Batimastat, a Synthetic Inhibitor of Matrix Metalloproteinases, Potentiates the Antitumor Activity of Cisplatin in Ovarian Carcinoma Xenografts

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ABSTRACT

Batimastat (also known as BB-94) is a synthetic matrix metalloproteinase inhibitor that has shown antineoplastic and antiangiogenic activity in various tumor models. In this study, two human ovarian carcinoma (HOC) xenografts (HOC22 and HOC8) were used to investigate the effect of batimastat on the antineoplastic activity of cisplatin. Both xenografts produced ascites and solid lesions in the peritoneal cavity of nude mice. HOC cells were inoculated i.p. in nude mice, and treatment was started at different stages of the disease. Batimastat was administered alone or concurrently with or subsequent to cisplatin therapy. In all of the protocols, the response of HOC xenografts was confirmed by cytological analysis of ascites and histological examination of the organs in the peritoneal cavity.

Treatment of nude mice bearing early-stage (3 days after tumor implantation) HOC22 or HOC8 with cisplatin or batimastat alone delayed tumor growth and increased the survival time of the mice, although all animals eventually died. In contrast, treatment with batimastat (60 mg/kg i.p. every other day, for a total of eight injections) concomitantly with cisplatin (4 mg/kg i.v., every 7 days for a total of three injections) completely prevented growth and spread of both xenografts, and all animals were alive and healthy on day 200. The potentiation of cisplatin's activity by batimastat was dose dependent and was observed in the treatment of both advanced (7 days after tumor inoculation) and late-stage (20 days after inoculation) tumor. The administration of batimastat following cisplatin therapy also led to significant improvement in the survival of mice compared to treatment with cisplatin alone. These results suggest a potentiation of the antineoplastic activity of cisplatin by batimastat and support the use of the two agents in combination in the treatment of ovarian cancer patients.

INTRODUCTION

MMPs' expression and activation appear to play an important role in the process of tumor progression (1, 2). This process involves local tumor expansion through adjacent normal tissues, invasion by metastatic cells of blood vessels and lymphatics, and extravasation at distant sites. Moreover, MMPs appear to be involved in the process of angiogenesis, mediating the remodeling and penetration of extracellular matrix by new capillaries (3–5). An MMP inhibitor should, therefore, have the potential to inhibit both tumor growth and spread (6–8).

Batimastat, also known as BB-94, is a synthetic, low molecular weight broad-spectrum MMP inhibitor (9). Designed as an anti-invasive and antimetastatic drug, batimastat has been shown to inhibit metastasis of the murine B16 melanoma (10) and local and metastatic spread of human colon and breast cancers in nude mice (11–13). Recently, we reported that batimastat reduced the growth of experimental murine hemangiomma in nude mice and demonstrated the antiangiogenic potential for this drug (14). Davies and coworkers (15) have shown that batimastat given to nude mice bearing HOC reduced ascites and prolonged the survival of the mice. They reported a change in the tumor stroma composition with the formation of avascular tumors, supporting the hypothesis of an antiangiogenic potential for batimastat (15). In all of these studies, batimastat delayed and inhibited tumor growth without, however, eradicating the tumors. This might be expected of a drug that, in contrast to conventional chemotherapeutic agents, is not toxic to tumor cells (10, 11, 14) but exerts its effects through inhibition of extracellular matrix breakdown and neovascularization.

Cisplatin and related analogues are the first choice of treatment for ovarian carcinoma patients (16). However, the remission that results from this cytotoxic treatment is often short, and patients frequently develop resistance after exposure to the drugs (17). In addition, systemic side effects limit the continuation of therapy. Given these circumstances, novel therapies directed to diverse molecular targets, administered in combination with standard treatments, should provide new op-
Fig. 1 Experimental protocols. Nude mice were inoculated i.p. with HOC cells (Day 0), and treatments were started at Day 3 (early-stage tumor), Day 7 (advanced-stage tumor), or Day 20 (late-stage tumor). Cisplatin and doxorubicin (●, i.v. injections) and batimastat (□, i.p. injections) were administered at doses and schedules as detailed in “Results.” †, beginning of treatment; ‡, tumor burden evaluation at the end of treatment period; . . . . . , mice left for survival (n = 8).

portunities for anticancer therapy without exacerbation of acute systemic or cumulative toxicity (18).

