Transdermal Administration of Bromocriptine

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Bromocriptine (BRC) has been mainly used for the inhibition of lactation, treatment of menstrual disorders, Parkinson disease, breast tumours, infertility and brain tumours as a dopamine agonist in clinics. But current BRC formulations have some side effects and bioavailability problems because of hepatic first pass effect. Transdermal application could be an alternative route to overcome all these problem and penetration properties of BRC has not been studied yet. Therefore, it was aimed to investigate the effectiveness of transdermal formulation of BRC which is applicable to the skin. For this purpose, a number of BRC gel formulations (Carbopol-934 (C-934), chitosan (CH) and Gantrez-SP215 (G-SP215) were developed and the effectiveness and bioavailability of the formulations were compared in rabbits. Commercial BRC tablets (Parlodel®) were also given to rabbits orally and plasma levels were compared. The effects of two different penetration enhancers, sodium taurocholate (ST) and ethoxydiglycol-Transcutol® (TR) on the BRC penetration were also investigated. The skin samples from the dorsal part of the rabbit were removed after CH gel application and investigated under electron microscope to understand the effects of the gel on the penetration and the possible penetration mechanisms through skin were also discussed. In conclusion, CH gel formulation was found to be the best formulation and comparable blood BRC concentrations were obtained when applied to the rabbit skin. Higher blood levels were obtained with the use of CH. The main penetration process was found to be through transcellular route but some other mechanisms were also found to be incorporated, after microscopic investigation. CH gel was found to be a useful carrier for BRC administration through dermal route and the penetration enhancing effect and the mechanism of CH gel were first established in this study. It was concluded that transdermal delivery of BRC may be a very promising alternative route to the oral route for the treatment.

Key words bromocriptine; chitosan; sodium taurocholate; transcutol; transdermal application; penetration enhancer

Bromocriptine (BRC) is a semi-synthetic ergot alkaloid and it has been used for the treatment of diseases caused by hyperprolactinemia and for some neurological disorders as a dopamine agonist in clinics.¹⁾ BRC is mainly used for the inhibition of lactation,²⁾ treatment of menstrual disorders,³⁾ Parkinson disease,⁴⁾ breast tumours,⁵⁾ infertility⁶⁾ and brain tumours.⁷⁾

Bromocriptine is well absorbed through the GI tract but it has low bioavailability because of the hepatic first pass effect, but many patients have complained with GI side effects, headache and dizziness.⁴⁾ The long acting injectable form of BRC has been developed²⁾ and it avoids the gastrointestinal side effects but it is expensive and cannot be self-administered.

An alternative delivery route such as vaginal application of BRC has been reported.^{8,9)} Bromocriptine is well absorbed from vagina. Acartürk and Altuğ have studied the vaginal ring formulation of BRC with polydimethylsiloxane *in vitro* and *in vivo* in rabbits.^{10,11)} It was shown that the controlled release intravaginal ring containing BRC was found to be effective to decrease the plasma prolactin level in rabbits.

BRC has also used for the treatment of Parkinson disease and the transdermal administration may be more useful than vaginal administration, being not gender limited. Transdermal delivery of drugs is also very convenient from the application point of view and it offers several advantages over the other routes of drug administration and termination of the drug input is possible in problematic cases.^{12,13)}

Transdermal systems represent one of the most successful non-oral systemic drug delivery system. However, because of limitations of the technology, the physical, chemical nature of the compounds and also lack of the knowledge about pen-

etration mechanisms, transdermal drug delivery is not suited to all drugs and it needs to be justified for the therapies.¹⁴⁾ The penetration mechanisms, histopathological hazards of the enhancers and vehicles need to be clarified.

The aim of this study was to investigate an alternative delivery route for BRC. Several topical BRC gel formulations were developed using Carbopol (C-934), Daichitosan (CH) and Gantrez-SP215 (G-SP215) and applied to rabbits. Commercially available BRC tablets (Parlodel®) were also given to rabbits orally and plasma levels were compared. Two different penetration enhancers, sodium taurocholate (ST) and ethoxydiglycol-Transcutol® (TR) were also used to improve BRC penetration.

