Rectal Absorption of [Bis (acetato) ammine dichloro (cyclohexylamine) platinum(IV)] (BMS-182751), a New Anti-tumor Agent, in Rats

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The rectal absorption of a platinum anti-tumor agent, [bis (acetato) ammine dichloro (cyclohexylamine) platinum(IV)] (BMS-182751), was investigated in rats. BMS-182751 was co-ground with various carriers to improve its poor aqueous solubility. The highest drug dissolution was observed for the co-ground mixture of BMS-182751 and low molecular (LM) gelatin (1:9, w/w), followed by β-cyclodextrin and polyvinylpyrrolidone. The influence of a suppository base or additive on the rectal absorption of BMS-182751 in the drug state of crystalline powder or co-ground mixture was examined in vitro using excised rat rectum. A macrogol base gave much higher BMS-182751 permeation across the rat rectum than that from a Pharmasol base. The addition of sodium caprylate or caprylic acid to the macrogol base markedly enhanced the drug permeation, and a 3% addition of sodium caprylate to the base afforded maximum drug permeation. Two rectal formulations, the co-ground mixture with LM-gelatin plus 3% sodium caprylate in macrogol and the crystalline drug alone plus 3% sodium caprylate in macrogol, as well as an oral aqueous drug suspension, were administered to rats. The \( C_{\text{max}} \) and \( AUC_{0-\infty} \) values for platinum from the former suppository were 5.1- and 4.1-fold greater than those from the oral suspension, respectively. The values from the latter suppository were almost comparable to those from the suspension. These results suggest that the suppository may provide a promising therapeutic means for cancer treatment using this platinum agent.

Key words: anti-tumor agent; BMS-182751 suppository; rectal absorption; sodium caprylate; co-ground mixture; rat

Cisplatin and carboplatin, the existing clinical platinum agents, are extensively used in cancer chemotherapy. Both agents are typically given intravenously due to their limited gastrointestinal absorption.\(^{1}\)

[Bis (acetato) ammine dichloro (cyclohexylamine) platinum(IV)]\(^{2}\) (BMS-182751, Fig. 1) was newly synthesized in the expectation of improving such poor gastrointestinal absorption of cisplatin and carboplatin. This drug is currently undergoing clinical study in an oral capsule form. Recent preclinical studies have demonstrated that BMS-182751 is absorbed from the gastrointestinal tract in mice, and exhibits anti-tumor activity superior to that of cisplatin or carboplatin and, unlike cisplatin, is devoid of renal toxicity and peripheral neurotoxicity.\(^{3}\) In the clinical Phase I studies in patients, however, nonlinear absorption and greater interpatient pharmacokinetic variability of BMS-182751 was seen with a dose escalation of \( \geq 200 \text{ mg/m}^2 \), likely due to its poor aqueous solubility \((<0.3 \text{ mg/ml})\).\(^{4}\) Although the oral dosage form of the capsule only is currently being developed for BMS-182751, the development of a new administration route by rectum is anticipated to expand the clinical use of this drug, since it would afford ease of administration to pre- and post-operative patients experiencing difficulties in oral dosing.

The present study was undertaken to develop a bioavailable rectal formulation as an additional dosage form to the currently developing oral capsule. BMS-182751 was co-ground with various additives as a carrier for the poorly water-soluble drug to explore the feasibility of improving its aqueous solubility, then the preparations were incorporated into a fatty or water-soluble suppository base. The influence of the suppository base, sodium caprylate or caprylic acid as an additive, on the rectal absorption of BMS-182751 was examined in vitro utilizing excised rat rectum. The formulations finally selected by the in vitro absorption tests were subjected to an in vivo absorption experiment to determine the rectal absorption of BMS-182751 in rats.

