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# Preclinical oral antitumor activity of BMS-185660, a paclitaxel derivative

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**Abstract** *Purpose*: A water soluble paclitaxel derivative, BMS-185660, identified previously as having parenteral activity comparable with that of the parent drug, was evaluated for antitumor activity when given orally. *Methods*: Staged subcutaneous (s.c.) tumor models of both murine and human origin were used for this purpose. *Results*: BMS-185660 achieved levels of activity following oral administration which were comparable with those maximum effects obtained using intravenous (i.v.) paclitaxel. Consecutive daily oral administrations of BMS-185660 resulted in maximum gross log cell kill (LCK) values of 1.7–2.0 in two experiments involving the s.c. Madison 109 murine lung tumor model, which were comparable with the best effects of the derivative injected intravenously, and 0.3 to 0.9 LCK greater than the maximum effects obtained with i.v. paclitaxel; paclitaxel given orally was inactive. Against a human ovarian tumor model with developed resistance to cisplatin (A2780/cDDP), oral BMS-185660 achieved a maximum LCK of 1.8 compared with i.v. paclitaxel, which produced a maximum 2.4 LCK. Also, in the human HCT-116 colon carcinoma model, oral BMS-185660 cured a maximum of seven of eight mice compared with six of seven mice cured with i.v. paclitaxel. The loss in potency between comparably effective intravenously and orally administered doses of BMS-185660 was about four- to five-fold, but since no drug-associated lethality was ever observed following the oral administration of the highest doses of BMS-185660, further dose escalation may have been tolerated. The intermediate metabolite between BMS-185660 and paclitaxel is BMS-181681. This compound was also evaluated orally and found not to be active versus s.c. M109, despite demonstrating good activity by the i.v. route. *Conclusion*: The comparable activities of both intravenously and orally administered BMS-185660 to intravenously administered paclitaxel, combined with the attribute of improved water solubility, provides a good basis for further derivative development.

**Key words** Taxol · Taxane · Anticancer drugs

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## Introduction

The dramatic impact of Taxol (paclitaxel) upon selected neoplastic diseases [2, 4, 13] has occurred despite the presence of cremophor and ethanol in the injection vehicle. The toxicities associated with excipients such as cremophor and Tween 80 [1, 3, 7] may prevent the full therapeutic potential of paclitaxel and other water insoluble derivatives (e.g., Taxotere) from being realized. Thus, the recent availability of water soluble paclitaxel derivatives possessing good preclinical antitumor activity represents an important achievement and opportunity [12]. The lead candidate identified from among those derivatives was BMS-185660. While exploring further the antitumor potential of BMS-185660, we found it to be active (unlike paclitaxel) when given orally. The prospect of both a water soluble and orally active paclitaxel derivative has important implications with regard to patient care and reduced attendant costs of protracted treatment regimens. Accordingly, we present herein a description of the antitumor testing conducted using orally administered BMS-185660 and intravenously administered paclitaxel against several distal site tumor models of both murine and human origin.

## Materials and methods

### Mice

Balb/c and (Balb/c × DBA/2)$F_1$ hybrid (CDF1) conventional mice and Balb/c-background athymic mice (16–20 g), were purchased

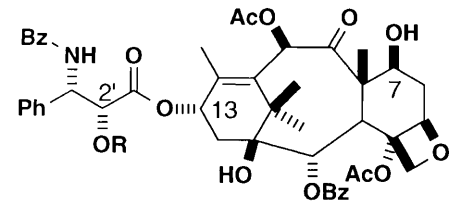
from Harlan Sprague-Dawley (Indianapolis, Ind., USA). They were provided with food and water ad libitum. All studies involving these animals were conducted in accordance with our NIH and Bristol-Myers Squibb (BMS) animal care and use guidelines.

## Tumors

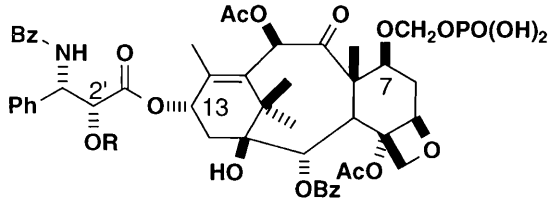
The murine lung carcinoma, M109 [8], was passaged subcutaneously biweekly in Balb/c mice and implanted subcutaneously into $CDF_1$ mice for antitumor evaluations. The human A2780 ovarian carcinoma resistant to cisplatin, A2780/cDDP [11], and HCT-116 colon carcinoma [10] were grown subcutaneously in athymic mice for both passage (every 2 to 3 weeks) and therapy experiments.

