Effects of Social Housing Condition on the Response of the Shionogi Mouse Mammary Carcinoma (SC115) to Chemotherapy

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ABSTRACT
In the present study, we demonstrate that social housing conditions significantly alter the response of the transplantable androgen-responsive Shionogi mouse mammary tumor (SC115) to chemotherapy. Mice were reared either in groups (G) or as individuals (I). Immediately following tumor cell or vehicle injection, mice were rehoused from group to individual (GI) or from individual to group (IG) conditions. A combination of Adriamycin (4 mg/kg) and cyclophosphamide (61.5 mg/kg), in a series of three i.p. injections 7 days apart, was initiated when mean tumor weights within a housing condition (GI or IG) reached 1 g. Survival probability was significantly greater in mice in the IG housing condition compared to those in the GI housing condition (47% versus 19%, respectively). Additionally, the median survival time following the initiation of chemotherapy was greater for mice in the IG than for mice in the GI condition (24.5 days versus 15.0 days, respectively). These findings suggest that a psychosocial stressor, social housing condition, can significantly influence chemotherapeutic efficacy.

INTRODUCTION
A number of human studies have demonstrated that stressful life events and the ability to cope with stress may play a role in cancer growth and metastasis. Psychosocial stressors such as divorce or bereavement have been associated with increased cancer risk (1–5) and increased probability of metastasis (6–8). Studies have also shown that social involvement with support groups or family members may be related to an increase in survival time (9–12) and a decrease in the rate of metastasis (13, 14), possibly by providing the patient with a form of coping strategy (15–17). There are, however, several studies that have reported no association among psychosocial stressors, coping strategies, and survival time (18–22) or rate of metastasis (23, 24). A number of issues may affect the interpretation of these data. For example, in retrospective studies, the diagnosis of cancer may distort the patient’s perception of past stressful events due to the knowledge that she/he has cancer. Also, the clinical manifestation of cancer may occur years following neoplastic change and, therefore, the onset of the disease may have occurred prior to the stressful event.

Animal models allow investigation of the relationship between psychosocial stressors and tumor growth under more controlled conditions. However, even in animal studies, the data are complex. Factors such as chronicity and timing of the stressor and the type of tumor being examined can influence stressor effects on tumor growth and metastasis (25–31). Psychosocial stressors such as housing condition or psychological stressors such as forced restraint have been shown to affect tumor growth and/or metastasis of either transplantable (32–34) or chemically induced tumors (33, 35, 36). Of relevance to this report, group-housed animals typically have smaller tumors and increased rates of tumor regression or rejection than individually housed animals (27, 28). In addition, a change in housing condition may increase tumor growth compared to that found in individually or group-housed animals that do not experience change (32). Interestingly, it has been suggested that the adverse consequences of housing change on tumor growth may be reduced by fighting (25, 32), which may act as a coping mechanism and therefore reduce the impact of housing change on the animals.

We have developed an animal-tumor model that demonstrates that a change in social housing condition can dramatically affect the growth of the transplantable, androgen-responsive, Shionogi mouse mammary tumor (SC115; Ref. 34). Moreover, we have shown that the direction of change in housing condition significantly influences tumor growth rate (34, 37). Being reared in a group and then individually housed (GI) following tumor cell injection increases tumor growth rate, whereas being reared individually and then group housed (IG) reduces tumor growth rate, compared to that in mice remaining in their group-rearing condition (GG).

Recently, epidemiological evidence suggests that psychosocial stressors may affect not only tumor growth and metastasis but also tumor response to chemotherapy. Psychosocial stressors such as divorce or bereavement have been associated with decreased efficacy of cancer therapies (38, 39). Reducing the impact of psychosocial stressors through social support or psychosocial intervention can extend survival time and decrease the toxic side effects of chemotherapy in cancer patients (13, 40–42). For example, in patients with metastatic non-small cell lung cancer, the combination of supportive care and chemotherapy offers a survival advantage over either modality alone (43). In breast cancer patients, the combination of chemotherapy and psychotherapy (41) or psychosocial support (14) increases survival time over chemotherapy alone. Recent animal studies (44–47) support these findings. It has been shown that exposure to rotational stress decreases the antitumor effects of p.o. administered cyclophosphamide or razoxane in mice bearing Lewis lung carcinoma in terms of tumor burden, extent of metastasis, and survival time (46, 47).