Batimastat and other MMP inhibitors have now entered clinical trials in cancer patients, and there is increasing interest in using these inhibitors in combination with existing cytotoxic chemotherapies. Recently, the potentiation of cytotoxic cancer therapies by the antiangiogenic compounds (19) and a gelatinase-selective MMP inhibitor (20) have been reported in murine tumor models.

Here, two HOC xenografts (HOC22 and HOC8) transplanted in the peritoneal cavity of nude mice (21, 22) were used to investigate the effect of batimastat on the antineoplastic activity of cisplatin.

MATERIALS AND METHODS

Animals. Female NCr-nu/nu mice were obtained from the animal production colony of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). Mice were used when they were 8 to 10 weeks old and were weight matched. Throughout this study, nude mice were housed in filtered-air laminar flow cabinets and manipulated following aseptic procedures. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; and NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

Cell Lines. HOC xenografts HOC22 and HOC8 were established and maintained i.p. in nude mice as described previously (21, 22). The doubling times of HOC22 and HOC8 growing in the peritoneal cavity were 5.1 ± 1.5 and 6.2 ± 0.5 days, respectively. HOC cell suspensions grow in the peritoneal cavity of all injected mice, producing ascites and solid lesions on abdominal organs, primarily the diaphragm, omentum, and liver.

Drug Treatment. Batimastat (also known as BB-94; [4-[(N-hydroxyamino)-2R-isobutyl-3S-(thienylthiomethyl)-succinyll]-L-phenylalanine-N-methylamide; M, 478), its enantiomer BB-1722, and vehicle were provided by British Biotech Pharmaceuticals Ltd. (Cowley, Oxford, United Kingdom). Batimastat was formulated as for use in the clinic, namely as a 20 mg/ml suspension in 2.5% ethanol, 2.5% polyethylene glycol 400, and 1% methyl cellulose. Batimastat or vehicle was further diluted in glucose 5% (1:3) immediately before administration. In the experiments in which BB-94 and BB-1722 activities were compared, the inhibitors were both suspended by sonication in PBS containing 0.01% Tween 80 (pH 7.2). These preparations were injected i.p. into mice at the concentrations and schedules indicated in the respective experiments. Cisplatin (also known as DDP; provided by Bristol Myers-Squibb, Wallingford, CT) and doxorubicin (also known as ADM; provided by Pharmacia-Upjohn, Nerviano, Italy) were dissolved in distilled water and given by i.v. administration. Experimental protocols are described in Fig. 1, and doses and schedules of treatments are detailed in “Results.” Appropriate vehicles (defined as “vehicle-BB-94” and “vehicle-DDP”) were injected, using the same schedule and route of injection as the active therapies.

Treatment Evaluation. HOC8 and HOC22 ascites were injected i.p. as a cell suspension to 10 nude mice (approximately 10 × 10⁶ cells/animal) for each experimental group. Two mice per group were used to determine tumor burden at the end of treatment period (see below), and eight mice were left for evaluation of survival (Fig. 1). Treatment was started 3 (early stage), 7 (advanced stage), or 20 days (late stage) after tumor inoculation; tumor burden was evaluated in representative mice at the beginning of treatments, as shown in “Results.” Mice were monitored twice a week for body weight loss, and tumor formation in the peritoneal cavity (abdominal distension) and survival time were recorded. At autopsy, the peritoneal cavity was macroscopically examined to ascertain the presence of tumor. Results are plotted as percentage survival of animals versus days after tumor transplant. The percentage ILS was calculated as 100 × [(median survival day of treated group – median survival day of control group)/median survival day of control group]. Differences in survival time were analyzed by the log-rank test. Surviving animals that did not present gross evidence of tumors in the peritoneal cavity were killed and autopsied no earlier than 90 days after the death of the last control animal. The absence of tumor in “cured” mice was confirmed by cytohistological examination, as described below.
concurrently with vehicle-DDP (vehicle). cisplatin given concurrently with vehicle-BB-94 (DDP); batimastat given concurrently with vehicle-DDP (vehicle). BB-94 (60 mg/kg) and vehicle-BB-94 were administered i.p. every other day for eight injections; DDP (4 mg/kg) and vehicle-DDP were administered i.v. every 7 days for three injections. n = 8 mice per group. Results are representative of five independent experiments.