MATERIALS AND METHODS

Materials BMS-182751 was synthesized at Johnson Matthey Technology Centre (Reading, UK) and obtained from Bristol-Myers Squibb Co. (New Jersey, U.S.A.). The fraction of BMS-182751 powder passed through a 100 mesh screen was always used. The pharmaceutical additives used for the preparation of co-ground mixtures were gelatin (acid treated) from Miyagi Kagaku Co. (Miyagi, Japan), low molecular gelatin (LM-gelatin, acid treated) with an average molecular weight of 7000 from Nippon Co. (Tokyo, Japan), polyvinylpyrrolidone (PVP, K-30) from GAF Chemicals Co. (New Jersey, U.S.A.), hydroxypropylmethyl cellulose (HPMC, 3 cps) from Shin-Etsu Chemicals Industries (Tokyo, Japan), and β-cyclodextrin from Nihon Shokuhin Kako Co. (Tokyo, Japan). Avisol (RC-591NF) for drug suspension in water was obtained from Asahi Chemical Industry Co. (Tokyo, Japan). The fatty base used for the rectal formulation was Pharmasol (type B-112, nearly equivalent to Witapsol H-15 in fatty acid composition) from Nippon Oil & Fats., Co. (Tokyo, Japan).

Fig. 1. Chemical Structure of BMS-182751

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Macrogel 400, 1500 and 4000 were obtained from Nippon Oil & Fats Co. (Tokyo, Japan). All other chemicals were of reagent grade. All experiments were performed under subdued light to prevent light degradation of BMS-182751.

**Solubility Studies** Solubility measurements were carried out according to the method of Higuchi and Connors. An excess amount of BMS-182751 (100 mg) was placed in 20 ml of a 1/15 m phosphate buffer (pH 7.5). The solution was shaken in a water bath at 37 ± 0.5 °C for 24 h. After equilibration was attained, an aliquot was withdrawn using a 0.22 μm membrane filter (Advantec Toyo, Tokyo, Japan). One milliliter of the filtrate was appropriately diluted with a 10% (v/v) acetonitrile aqueous solution and analyzed by HPLC. The chromatographic operating conditions were as follows: C18 reversed phase column (Hypersil ODS-5 μm, length: 4.6×250 mm); acetonitrile:water (25:75, v/v); flow rate of 1.3 ml/min; 210 nm detector (Shimadzu Seisakusho Co., Ltd., Kyoto, Japan).

**Preparation of Co-ground Mixtures** Three grams of the mixture of BMS-182751 and each of the carriers mixed at weight ratios of 1:2, 1:4 and 1:9 were triturated for 5 h using an automatic triturator (Aichi Denki Co., Aichi, Japan) with a mortar (13 cm i.d.) and pestle. The trituration processes were performed at room temperature. The co-ground mixtures of BMS-182751 with carriers for dissolution testing were all prepared at a 1:9 mixing ratio, since the amorphous state of the drug was obtained at ratio of 1:9 in the BMS-182751:LM-gelatin mixture. The physical mixture of BMS-182751 and LM-gelatin was prepared by tumbling each powder (<100 mesh) for 1 min in a bottle.

**Powder X-Ray Diffraction** A powder X-ray diffractometer (RAD-IC; Rigaku Denki Co., Ltd., Tokyo, Japan) was operated under the following conditions: target Cu, filter Ni, voltage 30 kV, current 20 mA, scanning speed 4°/min.

**Dissolution Study** A powder sample containing 600 mg of BMS-182751 in each co-ground mixture was directly transferred into 300 ml of phosphate buffer (1/15 m, pH 7.5) kept at 37 ± 0.5 °C and was stirred with a magnetic stirrer bar at 300 rpm. The beaker had a 500 ml capacity and was 85 mm in diameter. An aliquot of the solution (1 ml) was removed at definite time intervals with a syringe and filtered through a membrane filter (pore size: 0.22 μm). The filtrate was diluted with a 10% (v/v) acetonitrile aqueous solution and analyzed for drug concentration by HPLC. The chromatographic operating conditions were in accordance with those described in the solubility studies of this paper.

