AAPS PharmSciTech, Vol. 9, No. 3, September 2008 (© 2008)
DOI: 10.1208/s12249-008-9096-y

Brief / Technical Note

Bioavailability Enhancement and Targeting of Stomach Tumors Using Gastro-Retentive Floating Drug Delivery System of Curcumin—"A Technical Note"

Shishu,[1,2] Neeta Gupta,[1] and Nidhi Aggarwal[1]

Received 15 September 2007; accepted 10 April 2008; published online 4 July 2008

KEY WORDS: benzo(a)pyrene; buoyancy; curcumin; effervescent agents; floating drug delivery systems; gastric residence time.

INTRODUCTION

Effective delivery of the drugs to the stomach, for local action and treatment of gastric disorders such as gastric cancer, gastric ulcers can be achieved by floating dosage forms like single and multiple unit gas generating systems, hollow microspheres, hydrodynamically balanced systems, swelling or expanding systems, mucoadhesive systems and other gastroretentive dosage forms (1). Curcumin is a major pigment of the *Curcuma* species, commonly used as a yellow coloring and flavoring agent in foods particularly in South Asia (2). Use of curcumin in traditional medicine and as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders; rheumatism and sinusitis (3) has been well documented. The novel trends in medicine have led to extensive investigations to establish the wide spectrum of biological and pharmacological actions of this phytochemical. Curcumin has been reported to possess anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antiarthritic, antibacterial, antifungal, antiprotozoal, antiviral, anti-Alzheimer, anti-psoriatic and neuroprotective activities (4).

The efficacy, pharmacological safety, cost effectiveness of curcumin and no-dose limiting toxicity (4) has also prompted many researchers to further investigate this molecule. However, it has also been recognized that the therapeutic effectiveness of curcumin is limited due to its poor circulating bioavailability and absorption from the gastrointestinal tract.

Numerous attempts have been made to improve the solubility of curcumin by complex formation or interaction with various macromolecules like gelatin, polysaccharides and cyclodextrins (5,6) preparation of solid dispersions with polyvinyl pyrrolidone (7) and prodrugs of curcumin (8).

Present work was designed to improve the aqueous solubility of curcumin at acidic pH by incorporating it into β-cyclodextrin (β-CD) complex. Further to improve absorption of curcumin–β-CD complex from stomach, to target stomach tumors and to prevent degradation of curcumin in the alkaline environment of intestine, a floating drug delivery system (FDDS) with sustained release characteristics was developed.

MATERIALS AND METHODS

Materials

Curcumin was purchased from Sigma Chemical Company, USA, Hydroxypropyl methylcellulose (HPMC) K15M was obtained as a gift sample from Colorcon Asia Pvt. Ltd., Mumbai, India and Carbopol 934P was a gift from BF Goodrich, Germany. All other reagents and chemicals used were of analytical grade.

Preparation and Evaluation of Curcumin β-cyclodextrin Complex

Kneading method was used to prepare 2:1 host–guest molecular complex of curcumin with β-CD which was confirmed by Fourier transform infrared method (9).

Preparation of Floating Tablets of Curcumin

The floating tablets of curcumin (CI) and curcumin β-cyclodextrin complex (CII) were compressed using HPMC K15M (200 mg), dicalcium phosphate (20 mg), sodium carbonate (40 mg), citric acid (20 mg), Carbopol 934P (25 mg) and magnesium stearate (10 mg). The formula used was previously optimized for the various parameters like buoyancy, crushing strength, floating lag time, swelling index etc by varying amounts of HPMC and using different fillers like lactose, microcrystalline cellulose and dicalcium phosphate keeping amount of other ingredients constant (10). All the ingredients except Carbopol and magnesium stearate were blended homogenously for sufficient time and wet granulated using 95% *v*/*v* ethanol. The dried granules of

[1] University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, 160014, India.
[2] To whom correspondence should be addressed. (e-mail: shishugoindi@yahoo.co.in)

16–20 # fractions were mixed with Carbopol and magnesium stearate. This mixture was compressed into tablets using flat faced, round punches of 13.1 mm diameter in a single punch tableting machine (11).

Evaluation of Floating Tablets

The prepared tablets were evaluated for drug content, weight variation, hardness, thickness and diameter uniformity.