MATERIALS AND METHODS

Materials Bromocriptine mesilate was a gift from Novartis Co. (İstanbul, Turkey). Sodium taurocholate (ST) was purchased from Sigma. Ethoxydiglycol-Transcutol® (TR) was donated by Gattefosse (France), Carbopol (C-934) was from BF Goodrich Company (U.S.A.). Daichitosan (CH) was obtained from Dainichiseika Color & Chemicals Mfg., Co. Ltd. (Japan). Gantrez-SP215 (G-SP215) was obtained from ISP, New Jersey (U.S.A.). Parlodel® tablets (Novartis Co., İstanbul, Turkey) were purchased from the market.

Preparation of Gel Formulations For formation of the gel, CH or C-934 was dispersed in water then acetic acid or 0.01 N NaOH was added, respectively. BRC was dissolved in absolute ethanol and then mixed with the gel. TR or ST was added for the preparation of CH-ST or CH-TR gel. G-SP215 solution in ethanol was neutralised by triethanolamine and mixed with BRC solution in ethanol. Table 1 shows the con-

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Table 1. Contents of the Gels

Gels	CH (%)	C-934 (%)	G-SP215 (%)	Triethanolamine (%)	ST (%)	TR (%)	Ethanol (%)	BRC (%)
C-934	—	1	—	—	—	—	50	2.5
G-SP215	—	—	22	6.5	—	—	69	2.5
CH	1	—	—	—	—	—	50	2.5
CH-ST	1	—	—	—	1	—	50	2.5
CH-TR	1	—	—	—	—	37.5	12.5	2.5

tent of the gels. The pH of the gel was found to be 4.7, 6.7 and 6.9 for CH, C-934 and G-SP215 gels, respectively.

Determination of Apparent Octanol/Water Partition Coefficient of BRC The shake-flask method was used to determine the apparent octanol/water partition coefficient of BRC known as log D. Octanol (50 ml) was mixed with 5 ml of water for overnight period using magnetic stirrer at 25 °C before the experiment to ensure that octanol was saturated with water. Twenty mg of BRC was dissolved in a ml of water saturated octanol then, 2 ml of pH adjusted water (using phosphate or citrate buffer) and stirred together for another overnight period at the same temperature. Water phase was taken and analysed by HPLC. The log D values were then calculated at various pHs. These values were plotted against pH.

Rabbit Experiments New Zealand white rabbits were used weighing 3 to 3.5 kg and water available ad lib throughout the study. The Ankara University Veterinary Faculty Ethical Committee in accordance with internationally accepted principles approved the experimental protocol. Dorsal part of the hair was gently cut and removed by hair clippers without caused any skin damage and the area was marked (radius of the circular area was 5.5 cm). Rabbits were heparinized with 20 IU/kg heparin, 10 mins later, tablets (5 mg BRC) were given orally or gel formulations (1 g) were applied to the skin. Blood samples ($\approx$ 2 ml) were taken for the period of 6 h from the ear vein and their plasma were analysed by HPLC.

HPLC Analysis An HPLC method was adapted and modified from the literature.¹⁴⁾ Briefly, 1.5 to 2 ml of blood samples were taken and centrifuged ($1000\times g$ for 3 min). Internal standard, 100 μ l of ergotamine tartarate (ER) (6 μ g/ml) was added to 0.5 ml of plasma and 200 μ l of potassium carbonate (2.5 M) was added and this mixture was extracted with 3 ml of diethyl ether for a minute using vortex mixer. Organic phase (2 ml) was taken and 100 μ l of H₂SO₄ (0.05 M) was added and vortexed for a minute. Aqueous phase was taken and injected directly to the HPLC. The conditions of the HPLC were as follows:

Mobile phase: methanol : water : acetonitrile (280 : 180 : 40) containing 0.2 g. heptane sulphonic acid sodium salts

Flow rate: 1 ml/min

Column: Hypersil, ODS, RP-C18 (25 $\times$ 0.4 cm, particle size 5 μ m)

Injection volume: 50 μ l

Detection: 254 nm

Retention time: 7 min (ER) 13 min (BRC)

Microscopic (Histological) Investigation The skin samples from rabbits were taken from the CH gel applied area, six hours after from the application of BRC gels, the skin samples for the control were obtained from other part of the

Fig. 1. Blood BRC Concentrations after Application of BRC Gels to the Skin and Administration of BRC Tablets during the Period of 6 h (Mean $\pm$ S.E.M., $n=5$)

dorsal area. Samples were fixed using 2.5% glutaraldehyde and 1% osmium tetroxide and samples were prepared using routine sample preparation techniques for the electron microscopy. Thin semi sectional skin preparations were observed under Zeiss 900 EM and changes of the skin layer cells were evaluated.