**Tumor Burden Evaluation.** Nude mice were euthanized by carbon dioxide inhalation. Peritoneal ascites was harvested by lavage with 3 ml of 0.9% NaCl, and the total volume and number of cells (due to cell clumps only; representative of tumor burden) for each mouse were determined. The cell suspension was spun in a cytocentrifuge, and the cells were fixed and stained according to the method of Papanicolaou. The specimen was spun in a cytocentrifuge, and the cells were fixed and stained according to the method of Papanicolaou. The ovary/uterus, pancreas, omentum, spleen, liver, diaphragm, and lung were collected, fixed in 10% phosphate-buffered formalin, and processed for standard histological analysis.

**RESULTS**

**Effect of Batimastat Given Concurrently with Cisplatin on Early-Stage HOC Xenografts.** The antitumor activity of batimastat was evaluated on HOC22 transplanted in the peritoneal cavity of nude mice. Treatment started 3 days after tumor cell injection (Fig. 1, protocol A) when mice showed minimal tumor burden with few tumor cells present in the peritoneal lavage and microscopic tumor deposits on the visceral organs (23). HOC22 induced tumor formation in all of the vehicle-treated mice, with a median survival time of 47 days (range, 35–51 days; Fig. 2). At autopsy, mice bearing HOC22 showed hemorrhagic ascites with gross tumor masses in the peritoneal cavity and in the pancreas, diaphragm, liver, and omentum. HOC22 was sensitive to cisplatin treatment, with an ILS of 162% (P < 0.001). Batimastat alone, though less active than cisplatin, also increased the survival time of the nude mice (ILS = 69%; P < 0.005). In contrast, batimastat given concurrently with cisplatin completely inhibited the growth of HOC22, and all mice were apparently tumor-free 200 days after tumor cell injection. The postmortem analysis revealed no evidence of tumor cells in the peritoneal lavages or of metastases in any of the organs examined, with the exception of three mice that showed micrometastases in the omentum. Mice treated with batimastat did not show sign of toxicity, and its administration in addition to cisplatin did not affect weight loss.

To investigate the effect of batimastat on the growth behavior of the tumor, two mice per group were killed and autopsied after the end of the treatment (Fig. 1, Day 20), and ascites and organs were collected for cytohistopathological analysis. Vehicle-treated mice had more ascitic fluid, which contained a higher number of tumor cells (mean = 170 × 10⁶ cells), than that of mice treated with either batimastat (mean = 63 × 10⁶ cells) or cisplatin (mean = 33 × 10⁶ cells). In contrast, few detectable cells (mean = 1.5 × 10⁶) were found in the peritoneal lavage harvested from mice treated with the combination of the two drugs. In addition, bulky masses of tumor cells were observed in the omentum, liver, diaphragm, pancreas, and lymph nodes of vehicle-treated mice, whereas only superficial micrometastases were observed in the omentum of one mouse treated with batimastat together with cisplatin. Animals treated with the individual therapies showed diffuse metastases in the omentum, diaphragm, pancreas, and liver, but to a lesser extent than did the vehicle-treated mice.

The potentiating effect of batimastat was dose dependent (Table 1). Batimastat, given at the maximum concentration of 60 mg/kg concomitantly with cisplatin, induced complete response in all of the mice. The combinations of cisplatin with 6 and 0.6 mg/kg of batimastat significantly (P < 0.001) increased the survival (ILS = 97 and 47%, respectively, compared to cisplatin-treated mice) and inhibited tumor growth in 50 and 25% of the mice, respectively. Batimastat alone at doses of 60 and 6 mg/kg marginally affected the survival of the mice but was not active at 0.6 mg/kg (data not shown). BB-1722 is the enantiomer of batimastat, and it is at least 1000-fold weaker in inhibiting MMPs. BB-1722, in combination with cisplatin, was active only at the highest dose of 60 mg/kg. However, BB-1722 was less effective than batimastat given at the same dose (40%
Table 1  Effect of different doses of batimastat and BB-1722 given concurrently with cisplatin

Nude mice were inoculated i.p. with HOC22 cells, and treatments started 3 days later (Fig. 1, protocol A).