In vitro release studies were carried out using USP XXIII Dissolution Apparatus II (paddle type). The floating tablets of CI and CII were dropped into 900 ml of HCl buffer pH 1.2 maintained at a temperature of 37±0.5°C and stirred at a speed of 50 rpm. At different time intervals, 10 ml aliquot was withdrawn and volume was replaced with an equivalent amount of fresh dissolution medium kept at 37°C. The collected samples were filtered and analyzed at λ_{max} 420 nm (12) using ultraviolet–visible spectrophotometer against HCl buffer pH 1.2 taken as blank. Also, pure curcumin and curcumin β-CD complex (100 mg each) were filled into hard gelatin capsules and subjected to dissolution studies in the manner described above to serve as Control I and Control II respectively. Drug release data were analyzed using ZOREL software (13) after correcting the values for the drug loss occurred during sampling. Based primarily on the algorithms proposed by Peppas and Sahlin (14) the software reports the values of the release exponent (n) indicating the kinetics of drug release, the kinetic constant (k), magnitudinal contributions of the Fickian diffusion (k_1) and polymer relaxation (k_2), respectively.

The time between the introduction of the tablet into the medium and buoyancy of the tablet to the upper one-third of the dissolution vessel (buoyancy lag time) and the time for which the formulation constantly floats on the surface of medium (duration of buoyancy) were measured simultaneously as a part of dissolution studies (15).

Radial swelling of the matrices was monitored by immersing the tablet in a beaker containing 250 ml of HCl buffer pH 1.2. The increase in the tablet diameter was measured at predefined time intervals over a period of 24 h.

Antitumor Activity

Albino female mice (Balb/C strain) of 8–9 weeks old weighing 20–30 g were used. The animals were kept under standard 12/12 light/dark cycle and were given food and water *ad libitum*. The animals were administered two doses of 3 mg of benzo(a)pyrene [B(a)P] in 0.25 ml of corn oil per oral at the gap of 2 weeks. The vehicle alone in an equal quantity was administered to the control group (16–18). The B(a)P treated mice were divided into four groups (n=12 per group). Group I served as non-treatment control, Group II, III and IV were given treatment CI, Control II and CII respectively per orally as 100 mg/kg corn oil suspension of the drug till the end of the experiment.

Tumor Determination

Animals were sacrificed after 18 weeks of last dose of B(a)P by cervical dislocation. The forestomach was separated and was cut longitudinally and fixed in 10% buffered formalin-phosphate. Stomach papillomas measuring 1.0 mm or larger were counted using magnifying glass (18). The

Table I. Drug Release Parameters of Various Floating Tablet Formulations of Curcumin

Formulation code[a]	Release exponent (n)	Kinetic constant (k)	Fickian diffusion constant (k_1)	Polymer relaxation constant (k_2)	$t_{50\%}$ (h)	$t_{80\%}$ (h)	Overall rate of drug release (mg/h) mean±SD (n=6)	Buoyancy lag time (min)	Duration of buoyancy (h)
Control I	0.6336	0.0054	–	–	–	–	0.340±0.537	–	–
Control II	0.4539	0.7998	–	–	1.1	1.8	23.824±3.42	–	–
CI	0.7511	0.0023	1.0008	0.0013	–	–	0.152±0.116	10–12	16–17
CII	0.7752	0.1347	1.1333	0.0475	5.465	11.688	1.008±0.828	10–12	16–17

[a] CI Curcumin floating tablet, CII curcumin β-CD complex floating tablet, Control I indicates pure curcumin; Control II indicates pure curcumin β-CD complex

Table II. Effect of Floating Tablet Formulations of Curcumin on B(a)P Induced Forestomach Tumors in Albino Female Mice

Treatment	Mice with tumors (%)	No. of tumors per tumor bearing mice	Tumorigenic index[a]
B(a)P	100	2.00	200.00 (100)[b]
B(a)P+CI	75	1.50	112.50 (56)
B(a)P+Control II	66	1.32	87.12 (43)
B(a)P+CII	50	1.00	50.00 (25)

[a] Tumorigenic index obtained by multiplying the percentage of mice with tumors times the mean number of tumors per tumor bearing mouse
[b] Number in parentheses indicate percentage of the control tumorigenic index

relative susceptibility to B(a)P induced tumors was expressed by the tumorigenic index as proposed by Shimkin (19).

$$\text{Tumorigenic index} = \text{Percentage of mice with tumors} \times \text{Mean number of tumors per tumor bearing mouse}$$

All the data was statistically analyzed by one way analysis of variance followed by Dunnett's method.

RESULTS AND DISCUSSION

The average weight of tablets for both the formulations (CI–CII) was found to be 414.15±1.29. The thickness was 3.22±0.02 mm and hardness was found to be 2.50± 0.06 kg/cm^2. It was also observed that tablets of both the formulations possessed acceptable physical characteristics.

Dissolution Studies

Table I enlists the various dissolution parameters computed for all the formulations. The critical value of release exponent (n) calculated as per algorithm proposed by Peppas and Sahlin, was 0.75 and 0.77 for Formulations CI and CII respectively. In general the release pattern of curcumin from floating tablets was found to be non-Fickian. Much higher values of k_1 (1.001–1.133) compared to k_2 (0.001–0.048) clearly indicate that the drug release was governed predominantly by Fickian diffusion, with varying contribution of polymer relaxation mechanism as well.