## Compounds

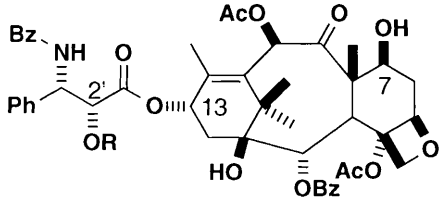
Paclitaxel was nearly always dissolved in cremophor/ethanol (50%/50%) and further diluted with saline, within 30 min of use, to a final vehicle proportion of cremophor (10%)/ethanol (10%)/saline (80%). The only exception to this procedure was when paclitaxel was suspended in water with a few drops of Tween 80 prior to its oral administration in one experiment. BMS-185660, a triethanolamine salt of >98% purity, was administered either intravenously in water, or orally in water with a few drops of Tween 80 added, and always within 30–60 min of its preparation. The limits of its solubility have been described previously [12]. (Note: a few drops of Tween 80 do not make an oral taxane.) BMS-181681, an insoluble paclitaxel derivative, and the intermediary metabolite between BMS-185660 and paclitaxel, was dissolved in dimethylsulfoxide (DMSO) and diluted with water prior to (and within 10 min of) its intravenous (i.v.) administration, or suspended in water with a few drops of Tween 80 for its oral administration. Each of these compounds is depicted in Fig. 1. Cisplatin was dissolved in saline. Compounds were administered either intravenously or orally in a volume of 0.01 ml or 0.02 ml/g of body weight; the smaller volume was used for vehicles containing either cremophor or DMSO. Both BMS-185660 and BMS-181681 were prepared in the Antitumor Chemistry Department, Bristol-Myers Squibb, Wallingford, Conn., USA.




**Paclitaxel R = H**




**BMS 185660 R = -COOEt**




**BMS 181681 R = -COOEt**

**Fig. 1** Structures of paclitaxel, BMS-185660, and BMS-181681. *Bz* benzoyl, *Ac* acetyl, *Ph* phenyl

## Assessment of antitumor activity

Detailed descriptions of the basic assay and evaluation procedures used for the tumor models and experiments contained herein have been reported [8, 10, 11, 12]. Briefly, therapeutic results are presented in terms of: (a) increases in life span reflected by the relative median survival time (MST) of treated (T) versus control (C) groups to derive percent increase in life span (%ILS) values in the M109 experiments, and any long-term survivors (typically determined on day 60 for subcutaneous (s.c.) M109 experiments and days 54–80 for human tumor xenograft studies), and (b) primary tumor growth inhibition determined by calculating the relative median times for T and C mice to grow tumors of a predetermined "target" size (1 g for M109 and A2780/cDDP tumors, 0.5 g for HCT-116 tumors) and expressed as T-C values (in days). Individual mice bearing tumor xenografts were killed soon after having grown tumors to the aforementioned target sizes. The dose of a compound which yielded the maximum therapeutic effect was termed the optimal dose (OD). When more than one therapeutic endpoint was determined (as in M109 experiments), it was possible to derive more than one OD value. Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test [5]. The activity criterion for increased life span in the s.c. M109 tumor model was a %ILS of ≥25% . The activity criterion for tumor inhibition/reduction was a delay in primary tumor growth consistent with 1 gross $\log_{10}$ cell kill (LCK). The absolute T-C value needed to attain this level of efficacy varied from experiment to experiment and depended upon the tumor volume doubling time (TVDT) of the control mice in each study (i.e., T-C/TVDT × 3.32).

Group sizes typically consisted of eight or nine mice. The treatment regimen for the s.c. M109 tumor tests was always five consecutive once-daily administrations beginning on day 4 post-tumor implant (i.e., qd × 5; d. 4). For the human tumor xenograft tests, compounds were given once daily, every other day for five administrations (i.e., q2d × 5), beginning when the tumors were staged to between 50 and 100 mg. These treatment schedules were selected based upon previously reported data describing them as optimal for paclitaxel in the same or comparable tumor models [9]. Compound-treated mice dying prior to having their tumors reach target size were considered to have died from drug toxicity. No control mice died bearing tumors less than target size. Groups of mice with more than one death attributable to drug toxicity were considered to have had excessively toxic treatments and their data were not used in the evaluation of a compound's antitumor efficacy. A few mice that died immediately upon receiving an i.v. injection were excluded as laboratory accidents. A maximum tolerated dose (MTD) was defined as one whose toxicity approached but did not attain the degree of lethality just described as being excessive. Operationally, when a particular dose of a compound caused excessive lethality, the MTD would be typically assigned the nearest concomitantly evaluated lower dose, which was not excessively lethal. The MTD was often synonymous with the OD. Compounds were typically evaluated at a minimum of three dose levels per experiment.

