, China) for 1 min. Centrifugation was conducted at 14 500 r/min for 10 min, and 20 μL of the supernatant was injected in HPLC.

Accuracy and precision of the method was determined by comparing three blank plasma samples of 20 μL ibuprofen solutions with concentrations of 6.4, 80, and 500 μg/mL. Intra- (5 samples per concentration) and inter-day (5 consecutive days) relative standard deviations (RSD) were also calculated.

2.2.5 Pharmacokinetic parameters

The pharmacokinetic parameters were calculated by DAS 2.0 (China), including maximum plasma concentration (C_{max}), corresponding time to peak concentration (T_{max}), area under the concentration-time curve ($AUC_{(0-11)}$ and $AUC_{(0-\infty)}$), and mean residence time (MRT). Relative bioavailability (F%) and bioequivalence were estimated against the above parameters.

2.3 Gamma-scintigraphy study

2.3.1 Capsule filling and radiolabelling

An amount of lactose (3 g) was weighed by a precision electronic balance (Mettler AE240) and manually mixed with a 44.4 MBq ^{99m}Tc concentrated solution on the day of the experiment. The mixture was dried in an oven. The capsules weighed 300 mg, and the mean level of radioactivity during administration was 3.75 MBq.

2.3.2 Selection of subjects

Eight healthy volunteers (5 males and 3 females) between the ages of 22 and 26 participated in the study. All of the subjects were in the standard weight range and had normal gastrointestinal function. They did not touch or administer any other radioactive substances for one week before the studies, and abstained from smoking and drinking alcohol during the study. All of the volunteers were informed about the potential risks, and written informed consent to participate was obtained.

2.3.3 Experimental procedure

The experiment was a random, four-cycle, cross-over and double blinded design. All the practices followed the safety guidelines of the radioactive laboratory and were monitored by the doctors and nurses. The subjects fasted for at least 12 h before the study, and after that the capsules were administered orally with 180 mL of water. The volunteers remained in an upright position for 20 s and then the disintegration was traced by a Gamma camera at 60 s intervals over 30 min. The images were processed by a computer system, and the time at which the radioactive marker started to spread in the images was considered the initial disintegration time. The eliminating time was one week for each type of capsules.

3 RESULT AND DISCUSSION

3.1 Bioavailability and bioequivalence study

3.1.1 HPLC behavior

The method resulted in good peak shapes for ibuprofen and indometacin at the described conditions. The drug and internal standard were completely isolated from the endogenous substances in the plasma. The results show that the retention time of indometacin was 7.731 min, and that of ibuprofen was 8.497 min. Fig.1 shows the chromatograph profiles of a blank plasma sample and the control.

3.1.2 Accuracy and precision assays

The plasma sample analytical method had a detection limit of 0.16 μg/mL, intra-day RSD of <3.99%, and inter-day RSD of <4.89% (see Table 1). In addition, it had good linearity ($y=0.023\ 4x-0.005$, $R^2=0.999\ 9$). Therefore, it can be concluded that the method was accurate and precise, and thus can be used as a standard method for the measurement of bioavailability.

3.1.3 Pharmacokinetic parameters

All 12 participants completed the study. The pharmacokinetic parameters were summarized in

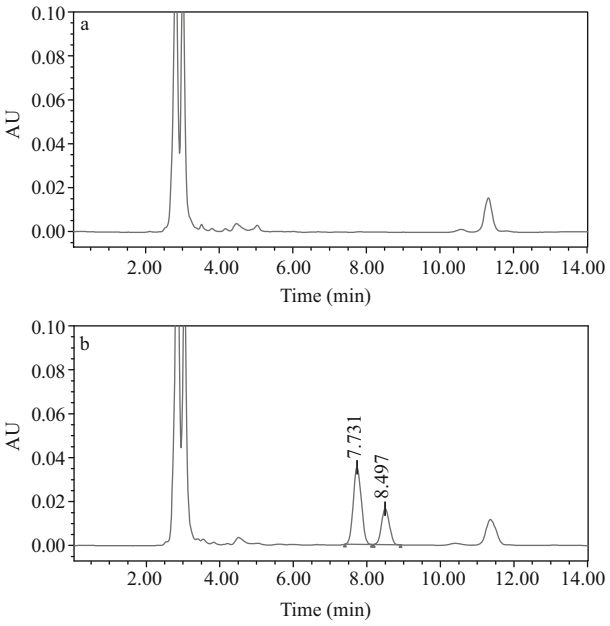

Fig.1 Chromatographic profiles of blank plasma (a) and a plasma sample containing the ibuprofen standard and indometacin (b)

