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In-vitro and in-vivo evaluation of a matrix-controlled bromocriptine mesilate-releasing vaginal ring

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Abstract

The aim of this study was to evaluate the in-vitro release rates and in-vivo effectiveness of a controlled release, intravaginal dosage form of bromocriptine mesilate. The dimeticone (poly(dimethylsiloxane)) elastomer vaginal ring contained 3 mg bromocriptine mesilate and low molecular weight gelatin in a ratio of 1 : 3, and 10 % propylene glycol. The daily release rate of the drug was designed to be 10 % of the total administered dose and was confirmed with release experiments under sink conditions. The effect of the vaginal ring preparation on plasma prolactin level in the rabbit was investigated over a10-day period. The results showed that the vaginal ring of bromocriptine mesilate significantly decreased the plasma prolactin level of the test group ($P < 0.001$–0.05) compared with the control and placebo groups. It was concluded that bromocriptine mesilate was effectively absorbed from rabbit vagina and that this controlled release intravaginal ring preparation of bromocriptine mesilate was effective in decreasing the plasma prolactin level in rabbits for ten days.

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Introduction

Vaginal drug delivery systems are traditionally used to deliver contraceptives and drugs for treatment of vaginal infections (Brannon-Peppas 1993; Robinson & Bologna 1994). The vaginal mucosa is a good absorption site for systemic action due to its surface area and rich vascularity. Vaginal drug delivery is also an alternative delivery route for drugs with poor oral bioavailability due to extensive hepatic metabolism.

The semi-synthetic ergot alkaloid bromocriptine mesilate is a dopamine agonist and has been used in the treatment of hyperprolactinaemic disorders, acromegaly and Parkinson's disease (Parkes 1979; Vance et al 1984). It is extensively metabolized with a first-pass effect that yields an oral bioavailability of only 4.2 %. The oral administration of bromocriptine mesilate also causes some side effects in approximately 50–70 % of patients, and at least 10 % of patients may discontinue therapy (Cedarbaum 1987). These side effects are most commonly nausea, vomiting, headache and dizziness. The long-acting injectable form of bromocriptine mesilate has been developed to improve the efficacy and tolerability of bromocriptine mesilate treatment and to eliminate compliance problems (Defoort et al 1987). Although the new preparation avoids the gastrointestinal side effects, it is expensive and cannot be self-administered.

It has been reported recently that bromocriptine mesilate is absorbed well from the vagina, and that vaginal administration of bromocriptine tablets effectively

reduced serum prolactin levels in normal ovulatory women and patients with hyperprolactinaemia (Vermesh et al 1988; Kletzky & Vermesh 1989; Katz et al 1989; Ginsburg et al 1991). The intravaginal route has lower gastrointestinal side effects compared with the oral route and is a safe alternative in those patients with intolerance to oral bromocriptine (Dash et al 1994; Carranza-Lira et al 1999). A controlled release intravaginal dosage form of bromocriptine mesilate may therefore be a useful alternative to oral therapy to improve patient compliance.

Medical grade dimeticone (poly(dimethylsiloxane) polymers can be formulated with a variety of drugs and are used as matrix materials for transdermal, vaginal and implanted controlled drug delivery systems (Hsieh et al 1985; Ritschel & Nayak 1987; Hoth & Merkle 1991). Various types of ring-shaped vaginal controlled release devices with dimeticone have been designed. These devices have the advantage that the ring can be conveniently inserted and removed by the woman herself (Sam 1992). The vaginal rings have an outer diameter of 55–80 mm and a thickness of 4.9–9.5 mm, and are typically fabricated from silicone rubber (Silastic 382) (Mishell & Lumkin 1970; Olsson & Odlind 1990). Progestogens (Mishell et al 1970; Victor & Johansson 1976), combinations of progestogen and estrogen (Elkik et al 1986; Leede et al 1986)), and prostaglandins (Lauersen & Wilson 1976) have been used as drugs in various vaginal ring formulations.