**Preparation of Rectal and Oral Formulations** For a fatty suppository base, BMS-182751 alone or the co-ground mixture of BMS-182751 with a carrier was suspended in a Pharmasol base with occasional stirring after the base had been melted at 50 °C. For a water-soluble suppository base, the drug sample was suspended in the macrogol base after a mixture of macrogel 400, 1500 and 4000 (5:1:2, w/w) had been melted at 70 °C. The molten mass was then sucked up into a plastic tube (0.4 cm i.d.) and allowed to solidify at room temperature. Sodium caprylate powder passed through a 100 mesh screen or liquid caprylic acid was added to the melted base at a concentration of 1—5% (w/w) to the weight of the base. The BMS-182751 content in each formulation was 2 mg per about 100 mg of total weight of the formulation. These prepared formulations were stored in a refrigerator (5 °C) until use. For an oral formulation, BMS-182751 crystalline powder was suspended at a concentration of 2 mg per milliliter in water containing 1.5% (w/w) Avicel, using a glass homogenizer (Iwaki Glass Co., Ltd., Tokyo, Japan). The aqueous drug suspension was administered immediately after preparation.

**In Vitro Release of BMS-182751 from Rectal Formulations** The release test was performed using a Franz-type diffusion cell according to the method reported previously, with some modifications. A 200 mesh stainless steel screen was placed at the top of the receptor chamber, then sandwiched between the donor and receptor chambers. Phosphate buffer (1/15 m, pH 7.5, 27 ml) was poured into the assembly so that the surface of the buffer was 0.5 cm higher than the mesh. The receptor phase was stirred at 350 rpm with a magnetic stirrer bar. The assembly cell was placed in a 37 ± 1 °C environmental chamber. The release experiment was initiated by placing a unit of the rectal formulation (about 100 mg) in the donor phase. The top of the buffer in the donor was sealed with a glass slip to prevent the evaporation of water. An aliquot of the buffer (0.3 ml) was removed at specified time intervals, and the volume was replaced by fresh buffer equilibrated at the same temperature. The drug concentration in the receptor solution was determined by HPLC. Chromatographic operating conditions were in accordance with those described in the solubility studies of this paper.

**In Vitro Rectal Absorption Experiment** An in vitro absorption experiment using excised rat rectum mounted on a Franz diffusion cell was performed according to the method reported by Ogiso et al. The rectum was freshly excised from each male SD rat (9 weeks old, 300—320 g). The surface mucosal area exposed for absorption was 0.385 cm² (diameter: 0.7 cm). In this study, each rectal formulation containing 2 mg of BMS-182751 was applied to the mucosal side after placing 0.5 ml of the receptor medium (37 °C) on the mucosal membrane. An aliquot (0.1 ml) of receptor medium was withdrawn periodically for 23 h. The receptor medium was bubbled with a gas mixture of oxygen—carbon dioxide (95:5, v/v). The concentration of BMS-182751 in the receptor medium was determined by HPLC. The chromatographic operating conditions were in accordance with those described in the solubility studies of this paper. Statistical evaluation of the data was carried out using one-way ANOVA. A probability value smaller than 0.05 was considered statistically significant.

Histological evaluation was performed on the rectal tissues immediately after excision (t=0 h) and after 12 and 23 h of incubation in the receptor medium. The tissues were examined using light microscopy (Nikon Co., model: optiphot, Tokyo, Japan), after being fixed in 10% (w/v) buffered formalin and stained with hematoxylin and eosin.

**In Vivo Absorption Study** Male SD rats (9 weeks old, 300—320 g) were fasted but had free access to water for 24 h before and during the rectal and oral administration experiments. The BMS-182751 suppositories were inserted to a depth of 1 cm from the anus at a dose of 6 mg/kg, and the anus was closed with an adhesive, Aron Alpha (Toakasei Kogyo Co., Tokyo, Japan). The aqueous suspension of BMS-182751 was administered orally at a dose of 6 mg/kg using a sonde. Blood samples were withdrawn from the tail vein periodically at 0.5, 1, 2, 4, 8 and 24 h post drug administration.
Platinum concentrations in plasma were determined according to the method reported by McKeage et al. Platinum analysis was undertaken by flameless atomic absorption spectrometry using a Hitachi Spectrometer (model: Z-8200, Tokyo, Japan). The absorption was measured at 265.9 nm. Standard curves for platinum were measured over a range of 30–1000 ng Pt/ml and shown to be linear. Linear regression analysis of these data consistently gave correlation coefficients >0.995. The lower limit of quantitation for platinum in plasma was 30 ng Pt/ml. The maximum plasma platinum concentration (C_{max}) and the time of its occurrence (T_{max}) after rectal or oral administration was observed from each plasma concentration–time curve. The area under plasma concentration–time curves from 0 to 24 h ([AUC]_{0-24h}) after administration was calculated by the trapezoidal rule. Statistical analysis was performed using one-way ANOVA, and the significance level adopted was p<0.05.

