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The overall goal of these pre-clinical studies is to optimize the therapeutic potential of the novel agent, swainsonine, for utilization in the management of myelosuppression associated with high-dose chemotherapy, a method of treatment currently being used for human metastatic and high-risk primary breast cancer. Our initial studies have focused on determining the optimal dose, schedule and timing of swainsonine treatment to protect against the toxicity associated with multiple cycles of chemotherapy, administered similar to that used in humans. Our studies have clearly demonstrated, for the first time, that survival in mice receiving methotrexate and swainsonine is 7-fold greater than that in mice receiving methotrexate only. Thus swainsonine is able to significantly protect mice from the chemotoxicity associated with up to 3 cycles of methotrexate. The relationship between this increase in survival and swainsonine's known effect on hematopoietic cells is currently under study in non-tumor bearing and tumor-bearing mice.

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INTRODUCTION

Breast cancer is now the most common disease for which high-dose chemotherapy and autologous bone marrow transplantation is performed in the United States, surpassing non-Hodgkin's and Hodgkin's disease as an indication. Increasingly, this therapeutic modality is being used earlier in the disease, particularly in the high-risk adjuvant setting. During the past decade, dose intensification has been a recurrent theme in the attempt to devise new clinical approaches to various neoplasms, particularly the solid tumors such as breast (1,2). Whereas adjuvant chemotherapy has been demonstrated to produce modest but consistent improvement in long-term disease-free and overall survival, in patients with primary breast cancer (3), these improvements are achieved with chemotherapy programs that are not curative in metastatic disease. Further, there is increasing evidence both from retrospective analyses (4) and from prospective randomized clinical trials that dose intensification is sometimes associated with superior outcomes in metastatic (5-7) and primary breast cancer (8). The concept of high-dose chemotherapy with autologous bone marrow (BM) support was initially developed to circumvent the problem of chemotherapy dose related myelosuppression. The use of autologous marrow allows intensification of chemotherapy doses so that the maximal tolerated dose of a chemotherapeutic regimen is determined not by extent of myelosuppression but rather by effects on the patient's nonhematopoietic organs. However, autologous bone marrow transplantation has its limitations. Despite the use of autologous BM, high-dose chemotherapy is still associated with prolonged periods of myelosuppression during which time the patient is highly susceptible to a variety of bacterial and fungal processes (9). Recently, recombinant colony stimulating factors [CSF] (10-12), particularly when coupled with CSF-primed peripheral blood progenitor cells (13), have enabled a reduction in the duration of severe leukopenia and in the morbidity associated with bone marrow transplantation. However, these colony stimulating factors have not been as effective in ameliorating the therapy related thrombocytopenia and subsequent requirement for platelet transfusions (13). Phase I clinical trials are currently in progress using IL-11 to stimulate megakaryocyte differentiation, however a possible side effect of this therapy is known to be anemia. Consequently, considerable effort is continuously being expended to develop therapeutic strategies employing new agents and/or combinations of cytokines which will have the capability of not only further eradicating the neutropenia but also the thrombocytopenia associated with high-dose chemotherapy.

Over the past several years, our laboratory has focused on the biological mechanism(s) underlying the myeloproliferative properties of **swainsonine**, an indolizidine alkaloid which has been reported by our laboratory and others to mediate varied biological effects including the inhibition of tumor growth (14,15) and metastasis (16-19). Most exciting are the recent findings which demonstrate that swainsonine mediates an array of immunomodulatory activities including the augmentation of cytotoxicity by natural killer (13,20) and lymphokine-activated killer cells against allogeneic (21-23) and autologous tumor cells (24). In further studies, we have demonstrated that swainsonine stimulates macrophage tumoricidal activity and the stimulation of tumoricidal activity is associated with increased secretion of interleukin-1 and expression of Class II major histocompatibility antigen (25). Swainsonine's

ability to activate these cytotoxic effector cells are suggestive of its potential as a cancer therapeutic agent.

Swainsonine was felt to be a logical candidate of exogenous stimulation of bone marrow proliferation because of its ability to elicit endogenous production of IL-1 (25), IL-2 (26), tumor necrosis factor (27) and possibly other polypeptides that regulate growth and differentiation. We have shown that systemically administered swainsonine promotes the proliferation of murine bone marrow cells ***in vivo,*** as measured by increase in bone marrow cellularity, engraftment efficiency and colony forming unit activity. Bone marrow cells from swainsonine-treated animals also exhibited an increase in [^{3}H]-thymidine incorporation, suggesting that a larger fraction of the cells were in the S-phase of the cell cycle (28). Similar findings have been reported for swainsonine stimulated murine (29) and human (30) lymphocytes. As expected, in a non-tumor bearing murine model, swainsonine proved to be highly efficacious in preventing the bone marrow toxicity associated with the intravenous administration of high doses of chemotherapeutic agents commonly used to treat human tumors [e.g. methotrexate, 5-fluorouracil, cyclophosphamide, and doxorubicin] (31). The protective effect could also be demonstrated with both murine and human bone marrow cells exposed to 3'-azido-3'deoxythymidine under ***in vitro*** culture conditions (27,32). Interestingly, a Phase I clinical trial in Canada suggests that swainsonine produces minimal toxicity when administered intravenously to cancer patients at dosages that inhibit both Golgi 'α-mannosidase II and lysosomal'-mannosidases. However, more significant toxicity was observed in patients with hepatic metastasis or liver enzyme abnormalities (31).

The studies cited above demonstrate that swainsonine is a novel immunomodulatory agent that may have immediate clinical application. The antimetastatic and myeloproliferative properties of swainsonine, coupled with its ability to stimulate the cytotoxicity of non-specific immune effector cells, strongly support its potential usefulness in high-dose chemotherapy programs in humans.