<table>
<thead>
<tr>
<th>Treatment (dose in mg/kg)</th>
<th>No. of mice with tumor/no. of tumor-transplanted mice</th>
<th>MST* (range)</th>
<th>% ILS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-DDP + vehicle-BB-94</td>
<td>8/8</td>
<td>34 (32–35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDP + vehicle-BB-94</td>
<td>8/8</td>
<td>74 (68–80)</td>
<td>117*</td>
<td>0.001</td>
</tr>
<tr>
<td>DDP + BB-94 (60)</td>
<td>0/8</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>DDP + BB-94 (6)</td>
<td>4/8*</td>
<td>146 (107–161)</td>
<td>97*</td>
<td>0.001</td>
</tr>
<tr>
<td>DDP + BB-94 (0.6)</td>
<td>6/8*</td>
<td>109 (92–126)</td>
<td>47*</td>
<td>0.001</td>
</tr>
<tr>
<td>DDP + BB-1722 (60)</td>
<td>2/5*</td>
<td>108 (108–108)</td>
<td>45*</td>
<td>NC</td>
</tr>
<tr>
<td>DDP + BB-1722 (6)</td>
<td>5/5</td>
<td>75 (63–97)</td>
<td>1.3*</td>
<td>NS</td>
</tr>
<tr>
<td>DDP + BB-1722 (0.6)</td>
<td>5/5</td>
<td>75 (63–82)</td>
<td>1.3*</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Batimastat (BB-94) and BB-1722 at different concentrations and corresponding vehicle were administered i.p. every other day for eight injections; cisplatin (DDP 4 mg/kg) and corresponding vehicle were administered i.v. every 7 days for three injections.

** Evaluated 150 days after tumor cell injection.

*MST, median survival time.

NC, not calculable; NS, not significant.

Calculated on tumor-bearing mice.

** versus vehicle-treated mice.

Fig. 3  Response of early-stage HOC8 xenograft to batimastat given concurrently with cisplatin. Nude mice were inoculated i.p. with HOC8 cells, and treatments were started 3 days after the injection of tumor cells (Fig. 1, protocol A). Treatment groups were as in Fig. 2. Results are representative of two independent experiments.

tumor growth inhibition), and at doses of 6 and 0.6 mg/kg, it did not improve the response to cisplatin (Table 1).

The effect of batimastat was investigated on a second xenograft model, HOC8, established from a different patient with ovarian carcinoma (21). Like HOC22, HOC8 forms ascites and spreads to the visceral organs of the peritoneal cavity in nude mice. Fig. 3 shows that all of the vehicle-treated mice developed tumor in the peritoneal cavity with a median survival time of 95 days (range, 59–130). At autopsy, mice showed ascites and metastases to the liver, omentum, and diaphragm. As described previously (21), HOC8 was only marginally responsive to cisplatin, with this agent giving an ILS of 25% (P < 0.05), whereas batimastat alone did not significantly increase the survival time (ILS = 6.8%). The limited response to cisplatin and batimastat as single therapies was confirmed by the evaluation of tumor burden in two representative mice at the end of treatment (mean = 75 × 10⁶, 55 × 10⁶, and 30 × 10⁶ cells in the ascites of mice treated with vehicle, batimastat, and cisplatin, respectively). However, as with HOC22, concomitant treatment of batimastat with cisplatin (Fig. 1, protocol A) in the HOC8 model inhibited tumor growth (undetectable tumor cells in the ascites at the end of treatment), and all mice were tumor free 200 days after tumor cell injection (Fig. 3). The autopsy confirmed the absence of tumors in the peritoneal cavity of all of the mice, and the microscopic analysis revealed micrometastases in the omentum of only one mouse.

Effect of Batimastat Given Concurrently with Cisplatin on Advanced and Late-Stage HOC Xenografts. To determine the influence of tumor burden on the antitumor activity of the batimastat-cisplatin combination, mice bearing the HOC22 xenograft were treated at different stages of tumor development (Fig. 1, protocols B and C). When representative mice were autopsied at the beginning of treatments, 7 days after HOC22 inoculation (advanced tumor), ascites (mean = 15 × 10⁶ cells) and microscopic tumor deposits (omentum and liver) were detected; mice autopsied 20 days after HOC22 inoculation (late-stage tumor) showed a greater accumulation of ascites (mean = 100 × 10⁶ cells) and macroscopic tumor masses in the peritoneum with metastases in the omentum, diaphragm, pancreas, and liver (23).
Table 2  Effect of BB-94 given in combination with doxorubicin or cisplatin on advanced-stage HOC22 xenografts