## Results

Paclitaxel and BMS-185660 were administered by the oral and i.v. routes to mice bearing s.c. tumors of murine and human origin. BMS-181681, the insoluble intermediary metabolite between BMS-185660 and paclitaxel, was evaluated in a few of the same experiments. The initial discovery of the oral activity of BMS-185660 was made in the murine M109 tumor model. A summary of the optimal effects obtained in that model is provided in Table 1. In the first experiment conducted, no. 249, both paclitaxel and BMS-185660 were administered orally (p.o.) as a slurry in a water plus Tween 80 vehicle. At the highest doses tested (160 mg/kg · inj, p.o.), paclitaxel was inactive but BMS-185660 produced a maximum ILS of 58%, accompanied by a 22.8-day T-C value. Considering the 3.5-day TVDT in this study, the delay in tumor growth observed was reflective of 2.0 LCK. In comparison, BMS-185660 delivered intravenously produced 1.6 LCK at an OD of 36 mg/kg · inj (ILS of 55% and 19.0 day T-C), and i.v. paclitaxel caused a 13.0-day T-C and 32% ILS for a 1.1 LCK. The delay in tumor growth caused by oral BMS-185660 represented a significant ($P < 0.001$) improvement of nearly 1 LCK over the best effect caused by concomitantly evaluated i.v. paclitaxel therapy.

The unexpected oral activity observed had to be confirmed and this was accomplished in experiment no. 254. Paclitaxel was given orally in the same vehicle used for its i.v. administration, which allowed for its dissolution but limited the amount that could be delivered in soluble form per administration. The highest dose tested (60 mg of paclitaxel per kg/inj), as well as all other doses given, failed to produce an active result with regard to either life span increase or tumor growth delay. Intravenously administered paclitaxel was active however, producing a maximum ILS of 67% and 14.0-day T-C; this delay was equivalent to a 1.4 LCK given the 3.0-day TVDT in this experiment. BMS-185660, in comparison, achieved identical maximum 17.0-day T-C values following therapy with either 200 mg/kg · inj (p.o.; highest dose evaluated), or 48 mg/kg · inj, (i.v.). The maximum ILS values were 72% and 70%, respectively, and the LCK value associated with the observed T-C values was 1.7.

In M109 experiment no. 257, paclitaxel, evaluated only by the i.v. route, produced a maximum ILS of 39% accompanied by a 13.3-day T-C. Based on the 2.5-day TVDT in this study, paclitaxel had caused the equivalent of a 1.6 LCK. Evaluated in parallel, the insoluble derivative, BMS-181681, performed like paclitaxel in that it was quite active when given intravenously (maximum ILS of 30% and 13.0-day T-C at its MTD and OD of 30 mg/kg · inj, reflective of a 1.6 LCK), but inactive when given p.o., even at the highest dose tested (200 mg/kg · inj).

The oral activity of BMS-185660 was further explored in two staged s.c. human tumor xenograft models, A2780/cDDP and HCT-116, and comparisons of the optimal effects associated with oral administration of the derivative relative to optimal i.v. administration of paclitaxel are shown in Figs. 2 and 3. Against A2780/cDDP, all treatments were delayed until day 11 post-tumor implant (Fig. 2). Optimal treatment with i.v. paclitaxel, and i.v. BMS-185660, both at 36 mg/kg · inj, resulted in comparable maximum T-C values of 26.3 days and 29.8 days, respectively. Considering the TVDT of 3.3 days in this study, these delays in tumor growth were reflective of activity equivalent to 2.4 LCK and 2.7 LCK, respectively. In comparison, optimal oral treatment with 160 mg/kg · inj of BMS-185660 yielded a maximum T-C of 20.0 days, or 1.8 LCK. A 50% higher dose of the derivative was also evaluated orally in this experiment and, although tolerated, it did not produce a superior antitumor effect. Cisplatin was included in the experiment too; it was inactive (0.3 LCK) at its MTD (7.5 mg/kg · inj, i.v., administered q3d × 3), thereby confirming the resistance of this tumor model toward the drug.