Because breast cancer is such a common disease, the approach of high dose therapy with bone marrow support is too complicated and expensive to be widely utilized. Unless we understand the toxicities and develop strategies to minimize them, even the demonstration of an effective treatment method will unlikely be of major value to a significant number of patients. The need for the development of modalities of treatment utilizing combinations of early- and late-acting cytokines which will mimic the "natural maturation process" as well as the development of new, non-toxic, inexpensive agents which are effective enhancers of hematopoietic engraftment and ***ex vivo*** growth are of high priority. The pre-clinical studies undertaken in this grant focus on optimizing the therapeutic potential in human cancer of the agent swainsonine and providing the requisite data supportive of getting swainsonine entered into clinical trials here in the United States.

BODY

Methods and Procedures:

Animals: Pathogen-free B6C3F1 female mice, obtained from Charles River Laboratories, were 18-20 grams when received and allowed to rest for one week before use in experiments. Mice were maintained in isolation cubicles in filter top cages, according to all institutional ethical guidelines.

Drugs: Methotrexate (MTX) was obtained from Immunex(R), Lederle Parentals, Inc. Carolina, PR and doxorubicin/adriamycin (ADR) from Pharmacia S.P.A., Milan Italy. Both chemotherapeutic agents were dispensed by the pharmacy here at Duke University. Swainsonine was purchased from Toronto Research Chemical, Inc., Toronto, CN.

Determination of LD_{50}: Groups of 10 female mice were given equal logarithmic-spaced doses of single-bolus intraperitoneal injections of chemotherapeutic drugs (MTX) and adriamycin (ADR). The number of deaths was recorded for each drug over an observtion period of 14 days. The median lethal dose (LD_{50}) was obtained for these two drugs, MTX and ADR, from semilogarithmic plots of percent death versus dose; the median lethal dose is defined as the amount of drug resulting in 50% killing within 14 days.

Swainsonine Treatment Protocols: To investigate the ability of swainsonine to abrogate lethality following administration of 2-3 cycles of a single bolus LD_{50} of MTX or ADR, mice were individually weighed and numbered and the amount of chemotherapy calculated for each mouse based on kilogram body weight. This was done each time chemotherapy was administered. We utilized our previously determined schedules for time of initiation and duration of swainsonine treatment (31). Twice daily intraperitoneal injections (20ug/day) of swainsonine were initiated on day +2, with administration of a single bolus dose of methotrexate on day 0. Swainsonine was administered for 10 consecutive days and the survival of control groups and swainsonine-treated groups was monitored. Since the present studies, for the first time, involved multiple cycles of chemotherapy drugs, we varied the interim time (rest period) between cycles of MTX and swainsonine, to determine the optimal schedule for overall survival. Following a rest period of 7, 10 or 14 days, the cycle of MTX, day 0, followed by swainsonine on days +2 to +12 was repeated. Whereas the calculation of the LD_{50} was based on a 14-day end point, survival for experiments was determined based on a 30-day end point following the last MTX injection. In all of the experiments to be described, two groups of control animals were used. The first group received twice-daily intraperitoneal injections of MTX plus isotonic saline (plain vehicle), and the second group received isotonic saline without therapeutic drug.. Generally, 50% or more of the mice in the drug treated control group died during the course of the experiment, whereas none of the animals in the saline-only control group died. All experiments were terminated 30 days following the last injection of the chemotherapeutic agent.

Tumors: The murine mammary tumor MC-2 was obtained as a frozen stock tissue culture from Dr. Jan Vaage of Roswell Park Cancer Institute, Buffalo, NY. Cells were thawed and maintained in RPMI 1640 tissue culture media, supplemented with 10% fetal bovine serum, L-glutamine (2mM/ml), penicillin (100U/ml) and streptomycin (100ug/ml), in a 37 C humidified atmosphere of 5% CO_2, 95% air. To determine the ability of these cells to grow *in vivo* and the number of cells needed to obtain tumors 0.5-1.0 cm in diameter, MC-2 cells, which had been expanded in cell culture, were washed once to remove all serum containing medium, then resuspended in sterile phosphate buffered saline and varied numbers of cells injected subcutaneously into B6C3F1 female mice weighing 18-20g. Mice were observed daily to determine the rate and size of tumor growth. Experiments were terminated at 21 days.

Blood Analysis: Samples of blood were obtained by intraorbital bleeding of mice with a capillary tube. One drop was added to a slide for differential analysis and the complete blood count (CBC) was done automatically on a System 9000 blood analyzer (Serono-Baker Diagnostic, Inc, Allentown, PA.). All analysis were performed by the Diagnostic Services Laboratory of the Duke University Medical Center Vivarium.

Results and Discussion:

LD_{50} determinations: In our laboratory, we had previously determined the LD_{50} for both methotrexate and adriamycin, however, these experiments were done with different sources of drugs and most importantly utilized a different strain of mice (C57BL/6) The mice used in our present studies are B6C3F1, which is the F1 cross of C57BL/6 and C3H. Thus, it was essential that we determine the LD_{50} for each drug in these mice. Forty female mice, 8-12 weeks of age (18-20g) were divided into groups of 10 and were given equal logarithmic-spaced doses of a single-bolus intraperitoneal injection of either methotrexate (MTX) or doxorubicin/adriamycin (ADR). Mice were observed twice daily for 14 days and the number of deaths recorded. The median lethal dose (LD_{50}) was obtained for these two drugs, MTX and ADR, from semilogarithmic plots of percent death versus dose; the median lethal dose is defined as the amount of drug resulting in 50% killing within 14 days. A number of experiments were performed to obtain the appropriate range of drug to use to determine the LD_{50}. Initially we observed significant variation from experiment to experiment relative to effective doses, however when we changed vendors for the source of our mice the results stabilized somewhat. In several experiments we ended up with only two points to determine our LD_{50}. We consulted with the head of the Phamacology Monitoring Lab in our Bone Marrow Transplant Program and was advised that our results for MTX were not uncommon and duplicates were within the range of experimental error for the agent. Figures 1 and 2 illustrate representative experiments for determining the LD_{50} for MTX and ADR. In Table I we have summarized our data which indicates that the calculated LD_{50} doses for MTX and ADR are 1965mg/kg and 34.25mg/kg respectively.