Nude mice were inoculated i.p. with HOC22 cells, and treatments started 7 days later (Fig. 1, protocol B).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice with tumor</th>
<th>MSTa (range)</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8/8</td>
<td>62 (56-74)</td>
<td></td>
</tr>
<tr>
<td>DDP</td>
<td>8/8</td>
<td>74 (72-86)</td>
<td>19‡</td>
</tr>
<tr>
<td>ADM</td>
<td>0/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB-94</td>
<td>8/8</td>
<td>69 (57-81)</td>
<td>11d</td>
</tr>
<tr>
<td>BB-94</td>
<td>8/8</td>
<td>66 (57-85)</td>
<td>6d</td>
</tr>
</tbody>
</table>

a BB-94 (60 mg/kg) or vehicle-BB-94 were administered i.p. concurrently with cisplatin (DDP; 3 mg/kg, i.v.) or doxorubicin (ADM; 4.6 mg/kg, i.v.) three times, every 4 days.

* MST, median survival time.

† Evaluated 160 days after tumor cell injection.

‡ Not significantly different from vehicle-treated mice.

Concomitant administration of cisplatin with batimastat resulted in complete inhibition of the advanced tumors, with all mice surviving for more than 160 days (Table 2). Treatment of advanced tumors with cisplatin alone resulted in only a modest nonsignificant increase in survival (ILS = 19%; Table 2). The combined therapy was less effective in mice bearing late-stage tumors but still resulted in a significant increase in survival (ILS = 48%; P < 0.01; Fig. 4). In this group of treatment, neoplastic cells were indeed present in the ascites of the two representative mice autopsied at the end of treatment (mean = 150 × 10⁶ cells) with metastases to the peritoneal organs. Neither agent, when used alone, was effective in increasing survival relative to the vehicle control animals.

The experiment with the advanced tumors for which treatment began on day 7 was also used to test whether batimastat would potentiate the antineoplastic activity of another commonly used cytotoxic therapy, doxorubicin. Doxorubicin administered alone resulted in a small but nonsignificant increase in survival (ILS = 11%; Table 2), comparable to the activity shown by cisplatin in the advanced tumors (ILS = 19%). However, whereas the cisplatin-batimastat combination resulted in complete inhibition of tumor development, the doxorubicin-batimastat combination was no more active (ILS = 6%; Table 2) than doxorubicin alone. Autopsy of two mice from the doxorubicin-batimastat group at completion of therapy revealed the absence of tumor cells (mean = 30 × 10⁶ cells) in the ascites and visceral tumor deposits.

Effect of Batimastat Administration Subsequent to Cisplatin Therapy on HOC Xenografts. Because the treatment of mice bearing HOC22 with cisplatin alone inhibited tumor growth and increased the survival of mice, we investigated whether batimastat treatment administered after reductive therapy with cisplatin could potentiate its effect (Fig. 1, protocol D). This is analogous to the way batimastat has been proposed to be administered to patients with ovarian malignant ascites (24).

Treatment with batimastat started on day 20, 3 days after the end of therapy with cisplatin, and continued for an additional 2 weeks. Fig. 5 shows that batimastat administered after cisplatin delayed tumor growth and significantly increased the survival of the mice (ILS = 158%; P < 0.001), whereas batimastat given to mice that were not pretreated with cisplatin was inactive. This sequential treatment of batimastat after cisplatin was significantly more active than cisplatin given alone (ILS = 64%; P < 0.01), and two of the eight mice did not show any sign of tumor 200 days after tumor injection (5 months after treatment). Cytological analysis confirmed the absence of tumor cells in the peritoneal lavage of these two mice, but the histological analysis revealed metastasis to omentum, diaphragm, and liver.

Similar results were obtained with the sequential treatment of HOC8 tumors, although the improvements in survival were more modest probably due to the relative insensitivity of this xenograft to platinum therapy (cisplatin alone, ILS = 25%, and sequential cisplatin and batimastat, ILS = 52%, P < 0.001; Fig. 6). The median survival time of mice treated with batimastat but not receiving previous cisplatin treatment was not different from that of vehicle-treated mice.