The present study, using our animal-tumor model, extends these previous data in three important areas: (a) we used a psychosocial stressor, change in social housing condition from group to individual (GI) or individual to group (IG); (b) we used a mouse mammary carcinoma that has similarities to hormone-responsive human cancers; and (c) we used a chemotherapeutic regimen consisting of AD and CY administered i.p.

MATERIALS AND METHODS
Tumor Propagation and Experimental Animals. The Shionogi mammary carcinoma (SC115) was derived from a mammary tumor that spontaneously arose in a female mouse of the DDS strain. After 19 passages in male mice, the SC115 androgen-responsive variant arose that grows more rapidly in males than in females (48, 49). This androgen-responsive subline designated SC115 Class A (48), used in the present study, was maintained by serial transplantation in male mice of the DDS strain as described previously (50). Briefly, tumors weighing approximately 2 g were dissociated to single cells according to our standard protocol (34), and mice were injected s.c. in the interscapular region with 2 x 10⁶ cells suspended in 0.1 ml DMEM (Terry Fox Laboratory, Vancouver, British Columbia, Canada).
Male DD/S mice (n = 112) between 2 and 4 months of age were the experimental subjects in this study. Mice were reared either individually housed (I) or group housed (G). Immediately following tumor cell injection (s.c. injection of 2 x 10^6 cells suspended in 0.1 ml DMEM) or tumor cell-vehicle injection (s.c. injection of 0.1 ml DMEM), those mice reared in groups were rehoused individually (GI) and those reared individually were rehoused in groups (IG), according to our published protocol (34). Animals within each housing condition were randomly assigned into tumor cell injection groups receiving either chemotherapy (n = 21 GI, n = 30 IG) or drug vehicle alone (n = 20 GI, n = 25 IG), or into tumor cell-vehicle injection groups receiving chemotherapy (n = 6 GI, n = 10 IG; Fig. 1). Beginning the day following tumor cell or tumor cell-vehicle injection, all animals were exposed to an acute daily stressor consisting of exposure for 15 min/day, 5 days/week to one of five different novel environments, a treatment that we have shown enhances tumor growth rate differences between experimental groups (34). Mice were palpated every second day; once tumors were measurable (approximately 8–10 days), caliper measurements were taken. Tumor weights were calculated according to the formula (51):

\[ \text{Tumor weight (g)} = \frac{\text{Length (cm)} \times \text{Width (cm)}}{2} \]

Chemotherapy. Chemotherapy or drug vehicle administration (9 g of NaCl per 100 ml of distilled water) was initiated when the mean tumor weight of mice within each housing condition (i.e., mean tumor weight among mice of a previously grouped condition (GI) or within each newly formed group (IG)) reached 1.0 ± 0.2 g (Fig. 1). Chemotherapy consisted of a combination of AD (Adria Laboratories of Canada Ltd., Mississauga, Ontario, Canada) at 40.0 mg/kg and CY (Procytox; Homer, Montreal, Quebec, Canada) at 61.5 mg/kg in NaCl solution. Drugs were administered i.p. every 7 days for a total of three injection rounds. Mice in the no tumor control condition received the chemotherapeutic regimen starting at 16 days after tumor cell-vehicle injection for mice in the GI condition (mean time for GI tumor-bearing mice to reach 1 g) or 20 days after tumor cell-vehicle injection for mice in the IG condition (mean time for IG tumor-bearing mice to reach 1 g). The doses of drugs selected for this study have been shown to be optimal for SC115 tumor regression with minimal toxic side effects (50). Mice were monitored daily for drug toxicity as assessed by morbidity and mortality. Body weights were measured every second day, concurrent with tumor weight measurements.

Statistical Analysis. Tumor response to chemotherapy in GI- and IG-housed mice was analyzed using survival probability and TGD. Survival probability was determined using Kaplan Meier plots and Cox proportional hazards regression (52, 53). Death, regardless of cause, was considered an event (i.e., mice were sacrificed when tumor weight exceeded 3 g or mice were found dead, presumably from the toxic side effects of chemotherapy). Mice that were still alive at 30 days following the first round of chemotherapy were considered censored. TGD is defined as the mean time for tumors of chemotherapy-treated mice to reach a specific weight minus the mean time for tumors of drug vehicle-treated mice to reach the same weight. Differences in TGD of mice in the experimental housing conditions was analyzed by ANOVA for the factors of Group and Weight. Tumor growth in drug vehicle-treated animals was also analyzed by ANOVA for the factors of Group and Days with repeated measures on Days; interactions were further analyzed by Tukey’s post-hoc tests. Differences in the median survival times of mice in the two experimental housing conditions following chemotherapy initiation was analyzed by Student’s t test.