Chemoprotective effects of swainsonine in mice receiving multiple cycles of methotrexate: In previous experiments, we have demonstrated the ability of swainsonine to decrease the lethality of both cell-cycle specific (methotrexate and fluorouracil) and non-cell

cycle specific (cyclophosphamide and doxorubicin/adriamycin) chemotherapeutic agents (31). However, in these earlier studies only one schedule of administration was used. A single bolus injection (1 LD_{50}) of the chemotherapeutic agent was given and swainsonine was started either one day before or on the day of administration, in the case of non-cycle specific agents and two days after administration of the drug for cycle specific agents. In all cases, swainsonine was given intraperitoneally for 10 consecutive days. In the present studies, we are focusing on modeling the administration of chemotherapeutic agents to more closely correlate with regimens used with human patients. Our initial studies have focused on methotrexate. We have administered 2 cycles of methotrexate ($1LD_{50}$ of drug/cycle) and swainsonine, with each cycle being separated by a period of recovery. The day of initiation of swainsonine as well as the schedule of administering for 10 consecutive days, has remained constant. The first set of experiments, summarized in FIG 3, were designed to determine three things: i) whether mice could in fact survive 2 cycles of chemotherapy; ii) if swainsonine was protective; iii) and to determine the optimal recovery period. We tried 7, 10 and 14 day recovery periods and found 10 days to be optimum. B6C3/F1 mice were divided into 2 groups of 46 mice each for experimental groups, MTX + Saline and MTX + Swainsonine. Control groups receiving Saline Only and Swainsonine + Saline consisted of 5-8 mice. In the experiment shown in Fig 3, mice received 1 LD_{50} of MTX on day 0 and on day 2, either saline or swainsonine (20ug/day) was administered twice daily intraperitoneally, for 10 consecutive days. Mice were then allowed a ten (10) day recovery period before the cycle of MTX and swainsonine was repeated. At the end of cycle one 28 % (33/46) of mice receiving MTX + saline survived as compared to 80% (9/46) mice receiving swainsonine + saline, a 3-fold increase in survival. These results were expected and are supportive of our previously published findings (33). Most important, are our findings of the effect of swainsonine on two (2) cycles of MTX. As can be seen, when mice received a second cycle of MTX, the cumulative survival was 15% (39/46). Swainsonine was able to increase the cumulative survival to 80% (37/46).

In a second set of experiments in which we gave 2-cycles of MTX, followed by swainsonine and a 10-day recovery period, and observed similar results to those above, we decided to administer a third cycle of MTX to the survivors, though the numbers in the MTX treated groups were small. As can be seen in Fig 4, swainsonine was able to protect significantly against 3 cycles of MTX chemotherapy. One (1) cycle of MTX produced only a survival rate of 33% (15/45), as compared to 82% (37/45) for mice receiving swainsonine + MTX, a 2.5-fold increase in survival. Following the second cycle of MTX 91% (11/15) of the mice surviving cycle one also survived cycle two, resulting in an **overall** survival rate of 24% (11/45). Finally, after 3 cycles of MTX chemotherapy, 5/45 or 11% of the mice survived **overall**, whereas 37/45 or 80% of mice receiving swainsonine + MTX were survived. Greater than a 7-fold increase in survival following 3 cycles of MTX chemotherapy. These data very strongly support the chemoprotective role of swainsonine following multiple cycles of MTX. As was observed in the previously discussed 2-cycle experiment, with each successive cycle of MTX their was a decrease in lethality of mice receiving MTX only. For example, 33% of mice survived one cycle of swainsonine, of these survivors, 91% survived cycle two and of cycle two survivors, 89% survived 3 cycles of MTX. These findings are consistent with the concept of development of resistance to chemotherapy.

Now that we have demonstrated the ability of swainsonine to protect against 3 cycles of MTX, we are initiating studies to verify its effectiveness with adriamycin (doxorubicin) ADR, a non-cycle specific chemotherapeutic agent. Concurrently, we are proceeding with the MTX model and designing experiments which will allow us to determine what effect swainsonine has on hematopoietic cells. We will obtain blood and bone marrow specimens, at different time points as mice progress through the treatment schedule, for Complete Blood Count (**CBC**) as well as peripheral blood differential analysis and to monitor stem cell and committed progenitor cell numbers. Peripheral blood analysis will be done to monitor neutrophil and platelet counts. This data will assist in determining whether swainsonine affects the kinetics of appearance of different populations of progenitor cells. We have done one preliminary blood analysis assessment and it will be discussed in the next section. Stem cells and committed progenitor cells that are lineage committed will be assessed by the high proliferative potential colony forming cell assay (HPP-CFU) and colony forming unit (CFU-c) assays, respectively. It is important to the potential usefulness of swainsonine that we obtain an understanding as to which subpopulation(s) of bone marrow cells is (are) being stimulated by swainsonine. For example, if its chemoprotective capability is related to its ability to recruit committed progenitors, then these progenitors will lack the self-renewal capacity of stem cells and thus will support only short term repopulation. We will also monitor bone marrow cellularity as a measurement of myeloproliferation.