RESULTS AND DISCUSSION

Characterization of BMS-182751 in BMS-182751 : LM-Gelatin Mixture by X-Ray Diffraction

Figure 2 shows the X-ray diffraction patterns of BMS-182751 crystalline powder alone, a physical mixture of BMS-182751 with LM-gelatin (1:9), and the co-ground mixtures of BMS-182751 with LM-gelatin at weight ratios of 1:2, 1:4 and 1:9. Major diffraction peaks of 6.4, 12.8 and 16.7° 2θ were regarded as characteristic peaks of BMS-182751 crystal in the mixture. The co-ground mixtures showed a decrease of the diffraction peaks with an increase in the amount of carrier, implying that the crystallinity of the drug was lost. The diffraction peaks of BMS-182751 disappeared almost completely at a ratio of 1:9, indicating that BMS-182751 was present as an amorphous state in the mixture.

Dissolution Rate of BMS-182751 from Co-Ground Mixtures

Figure 3 shows the dissolution profiles of BMS-182751 from crystalline drug powder alone, a physical mixture of BMS-182751 with LM-gelatin (1:9) and the co-ground mixtures of BMS-182751 with various carriers (1:9). The co-ground mixtures all exhibited remarkably sharp increases in BMS-182751 dissolution rate; the amounts which dissolved in 3 min were increased at least 2.4-fold compared to that from crystalline drug alone or its physical mixture. The drug solubility in the dissolution medium at 37°C was 0.67±0.03 mg/ml (mean±S.E.; n=3) in this study. All drugs in the dispersed systems by co-grinding exceeded this solubility level, and the dissolved amounts of BMS-182751 were decreased with time, particularly in the mixtures co-ground with LM-gelatin, β-cyclodextrin or PVP showing a typical supersaturation phenomenon. Among the carriers investigated, the maximum dissolved amount of BMS-182751, 1.35 mg/ml in 3 min, was observed for the co-ground mixture with LM-gelatin, followed by β-cyclodextrin and PVP. This dissolution enhancing effect by the co-grinding process was likely due to a primary contribution factor of the reduction of drug particle size, resulting in an efficient exposure of the drug to water and dispersion. LM-Gelatin exhibited 1.8-fold greater drug dissolution in 3 min than that from the original high molecular gelatin. This is probably because LM-gelatin may possess a higher wetting or dissolving ability in water, thereby contributing to the faster drug dissolution. Thus, the co-grinding of BMS-182751 with LM-gelatin was found to be a promising pharmaceutical medium to make this drug sufficiently available for higher aqueous solubility.

In Vitro Release of BMS-182751 from Rectal Formulations

The drug can be absorbed after it is released from the suppository base, and the absorption is considered dependent on the nature of the base, additives, particle size or the solubility of the drug in the base. The drug release was therefore examined using two types of suppository bases, fatty Pharma-sol and water-soluble macrogol bases, with and without sodium caprylate. In this release study, the co-ground mixture of BMS-182751 and LM-gelatin (1:9) was chosen as a representative co-ground preparation, since it showed the highest drug dissolution (Fig. 3). Figure 4 shows the release profiles of BMS-182751 from various rectal formulations. The macrogol base released 77% and 63% of the amount of BMS-182751 in 20 min from the crystalline drug powder and the co-ground mixture, respectively, while Pharma-sol released only 2% of the drug amount from the crystalline drug powder and 3% from the co-ground mixture. This indicates that macrogol is decidedly the most favorable base for BMS-182751 release. The addition of sodium caprylate to the bases had little influence on the drug release rate. When dispersed in macrogol in the crystalline powder state, the release rate of BMS-182751 was unexpectedly enhanced to reach the release level of the co-ground mixture in macrogol.
It has been reported that a number of drugs such as griseofulvin, prednisolone and dicumarol incorporated into a macrogol matrix by melting techniques, enhanced drug dissolution due to the molecular dispersion of the drug in the water soluble carrier forming a solid dispersion. Similar to these solid dispersion systems, this faster release from the BMS-182751 crystalline powder may also be due to such finely dispersed or partly dissolved drug conditions in the macrogol base.

