The Influence of Ovarian Cancer Induced Peritoneal Carcinomatosis on the Pharmacokinetics of Albendazole in Nude Mice

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Abstract. *Angiogenesis in the peritoneal cavity arising from ovarian cancer leads to peritoneal carcinomatosis, malignant ascites formation, morbidity and high mortality. Recent studies in our laboratory have shown albendazole (ABZ) to be a potent inhibitor of angiogenesis and malignant ascites formation. The current study was designed to find the pharmacokinetics of the drug and its major metabolites albendazole sulfoxide (ABZSO) and albendazole sulfone ($ABZSO_2$) in an experimental model of ovarian cancer. Additionally, we sought to investigate if the cancer-induced changes in the peritoneal cavity would affect the kinetics of ABZ. On this basis, ABZ was administered 150 mg/kg intraperitoneally to groups of mice bearing peritoneal carcinomatosis and also to groups of healthy mice with no tumor. Blood and peritoneal wash samples were collected for up to 72 h. Concentration of ABZ and its metabolites in the samples were analysed by an established high performance liquid chromatography method. In the healthy mice, drug and metabolite concentrations were found to be low to undetectable. On the contrary, tumor-bearing mice had higher levels of ABZSO in both the plasma and their peritoneal wash. This may at least in part be attributed to the high vascular endothelial growth factor levels present in the peritoneal cavity of the diseased mice. The data obtained in this study suggest that peritoneal carcinomatosis changes ABZ absorption from the peritoneal cavity.*

Abbreviations: ABZ, Albendazole; ABZSO, albendazole sulfoxide; $ABZSO_2$, albendazole sulfone; HPLC, high performance liquid chromatography; VEGF, vascular endothelial growth factor.

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Key Words: Albendazole, peritoneal carcinomatosis, angiogenesis, ovarian cancer, vascular endothelial growth factor.

Ovarian cancer is the sixth most commonly diagnosed cancer among women in the world and on an estimated basis 204,000 new cases are diagnosed and 125,000 women die of the disease each year (1). The majority of patients with ovarian cancer present late with advanced disease (FIGO stage III-IV) and in this group of patients, despite multimodality treatment with surgical debulking followed by platinum-taxane combination chemotherapy, the 5-year survival rate is dramatically poor (2). The disease is characterized by widespread intraperitoneal carcinomatosis and the formation of large volume of malignant ascites (3, 4). Vascular endothelial growth factor (VEGF; initially known as vascular permeability factor) is thought to play a major role in the progression of ovarian cancer by promoting neovascularization and subsequent growth of intraperitoneal tumors and by inducing ascites formation through enhancement of the vascular permeability (5, 6). Both pre-clinical and clinical studies support the key involvement of VEGF in the pathophysiology of ovarian cancer (7). On this basis, new treatment approaches utilizing agents which target VEGF are under study in the treatment of ovarian cancer.

ABZ (ABZ; methyl 5-propylthio-1H-benzimidazol-2-yl carbamate) is a potent broad-spectrum anthelmintic drug widely used in human and veterinary medicine with a good safety profile (8). Used at higher dosages and for long durations, ranging from a few weeks for cystic echinococcis to life-long for alveolar echinococcosis, benzimidazoles have deeply modified the management of these patients and their life expectancy (9). After oral administration, ABZ is extensively metabolized by a sequence of oxidations on the sulphur atom leading to the formation of the active metabolite, ABZSO part of which is further oxidized to an inactive metabolite albendazole sulfone ($ABZSO_2$) (10). We have shown that in comparison to oral administration, intraperitoneal administration of ABZ leads to a slower and more sustained absorption of ABZ (11). In recent years, we have shown ABZ to be a potent antiproliferative and antitumor agent in a number of cancer types including hepatocellular

(12) colorectal (13) and also in leukemia cells resistant to paclitaxel and epothilone (14). Additionally, in pre-clinical studies, ABZ has been found to suppress VEGF and abrogate malignant ascites fomation (15, 16). Other benzimidazole carbamates such as mebendazole have also been shown to possess potential anticancer therapeutic properties (17-19).