In accordance with our earlier proposed Statement of Work we are proceeding with studies to compare parenteral administration of swainsonine with continuous administration, over a period of 10 days, by implantation of ALZET$^{(R)}$ osmotic pumps. This data will be compared with the survival data of above reported experiments where swainsonine has been administered intraperitoneally, twice daily. We have done preliminary work to ascertain our surgical proficiency with inserting the pumps and verified the emptying of the contents of the pumps. Initial experiments are now in progress.

Effect of swainsonine on peripheral blood cells: As discussed above, we are initiating studies to determine whether swainsonine affects the kinetics of appearance of different populations of progenitor cells. We have done one (1) preliminary blood analysis on day 5 of their rest period after mice completed 2 cycles of MTX chemotherapy and prior to commencing their third cycle of chemotherapy. Two mice each were obtained from the control groups which received saline only or MTX + saline, three mice were tested from the MTX + swainsonine group. Blood samples were obtained by intraorbital bleeding, a drop placed on a slide for differential analysis and the remainder used for automatic analysis by a System 9000 Blood Analyzer (Table II). In this initial experiment no basic differences were observed. In the swainsoonine treated group, there was a 14% increase in polymorphonuclear cells (PMN), however, ironically, the platelet count was greater in groups without swainsonine (Table II). I should emphasize that these findings are **very** preliminary and represent only one (1) time point. Quite likely when more definitive studies are done, significant findings will be observed.

Growth of MC-2 murine mammary tumor cells in mice: Although swainsonine has been shown to be protective of hematopoietic progenitor cells in normal mice, we have outlined in our Statement of Work the necessity for investigating its efficacy as a hemarestorative agent in tumor bearing mice. If swainsonine is to have applicability in a human therapy setting, then it must be able to protect hematopoietic progenitor cells from toxicities associated with the chemotherapy agent in the presence of tumor and it must not hinder the anti-tumor effects of the chemotherapy treatment. As a precursor to studies whose aim is to test whether swainsonine can protect hematopoietic cells from high-dose chemotherapy toxicity in tumor-bearing mice and to determine if the administration of swainsonine will in anyway interfere with the anti-tumor activity of the chemotherapy agents, it was necessary for us to determine certain growth characteristics of the tumor cells *in vivo*. Previously, we had proposed using the mammary adenocarcinoma tumor cell line 16/C but are using the mammary tumor line MC-2 instead, because of it was readily accessible and at no cost. The three main facts which we needed to learn about these cells were: a) if the cells would grow in mice after having been maintained in culture; b) the number of cells it would take to produce tumors 0.5-1.0 cm in size; c) and the growth rate of these tumors. To determine these findings, MC-2 cells were expanded in cell culture, washed and varying numbers of cells injected subcutaneously into 8-12 week old (18-20g) female B6C3/F1 mice. The mice were checked daily for the appearance of tumor and tumor growth. We wanted to obtain a cell number which would produce tumors in 7-10 days that were 0.5-1.0 cm in size. We did not want larger, more agressive tumors that potentially could overload the system and then not give a window in which to be responsive to the chemotherapeutic drug. In repeated experiments (Table III) 5×10^7 cells produced tumors of desirable size. Thus this is the dose we will use in the studies to determine the chemoprotective properties of swainsonine in tumor bearing animals.

Recommendations:

In general, we feel that our studies are progressing on task with our Statement of Work and our recommendations are to proceed with the Statement of Work as submitted in the original proposal.. We have established for one chemotherapeutic agent, methotrexate, the ability of swainsonine to protect against several cycles of chemotherapy, and have determined the LD_{50} of the second, doxorubicin/adriamycin, and are ready to proceed with studies utilizing that agent. Furthermore, we are now ready to test and compare the efficacy of continuous administration of swainsonine, using Alzet osmotic pumps, to that which we have obtained with parenteral administration of swainsonine. We have switched tumor models, from the mammary adenocarcinoma 16/c to MC-2 which is also a mammary tumor. This switch was primarily based on the availability of the latter. We have established the ability of MC-2 to grow in mice and recommend that we proceed with establishing it *in vivo* for utilization in experiments investigating the ability of swainsonine to protect from chemotherapy associated toxicity in a tumor-bearing model. We have not achieved as much as we had hoped relative to studies on monitoring the stem cell and progenitor cell numbers, however the technical and personnel problems which slowed this aspect of the project have been addressed and we are aggressively moving forward with these studies.

CONCLUSIONS

At the end of the first year of our two-year project, we have completed many of the baseline studies which are essential for the major undertaking which we hope to accomplish in the final year on this project. In this first year of study, our most significant findings have related to the evidence supporting the ability of swainsonine to protect against the toxicity of multiple cycles of chemotherapy, specifically methotrexate. This is a very significant finding and one which relates directly to one of the aims of these studies; that being, to demonstrate, in a model in which chemotherapy is administered more analogous to the human, the chemoprotective properties of swainsonine. These positive findings are another link in the supportive chain for the clinical potential of swainsonine.