The characteristics of silicone polymer–bromocriptine mesilate matrices and in-vitro release of bromocriptine mesilate from such dimeticone matrices were investigated by Acartürk & Altug (2000). It was found that cylinder-shaped drug–polymer matrices containing silicone elastomer (MDX-4-4210), 10% propylene glycol and a kneaded mixture of drug and low molecular weight gelatin in a 1:3 ratio showed the best drug release profile.

The objectives of this study were to prepare a vaginal ring device of bromocriptine mesilate with dimeticone and to evaluate the effect of this formulation on plasma prolactin level in rabbits.

Materials and Methods

Materials

Bromocriptine mesilate was a gift from Novartis Co., Turkey. Dimeticone elastomer (Silastic MDX-4-4210) was kindly provided by Dow Corning, USA. The low-molecular-weight-gelatin (LMWG) (MW $\cong$ 5000) was donated by Nitta Gelatin Co. Ltd, Osaka, Japan. The other materials were of analytical grade obtained commercially.

Plotting of the target profile

A target profile (amount released vs time) was plotted (Robinson & Eriksen 1970) on the basis of the following pharmacokinetic parameters of bromocriptine mesilate: distribution volume Vd = 4860 L, effective plasma concentration $C_p = 365$ pg mL^{-1}, vaginal absorption constant $k_a = 0.1931$ h^{-1}, disposition constant $k_d = 0.0262$ h^{-1}, area under curve $AUC_{0-\infty} = 19600$ pg h mL^{-1}, and a dosing interval of 10 days. The k_a, k_d and $AUC_{0-\infty}$ values were calculated from the plasma-time data, as reported by Vermesh et al (1988). The release rate of the drug was designed to be 10% of initial drug content per day based on the target profile.

Preparation of the vaginal ring

The solubility of bromocriptine mesilate in pH 5 phosphate–citrate buffer (mixture of 0.102 M Na_2HPO_4 2 H_2O and 0.049 M $C_6H_8O_7$ 1 H_2O) and silicone elastomer was found to be 0.0251 ± 0.0018 mg mL^{-1} (mean $\pm$ 95% confidence interval) and 0.742 ± 0.390 mg mL^{-1}, respectively. Octanol/buffer and silicone polymer/buffer partition coefficients of bromocriptine mesilate were measured as 7.58 ± 4.39 and 29.5 ± 15.6, respectively. Determination of these parameters was described by Acartürk & Altug (2000).

The oral dose of bromocriptine mesilate was calculated to be 0.2–0.3 mg/day for rabbits. Therefore, a total of 3 mg bromocriptine mesilate was used for the vaginal ring tested over 10 days in in-vivo studies. When administered vaginally a much lower dose of bromocriptine mesilate would be required compared with the oral dose, because when administered orally bromocriptine mesilate is extensively metabolized in the liver. However, in reported vaginal studies the daily oral dose of bromocriptine mesilate has been used in women (Vermesh et al 1988; Katz et al 1989; Carranza-Lira et al 1999). Therefore, in this study the daily dose of bromocriptine mesilate to be administered vaginally was identical to the oral dose for rabbits. The composition of the vaginal ring was dimeticone elastomer, 12 mg bromocriptine mesilate–LMWG as a kneaded 1:3 (by weight) mixture (this mixture contained 3 mg bromocriptine mesilate) and 10% propylene glycol. Firstly, 10 parts silicone elastomer was blended with one

part of curing agent. This mixture was then mixed with the propylene glycol and drug–LMWG mixture. This final mixture was placed into a ring-shaped Teflon mould 5 mm thick with a total diameter of 15 mm and allowed to cure at room temperature. The total calculated surface area of the vaginal ring was 494 mm^2. Placebo rings were prepared for comparison of the results.

The drug–LMWG mixtures were prepared by the method of Acartürk et al (1992). Briefly, the required amounts of drug and polymer were weighed and placed in a mortar and then the mixtures were kneaded for 1 h with 1.5-times their weight of water. The mixtures were dried under vacuum at room temperature for 48 h and then screened through a 25-mesh sieve.

In-vitro release studies

Release experiments were performed under sink conditions in an Erlenmeyer flask containing 100 mL phosphate–citrate buffer at pH 5. The flasks were shaken in a water bath controlled at 37°C over a 10-day period. The samples were withdrawn at the same time of day and assayed spectrophotometrically at 310 nm. The buffer was replaced daily.