**In Vitro Rectal Absorption** The influence of the suppository base and the additive of sodium caprylate or caprylic acid on the BMS-182751 rectal absorption was estimated *in vitro* using excised rat rectum. The co-ground mixture of BMS-182751 and LM-gelatin (1:9) was chosen for this study since it showed good dissolution and release rates from suppository bases (Figs. 3 and 4). Figure 5A shows the permeation profiles of BMS-182751 from crystalline drug powder alone or its co-ground mixture incorporated into a macrogol or Pharmasol base. In the suppositories containing BMS-182751 crystalline powder, the cumulative amount of drug which permeated through the rectum after 23 h was 203 nmol/cm² in the macrogol base, while none of the drug was permeated from the Pharmasol base. This higher drug permeation from the macrogol base is most certainly due to the cosolvent effect of macrogel by increasing the drug solubility in both the base and the mucosal fluid or by rapid drug migration from the base to the surface of the mucosal membrane as supported by the *in vitro* release data (Fig. 4). In the suppositories containing the co-ground mixture, the cumulative amount of drug after 23 h was increased by the co-grinding to 236 nmol/cm² in a macrogol base and 28 nmol/cm² in a Pharmasol base. Figure 5B shows the BMS-182751 permeation from the suppositories containing 1—5% of sodium caprylate in macrogol, 3% of sodium caprylate in the Pharmasol base and 3% of caprylic acid in macrogol. BMS-182751 permeation was markedly enhanced by these additives, and the sodium caprylate brought a slightly higher

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**Fig. 3.** Dissolution Profiles of BMS-182751 from the BMS-182751-Carrier Systems in pH 7.5, 1/15% Phosphate Buffer at 37°C

Co-ground mixture of BMS-182751:carrier (1:9, w/w); ●, BMS-182751:LM-gelatin; ○, BMS-182751:β-cyclodextrin; ●, BMS-182751:PVP; ×, BMS-182751:HPMC; □, BMS-182751:gelatin; ▲, physical mixture of BMS-182751:LM-gelatin (1:9, w/w); ∆, BMS-182751 crystalline powder. Dotted line: solubility level of BMS-182751. Each point represents the mean ± S.E. of three or four experiments.

**Fig. 4.** Release Profiles of BMS-182751 from Suppositories in pH 7.5, 1/15% Phosphate Buffer at 37°C

In macrogol base: ▲, BMS-182751 crystalline powder+3% w/w sodium caprylate; Δ, BMS-182751 crystalline powder; ●, BMS-182751:LM-gelatin (1:9, w/w) co-ground mixture+3% w/w sodium caprylate; ○, BMS-182751:LM-gelatin (1:9, w/w) co-ground mixture. In Pharmasol base: ●, BMS-182751:LM-gelatin (1:9, w/w) co-ground mixture+3% w/w sodium caprylate; □, BMS-182751:LM-gelatin (1:9, w/w) co-ground mixture; ×, BMS-182751 crystalline powder. Each point represents the mean ± S.E. of three experiments.

**Fig. 5.** Rectal Membrane Permeation Profiles of BMS-182751 Following Application of Suppositories without (A) or with (B) Sodium Caprylate