REFERENCES

1. Frei E, Canellos GC: Dose - a critical factor in cancer therapy. Am J Med 69:585-584, 1980.
2. Peters WP: High-dose chemotherapy and autologous bone marrow support for breast cancer. In, Important Advances in Oncology eds. DeVita, VT, Hellman S, Rosenberg SA. Philadelphia: J.B. Lippincott Co., pp.135-150, 1991.
3. Early Breast Cancer Collaborative Group:Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. Lancet 339:1-15, 71-85, 1992.
4. Hryniuk W, Bush H: The importance of dose intensity in chemotherapy of metaststic breast cancer. J Clin Oncol 2:1281-1288, 1984.
5. Hoogstratten B, George S, Samal B: Combination chemotherapy and Adriamycin in patients with advanced breast cancer. Cancer 38:13-20, 1976.
6. Smalley RV, Murphy S, Huguley CM: Combination versus sequential five-drug chemotherapy in metastatic carcinoma of the breast. Cancer Res 36:3911-3916, 1976.
7. Tannock I, Boyd N, DeBoer G, et al:A randomized trial of two dose levele of cyclophosphamide, methotrexate, and fluorouracil chemotherapy for patients with metastatic breast cancer. J Clin Onc 6:1377-1387, 1988.
8. Wood WC, Budman DR, Korzun AH et at.: Dose and dose intensity of adjuvant chemotherapy for stage II, node-positive breast carcinoma. N Engl J MEd 330:1253-1259, 1994.
9. Petros WP and Peters WP: Hematopoietic colony-stimulating factors and dose intensity. Seminars In Oncol. 20:94-99, 1993.
10. Nemunaitis,J, Rabinowe SN, Singer JW, Bierman PJ, Vose JM, Freedman AS, Onetto N, Gillis S, Oette D, Gold M, Buckner CD Hansen JA, Ritz J,Appelbaum FR, Armitage JO, Nadler LM: Recombinant granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for lymphoid cancer. N. Engl. J. Med. 324:1773-1778, 1991.
11. Mertelsmann R, Herrmann F, Hecht T, Schulz G: Hematopoietic growth factors in bone marrow transplantation. Bone Marrow Transplant. 6:73-??, 1990.
12. Brandt SJ, Peters WP, Atwater SK, Kurtzberg J, Borowitz MJ, Jones RB, Shpall EJ, Gilbert CJ, Bast RC JR, Oette DH: Effect of recombinant human granulocyte

macrophage colony-stimulating factor on hematopoietic reconstitution after high-dose chemotherapy and autologous bone marrow transplantation. N Engl. J. Med. 318:869-876, 1988.

13. Peters WP, Ross M, Vrdenburgh J, et al.: Role of cytokines in autologous bone marrow transplantation. Hematol Oncol Clin North Am 7:737-47, 1993.
14. Dennis JW, Koch K, Beckner D: Inhibition of human HT29 colon carcinoma growth in vitro and in vivo by swainsonine and human interferon-α2. J. Natl Cancer Inst. 81:1028-1033, 1989.
15. Mohla S, White SL, Grzegorzewski K, Nielson D, Dunston GM, Dickson L, Cha JK, Asseffa A; Olden K: Inhibition of growth of subcutaneous xenografts and metastasis of human breast carcinoma by swainsonine: Modulation of tumor cell HLA class I antigens and host immune effector mechanisms. Anticancer Res 10:1515-1522, 1990.
16. Humphries MJ, Matsumoto K, White SL, Olden, K: Oligosaccharide modification by swainsonine treatment inhibits pulmonary colonization of B16-F1 murine melanoma cells. Proc. Natl Acad Sci USA 83:1752-1756, 1986.
17. Pulverer G, Beuth J, Ko HL, Yassin A, Ohshima Y, Roszkowski K, Uhlenbeck G: Glycoprotein modifications of sarcoma L-1 tumor cells by tunicamycin, swainsonine, bromoconduritol or 1-desoxynojirimycin treatment inhibits their metaststic lung colonization in Balb/c-mice. J Cancer Res Clin Oncol 114:217-220, 1988.
18. Newton SA, White SL, Humphries MJ, Olden K: Swainsonine inhibition of spontaneous metastasis. J Natl Cancer Inst 81:1024-1029, 1989.
19. Humphries M, Olden K: Asparagine-linked oliogosaccharides and tumor metastasis. Pharmacol Ther 44:85-105, 1989.
20. Tulsiani DRP, Touster O: Swainsonine causes the production of hybrid glycoproteins by human skin fibroblasts and rat liver Golgi preparations. J Biol Chem 258:7578-7585, 1983.
21. Bowlin TL, McKown BJ, Kang MS, Sunkara SP: Potentiation of human lymphokine activated killer cell activity by SW, an inhibitor of glycoprotein processing. Cancer Res. 49:4109-4113, 1989.
22. Yagita M, Saksela E: SW, an inhibitor of glycoprotein processing, enhances cytotoxicity of large granular lymphocytes.Scand J Immunol 31:275-282,1990
23. Kang, MS, Bowlin TL, Vijay IK, Sunkara SP: Accumulation of pentamannose oligosaccharides in human mononuclear leukocytes by action of swainsonine, an inhibitor of glycoprotein processing. Carbohydr Res. 248:327-37, 1993.
24. Fujieda S, Noda I, Saito H et al: Swainsonine augments the cytotoxicity of human lymphokine-activated killer cells against autologous thyroid cancer cells.Arch Otolaryngol Head Neck Surg 120:389-394, 1994.
25. Grzegorzewski K, Newton SA, Akiyama SK, Sharrow S, Olden K, White SL: Induction of macrophage tumoricidal activity, major histocompatibility complex class II antigen (Ia^k) expression, and interleukin-1 production by swainsonine. Cancer Commun 1:373-379, 1989.
26. Bowlin TL, Sunkara PS: Swainsonine, an inhibitor of glycoprotein processing, enhances mitogen induced interleukin 2 production and receptor expression in human

lymphocytes. Biochem Biophys Res Commun 151:859-864, 1988.