On comparison between *in vitro* release pattern of Control I and CI (Fig. 1) it was found that, both the profiles were almost superimposable. Moreover, a very low percent drug release value (2.25% in 24 h) indicates the poor solubility characteristics of curcumin in the gastric pH. However, inclusion of curcumin into β-CD drastically improved the solubility of curcumin in the acidic environment and this is evident from the release pattern of Control II (Fig. 1). Almost 50% of curcumin was released in 1 h and more than 80% was released in 2 h from Control II. The overall release rate constant from Control II was observed to be 0.7998 which is significantly different and greater than Control I. Further, results indicate that buoyant Formulation CII could sufficiently sustain release of curcumin from the matrix (k=0.1347) and only 50% of curcumin was released in 5 h and almost 100% release was achieved in 24 h.

An initial lag time of 10–12 min was observed for floating tablets of both the Formulations (CI and CII) of curcumin to come on the surface after that the tablets remained buoyant up to 16 h without disintegration.

Swelling characteristics of Formulation CII was estimated in HCl buffer pH 1.2 for 24 h. The size of the tablet was found to increase 1.5 times the initial diameter after 15 h. This increase in size may also prevent the exit of floating tablet from pyloric sphincter and help to improve its gastric retention property.

Antitumorigenic Effects

The studies indicate that treatment of mice with B(a)P resulted in 100% incidence of forestomach tumors after 10 weeks with an average of 2.0 tumors per mouse compared to corn oil-treated control animals (Table II).

The treatment of mice with CI, Control II and CII after last dose of B(a)P i.e., during the initiation period, resulted in 25%, 35% and 50% reduction in tumor incidence respectively (Table II). The overall rate of tumor incidence and number of tumors/mouse were greater in animals which were treated with CI than CII. Thus, site-specific delivery of curcumin through FDDS produced statistically significant (P<0.05) reduction in number of tumors. The insignificant reduction in number of tumors by CI may be attributed to low solubility of curcumin in acidic pH of stomach (6), which might have prevented its solubilization in stomach fluids to give the

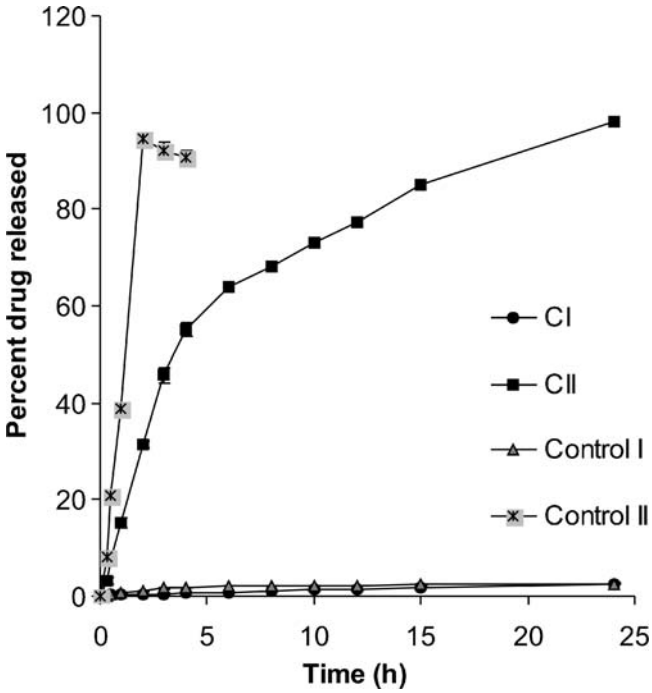

Fig. 1. Comparison of *in vitro* dissolution profiles of different floating tablet formulations with control formulations of curcumin

required therapeutic concentration in body. The combined effect of complexation of curcumin with β-CD and formulation of resulting complex into FDDS might have improved solubility of curcumin along with site-specific delivery to gastric tumor giving the required therapeutic effect in mice.

Results of *in vivo* antitumor studies were in agreement with *in vitro* release studies of curcumin from floating tablets. Due to the low solubility of curcumin at acidic pH (6), the Formulation CI offered no significant advantage over Control I. The amount of curcumin released from Control I (2.62% in 24 h) and CI (2.44% in 24 h) was very low. However, 98% of curcumin was released in 24 h from CII due to the increase in the water solubility of curcumin by β-CD.

