Negative Impact of Cancer Chemotherapy on Protein Metabolism in Healthy and Tumor-Bearing Rats

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Although chemotherapeutic agents are widely used in the treatment of cancer, few experimental data are available on their effects on host N metabolism. We studied the effects of a single intraperitoneal (IP) injection of cyclophosphamide ([CYP] 120 mg/kg), 5-fluorouracil ([5-FU], 50 mg/kg), cisplatinum ([CDDP], 5 mg/kg), or methotrexate ([MTX], 30 mg/kg). N balance was studied for 6 days following chemotherapy in healthy rats (n = 40) and in rats bearing Morris Hepatoma 7777 ([MH7777] n = 40) in a situation comparable to that of human cancer (tumor burden <0.2% of body weight, moderate anorexia, and weight loss). In healthy rats, all drugs induced transient body weight loss, anorexia, and poor N balance. At day 6 posttreatment, all animals had resumed normal feed intake and positive N balance except CDDP-treated rats, which showed continued weight loss and poor N balance. CDDP and MTX exhibited antitumor activity; however, CDDP induced diarrhea in six of eight tumor-bearing rats. Drug-induced anorexia was more severe in tumor-bearing than in healthy treated rats. N balance was more severely decreased in MH7777-bearing rats than in healthy treated animals in response to 5-FU (159 ± 36 *v* 273 ± 27 mg N/2 d) and MTX (−66 ± 36 *v* 153 ± 37 mg N/2 d) at days 3 to 4 postinjection. These results establish the presence of drug-specific effects on host N balance and the existence of a drug-tumor interaction for N metabolism in the tumor-bearing host.

EXTENSIVE WASTING (cachexia) including body and tissue protein loss and poor or negative nitrogen (N) balance is frequently observed in cancer patients.[1] This form of malnutrition contributes directly to increase mortality and morbidity, and limits cancer treatment tolerance. Cachexia is induced by the disease through a variety of processes[2] that have been studied and characterized in untreated animals frequently bearing large tumors comprising up to 30% of body mass.[3,4] Extrapolation to the human situation is therefore difficult, since specific antitumor treatments are provided to cancer patients at the time of diagnosis when tumor mass is usually small (eg, at an early stage of the disease). We suggest that cancer treatments, especially chemotherapy, might represent a major factor involved in the development of cancer cachexia. Many chemotherapeutic agents induce anorexia, diarrhea, or nausea.[5] Although those effects might decrease N balance and contribute to malnutrition, there are no published systematic studies of the effects of different chemotherapeutic agents on host nutritional status. In addition, since these compounds are chemically reactive, they interact with intracellular proteins and/or DNA synthesis of tumor cells, but also of host cells.[6] Consequently, chemotherapy might directly modify host protein metabolism at the tissue level.

Little consideration has been given to the effect of selective chemotherapeutic agents on protein metabolism in experimental studies.[7,8] In healthy rats treated with daily injection of 5-fluorouracil (5-FU), N balance decreased after initiation of treatment and became progressively negative.[8] Similarly, a single injection of 4-epidoxorubicin reduced N balance by 55% over a 5-day study in tumor-bearing rats, but not in healthy animals.[7] We therefore studied the effects of four chemotherapeutic agents widely used in the treatment of different types of human cancer, based on their different biochemical mechanisms of action: cyclophosphamide ([CYP] alkylation of DNA), 5-FU (incorporation into RNA and DNA), cisplatinum ([CDDP] incorporation into DNA), and methotrexate ([MTX] antifolate).[9] Effects of chemotherapy were studied in healthy rats and in rats bearing subcutaneous Morris Hepatoma 7777 (MH7777)[4] at a limited stage of disease progression. At the whole-body level, N metabolism was studied through continuous measurements of N intake to assess the anorexogenic effect of the drug, fecal N excretion to calculate apparent digestibility of N, and urinary N excretion that reflects mainly amino acid oxidation, and N balance was calculated from these. Effects of chemotherapy at the tissue level were assessed in liver, small intestine, and skeletal muscle through determination of weight and protein mass.