27. Olden K, Breton P Grzegorzewski K, Yasuda Y, Gause BL, Oredepe OA, Newton SA, White, SL: The potential importance of swainsonine in therapy for cancers and immunology. Pharmac Ther 50:285-290, 1991.
28. White SL, Nagai T, Akiyama SK, Grzegorzewski K, Olden K: Swainsonine stimulation of the proliferation and colony forming activity of murine bone marrow. Cancer Commun 3:83-91.
29. White SL, Schweitzer K, Humphries MJ, Olden K: Stimulation of DNA synthesis in murine lymphocytes by the drug swainsonine: immunomodulatory properties. Biochem. biophys. Res. Commun. 150:615-625, 1988.
30. Myc A, Kunicka JE, Melamed MR, Darzynkiewicz A: Effect of swainsonine on stimulation of cell cycle progression of human lymphocytes.Cancer Res.49:2879-2883, 1989.
31. Oredipe OA, White SL, Grzegorzewski K et al.: Protective effect of swainsonine on murine survival and bone marrow proliferation during cytotoxic chemotherapy. J Natl Cancer Inst 83:1149-1156, 1991.
32. Klein, J-L, Roberts JD, George MD, Kurtzberg J. et al: Swainsonine overcomes the hematopoietic toxicity of AZT by stimulating bone marrow progenitor cells from humans and mice. (Personal communication).
33. Goss, PE, Baptiste J, Fernandes B, Baker M and Dennis JW: A Phase I study of swainsonine in patients with advanced malignancies. Cancer Res. 54:1450-1457, 1994.

APPENDICES

GRANT NUMBER DAMD17-96-1-6074

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Table I

Summary of LD_{50} Experiments
Methotrexate (MTX) and Adriamycine (ADRIA)

Calculated LD_{50} dose

MTX		ADRIA	
Expt. # 1 :			
----	----	0.72 mg/20 g	36 mg/kg
Expt. # 2 :			
36.8 mg/20 g	1840 mg/kg	----	----
Expt. # 3 & 4 :			
41.8 mg/20 g	2090 mg/kg	0.65 mg/20 g	32.5 mg/kg
Mean average (n = 2) :			
39.3 mg/20 g B.W.	1965 mg/kg	0.685 mg/20 g B.W.	34.25 mg/kg

Table II

Blood Analysis of Methotrexate Treated Mice

Expt. Grp	WBC x 10^3	RBC x 10^3	Platelet x 10^3	PMN %	Imm PMN %	Lymp %	Mono %	Eosin %
Saline n=2	11.8± .98	9.12±.31	1328 ± 134	30.5 ± 10.6	2.5± .7	61±7	5±1.4	1±1.4
MTX n=2	9.1±2.4	9.08±.21	1448±28	30.5 ±13.4	1.5±2.1	61.5 ±14.8	3.5±2	3±1.4
MTX + Swainso n=3	10.1±3.4	9.54±.45	889±39	35.3±9.7	2±1	57.6 ±9.5	1.6±.58	3.33±.57

WBC=white blood count, RBC=red blood count, PMN=polymorphonuclear, Imm. PMN= immature polymorphonuclear cells, Lymp=lymphocyte, mono=monocyte, eosin=eosinophil, and swainso=swainsonine, Mice received MTX on day 0 and swainsonine (20ug/day) twice daily IP beginning on Day 2 and continuing for 10 consecutive days. Blood samples were obtained by intraorbital bleeding on the fifth day after swainsonine treatment ended. This was midway between the first and second cycle of chemotherapy.

Table III

Growth of MC-2 Mammary Tumor Cells In Mice

	Tumor Size (cm) 10 Days After Injections of Tumor Cells				
Cells Inj	*1st Day	0.1 - 0.5	0.6-1.0	1.0-2.0	2.0 - 3.0
Expt #1					
1×10^6	18	---	---	----	----
5×10^6	5	$1/3^{++}$	2/3	----	----
1×10^7	5	2/3	1/3	----	----
5×10^7	4	1/3	2/3	----	----
1×10^8	4	1/3	1/3	1/3	----
Expt # 2					
5×10^6	6	3/6	1/6	----	----
1×10^7	6	5/6	0/6	----	----
5×10^7	4	3/6	2/6	----	----

* First day of tumor appearance
$^{++}$ number of mice with tumors/number of mice injected