**Table 1** Comparative antitumor activity of orally and intravenously administered paclitaxel derivatives versus staged s.c. M109 lung carcinoma. *OD* optimal dose, *ILS* increase in life span, *T-C* relative delay (in days) of treated versus control groups of mice to reach a median tumor target of 1 g, *LCK* gross log cell kill, *ND* not done

| Compound | Experiment number | Maximum effects | | | | | |
|---|---|---|---|---|---|---|---|
| | | Orally | | | Intravenously | | |
| | | OD[a,b] | %ILS | T-C (days) [LCK] | OD[a] | %ILS | T-C (days) [LCK] |
| Paclitaxel | 249 | 160 | −2 | 0.3 [0] | 18/36 | 32 | 13.0 [1.1] |
| 185660 | 249 | 160 | 58 | 22.8 [2.0] | 36 | 55 | 19.0 [1.6] |
| Paclitaxel | 254 | 60 | 19 | 0.5 [0.1] | 48/36 | 67 | 14.0 [1.4] |
| 185660 | 254 | 200 | 72 | 17.0 [1.7] | 48 | 70 | 17.0 [1.7] |
| Paclitaxel | 257 | ND | ND | ND | 36 | 39 | 13.3 [1.6] |
| 181681 | 257 | 200 | −5 | 1.8 [0.2] | 30 | 30 | 13.0 [1.6] |

[a] OD in mg/kg · inj (or maximal tolerated or highest dose evaluated, whichever is less, if compound was not active) administered once daily for 5 consecutive days beginning on day 4 post-tumor implant. Split dose configuration signifies numerator yielded maximum percent increase in life span (%ILS), denominator yielded best T-C value

[b] All orally administered compounds were suspended in water + Tween 80, except for paclitaxel in experiment no. 254, where it was given in cremophor/ethanol/saline (as used for i.v. injections)

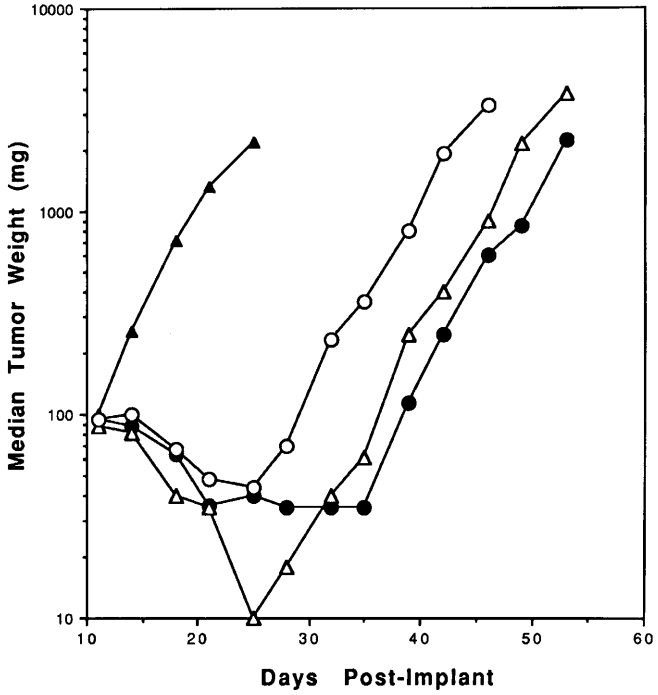



**Fig. 2** Activities of intravenously and orally administered BMS-185660 relative to intravenous (*i.v.*) paclitaxel versus staged s.c. A2780/cDDP human ovarian carcinoma. The following optimal treatments are depicted: ● 36 mg BMS-185660/kg · inj, i.v., ○ 160 mg BMS-185660/kg · inj, p.o., △ 36 mg paclitaxel/kg · inj, i.v., ▲ tumor-bearing controls. All compound administrations were made on a q2d-x-5 schedule beginning on day 11 post-tumor implant