MATERIALS AND METHODS

Animals and Diets

Experiments were conducted according to the guidelines of the Canadian Council on Animal Care. Mature female Sprague-Dawley rats of the Buffalo strain from a colony maintained in our laboratory were used in this study. Experiments were conducted in a temperature (24°C)- and humidity (80%)-controlled room with a 12-hour light cycle. One week before the beginning of experiments, rats were housed in individual metabolic cages to adapt to their new environment. Rats had free access to tap water and food. The diet was based on corn and soybean meal, and was formulated to exceed the requirements of growing rats as specified by the National Research Council.[10] Diets contained 20.6% crude protein and 3,190 kcal/kg diet.

Study Design

Experiment A (healthy rats). Forty rats were separated into five groups (n = 8 per group) such that the mean ± SD of the body

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weight was similar between groups (185 ± 4 g): sham treatment (vehicle only), CYP, 5-FU, CDDP, and MTX (Sigma, St Louis, MO). Drugs were administered intraperitoneally (IP) as a single injection in 1 mL sterile NaCl 0.9%: CYP 120 mg/kg,[11] 5-FU 50 mg/kg,[12] MTX 30 mg/kg,[13] and CDDP 5 mg/kg.[14] Rats were killed by CO_2 asphyxiation 6 days after drug injection.

Experiment B (tumor-bearing rats). Forty rats were separated into five groups (n = 8 per group) such that the mean ± SD of the body weight was similar between groups (200 ± 5 g). All rats were implanted with MH7777 subcutaneously on the flank with 25 μL finely chopped tumor cells from a single donor.[4] When implanted subcutaneously in rats, MH7777 is a slow-growing tumor that induces progressive anorexia and body wasting starting approximately 7 days after implantation.[4] Tumor cells are maintained as frozen stock in our laboratory and propagated by serial transplantation in rats of the same sex and strain. Tumors were allowed to grow for 7 days, by which time the tumor became just palpable, and chemotherapy was initiated as described earlier. Rats were killed by CO_2 asphyxiation 6 days after drug injection (13 days after tumor implantation).

Tolerance and Effectiveness of the Chemotherapy

Signs of chemotherapy-related toxicity including diarrhea, piloerection, and lethargy were monitored daily after drug injections. The presence of diarrhea was expressed as the number of animals exhibiting diarrhea and its duration (in days). The day of killing, the tumor was excised and weighed in both treated and untreated rats.

Analysis

Food intake and body weight. Food intake was recorded daily starting 2 days before the beginning of chemotherapy in both experiments. N intake was calculated from daily food intake for calculations of N balance and N apparent digestibility. In experiment A, body weight was recorded daily starting on the day of drug injection. In experiment B, body weight was recorded the day of tumor implantation and then daily starting on the day of drug injection.

N balance and apparent digestibility of N. Urine and feces were collected for periods of 2 days, starting 2 days before the beginning of chemotherapy and for 6 days after (one prechemotherapy and three postchemotherapy periods). In case of severe diarrhea, fecal collection in individual animals was not performed. Sulfuric acid 1N (1 mL) was added to urine containers to prevent bacterial proliferation. Urine was conserved at −20°C before N analysis by the Kjeldahl method.[15] Feces were dried at 60°C for 4 days and stored at −20°C before N analysis. Forty-eight-hour N balance was calculated from N intake and N excretion (urinary plus fecal); apparent N digestibility was calculated from N intake and fecal N excretion.

Tissue weights and N contents. On the day of death, the liver, entire small intestine (from stomach to cecum), and three different skeletal muscles based on fiber types (mixed, epitrochlearis [EPI]; fast-twitch, extensor digitorum longus [EDL]; and slow-twitch, soleus) were dissected from each animal and weighed. Small intestine was washed clean, and all tissues were stored at −20°C before analysis. N contents of liver and small intestine were determined by the Kjeldahl method.[15] N content of muscles was determined by the combustion method with N detection by thermal conductivity using a LECO analyzer (model FP428; Leco Corp, St Joseph, MI).[16]

Statistical Analysis

Data obtained were tested by one- and two-way ANOVA. Data are presented as the group mean ± SEM. The criterion for significance was a probability of .05.