The current study was thus designed to gain insight into the pharmacokinetics of ABZ in the OVCAR-3 nude mice experimental model of ovarian cancer-induced peritoneal carcinomatosis. Additionally, by investigating the pharmacokinetics of ABZ in normal mice (no tumor or disease), we sought to gain an idea of how the ovarian cancer-induced pathophysiological processes in the peritoneal cavity may influence the ABZ kinetics. Compared to normal mice, mice with peritoneal carcinomatosis had higher concentrations of ABZSO present in both their plasma and their peritoneal wash. These results reveal for the first time that underlying pathological condition (excessive peritoneal angiogenesis) may have an impact on ABZ/ABZSO kinetics. These findings may have significant clinical implications.

Materials and Methods

Drug preparation. ABZ was prepared as a 3 mg/ml suspension in 0.5% hydroperoxy methylcellulose (HPMC; Sigma-Aldrich, Sydney Australia). All vehicle and drugs were administered intraperitoneally (*i.p.*) as 1 ml/20 g of body weight and the volume of injection was adjusted so that each test animal received the specified dosage.

Development of intraperitoneal tumors. Female, 6- to 8-weeks-old nude athymic BALB/c *nu/nu* mice (Animal Resources Centre, Perth, Western Australia) were used for all experiments. Mice were housed under complete aseptic conditions and were fed autoclaved pellets and sterile water *ad libitum*. Health status of each animal was monitored daily and all animal procedures were conducted in conformity with institutional animal ethics committee guidelines (Animal Ethics Committee, University of New South Wales, Sydney Australia). The OVCAR-3 cells were originally obtained from the American Type Culture Collection (ATCC), and prepared for *in vivo* growth as previously described (15). Of the 90 mice used for this study, 60 were injected *i.p.* with 10 million OVCAR-3 cells suspended in 1 ml of the medium (RPMI 1640). Control mice (n=30) were given an equal volume of the medium without tumor cells. All animals were monitored ×3 weekly for 3 weeks.

Drug administration and sample collection. At the end of 3 weeks, mice in each group (n=2) were randomly assigned to one of the 6 treatment sub-groups (n=5). Before proceeding with drug or vehicle administration, all mice (control or peritoneal carcinomatosis) were subjected to peritoneal lavage (2 ml of sterile normal saline injected *i.p.* and aspirated immediately after kneading). Animals were then given an *i.p.* injection of the vehicle or ABZ suspension (150 mg/kg) and were euthanased at the predetermined time (0, 1, 6, 24, 48 and 72 h). Group 1 animals were treated with the vehicle and euthanized immediately to check established disease (VEGF levels and tumor growth). Group 2-6 animals were treated with ABZ (150 mg/kg) and euthanased at 1, 6, 24, 48 or 72 h post injection respectively. Similarly, control mice (no tumor) were treated with the vehicle and euthanased at the same indicated times. Before euthanasia, the peritoneal cavity was washed with 2 ml of normal saline and a blood sample was collected through cardiac puncture under anaesthesia. Aliquots of plasma and the centrifuged cell-free aspirate (peritoneal wash) were stored at –80˚C for subsequent analysis.

Determination of ABZ and metabolites in peritoneal wash and plasma. ABZ, ABZSO and $ABZSO_2$ concentrations in cell-free peritoneal wash and plasma were quantified using a validated high performance liquid chromatography (HPLC) method as previously described (11). Briefly, peritoneal wash and plasma samples were extracted by placing 200 µl of the sample in a glass tube. Sodium metabisulphite (100 µl) and 2 ml of ethyl acetate were added. Each tube was vortexed for 10 min followed by shaking gently for 20 min on a shaker. After centrifugation at 2000 rpm for 10 min, the organic layer was removed and evaporated in a Thermo Savant rotary vacuum chamber (Thermo Electron Corporation, Melbourne, Australia). The residue was resuspended in 200 µl of methanol for HPLC analysis. All data were recorded and analysed on a computer with Class-VP Chromatography Data System Software (Shimadzu, Sydney, Australia). Concentrations of ABZ, ABZSO and $ABZSO_2$ in the peritoneal wash and the plasma samples were determined by reference to corresponding standard curves, calculating the mass of samples, multiplying with appropriate dilution and recovery factor. Results are presented as mean±S.E.M.