In-vivo studies

The experimental protocol was approved by the Ankara University Veterinary Faculty Ethics Committee in accordance with internationally accepted principles.

Female New Zealand White rabbits (3–3.5 kg) were separated into three study groups: control, test and placebo. Food and water were freely available throughout the study. The vaginal ring was introduced under ketamine/xylazine anaesthesia into the vaginas of rabbits in the test and placebo groups. A silk suture was used to close the vagina after the insertion of the vaginal ring. Blood samples (1–1.5 mL) were taken from the ear vein at the same time of day for 10 days. Plasma was obtained by centrifuging the blood samples at 1000 *g* for 3 min.

Plasma prolactin levels of the rabbits were measured by a radioimmunoassay method (RIA) using a commercial kit (Radim, PRL limaCT, Rome, Italy). The results were compared with the control and placebo groups using one-way analysis of variance (GraphPad Software, Instat, version 2.04a.). Student–Newman–Keuls multiple comparison test was performed for post-hoc comparison of means.

Results

In-vitro release studies

The in-vitro release profile of bromocriptine from vaginal ring is shown in Figure 1. Approximately 90% of the bromocriptine incorporated into the ring was released over the 10-day test period. The release profile of the drug from vaginal ring was almost parallel to the target profile for six days. After six days, the release rate decreased.

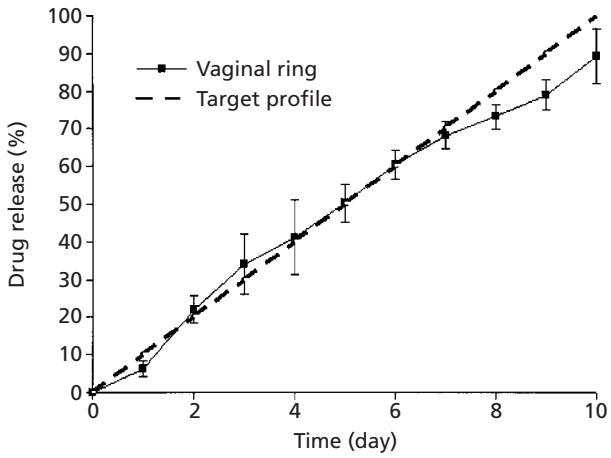

Figure 1 Cumulative release of bromocriptine from vaginal ring. Each point represents the mean ± confidence interval (n = 3).

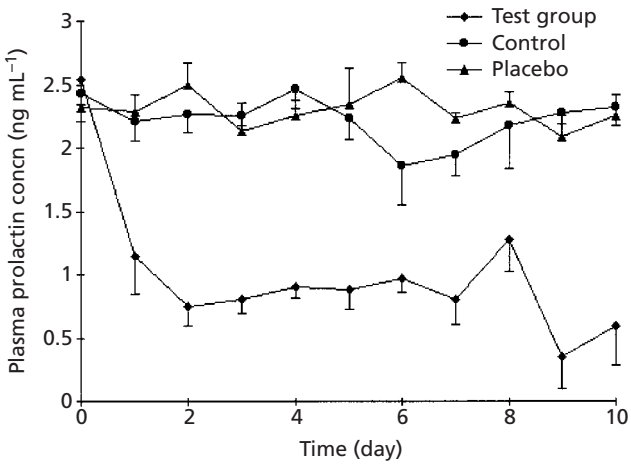

Figure 2 Plasma prolactin levels for test (n = 5), control (n = 3) and placebo (n = 3) groups in rabbits. Each point represents the mean ± s.e.m.

In-vivo studies

Figure 2 depicts the plasma prolactin levels of rabbits after the application of vaginal bromocriptine mesilate ring, placebo ring and for the control group. The plasma prolactin level in the test group fell by 24 h and was maintained for 10 days as intended. The mean ($\pm$95% confidence interval) prolactin levels of test, control and placebo groups at 24 h were 1.14 ± 0.82, 2.21 ± 0.68 and 2.28 ± 0.57 ng mL^{-1}, respectively. Statistical comparison of the results for each day showed that a significant difference was seen between the prolactin levels of the test group compared with the placebo and control group during each of the 10 days ($P < 0.001$–0.05).