SUMMARY AND CONCLUSION

In the present work floating tablets of curcumin β-CD complex were formulated to provide sustained release of drug with an aim to provide an effective therapy with enhanced solubility and bioavailability; targeted action and better absorption to treat stomach cancer. The Formulation CII exhibited maximum sustained release of curcumin with excellent floating and swelling properties. Also, *in vivo* antitumor studies confirmed that the overall rate of tumor incidence and number of tumors/mouse is less in animal group treated with CII compared to animals treated with CI and Control II in B (a)P induced tumor model of mice. These observations point to the potential of gastro-retentive form of curcumin β-CD complex as an effective delivery system to treat stomach cancers in animals and possibly in humans as well.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial assistance from the University Grants Commission, India, the gift HPMC by Colocron Asia Pvt. Ltd. Mumbai, Carbopol by B.F. Goodrich and ZOREL software from Dr. B.S. Bhoop, University Institute of Pharmaceutical Sciences, Panjab University (Chandigarh), India.

REFERENCES

1. L. H. Reddy, and R. S. Murthy. Floating dosage systems in drug delivery. *Crit Rev Ther Drug Carrier Syst.* **19**:553–585 (2002).
2. J. K. Lin, and S. Y. Lin-Shiau. Mechanism of cancer chemoprevention by curcumin. *Proc Natl Sci CouncROC (B).* **25**:59–66 (2001).
3. R. K. Banerjee, I. Chattopadhyay, K. Biswas, and U. Bandyopadhyay. Turmeric and curcumin: biological actions and medicinal applications. *Current Sci.* **87**:44–53 (2004).
4. B. B. Aggarwal, C. Sundaram, N. Malani, and H. Ichikawa. Curcumin: the Indian solid gold. *Adv. Exp. Med. Biol.* **595**:1–75 (2007).
5. T. Loftsson, H. H. Sigurdsson, M. Masson, and N. Schipper. Preparation of solid drug/cyclodextrin complexes of acidic and basic drugs. *Pharmazie.* **59**:25–29 (2004).
6. H. H. Tonnesen, M. Masson, and T. Loftsson. Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. *Int J Pharm.* **244**:127–135 (2002).
7. A. Paradkar, A. A. Ambike, B. K. Jadhav, and K. R. Mahadik. Characterization of curcumin–PVP solid dispersion obtained by spray drying. *Int J Pharm.* **271**:281–286 (2004).
8. P. Lu, Q. Tong, F. Jiang, L. Zheng, F. Chen, F. Zeng, J. Dong, and Y. Du. Preparation of curcumin prodrugs and their *in vitro* antitumor activities. *J Huazhong Univ Sci Technolog Med Sci.* **25**:732–734 (2005).
9. B. Tang, L. Ma, H. Wang, and W. Zhang. Study on the supramolecular interaction of curcumin and b-cyclodextrin by spectrophotometry and its analytical application. *J Agric Food Chem.* **50**:1355–1361 (2002).
10. Shishu, N. Gupta, and N. Aggarwal. A gastro-retentive floating delivery system for 5-fluorouracil. *Asian J Pharm Sci.* **2**:143–149 (2007).
11. A. O. Nur, and J. S. Zhang. Captopril floating and/or bioadhesive tablets: design and release kinetics. *Drug Dev Ind Pharm.* **26**:965–969 (2000).
12. C. R. Ireson, D. J. L. Jones, S. Orr, M. W. H. Coughtrie, D. J. Boocock, M. L. Williams, P. B. Farmer, W. P. Steward, and A. J. Gescher. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol Biomark Prev.* **11**:105–111 (2002).
13. B. Singh, and S. Singh. A comprehensive computer program for the study of drug release kinetics from compressed matrices. *Indian J Pharm Sci.* **60**:358–362 (1998).
14. N. A. Peppas, and J. J. Sahlin. A simple equation for the description of solute release, III: coupling of diffusion and relaxation. *Int J Pharm.* **57**:69–172 (1989).
15. S. Baumgartner, J. Kristl, F. Vrecer, F. Vodopivec, and B. Zorko. Optimization of floating matrix tablets and evaluation of their gastric residence time. *Int J Pharm.* **195**:125–135 (2000).
16. G. Deep, M. Dhiman, A. R. Rao, and R. K. Kale. Chemopreventive potential of triphala (a composite Indian drug) on benzo(a)pyrene induced forestomach tumorigenesis in murine tumor model system. *J Exp Clin Cancer Res.* **24**:555–563 (2005).
17. S. C. Gangar, R. Sandhir, D. V. Rai, and A. Koul. Modulatory effects of *Azadirachta indica* on benzo(a)pyrene-induced forestomach tumorigenesis in mice. *World J Gastroenterol.* **12**:2749–2755 (2006).
18. A. Triano, J. B. Simpson, M. Krathy, W. R. Lang, and A. J. Triolo. Protective effects of trifluralin on benzo(a) pyrene induced tumors in A/J mice. *Cancer Res.* **45**:601–607 (1985).
19. M. B. Shimkin. Induced pulmonary tumors in mice. II. Reaction of lung of strain A mice to carcinogenic hydrocarbons. *Arch Pathol.* **29**:239–255 (1940).