(A) ○, co-ground mixture/macrogol; △, BMS-182751 crystalline powder/macrogol; ○, co-ground mixture/Pharmasol; ×, BMS-182751 crystalline powder/Pharmasol. (B) ●, co-ground mixture+3% w/w sodium caprylate/macrogol; □, co-ground mixture+3% w/w sodium caprylate/Pharmasol; ▲, BMS-182751 crystalline powder+3% w/w sodium caprylate/macrogol; ●, BMS-182751 crystalline powder+3% w/w sodium caprylate/Pharmasol. The co-ground mixture herein indicates the co-ground preparation of BMS-182751:LM-gelatin (1:9, w/w). Each point represents the mean ± S.E. of three or four experiments.
BMS-182751 permeation than did caprylic acid. The maximum effective concentration of sodium caprylate for permeation promoting action was 3% in the macrogel base, whereas the amount of drug permeated decreased at a concentration of 5%. When 3% of sodium caprylate was added to the macrogel base, the cumulative amounts of BMS-182751 after 23 h increased further to 1276 and 1021 nmol/cm² in the co-ground mixture and in the crystalline drug, respectively. These results were confirmed by the fact that a flux was remarkably increased by sodium caprylate accompanying a shortening of lag time (Table 1). This permeation enhancement by sodium caprylate or caprylic acid may be due to the reduction of membrane resistance, improving the paracellular transport of drugs. These in vitro absorption data suggest that the combination of macrogel base and sodium caprylate or caprylic acid as an additive would provide a promising suppository formulation for absorption enhancement in a drug co-ground with LM-gelatin or even in the crystalline drug powder alone.

In this study, drug permeability was determined until 23 h utilizing excised rat rectum, while Ogiso et al. studied until 12 h. The histological observation for the rectum excised at 12 and 23 h revealed a partial removal of mucous epithelium from the lamina propria and a partial disruption of the underlying connective and muscle layers in comparison with the rectum at 0 h. However, no large difference in the histological change was observed between the 12 and 23 h tissues. Although the drug would be readily permeable under these changed tissue conditions, the flux data obtained are considered available for a rough screening of the formulation prior to an in vivo absorption assessment.

In Vivo Rectal Absorption The effect of co-grinding with LM-gelatin and the addition of sodium caprylate on the rectal absorption of platinum from BMS-182751 was investigated in rats. Figure 6 shows the platinum concentrations in plasma after the rectal administration of BMS-182751 crystalline powder in humans, rat, dog, and monkey with sodium caprylate in a macrogel base (suppository A), its co-ground mixture plus sodium caprylate in a macrogel base (suppository B) and after the oral administration of a BMS-182751 aqueous suspension. The pharmacokinetic values are given in Table 2. The C_{max} and AUC_{0-24h} values for platinum from the rectally administered suppository B were 5.1- and 4.1-fold larger than those from the oral suspension, respectively. The values from suppository A were almost comparable to those from the oral suspension. This higher plasma platinum concentration from suppository B may be explained by the viscous nature of LM-gelatin after being dissolved in the rectal fluid likely contributing to retaining a greater part of the formulation at the administration site, maintaining a high concentration of sodium caprylate for absorption enhancement in the area, and resulting in subsequent better rectal absorption. Thus, the suppository in which the co-ground mixture of BMS-182751 and LM-gelatin (1:9) was formulated in macrogel with 3% sodium caprylate was identified as the optimal formulation for rectal absorption.

In this study, platinum levels in plasma were determined for absorption assessment. Several studies have demonstrated that BMS-182751 is extensively converted into at least 6 different biotransformation products in human plasma. It has also been revealed that BMS-182751 following oral administration was never observed in plasma, although the metabolism in rat is unclear.

With regard to the rectal dosage form for cancer treatment, two rectal formulations, tegafur and 5-fluorouracil suppositories, are commercially available at present. Tegafur was de-
veloped for systemic cancer treatment, whereas 5-fluorouracil was for local cancer treatment limited to the rectum or colon. Both formulations have been used for the treatment of cancer disease, expanding the usefulness of treatment with these drugs. In terms of platinum agents, the application of a cisplatin suppository was recently reported to be effective for local cancer treatment, but none of the rectal formulations containing platinum agents have yet led to practical use.

In conclusion, platinum from a BMS-182751 suppository was found to be well absorbed through the rectal mucosa. The co-grinding of BMS-182751 with LM-gelatin was the most effective for the improvement of dissolution of the poorly water-soluble BMS-182751. The addition of sodium caprylate to the suppository in which the co-ground mixture was formulated in macrogol provided a drastic increase in rectal absorption. The suppository may provide a promising formulation for systemic cancer treatment with a platinum agent, and contribute to expanding clinical treatment using BMS-182751.

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