Statistical analysis. Pharmacokinetic and statistical data analyses were performed using GraphPad In Stat on Prism version 5.0 (GraphPad Software, San Diego, California, USA). Comparison of means was made using *t*-test. *P*-values of 0.05 and smaller were considered to be statistically significant.

Results

ABZ, ABZSO and $ABZSO_2$ concentrations in the peritoneal wash. In order to assess both the pharmacokinetics of ABZ and the impact of the disease on it, the study was conducted in both *i.p.* tumor bearing mice and in healthy normal mice at the same time. All mice inoculated with the tumor cells had developed tumors (186±47 mg) and malignant ascites (1.66±0.31 ml) confirming establishment of the disease. To further verify this, VEGF concentrations in the cell-free peritoneal wash were measured by means of a standard enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (Quantikine R& D systems, Minneapolis, USA). Compared to undetectable values in the control mice, at 3 weeks post cell inoculation, the VEGF concentration in the peritoneal wash of tumor-bearing mice were 2279±359.2 pg/ml (mean±S.D.).

ABZ, ABZSO and $ABZSO_2$ concentrations found in the peritoneal wash of mice at different time-points after ABZ administration are presented in Figures 1A to 1C respectively. As anticipated, the ABZ concentrations were extremely low in the control group and even undetectable in the diseased mice. This is in line with the literature showing

Table I. *AUC values for ABZ, ABZSO and $ABZSO_2$ following i.p. administration of a single dose of ABZ (150 mg/kg).*

	Plasma			Peritoneal wash		
	ABZSO	$ABZSO_2$	ABZ	ABZSO	$ABZSO_2$	ABZ
Control mice	40.3	19.8	0.9	30.7	4.6	10.0
Mice with PC	86.5	29.0	-	35.0	6.7	-

AUC, Area under the concentration-time curve (μg/ml × h); ABZ, albendazole; ABZSO, albendazole sulfoxide; $ABZSO_2$, albendazole sulfone.

that ABZ is rapidly absorbed (20, 21). Comparison of the ABZSO concentrations between healthy and tumor bearing mice (Figure 1B) revealed a difference ($p<0.01$) between the two groups at 1 h post administration. However, the concentrations of ABZSO rapidly declined over 6 h. Concentrations of the sulfone metabolite ($ABZSO_2$) were also higher in the peritoneal cavity of mice with peritoneal carcinomatosis. The area under the concentration-time curve for ABZ, ABZSO and ABZSO2 are presented in Table I.

Plasma ABZ, ABZSO and $ABZSO_2$ concentrations. Most pharmacokinetic studies conducted in animals or man have failed to detect ABZ within the serum or the plasma of the treated subject (22, 23). This is because ABZ is highly metabolized in the liver with an extensive first-pass effect (24). Accordingly, here, in the majority of mice, plasma ABZ levels were either unreliably low or below the detection limit. Plasma ABZSO concentrations are presented in Figure 2B. An increase in ABZSO concentrations in mice with peritoneal carcinomatosis is obvious. In mice with peritoneal carcinomatosis, the concentration of ABZSO gradually decreased over 24 h. On contrary, in control mice, ABZSO concentration peaked at 6 h and then gradually decreases. This suggests that there is a difference in the metabolic rate between the two groups of mice. The $ABZSO_2$ plasma concentrations were also higher in the tumor bearing mice (Figure 2C). It is evident that the ABZSO concentrations are higher in both the peritoneal wash and the plasma of mice bearing peritoneal tumors (Table I). These unprecedented levels of ABZSO detected in the plasma and in particular in the peritoneal cavity of mice with cancer correlate with the high peritoneal VEGF levels.