In both experimental models, the autopsy of representative mice at the end of the sequential treatment confirmed the presence of tumor deposit and ascites in the peritoneal cavity, though to a lesser extent than in mice treated only with cisplatin.

**DISCUSSION**

The antitumor activity of cisplatin in HOC xenografts is well documented. Batimastat has also been shown to inhibit the development of ascites in two ovarian xenograft models (15). The results from this study extend these observations in showing that batimastat markedly potentiates the antitumor activity of cisplatin. Treatment of early-stage HOC22 xenograft with either cisplatin or batimastat alone caused a significant improvement in survival, although all animals were dead by day 130. Treatment of the same xenograft with the cisplatin-batimastat combination resulted in all animals surviving the 200-day observation period, without losing weight and in apparent good health. At necropsy, only three of eight animals showed evidence of disease, in each case in the form of micrometastases in the omentum. A similar response was observed with the HOC8 xenografts, with metastases detected in only one of eight mice at necropsy, although treatment with cisplatin or batimastat alone resulted in only a modest increase in survival. In both the HOC models, exacerbated body weight loss was not observed with combined therapy, thus indicating the lack of cumulative toxicity of batimastat treatment.

The "curative" effect of cisplatin-batimastat combination was also observed in the treatment of advanced HOC22 (day 7; Table 2), and even in the treatment of late-stage HOC22 (day 20; Fig. 4), the potentiation of cisplatin by batimastat was evident. Neither batimastat nor cisplatin alone were able to significantly alter the survival of the mice when treatment was initiated on day 20.

Batimastat significantly potentiated the antitumor activity of cisplatin, even at doses 10–100 fold less than the optimal dose of 60 mg/kg. In contrast, BB-1722 the enantiomer of batimastat, which is a weak inhibitor of the MMPs, potentiated the antitumor activity of cisplatin only at the highest dose, suggesting that the activity of batimastat is associated with its MMP inhibitory property. Furthermore, batimastat failed to potentiate the activity of doxorubicin, suggesting that the observed potentiation of the activity of cisplatin by batimastat is not a general feature of the combined use of MMP inhibitors with cytotoxic agents.
Fig. 4 Response of late-stage HOC22 xenograft to batimastat given concurrently with cisplatin. Nude mice were inoculated i.p. with HOC22 cells, and treatments were started 20 days later (Fig. 1, protocol C). All other details are as in Fig. 2. Results are representative of two independent experiments.

Fig. 5 Response of HOC22 xenograft to batimastat given after cisplatin. Nude mice were inoculated i.p. with HOC22 cells, and treatment with cisplatin started 3 days later, followed by batimastat at day 20 (Fig. 1, protocol D). Treatment groups were: cisplatin treatment starting on day 3 followed by batimastat treatment starting on day 20 (DDP→BB-94); cisplatin alone starting on day 3 (DDP); batimastat alone starting on day 20 (BB-94); and control mice treated with vehicle-DDP from day 3, followed by vehicle-BB-94 from day 20 (vehicle). BB-94 (60 mg/kg) and vehicle-BB-94 were given i.p. every other day for eight injections; and DDP (4 mg/kg) and vehicle-DDP were given i.v. every 7 days for three injections. n = 8 mice per group. Results are representative of two independent experiments.

One possible explanation for the potentiation of the antineoplastic activity of cisplatin is that the concomitant administration of batimastat alters the pharmacokinetics of cisplatin, increasing the circulating concentration of the cytotoxic therapy. The absence of obvious signs of toxicity (body weight and in-life signs) in mice receiving the combination argues against this, as does the observation that batimastat does not alter the LD_{50} for cisplatin in mice. Another possibility is that batimastat is sensitizing the ovarian carcinoma cells to cisplatin. Batimastat is designed to interact with the zinc metal atom in the active site of MMPs, and some nonspecific interactions with platinum or platinum-protein complex might alter the uptake or clearance of cisplatin by tumor cells. Again, this seems unlikely because the batimastat-zinc interaction is dependent on the precise conformation and stereospecificity of the metalloproteinase active site. Unfortunately, neither HOC22 nor HOC8 can be maintained in vitro, precluding many simple analyses of cytotoxicity. However, previous studies have shown that batimastat has little or no direct cytotoxic activity in its own right (10, 11, 13, 14). We explored this possibility using the HOC cell line SKOV-3, which has a sensitivity to cisplatin in vitro that was not significantly increased by the presence of batimastat (data not shown). These findings, together with the data correlating the therapeutic efficacy of the combination with low tumor burden, argue in favor of a cytostatic effect of batimastat.