Statistics Statistic analysis was performed using a computer programme called Graphpad Software version 2.04a (INSTAT). Anova test was used to determine significant variations.

RESULTS AND DISCUSSION

C934, CH and G-SP215 were used to prepare gel formulations for the topical application of BRC. Blood BRC concentrations obtained after the application of C-934 gel, CH gel, G-SP215 gel and commercially available BRC tablet were depicted in Fig. 1.

C934 was chosen because of its high stability, compatibility and low toxicity.^{15,16)} CH is a polycationic polymer and it has been used to prepare some gel formulations^{17,18)} and to fabricate transdermal devices for the both lipophilic and hydrophilic drugs.^{19,20)} A polymeric material namely Gantrez® has been used to prepare topical gel formulations for transdermal applications.²¹⁾ G-SP215 is a monoethyl ester of poly(methyl vinyl ether/maleic acid). G-SP215 monoester resins were designed for use in personal care products. They form a clear film, which exhibits tack-free adhesion, excellent substantivity and moisture resistance. The contact time of the CH and C-934 gels with the skin may be rather long

Table 2. Some Pharmacokinetic Parameters of the BRC after the Application of Gel Formulation and Oral Tablets (Mean $\pm$ S.E.M., $n=5$)

Gels	$C_{\max}$ ($\mu\text{g/ml}$)	$t_{\max}$ (h)	AUC ($\mu\text{g/ml} \cdot \text{h}$)	Relative bioavailability (%)
C-934	$0.21 \pm 0.05^{a)}$	$1.90 \pm 0.81^{a,f)}$	$0.72 \pm 0.17^{a,b,c,e,f)}$	7.50 ± 1.78
G-SP215	0.68 ± 0.12	$1.20 \pm 0.19^{b)}$	$2.05 \pm 0.26^{b,d)}$	21.36 ± 2.70
CH	$0.68 \pm 0.20^{b)}$	$0.60 \pm 0.10^{c,g)}$	$1.74 \pm 0.12^{a,d,g,h)}$	18.12 ± 1.26
CH-ST	0.63 ± 0.27	$4.00 \pm 0.45^{a,b,c,d,e)}$	$2.26 \pm 0.29^{c,g)}$	23.54 ± 3.02
CH-TR	$1.44 \pm 0.61^{a,b)}$	$1.67 \pm 0.54^{d,f,g)}$	$3.77 \pm 0.39^{f,h)}$	39.28 ± 4.06
Parlodel®	0.79 ± 0.36	$0.50 \pm 0.00^{e)}$	$1.92 \pm 0.26^{c,d)}$	—

$C_{\max}$: a, b) $p < 0.01$. $t_{\max}$: a—g) $p < 0.01$. AUC : a, c) $p < 0.05$; e, b) $p < 0.01$; d) $p > 0.01$; f) $p < 0.001$; g, h) $p > 0.05$.

due to their bioadhesive properties. On the other hand, following the G-SP 215 gel application, the solvent (ethanol) evaporates rapidly and relatively hard structure formed at the gel surface and this outer layer protects further evaporation from the inside of the gel. This alcoholic medium in the gel provides faster BRC penetration (occlusion effect) (Fig. 1).

Some pharmacokinetic parameters such as $C_{\max}$, $t_{\max}$, AUC and relative bioavailability were determined from the observed data after the application of BRC tablets and different BRC gels using their blood profiles and shown in Table 2.

Higher BRC concentrations were observed after the application of CH and G-SP215 gels comparing to C-934 gel. AUC value for CH gel and G-SP215 gel formulation were found to be about 2 and 3 folds higher than that of C-934 gel, respectively ($p < 0.05$, $p < 0.01$). C-934 is an anionic polymer whereas BRC is positively charged. There may be some electronic interactions reduce the release of BRC from the C-934 polymer. AUC values after Parlodel® tablet administration were found significantly higher than C-934 gel ($p < 0.05$). On the other hand, AUC values for Parlodel®, CH gel and G-SP215 gel were not found statistically significant from each other ($p > 0.01$) (Table 2).