Discussion

In this study, we have compared the effect of peritoneal carcinomatosis on the pharmacokinetics of ABZ. Similar to other reports, ABZ was rapidly metabolized hence the level of detectable ABZ was very low. We found higher concentrations of ABZSO both in plasma and peritoneal wash samples collected from animals bearing tumors.

It was previously shown that *i.p.* administration of chemotherapeutic drugs lowers systemic toxicity (25, 26). Intraperitoneal administration of drugs allows direct instillation of the chemotherapy into the peritoneal cavity thus bypassing the cellular enclosure barriers preventing systemic chemotherapy to adequately reach the cavity. Consequently, some of the most commonly used agents in the chemotherapy of peritoneal carcinomatosis including the platinum salts, the taxanes, doxorubicin, gemcitabine and mitomycin-C are used intraperitoneally (27).

In addition to the physio-chemical properties of the drug in use, it has been shown that systemic drug absorption is dependent on the permeability of the peritoneal membrane, the concentration difference of chemotherapy in the peritoneal cavity *versus* the plasma, and the size of the peritoneal surface exposed to the chemotherapy (28). In patients with ovarian cancer and malignant ascites formation, increased cross sectional area of microvessels lining the peritoneal cavity and their role in producing surplus fluid has been shown (29). Additionally, the injured peritoneum is a rich source of cytokines and growth factors and in particular VEGF which plays a central role in promoting angiogenesis and vascular hyper-permeability (5, 30, 31). Under the influence of VEGF and other pro-angiogenic-inflammatory factors, the vessels become dilated, tortuous, leaky and lack the tight endothelial junctions of normal blood vessels (32). Consequently, permeability to water and macromolecules is increased and solute delivery to tissues is uncontrolled (33).

With ABZ being an oral anthelmintic therapy, ABZ pharmacokinetics after oral administration has been extensively studied in a number of species including mice (10). This however, is the first study to report ABZ kinetics in healthy or cancer bearing nude mice after *i.p.* administration. Most studies have found that after oral administration, ABZ is undetectable in plasma due to the high first-pass metabolism (34). This is because in the liver, ABZ is rapidly oxidized to ABZSO (active metabolite), part of which is later converted by a second oxidation to the inactive metabolite $ABZSO_2$ (22, 23). The oxidations are carried out by cytochrome P-450 and microsomal flavin-containing monooxygenases (23, 35). Due to undetectable plasma levels, the actual half-life of ABZ has never been reported. In line with the literature, it is evident from our results that, ABZ is rapidly metabolized thus leading to extremely low plasma levels. This trend was profoundly more evident in mice bearing peritoneal malignancy. It has been shown that, mice injected *i.p.* with tumor cells undergo extensive peritoneal vascularization with increased microvascular permeability, an effect that correlates well with VEGF levels (6, 36-39). The disease-induced vascular changes in the peritoneal cavity can on one hand lead to increased drug absorption and on the other, enables the secretion of the parent drug or its metabolites from blood