It must also be considered that batimastat is administered to

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4 British Biotech, personal communication.
plays a role in increasing vascular permeability (27), a process vascular tissue around clumps of ascitic cells, consistent with an batimastat treatment of ovarian carcinoma xenografts by Davies cells to activate MMP-2 that has been shown by in situ activator of MMP-2 (25). This may allow ovarian carcinoma batimastat as a single agent. A recent study has also shown that explain the greater sensitivity of the former to treatment with in HOC lular matrix by tumor-induced MMPs. In a previous study of combination, is that it acts by inhibiting the breakdown of extracel-

However, the results from the current study also show that sequential treatment with cisplatin followed by batimastat leads to an improvement in survival over cisplatin therapy alone. In this case, the benefits of sequential therapy were more apparent in HOC22 than in the HOC8 xenograft, which is less sensitive to cisplatin. These results suggest that the efficacy of batimastat might depend on an initial cytoreductive action by cisplatin.

The most straightforward mechanistic explanation for the efficacy of batimastat in these models, both alone and in combination, is that it acts by inhibiting the breakdown of extracellular matrix by tumor-induced MMPs. In a previous study of batimastat treatment of ovarian carcinoma xenografts by Davies et al. (15), there was evidence of accumulation of dense, avascular tissue around clumps of ascitic cells, consistent with an inhibition of extracellular matrix turnover. In this study, there was less evidence of stromal development, although a desmoplastic reaction was observed around some micrometastases (data not shown). The presence of the gelatinases MMP-2 and MMP-9 in the HOC xenografts has been demonstrated by zymography (data not shown). Indeed, HOC22 ascites was found to contain more gelatinase than that of HOC8, and this may explain the greater sensitivity of the former to treatment with batimastat as a single agent. A recent study has also shown that some HOC cell lines possess a plasma membrane-associated activator of MMP-2 (25). This may allow ovarian carcinoma cells to activate MMP-2 that has been shown by in situ hybridization to be present in the adjacent stroma (26). Furthermore, recent studies have shown that a 96-kDa gelatinolytic MMP plays a role in increasing vascular permeability (27), a process linked to ascites formation (28). Whether batimastat influences ascites formation by interfering with vascular permeability deserves further investigation.

Alternative explanations for the potency of the combination may lie in the interaction between MMPs and cytokines. MMP inhibitors have been shown to inhibit the release from cells of various cytokines, most notably TNF-α (29, 30). Because cytokines such as TNF-α and IL-1 are known to influence tumor progression (31, 32), it is possible that a cytokine-dependent mechanism underlies the increased activity of the cisplatin-batimastat combination. Again, the inability to maintain the HOC ovarian carcinoma cells in vitro makes further investigations of such putative interactions difficult. One approach may be to identify a HOC xenograft that can be studied in vitro and in vivo and to look for similar activity from the cisplatin-batimastat combination.

Results from this study of the combined use of cisplatin and batimastat in the HOC xenografts have obvious relevance to the clinical management of ovarian cancer. Approximately 60–80% of ovarian cancer patients with advanced disease will respond to platinum-based combination therapy, with “complete remissions” in 40–50% of patients. However, 5-year survival for ovarian cancer remains low, at 30–35%. The reason for treatment failure lies in residual disease that becomes progressively resistant to platinum chemotherapy with successive courses. The use of cisplatin and batimastat as sequential treatments has already been applied in the clinic (24, 33). This study raises two intriguing possibilities that warrant further investigation, both experimentally and clinically. The first is that concomitant administration of cisplatin and batimastat appears far more effective than sequential administration in increasing the survival of mice with early- or advanced-stage disease. The second is that the combination treatment is effective also on the poorly platinum-sensitive HOC xenografts. Although it is not possible from these limited experiments to tell whether this reflects restoration of platinum sensitivity, it would seem to justify further study.

REFERENCES

Batimastat, a synthetic inhibitor of matrix metalloproteinases, potentiates the antitumor activity of cisplatin in ovarian carcinoma xenografts.

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