RESULTS

Effectiveness and Tolerance of Chemotherapy

Tumor mass for individual animals is shown in Fig 1. At the end of the study, the mean tumor mass in animals receiving no chemotherapy was 365 mg (0.18% of body weight). CYP and 5-FU had no influence on tumor mass (mean tumor mass, 353 and 361 mg, respectively). CDDP and MTX were active against MH7777 with a mean tumor mass of 12 and 45 mg, respectively. CDDP and to a lesser extent MTX induced gastrointestinal toxicity. Mild diarrhea (soft feces) occurred in only two rats in experiment A (one CDDP and one MTX) and for a short period (1 day). In experiment B, six tumor-bearing rats treated with CDDP experienced severe diarrhea during the last 2 days of the study, and feces were not collected during this period (see above). Other signs of drug-related toxicity (piloerection and lethargy) were observed in approximately 100% of MTX- and CDDP-treated rats from day 2 postchemotherapy to day 5 (MTX) or 6 (CDDP).

Body Weight

In healthy untreated rats, body weight increased slightly but significantly over the study period (Fig 2). In both CYP- and 5-FU–treated groups, body weight remained similar to pretreatment values throughout the study (Fig 2A). Rats treated with MTX and CDDP lost weight after drug injection (Fig 2B). Body weights of CDDP-treated rats decreased continuously, whereas body weights of MTX-treated rats increased toward pretreatment levels by the end of study. Cumulative body weight gain over the study

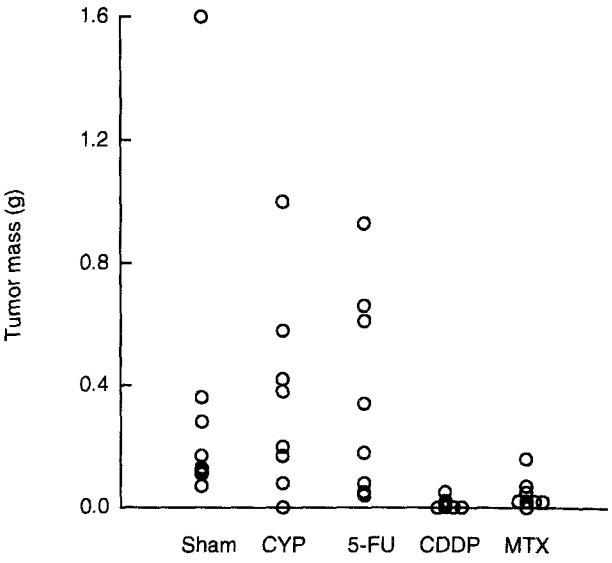

Fig 1. Individual tumor mass 6 days after beginning of chemotherapy.