Table 1 Data of intra- and inter-day relative standard deviation (RSD)

C (μg/mL)	Intra-day		Inter-day	
	AUC ($\times 10^3$)	RSD (%)	AUC ($\times 10^3$)	RSD (%)
50	637.27±16.90	2.65	639.62±30.42	4.76
8	90.27±2.62	2.90	98.42±1.72	1.75
0.64	7.28±0.29	3.99	7.57±0.37	4.89

C: concentration of ibuprofen; AUC: area under the curve; RSD: relative standard deviations.

Table 2. There are no statistically significant differences ($P>0.05$) in pharmacokinetic parameters amongst the three types of capsules except for the T_{max}. The Qualicaps® released the drug in the shortest time.

The mean plasma concentration-time curves of A, Q, and G are shown in Fig.2. The curves fit the typical profile of drug absorption, and the three curves do not greatly differ from each other. The plasma concentrations of ibuprofen were recorded and analyzed (data not shown). The results showed that there were considerable differences in the plasma concentrations of the same type of capsules between the 12 participants. This may be attributed to the different in-vivo conditions of the volunteers, such as the stomach pH, ionic concentration and ion types, which may affect the disintegration of the three types of shells corresponding to their different materials. In our previous study on the effect of pH on the in-vitro dissolution of capsules, we found that a pH similar to that of gastric juice did not influence the dissolution time of the drug, while the types and concentrations of ions did have an effect. For instance, APPCs did not disintegrate in the buffer containing potassium cations, but when the buffer contained sodium instead of potassium, their disintegration was not significantly different from that of gelatin capsules. Therefore, the physiological conditions of the subjects are a major factor affecting the absorption rate of the drug in the capsule shells.

3.1.4 Assessment of bioavailability and bioequivalence

Bioavailability indicates the degree of drug absorption after administration. It plays an important

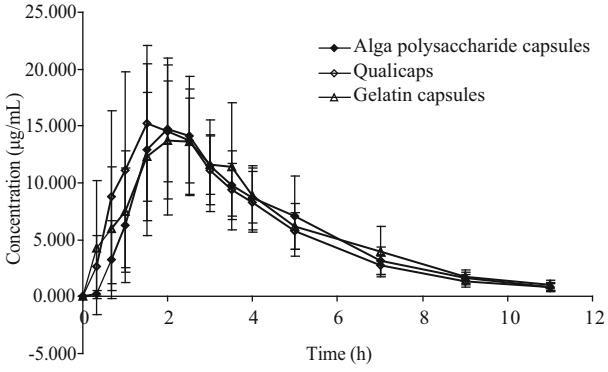

Fig.2 Mean plasma concentration-time curve of gelatin capsules, algal polysaccharide capsules, and Qualicaps®

Table 2 Calculated pharmacokinetic parameters following oral administration of APPC, Qualicaps® and gelatin capsules

	$T_{1/2}$ (h)	C_{max} (μg/mL)	T_{max} (h)	$AUC_{(0-11)}$ (μg/mL × h)	$AUC_{(0-\infty)}$ (μg/mL × h)	MRT (h)
APPCs	2.01±0.30	18.37±3.43	2.29±0.99	62.84±13.06	65.31±13.97	0.00
Qualicaps®	2.09±0.49	19.36±3.81	1.49±0.65	64.48±10.20	66.75±10.20	0.00
Gelatin Capsules	2.16±0.38	18.352±4.74	2.11±0.91	66.24±10.92	69.29±11.04	0.00

C: the plasma concentration of ibuprofen; AUC: area under the curve; MRT: mean residence time. $n=12$; data=mean±S.D.

role in the assessment of the medicament quality, and it may be influenced by storage temperature (Hakata et al., 1994), medium pH, ions, and some substances that interact with the drugs (Desai et al., 1994).