The $t_{\max}$ and $C_{\max}$ values after CH gel application were also found to be similar with the Parlodel (Table 2). Therefore, CH gel was chosen for further studies because all considerations mentioned above. It was also decided to add ST and TR to CH gel formulation to improve BRC penetration. G-SP215 was not chosen because of its high alcohol content. Hard and thick gel remained on the skin following the evaporation of the ethanol, after G-SP215 gel application.

The enhancer activity of CH on the buccal,²²⁾ nasal,²³⁾ rectal²⁴⁾ and intestinal mucosa²⁵⁾ have been reported. The mechanism of action of the CH was suggested to be a combination of bioadhesion and a transient widening of the tight junctions in the membrane.²³⁾ CH effects on the integrity of the epithelial cell membranes were found to be minimal when compared to the effects of known absorption enhancers.²⁶⁾ It was also reported that CH increases membrane permeability by affecting both paracellular and intracellular pathways.²⁷⁾ Although the enhancing effect of CH on the mucosal membranes is well established, there is no literature available for the CH skin penetration enhancing effect. In the case of using CH gel paracellular transport mechanism may be added to the transport of BRC through skin and a combined mechanism/effect could be effective.

Blood BRC concentrations after the application of CH gel, penetration enhancers (TR and ST) added CH gels were depicted in Fig. 2.

ST is a surface active agent and it increases the permeabil-

Fig. 2. Blood BRC Concentrations after Application of Enhancer Containing CH Gels during the Period of 6 h (Mean $\pm$ S.E.M., $n=5$)

ity of BRC through the skin by means of increasing solubility/partition of BRC (Fig. 2). The possible effects of ST on the substance penetration through the buccal, nasal and intestinal mucosa have been investigated^{28,29)} and the penetration enhancing effect was found to be important. The main penetration enhancing mechanism was found to be the effect of ST on tight junctions.

TR is a powerful solubilizing agent used in several dosage forms and it is very attractive as a penetration enhancer due to its non-toxicity, biocompatibility with the skin, miscibility with polar and non-polar solvents and optimal solubilizing properties for a number of compounds.³⁰⁾ It has been shown that TR significantly increases the percutaneous penetration of various active substances. It was shown that the enhancer mechanism of action occurs by making it possible to distinguish the contribution due to the improvement of drug solubility in the formulation, skin and drug concentration gradient in solution, from that due to interactions with the barrier structures of skin (stratum corneum) and to eventual skin depot effect.³¹⁾ TR was found to be capable to increase BRC penetration by providing an easier pathway during its own penetration and possibly forces BRC molecule to penetrate together. It also increases the solubility of BRC (Fig. 2). AUC values for CH-ST and CH-TR were found to be significantly higher than C-934 gel ($p < 0.01$, $p < 0.001$) but they were not found significantly higher than CH gel ($p > 0.05$) (Table 2).

Longer $t_{\max}$ values were obtained with CH-ST gel than the others ($p < 0.01$). Significantly higher $C_{\max}$ values were observed with CH-TR gel than both C-934 and CH gels ($p < 0.01$) (Table 2). The relative bioavailability values of G-SP215, CH, CH-ST and CH-TR gel were also found to be higher than C-934 gel. The highest value was obtained with CH-TR gel (Table 2).

Fig. 3. The Epidermis–Dermis Junction of the Rabbit Skin under Electron Microscope

Control group, $\times 20000$; De, desmosome; $\rightarrow\leftarrow$ intercellular space; To, tonofibril.

Fig. 4. The Epidermis–Dermis Junction of the Rabbit Skin, 6 h after CH Gel Application, under Electron Microscope

CH gel applied group, $\times 20000$; Co, collagen tissue; He, hemidesmosome; Va, vacuole).