Discussion

The hydrophobic nature of silicone matrices ensures a slow long-term release of the drug. However, for vaginal application of dimeticone polymers a substantial fraction of the dose should be released within a few days after the administration of the system. Various liquid or solid excipients have been added to increase drug release from silicone matrices (Di Colo et al 1986; Ritschel & Nayak 1987; Rehula 1993). Although the solubility of bromocriptine mesilate in the polymer matrices was higher than that of pH 5 buffer, the octanol/buffer and polymer/buffer partition coefficients of bromocriptine mesilate were high also. Thus, some additives were necessary to increase the drug release from silicone matrices. Water-soluble materials can lead to the formulation of pores and cracks in the polymer matrix to enhance the release of drugs. Propylene glycol and low molecular weight gelatin were found to be suitable excipients for bromocriptine mesilate release (Acartürk & Altug 2000).

It has been reported that the release of coumarin was increased by propylene glycol (Ritschel & Nayak 1987). Rehula (1993) reported that the release of papaverine hydrochloride was increased by polyethylene glycol (Rehula 1993). In addition, granulation of papaverine hydrochloride before incorporation of silicone matrices enhanced the drug release. In our preparation, particle size was increased by the kneading process, demonstrating its utility in enhancing the porosity of the silicone matrices. Drug molecules can easily diffuse from the silicone matrices into the outer environment of the matrix as the pores develop with hydration.

Propylene glycol and LMWG may also improve the hydrophilicity of the drug by surrounding the drug particles as a film layer. Although the target drug release profile was plotted on the basis of zero-order release, we previously reported that the release kinetics of bromocriptine from silicone elastomer fitted $Q\sqrt{t}$ kinetics i.e. matrix kinetics (Acartürk & Altug 2000). The kinetic analysis of release data, as evaluated by a computer program DISSOL (Agabeyoglu 1984) are shown in Table 1. The determination coefficient (r^2) and the sum of the weighted squared deviations (SWSD) suggested that the release kinetics of the drug from the vaginal ring were described by $Q\sqrt{t}$ kinetics also.

The vaginal route of bromocriptine administration circumvents the gastrointestinal tract and provides the acidic environment essential for bromocriptine absorption. In the acidic environment of the vagina, bromocriptine mesilate is soluble and readily absorbed, therefore when given vaginally in low doses it induces a rapid and sustained reduction in serum prolactin. Vermesh et al (1988) reported that, compared with oral dosing, bromocriptine was absorbed more slowly but almost completely from human vagina, since less than 1% of the initial dose of the drug could be recovered from the vagina after 24 h. Daily vaginal administration of 2.5 mg was sufficient to maintain the levels of the serum pro-

Table 1 Kinetic assessment of release data.

Kinetic parameter								
Zero order			First order			$Q\sqrt{t}$		
k_o	r^2	SWSD	k_1	r^2	SWSD	k	r^2	SWSD
0.0108	0.978	0.0486	9.21×10^{-3}	0.930	0.401	1.75×10^{-5}	0.996	0.174

Summary of output obtained from the program DISSOL: k_0 is the zero-order release rate constant (mg h^{-1}); k_1 is the first-order release rate constant (h^{-1}); k is the rate constant obtained from the slope of the linear regression of cumulative amount released per unit area vs square root of time (mg cm^{-2} h$^{-1/2}$); r^2, the coefficient of determination; SWSD, the sum of weighted squared deviations.

lactin within the normal range (Kletzky & Vermesh 1989). Dash et al (1994) obtained 50% reduction of serum prolactin in patients vaginally administered 2.5 mg bromocriptine mesilate tablets. Vaginal administration of bromocriptine resulted in significantly higher and longer-lasting levels of circulating bromocriptine when compared with an equal oral dose. Ginsburg et al (1991) have successfully treated 31 hyperprolactinaemic women with daily vaginal bromocriptine for over two years. Carranza-Lira et al (1999) documented the colposcopic changes after six months of vaginal administration of bromocriptine mesilate, and they concluded that the vaginal route was a safe alternative to oral bromocriptine mesilate.