The relative bioavailability of Qualicaps® and APPC was calculated to be 97.4% and 94.3%, respectively, compared to gelatin capsules. On the basis of the pharmacokinetic parameters, we assessed the bioequivalence of the three types of capsules using the [1-2α] confidence interval method (Table 3). The results showed that they were all bioequivalent. In conclusion, the bioavailability of APPC is not significantly different from the Gelatin capsules and the Qualicaps®.

3.2 Gamma-scintigraphic studies

The radioactive markers of the four types of capsules were observed using a gamma camera when the volunteers were lying on the E-CT (Emission Computed Tomography), indicating that the four capsule types could all get through the esophagus into the stomach; therefore, we supposed that there were no significant differences in the oesophageal transit of APPCs compared to the Gelatin, Qualicaps® and Vegcaps® shells. This agrees with the conclusion of Cole et al. (2004), who claimed that gelatin capsules and HPMC capsules made from gellan could move down to the stomach rapidly within 20 s. Honkanen et al. (2004) reported that HPMC capsules made from carrageenan may adhere to the esophagus leading to a delay in capsule transit. However, the authors explained that the reason may be the lack of water on the first day, and no other such phenomenon was observed thereafter. All of the volunteers in the present study strictly took 180 mL of water and remained in an upright position for 20 s, so there was no delay observed.

Table 3 Results of relative bioavailability and bioequivalence of APPC and Qualicaps referenced to Gelatin shells

	Relative bioavailability (F%)	Bioequivalence	
		Bioequivalent standard	Confidence interval
APPC	94.3	80.0%–125%	88.3%–100.8%
Qualicaps	97.4	80.0%–125%	91.2%–104.1%

Fig.3 shows the gamma-scintigraphic images of subject 1. The three vegetable capsules had a similar disintegration process, while the gelatin capsules had a constant central marker, although its initial disintegration time was shorter than the other capsule types. This suggests that the gelatin may become sticky when it reacts with water, and hence hinder the dissolution of lactose.

The disintegration time of the eight subjects was summarized in Table 4. The mean disintegration time of gelatin capsules was the shortest, and the other three are similar; however, there were no statistical differences between the four ($P>0.05$). This is consistent with the previous reports (Brown et al., 1998; Cole et al., 2004; Tuleu et al., 2007), indicating that vegetable capsules have longer disintegration times than their counterparts but they have no statistical differences. It seems that this is not accordance to the bioavailability studies, which show Qualicaps® a shortest T_{max}. However, it is shown that the highlight of gelatin capsules exists till the end of the study, so we can conclude that the content of gelatin capsules did not be absorbed by the subjects, and consequently, although gelatin capsules have the shortest disintegration time, its T_{max} is not the same status in the bioavailability studies. This is accordant to the results of C_{max} in the study.

4 CONCLUSION

Based on the results of bioavailability and gamma-scintigraphic studies, we concluded that the new APPC we developed disintegrates rapidly in-vivo, and it does not affect or decrease the release and absorption of the drug. Moreover, it overcomes the shortages of high moisture, cross-linking and risk of transit of animal-original diseases of gelatin capsules,

Table 4 Data for gamma scintigraphy of four types of capsules

Capsules	Disintegration time of capsules of each subject (min)								
	1	2	3	4	5	6	7	8	Mean±S.D.
A	4	9	14	8	13	15	6	7	9.5±4.0
B	4	11	13	13	7	8	6	12	9.3±3.5
C	5	23	5	3	15	7	11	6	9.4±6.7
D	3	4	12	8	13	5	5	9	7.4±3.7

A refers to APPC; B to Qualicaps®; C to Vegcaps®; D to gelatin shells.