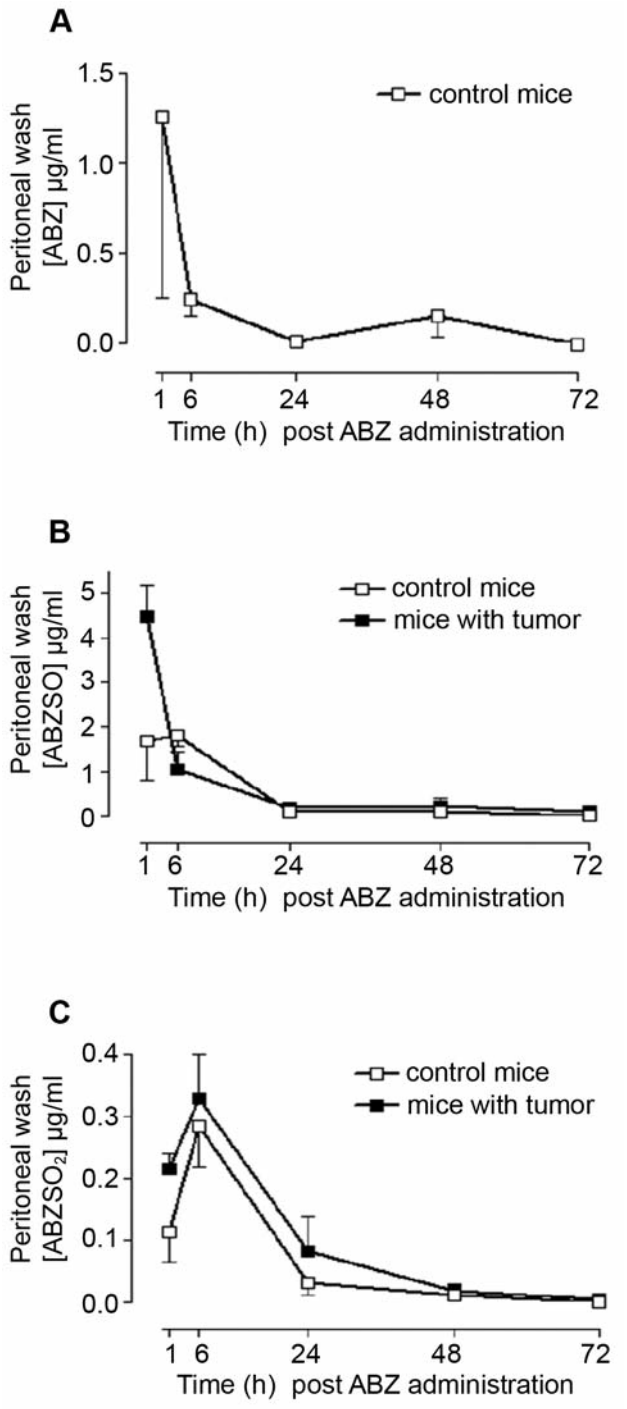

Figure 1. *Time courses of peritoneal concentrations (mean±S.E.M.) for ABZ (A) and its two metabolites ABZSO (B) and $ABZSO_2$ (C) after intraperitoneal administration of a single dose of albendazole (ABZ; 150 mg/kg) to healthy and mice with peritoneal carcinomatosis. Groups (n=5) of mice were euthanized at the predetermined times of 0, 1, 6, 24, 48 or 72 h post ABZ administration. Parent drug and metabolite concentrations in the aspirated cell free peritoneal wash were determined using an automated HPLC system. ABZ was not detectable in the peritoneal wash samples of mice with peritoneal carcinomatosis. Bars, S.E.M.*