Plasma prolactin level alone (Kletzky & Vermesh 1989; Dash et al 1994; Carranza-Lira et al 1999) or together with plasma bromocriptine mesilate level (Vermesh et al 1988; Katz et al 1989, 1991) have been measured to evaluate the effect of vaginal bromocriptine mesilate administration in-vivo. In our studies, we obtained a significant decrease in serum prolactin level of rabbit after the vaginal insertion of vaginal ring containing bromocriptine mesilate. This decrease remained unchanged over 10 days. High variation was seen during the experiments. Katz et al (1991) noted also that the circulating levels after vaginal administration of bromocriptine mesilate were more variable than levels after oral administration.

In our experiments, plasma prolactin level in the test group fell by 24 h and was maintained for 10 days. Vermesh et al (1988) reported that the mean plasma prolactin level decreased by 7 h after vaginal bromocriptine mesilate administration. The maximum prolactin decrease occurred 11 h after administration and the plasma prolactin levels changed little during the remaining 13 h. Katz et al (1991) showed that the plasma prolactin level decreased rapidly and reached the minimum by 4 h and remained unchanged during the next 8 h. It has been reported that the serum prolactin level decreased from a mean of 96 ± 28 ng mL^{-1} to 22 ± 5 ng mL^{-1}, and remained under the normal range during 35 days of daily vaginal administration of a 2.5-mg bromocriptine mesilate tablet in hyperprolactinaemic women (Kletzky & Vermesh 1989). Those studies showed that bromocriptine mesilate was absorbed slowly but completely from the human vagina.

The results showed that bromocriptine was effectively released from the vaginal ring preparation and absorbed by rabbit vagina. The controlled release vaginal ring preparation was effective in reducing plasma prolactin level in a rabbit model. The effectiveness of this formulation and dosing approach in women awaits subsequent clinical investigation, but it may be a useful alternative avoiding the adverse side effects of oral administration for long-term hyperprolactinaemia treatment and to improve patient compliance.

References

Acartürk, F., Altug, N. (2000) Preparation and evaluation of bromocriptine mesylate-polydimethylsiloxane matrices. *Pharmazie* **55**: 668–671

Acartürk, F., Kislal, Ö., Çelebi, N. (1992) The effect of some natural polymers on the solubility and dissolution characteristics of nifedipine. *Int. J. Pharm.* **85**: 1–6

Agabeyoglu, I. T. (1984) Un programme dans le langue basique de microcomputer pour la determination des donnes de dissolution. XVIII'eme Semaine Medicale Balkanique, Resumes II, 327, 30 Aout-4 Septembre, Istanbul

Brannon-Peppas, L. (1993) Novel vaginal drug release applications. *Adv. Drug. Deliv. Rev.* **11**: 169–177

Carranza-Lira, S., Gonzalez-Sanchez, J. L., Martinez-Chequer, J. C. (1999) Vaginal bromocriptine administration in patients with hyperprolactinemia. *Int. J. Gynecol. Obstet.* **65**: 77–78

Cedarbaum, J. M. (1987) Clinical pharmacokinetics of antiparkinsonian drugs. *Clin. Pharmacokinet.* **13**: 141–178

Dash, R. J., Ajmani, A. K., Sialy, R. (1994) Prolactin [PRL] response to oral or vaginal bromoergocriptine in hyperprolactinemic women. *Horm. Metab. Res.* **26**: 164

Defoort, P., Thiery, M., Baele, G., Clement, D., Dhont, M. (1987) Bromocriptine in an injectable retard form puerperal lactation suppression: comparison with estandron Prolangatum. *Obstet. Gynecol.* **70**: 866–869

Di Colo, G., Carelli, V., Nannipieri, M. F., Serafini, M. F., Vitale, D. (1986) Effect of water-soluble additives on drug release from silicone rubber matrices. II. Sustained release of prednisolone from non-swelling devices. *Int. J. Pharm.* **30**: 1–7