RESULTS

Consistent with our previous data (34), tumor growth rate in drug vehicle-treated mice was significantly faster in mice in the GI housing condition than in mice in the IG housing condition [F(1, 27) = 26.096, P < 0.001; Fig. 2]. Consequently, survival probability in these drug vehicle-treated mice was greater in the IG compared to the GI housing condition (\(\chi^2 = 6.482, P = 0.01\); Fig. 3). As expected, tumor-bearing, chemotherapy-treated mice survived longer than tumor-bearing, drug vehicle-treated mice, regardless of experimental housing condition (\(\chi^2 = 32.816, P < 0.001\)). As well, for all mice that received chemotherapy, survival probability was greater for non-tumor-bearing than for tumor-bearing mice (\(\chi^2 = 3.719, P = 0.05\); Fig. 3). Of importance is the observation that, similar to the drug vehicle condition, tumor-bearing chemotherapy-treated mice in the IG housing condition survived significantly longer than their counterparts in the GI housing condition (\(\chi^2 = 6.233, P = 0.01\); Fig. 3).

Further inspection of the data suggests that the increased survival probability in tumor-bearing mice in the IG compared to the GI housing condition was due to differential tumor responses to chemotherapy rather than to differences in the toxic side effects of chemotherapy: (a) the number of mice that were terminated due to tumor weights exceeding 3 g was greater for mice in the GI (n = 8; 38%) than in the IG (n = 4; 13%) housing condition, resulting in a greater number of IG (n = 14; 47%) than GI (n = 4; 19%) housed mice surviving to 30 days after chemotherapy or drug vehicle initiation.
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Fig. 3. Survival probability in tumor-bearing mice receiving chemotherapy (TC) or drug vehicle (TV) and in non-tumor-bearing mice receiving chemotherapy (NTC). Day 0 is the day of initiation of chemotherapy (AD at 4.0 mg/kg and CY at 61.5 mg/kg) or drug vehicle administration. The first symbol represents the time at which the first death(s) occurred in each condition. For tumor-bearing mice receiving either chemotherapy ($\chi^2 = 6.233$, $P = 0.01$) or drug vehicle ($\chi^2 = 6.482$, $P = 0.01$), survival probability was significantly greater for mice in the IG than for mice in the GI housing condition. As expected, tumor-bearing, chemotherapy-treated mice ($n = 21$ GI, $n = 30$ IG) had a significantly greater survival probability than tumor-bearing drug vehicle-treated mice ($n = 20$ GI, $n = 25$ IG; $\chi^2 = 32.816$, $P < 0.001$). Additionally, for all mice receiving chemotherapy, non-tumor-bearing mice had a significantly higher survival probability than tumor-bearing mice ($\chi^2 = 3.719$, $P = 0.05$).

DISCUSSION

The present data demonstrate that a psychosocial stressor, change in social housing condition, not only alters the SC115 tumor growth rate but also significantly affects the response of the SC115 tumor to chemotherapy. Data on tumor growth rate in drug vehicle-treated mice support and extend our previous findings (34); mice that experience a change from individual (GI) housing have a significantly faster tumor growth rate and a significantly reduced survival probability compared to mice that experience a change from individual to group (IG) housing. Importantly, the present data demonstrate that the direction of change in social housing condition also significantly influences survival probability following the initiation of chemotherapy. That is, chemotherapy-treated mice in the IG housing condition have a significantly greater survival probability than chemotherapy-treated mice in the GI housing condition. The data suggest that this difference in survival probability is due to differential tumor responses to chemotherapy, because the number of mice in the GI and IG conditions that died due to the toxic side effects of chemotherapy was similar in both the tumor-bearing and the non-tumor-bearing conditions.