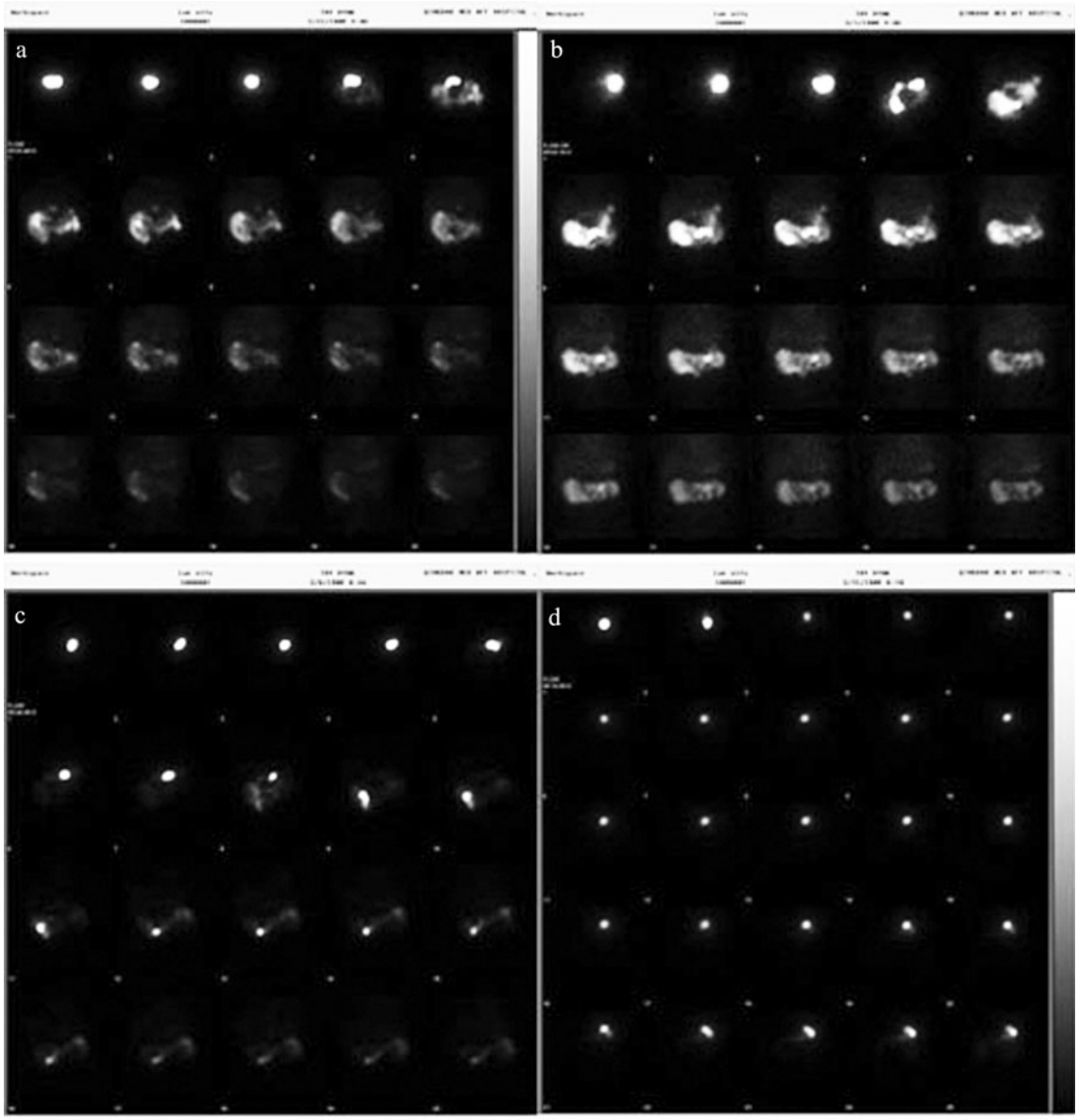

Fig.3 Gamma-scintigraphic images of four types of capsules for subject 1

a. APPCs; b. Qualicaps®; c. Vegcaps®; d. gelatin shells.

and comparing to its Japanese counterparts, it is low-costed. At last, the appropriate physicochemical properties render alga polysaccharide a good alternative to gelatin capsules.

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