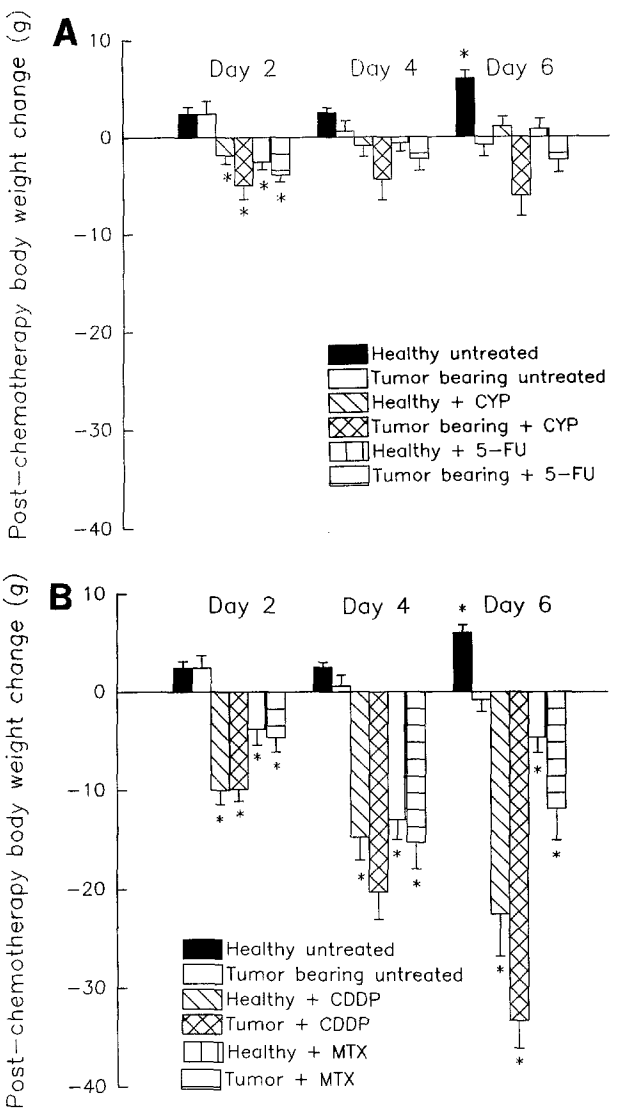

Fig 2. Effects of chemotherapy on body weight in healthy and tumor-bearing rats. (A) CYP and 5-FU; (B) CDDP and MTX. *Different from prechemotherapy values ($P < .05$).

was significantly decreased in CDDP- and MTX-treated rats (Table 1).

Untreated tumor-bearing rats exhibited no body weight change over the study period (Fig 2). All drug treatments induced significant weight loss during the first 48 hours after injection. Although it returned to pretreatment values in CYP- and 5-FU–treated rats (Fig 2A), body weight continued to decrease in other groups (Fig 2B). At the end of study, partial recovery was observed in MTX-treated rats, whereas body weight was at its minimum in the CDDP group. Cumulative body weight gain tended to be less in tumor-bearing rats as compared with healthy rats; in the case of CYP, this difference was significant (Table 1).

Food Intake

Food intake data (Table 1 and Fig 3) were normalized to body weight at the time of drug injection. In healthy rats, treatments with 5-FU, MTX, and CDDP (but not CYP) significantly reduced cumulative food consumption from −10% (5-FU) to −45% (CDDP) (Table 1). In tumor-bearing rats, cumulative food consumption was significantly decreased in all drug treatment groups from −20% (CYP) to −77% (CDDP) as compared with untreated rats. For each drug studied (except CDDP), anorexia was significantly more pronounced in tumor-bearing than in healthy rats.

Daily food intake of healthy treated rats (Fig 3) was significantly reduced during the first 3 postchemotherapy days. Food intake then returned to normal values on day 4 in 5-FU– and CYP-treated rats (Fig 3A), while remaining depressed until day 5 for MTX and day 6 for CDDP (Fig 3B). A significant increase in food intake was noted in MTX-treated rats as compared with prechemotherapy values on day 6 of the study (+8%, $P < .05$). Daily food intake in all tumor-bearing rats was lower than in healthy rats, and the time course of drug-induced anorexia was similar to that observed in healthy rats (Fig 3). Tumor-bearing rats treated with CYP, 5-FU, and MTX returned to pretreatment levels of food intake by the end of study (~6 g/100 g body weight), but none of them reached the level seen in healthy rats (eg, ~8 g/100 g body weight).

N Balance

N balance was not significantly different between healthy and tumor-bearing animals during the 2-day period immediately preceding chemotherapy (Table 2). After drug treatment, N balance in all treatment groups had a distinct temporal sequence. 5-FU induced no significant changes in N balance of healthy rats; N balance was significantly

Table 1. Cumulative Food Intake and Body Weight Gain Following Drug Injection in Healthy and Tumor-Bearing Rats

Parameter	Healthy	MH7777-Bearing
Cumulative body weight gain (g/6 d)		
Control (sham)	6 ± 1	−1 ± 1*
CYP	1 ± 1†	−6 ± 2*
5-FU	1 ± 1†	−2 ± 1
CDDP	−23 ± 4†	−33 ± 3†
MTX	−5 ± 2†	−12 ± 3†
Cumulative food intake (g/100 g body weight/6 d)		
Control (sham)	42 ± 1	36 ± 1*
CYP	40 ± 2	31 ± 1*†
5-FU	39 ± 1†	30 ± 1*†
CDDP	18 ± 5†	11 ± 1†
MTX	30 ± 2†	23 ± 2*†

NOTE. Data for food intake are expressed per unit of body weight at the time of drug injection. Healthy rats (n = 40) and rats bearing MH7777 (n = 40) were randomized to 5 treatment groups (n = 8 per group). Drugs were delivered as a single IP injection, and rats were studied for 6 days posttreatment.