The effects of ST and TR on the mucosal barriers are well characterised and studied but, the effect of CH has not been studied on the skin yet, therefore it was decided to investigate the CH enhancing effect on the skin using electron microscope. After the microscopic investigation, some changes on the skin layer cell were observed after CH gel application. Fig. 3 shows the epidermis-dermis junction of normal rabbit skin.

Figure 4 shows the electron microscope images of the skin cells six hours after CH gel application.

Under electron microscope, epidermal cells and intercellular space were observed in normal form in Fig. 3 (control group). Tonofibril bundles at the cytoplasmic region and desmosomes at the intercellular space were also observed clearly. Hemidesmosomes were observed at the edge of the epidermis-dermis junction. Intercellular space, connective tissue cells and normal basal lamina were observed. Collagen fibers and their explicit semi-sectional appearance were observed at the dermis region. In the CH gel applied group (Fig. 4), the tonofibril bundles were observed thinner and dense comparing with the control group. Desmosomes were

Fig. 5. The log D Values of BRC against to pH (Mean $\pm$ S.E.M., $n=5$)

not observed as many as seen in the control group at the intercellular region. It was also noticed that the dense plaque was present in some of the desmosomes and disappearance of tonofibrils were observed. A few tonofibrils were observed alone not as bundles. This observation was more explicit for hemidesmosomes. Some vesicles and vacuoles were also found in epidermis, the more vesicles were observed at the basal cells. There was no desmosomes and hemidesmosomes observed. The homogenisation of the dermis was also noticed. Similarly, it was reported in the literature that transmission electron micrographs of cells exposed to 0.1% CH for 30 min resulted in the appearance of large swollen intracellular vacuoles and endoplasmic reticulum cisternae.³²⁾

The penetration mechanism of BRC through skin was found to be complicated when CH gel was used. It was understood that there are several mechanisms may involve for instance the effect of alcohol, which is present in the gel, the effect of pH and possible enhancing effect of CH should be considered. Ethanol was used to dissolve BRC in the gel, because BRC has very poor water solubility (1 g/l to 0.0024 g/l for the pH range of 2 to 6).³³⁾ It acts as an enhancer by increasing the stratum corneum lipids fluidity and it increases solubility of the solute in the lipids.³⁴⁾ Ethanol facilitates the penetration and higher penetration values were observed when higher amount of ethanol was used in the formulation.

The pK_a of the BRC was reported³⁵⁾ with the value of 4.9 ± 0.05 in the literature. BRC may possibly be in non-ionised form with higher extend at the pH range of 4 to 5 (in CH gel) than other pHs. The log D values of the BRC were plotted against the pH and depicted in Fig. 5. The highest log D value was observed around pH 4 to 5.

The log D values were found to be lower before and after the pH range of 4 to 5. This finding suggests that the partition ability of BRC may be high at the pH range of 4 to 5, this range is also the pH range of the CH gel and skin; therefore BRC penetration was found to be faster when CH gel was used.

Although rabbit skin is known to be more permeable than human skin,³⁶⁾ our results show that it is possible to obtain faster penetration using CH gel when applied to the skin and it can be thought that the similar results can be obtained with the human skin, but further studies are necessary to develop an optimum formulation.

CONCLUSION

Our results showed that BRC can be penetrated through rabbit skin and it was possible to increase BRC penetration using CH and G-SP215 gel. It should also be considered that rabbit tissue is more permeable than human skin as generally appreciated, but it could be thought that CH and enhancer may work for human skin in a same way. The penetration process of BRC through skin was evaluated and concluded that there are several factors were found affecting BRC penetration when CH gel was used. The CH gel affected the skin cells and opened tight junctions and affected the connective cells and some clues were observed to suggest that BRC could be penetrated through skin in small vesicles. The observation of disappearance of the desmosomes and connective bundles and homogenisation of dermis indicate that CH gel affects the skin cells and provide faster penetration. The observation of vacuoles and vesicles may indicate that BRC also penetrates through the skin using other carrier system and these vesicles facilitate the process. All these observation suggest that the main penetration process for BRC occurs through intercellular pathway but the other mechanisms may also involve for transfer of the BRC through the skin.

It was concluded that the dermal application of BRC may be useful using CH gel and it was found to be an effective alternative route. Transdermal delivery of BRC may be a very promising alternative route to the oral route for the treatment.

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