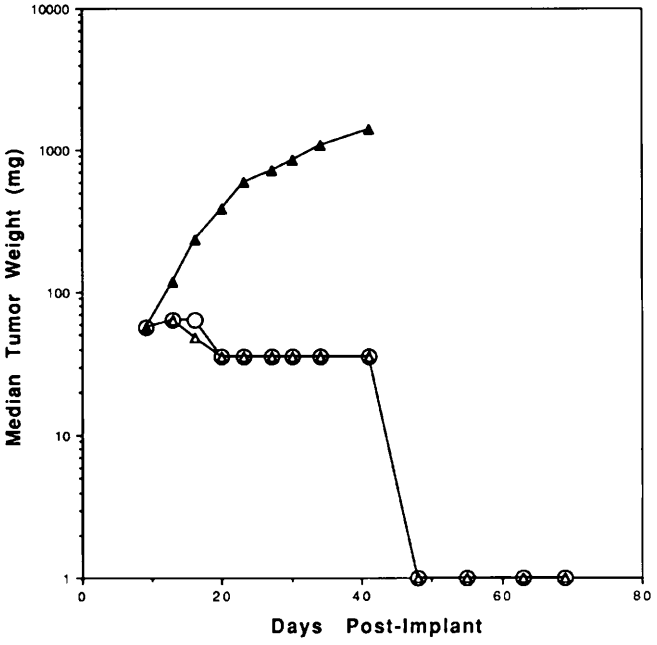



**Fig. 3** Activities of intravenous (*i.v.*) paclitaxel and orally administered BMS-185660 versus staged s.c. HCT-116 human colon carcinoma. The following optimal treatments are depicted: ○ 160 mg BMS-185660/kg · inj, p.o., △ 24 mg paclitaxel/kg · inj, i.v., ▲ tumor-bearing controls. All compound administrations were made on a q2d-x-5 schedule beginning on day 9 post-tumor implant

Lastly, the oral activity of BMS-185660 was compared with intravenously administered paclitaxel versus staged s.c. HCT-116 human colon carcinoma xenografts. The effects of both compounds given by the routes mentioned are depicted in Fig. 3. Despite the delay of treatment initiation (to day 9 post-tumor implant), the compounds were so effective as to cause many cures (tumor-free on day 80 post-implant) in the groups receiving optimal therapy. For example, treatment with 24 or 36 mg/kg · inj of paclitaxel, i.v., produced six cures of seven or eight treated mice, respectively, and 240 or 160 mg/kg · inj of BMS-185660, p.o., cured six or seven of eight treated mice, respectively. Orally administered BMS-181681 was also included in this experiment and it was inactive at doses up to 200 mg/kg · inj.

## Discussion

From among several water soluble derivatives of paclitaxel, BMS-185660 was recently identified and selected as one deserving of further evaluation [12]. That decision was based on its broad spectrum antitumor activity in several murine and human tumor models following parenteral administration, to a degree comparable with that of the parent drug. The potential advantage provided by a paclitaxel derivative that need not be administered parenterally in a vehicle containing cremophor or Tween 80 [1, 3, 7] was another reason for BMS-185660 being subjected to further study.

Now, in addition, we have demonstrated the oral activity of this same paclitaxel derivative. Most unexpectedly, upon its initial evaluation orally in the modestly staged and metastatic s.c. M109 tumor model, we found BMS-185660 to be not just active, but able to produce a significant improvement, of nearly 1 LCK, over the level of activity achieved using i.v. paclitaxel. Subsequent demonstrations of activity using the oral route of administration confirmed and extended this initial observation. We have found oral BMS-185660 to be as active (within 1 LCK or similar cure rates) as i.v. paclitaxel in all the tumor models in which it was evaluated (s.c. M109, s.c. A2780/cDDP, and s.c. HCT-116). The tumor models selected for study were representative of those known to be taxane-sensitive. As determined thus far, the oral OD for BMS-185660 has varied from 160 to 240 mg/kg · inj, and comparing these values to only those i.v. ODs obtained concomitantly (36–48 mg/kg · inj), the loss in potency (p.o. versus i.v.) was about four- to five-fold. It must be noted, however, that no MTD has yet been determined for orally administered BMS-185660. Within the scope of these efficacy studies, BMS-185660 was not observed to cause any atypical taxane toxicities.

We found too, that BMS-181681, like paclitaxel, was not active orally at the dose levels tested. For paclitaxel, this finding was true whether it was administered as a slurry, or as a solution in a cremophor/ethanol-based vehicle. Subsequent testing of paclitaxel orally at twice the dose level contained in the experiments described herein has yielded the same negative outcome (unpublished data). Since BMS-181681 is the intermediary metabolite on the biotransformation path toward paclitaxel from BMS-185660 via a proposed phosphatase action [6], it is likely that oral activity can be attributed to the phosphate group present on BMS-185660. BMS-185660 was not found in the plasma or liver of mice following its oral administration, but both BMS-181681 and paclitaxel were detected (J. Knipe, personal communication). Based on its antitumor activity, which was described as comparable to paclitaxel when both were administered parenterally [12], and the now disclosed oral activity of water soluble BMS-185660, future studies will be directed at evaluating other derivatives for yet greater improvements of these several features.

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