Elkik, F., Basdevant, A., Jackanicz, T. M., Guy-grand, B., Mercier-Bodard, C., Conard, J., Bardin, C. W., Corvol, P. (1986) Contraception in hypertensive women using a vaginal ring delivering estradiol and levonorgestrel. *J. Clin. Endocrinol. Metab.* **63**: 29–35

Ginsburg, J., Hardiman, P., Thomas, M. (1991) Vaginal bromocriptine. *The Lancet* **338**: 1205–1206

Hoth, M., Merkle, H. P. (1991) Formulation of silicone matrix systems for long term constant release of peptides. *Drug Dev. Ind. Pharm.* **17**: 985–999

Hsieh, D. S. T., Mann, K., Chien, Y. W. (1985) Enhanced release of drugs from silicone elastomers (I). Release kinetics of pineal and steroidal hormones. *Drug Dev. Ind. Pharm.* **11**: 1391–1410

Katz, E., Schran, H. F., Adashi, E. Y. (1989) Successful treatment of a prolactin-producing pituitary macro adenoma with intravaginal bromocriptine mesylate: a novel approach to intolerance of oral therapy. *Obstet. Gynecol.* **73**: 517–520

Katz, E., Schran, H. F., Weiss, B. E., Adashi, E. Y., Hassell, A. (1991) Increased circulating levels of bromocriptine after vaginal compared with oral administration. *Fertil. Steril.* **55**: 882–884

Kletzky, O. A., Vermesh, M. (1989) Effectiveness of vaginal bromocriptine in treating women with hyperprolactinemia. *Fertil. Steril.* **5**: 269–272

Lauersen, N. H., Wilson, K. H. (1976) The abortifacient effects of a

newly developed vaginal silastic device impregnated with a 0.5% concentration of 15(S)-15-methyl-prostaglandin $F_2\alpha$ methyl ester in the first trimester. *Contraception* **13**: 698–705

Leede, L. G. J., Govers, C. P. M., Nijs, H. (1986) A multi-compartment vaginal ring system for independently adjustable release of contraceptive steroids. *Contraception* **34**: 589–602

Mishell, D. R., Lumkin, M. E. (1970) Contraceptive effect of varying dosages of progestogen in silastic vaginal rings. *Fertil. Steril.* **21**: 99–103

Mishell, D. R., Talas, M., Parlow, A. F., Moyer, D. L. (1970) Contraception by means of a silastic vaginal ring impregnated with medroxyprogesterone acetate. *Gynecology* **107**: 100–107

Olsson, S. E., Odlind, V. (1990) Contraception with a vaginal ring releasing 3-keto desogestrel and ethinylestradiol. *Contraception* **42**: 563–572

Parkes, D. (1979) Drug therapy, bromocriptine. *N. Engl. J. Med.* **301**: 873–878

Rehula, M. (1993) Effect of polyethylene glycol on the pharmaceutical availability of papaverine hydrochloride from silicone matrices. *Pharmazie* **48**: 126–128

Ritschel, W. A., Nayak, P. M. (1987) Evaluation in vitro and in vivo dimeticone transdermal therapeutic systems. *Arzneimittelforschung* **37**: 302–306

Robinson, J. R., Eriksen, P. S. (1970) Theoretical approach to sustained-release multiple-dose therapy: noncumulative attainment of desired blood level. *J. Pharm. Sci.* **59**: 1796–1800

Robinson, J. R., Bologna, W. J. (1994) Vaginal and reproductive system treatments using a bioadhesive polymer. *J. Control. Release* **28**: 87–94

Sam, A. P. (1992) Controlled release contraceptive devices: a status report. *J. Control. Release* **22**: 35–46

Vance, M. L., Evans, W. S., Thorner, M. O. (1984) Bromocriptine. *Ann. Intern. Med.* **100**: 78–91

Vermesh, M., Fossum, G. T., Kletzky, O. A. (1988) Vaginal bromocriptine: pharmacology and effect on serum prolactin in normal women. *Obstet. Gynecol.* **72**: 693–698

Victor, A., Johansson, E. D. B. (1976) Plasma levels of d-norgestrel and ovarian function in women using intravaginal rings impregnated with dl-norgestrel for several cycles. *Contraception* **14**: 215–226