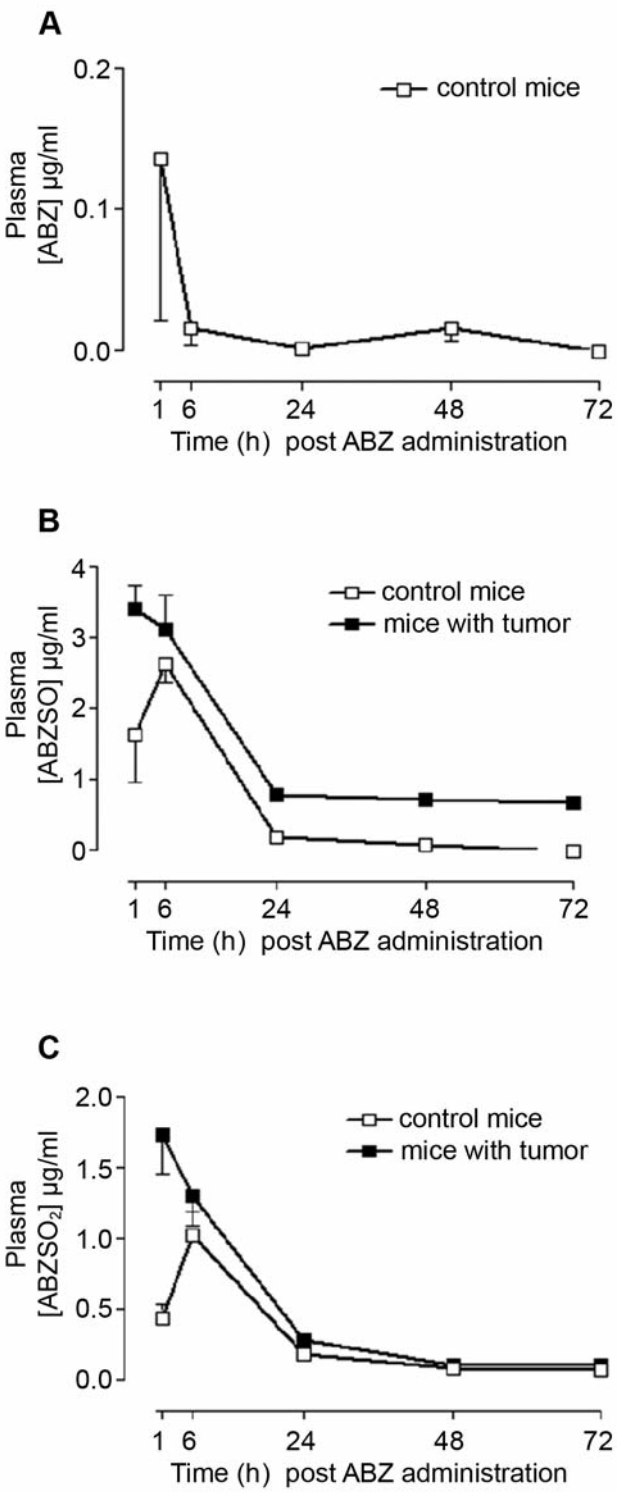

Figure 2. *Time courses of plasma concentrations (mean±S.E.M.) for ABZ (A), ABZSO (B) and $ABZSO_2$ (C) after the intraperitoneal administration of a single dose of albendazole (ABZ; 150 mg/kg, i.p.) to control and tumor-bearing mice. Groups (n=5) of mice were euthanized at the predetermined times of 0, 1, 6, 24, 48 or 72 h post ABZ administration. ABZ was not detectable in the peritoneal wash samples of mice with PC. Bars, S.E.M.*

back into the peritoneal cavity. The higher ABZSO concentrations in mice with peritoneal carcinomatosis may be explained by the vast vascularization of the peritoneal cavity plus the fact that under the influence of VEGF, these vessels become highly permeable thus allowing exchange of molecules between the two compartments (6, 37). Consequently, ABZSO entering the systemic circulation is secreted into the peritoneal cavity through highly permeable vessels within the peritoneal cavity. This is better illustrated when the plasma concentrations are also taken into consideration.

In summary, we have shown that in this model of ovarian cancer-induced peritoneal carcinomatosis, the peritoneal disease arising from the intraperitoneal tumor growth leads to changes in the pharmacokinetics of ABZ. High concentrations of the sulfoxide metabolite were detected in the peritoneal wash of the tumor bearing mice, a phenomenon never reported before. Moreover, a close correlation between plasma and peritoneal ABZSO concentrations was documented. These findings indicate that the tumor-induced pathological changes in the peritoneal cavity may influence the pharmacokinetics of *i.p.* administered drugs and hence the necessity for further investigations.

Acknowledgements

This work was supported in part by a grant-in-aid from the Lady Fairfax Foundation Sydney, NSW, Australia.

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Received November 30, 2009
Accepted January 11, 2010