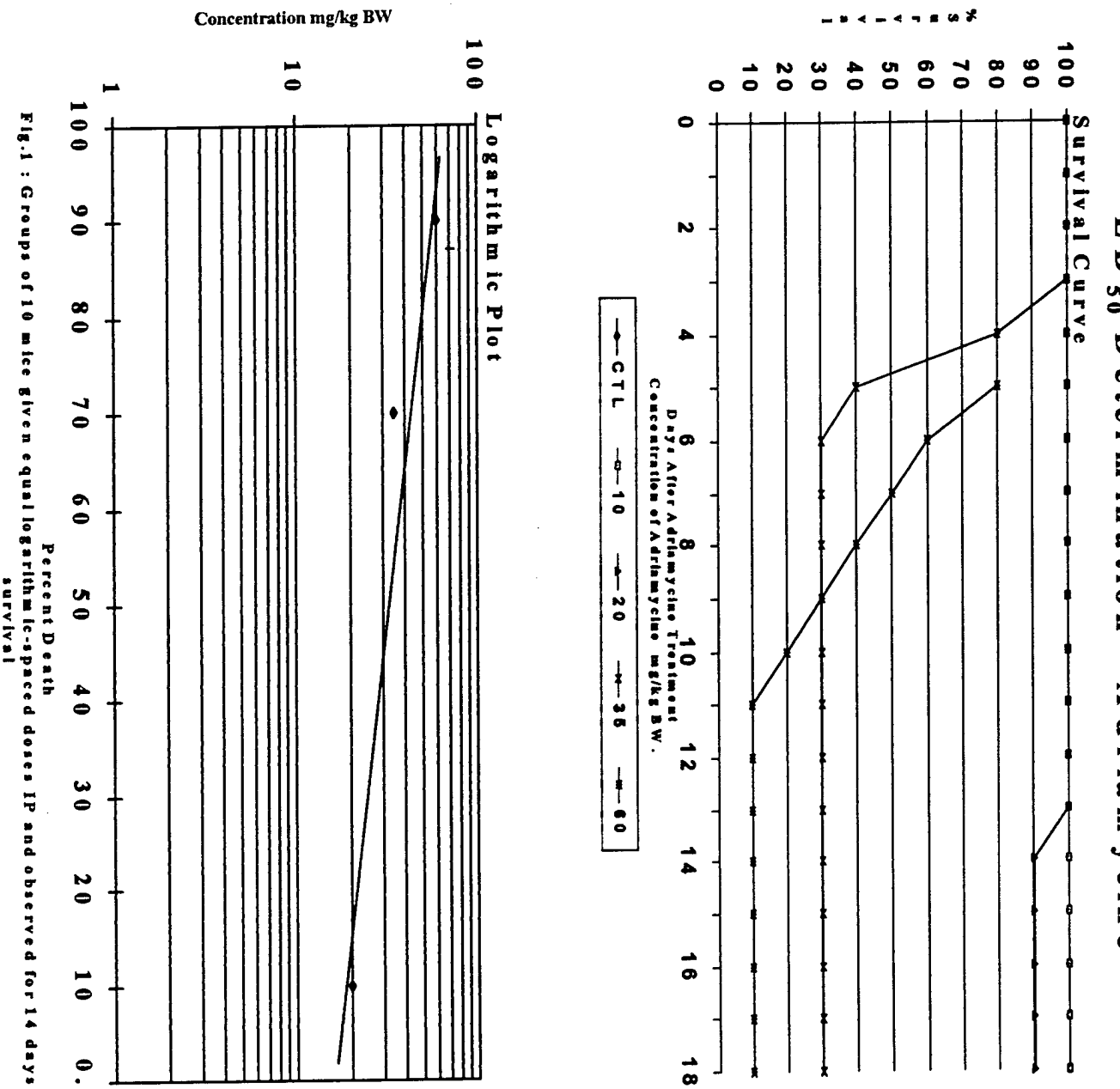
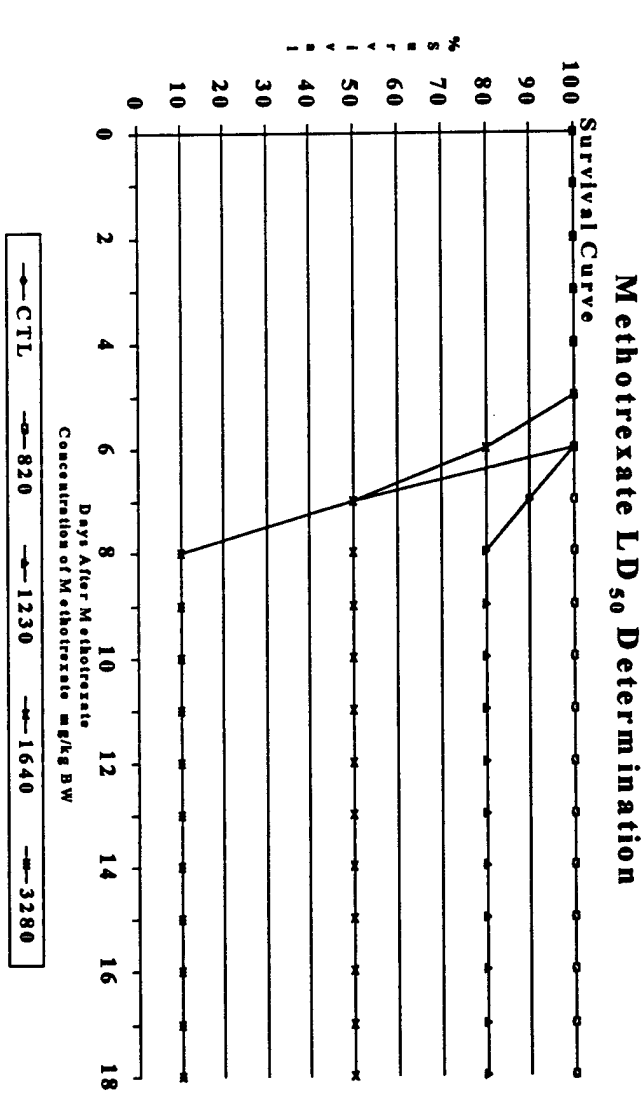


Fig.1 : Groups of 10 mice given equal logarithmic-spaced doses IP and observed for 14 days for survival