Although it is difficult to extrapolate data from animal studies to humans, our animal-tumor model has relevance to the human situation: (a) the androgen-responsive variant of the SC115 tumor, used in the present study, was derived from a mammary tumor that spontaneously arose in a female mouse (34, 48). This heterogeneous solid tumor is similar to some hormone-responsive cancers in humans in its sensitivity to different classes of steroid hormones, including androgens (49), estrogens (54), and glucocorticoids (55); (b) this mouse mammary tumor is immunogenic (56, 57), also characteristic of many human cancers; and (c) the SC115 mouse mammary tumor responds well to AD and CY, two chemotherapeutic agents used in the treatment of human cancers (50, 58, 59). Thus, our data suggesting a relationship between psychosocial stressors and tumor response to chemotherapy may have potential clinical relevance.

The mechanisms underlying the differential responses to chemotherapy observed in this study are unknown at present. One possibility is that stressor-induced and/or chemotherapy-induced changes in endocrine function are involved. We have shown previously that for male mice in our standard laboratory housing conditions (group housed and not subjected to daily novelty stressors), tumor response...
to AD and CY can be regulated by modulating the level of exogenous testosterone administered following castration (50). We have also demonstrated that the different housing conditions of our model are correlated with significant differences in basal testosterone and corticosterone levels (60). Basal levels of plasma testosterone are significantly higher, and basal levels of plasma corticosterone are significantly lower in GI- than in IG-housed mice over the first 7 days following tumor cell injection and rehousing. In the present study, it is not known if testosterone and/or corticosterone levels differ between mice in the two experimental housing conditions at the time when chemotherapy was initiated (between 14 and 20 days after tumor cell injection/rehousing) or during the course of chemotherapy. Studies investigating hormone levels at these times are in progress.

In addition to the possible effects of social housing condition on circulating hormone levels, there is also evidence that alkylating chemotherapeutic agents such as CY may reduce plasma levels of both testosterone and corticosterone (61—64). Although such effects have been demonstrated only following high, single-dose chemotherapy treatments (61—63), it is possible that the dose of CY used in the present study may differentially alter hormone levels in mice in the GI and IG conditions, which may in turn alter tumor response to chemotherapy.

Differential responses to chemotherapy in this study may also be mediated through changes in immune function. Such changes may occur either directly through psychosocial stressor- or chemotherapy-induced changes in immune function or indirectly through changes in hormonal activity that alter immunocompetence. We have shown that the SC115 tumor differentially stimulates NK cell activity in mice in the GI and IG conditions (56, 57). In addition to alterations in NK cell activity, preliminary evidence from our laboratory suggests that the SC115 tumor stimulates a tumor-specific cytolytic immune response. Immune rejection of tumors in human and murine studies has been shown to be mediated primarily by CTLs (65, 66). Several studies examining the effectiveness of tumor-specific CTLs introduced into a tumor-bearing host demonstrate that optimal treatment includes the addition of chemotherapeutic agents (67—72). On the other hand, chemotherapy itself may differentially affect the immune functions of mice in the two housing conditions. In animal studies, CY has been shown to alter cytokine levels (73—75), T-cell and NK cell activities (76, 77), and the accumulation of macrophages within the tumor (78, 79). Thus, it is possible that the greater chemotherapeutic efficacy observed in mice in the IG housing condition may be due to an increase in immunoactivity toward the tumor compared to that in mice in the GI housing condition. The interactions among host environment, chemotherapy treatment, and tumor growth are still to be elucidated.

In general, chemotherapy has been shown to be more effective against fast-growing than slow-growing tumors (80). Interestingly, our data indicate that chemotherapy is more effective against the slower-growing (mice in the IG condition) than the faster-growing (mice in the GI condition) tumors. It is possible that tumor growth rate is so rapid in mice in the GI housing condition that the tumor burden becomes too large for the chemotherapy to be effective. Because chemotherapy was initiated at a time when tumors, albeit at similar weights, were growing at different rates, it is difficult to determine if the differential responses to the chemotherapeutic drugs were due to different tumor growth rates, to psychosocial stressor-induced alterations in hormone levels and/or immune function, or to an interaction among these factors. Studies to resolve these questions are presently being conducted.

In summary, these data demonstrate that the psychosocial stressor of a change in social housing condition affects not only tumor growth rate but also tumor response to chemotherapy. These data highlight the possible impact of psychosocial stressors on the complex interrelationship among the host environment, tumor growth and progression, and the efficacy of chemotherapy.

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REFERENCES

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