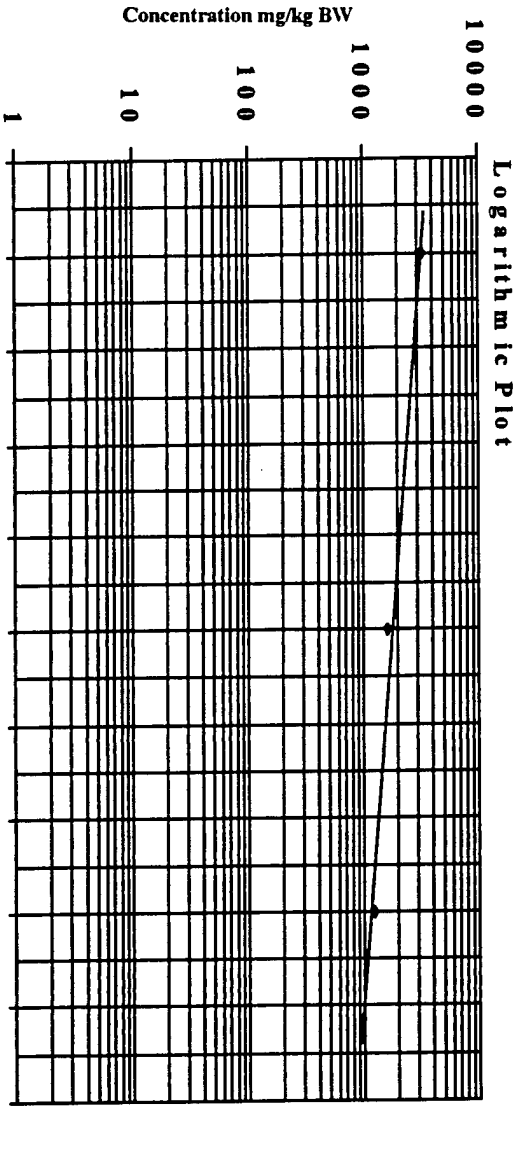


Fig.2 : Groups of 10 mice were given equal logarithmic-spaced doses IP and observed for 14 days for survival

Effect of Swainsonine on Survival of Normal B6C3F1 mice Following Two Cycles of Methotrexate

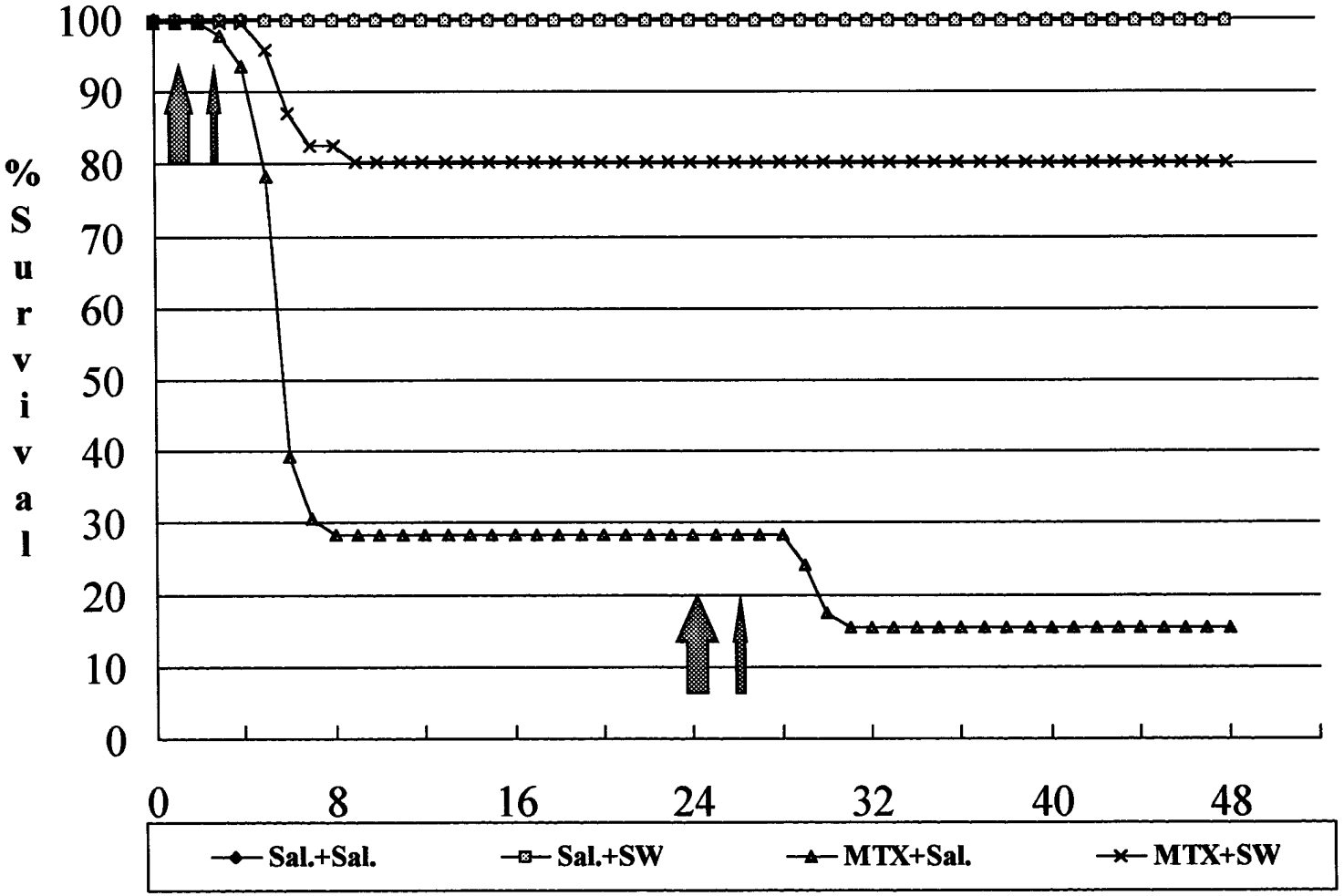


Fig. 3 : Mice received 1 LD_{50} of methotrexate (MTX) on day 0 followed by twice daily IP injection of swainsonine (20 ug/day) for 10 days. Mice were allowed to rest 10 days and the cycle of MTX and swainsonine was repeated. Left and right arrows indicate administration of MTX and beginning of 10 day swainsonine treatments respectively.