*Significantly different from healthy animals for each drug treatment group ($P < .05$).

†Significantly different from control for each parameter ($P < .05$).

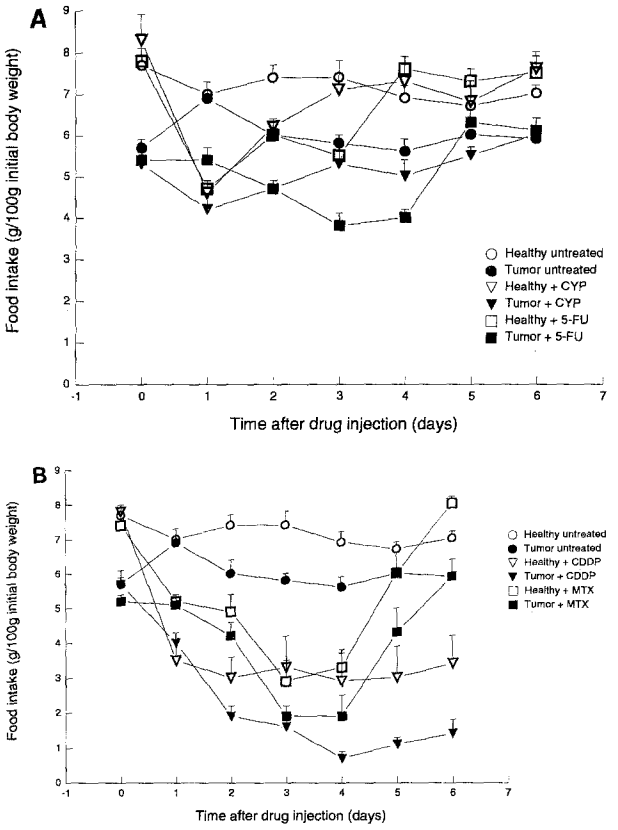

Fig 3. Effects of chemotherapy on food intake in healthy and tumor-bearing rats. (A) CYP and 5-FU; (B) CDDP and MTX.

decreased as compared with pretreatment values in healthy rats treated with CYP, MTX, and CDDP during the first 48 hours after drug injection (Table 2). Subsequently, CYP- and MTX-treated rats showed improved N balance. CDDP-treated rats showed the poorest N balance of all treated groups, and N balance in these animals remained decreased until the end of study (-70% *v* pretreatment values, $P < .05$). Similar results were noted for tumor-bearing rats (Table 2), but alterations in N balance were more pronounced than in healthy rats. For example, in the second period, tumor-bearing rats treated with 5-FU showed a significant reduction in N balance as compared with healthy rats treated with the same drug. This effect was particularly marked in tumor-bearing rats treated with MTX, which were not able to maintain positive N balance during this period. During the last period, N balance became more positive, and was above pretreatment values in rats treated with CYP and 5-FU ($P < .05$). In the CDDP group, N balance was not calculated during the last period because of severe diarrhea.

Apparent digestibility of N was not decreased by 5-FU, CYP, or MTX in both healthy and tumor-bearing rats (data not shown). Fecal N excretion (Table 2) closely paralleled food intake in all treatment groups, so that a constant proportion of dietary N was excreted in the feces. Differences in fecal N excretion therefore do not account for differences in N balance observed between treatments. Healthy rats treated with CDDP showed a slight reduction of N digestibility (76.3% *v* 84.1%, $P < .05$) during the first postchemotherapy period only. In tumor-bearing rats treated with CDDP, apparent digestibility of N was not calculated during the last period due to diarrhea.

Factors contributing to alterations in N balance (N intake and N excretion) varied with drug treatments (Table 2). CYP had minimal and transient effects on N balance in healthy and tumor-bearing rats even though they experienced a decrease in feed intake, because this loss was counterbalanced by reductions in urinary and fecal N. 5-FU had no significant effects on N balance in healthy rats. As for CYP, reduced N excretion compensated for reduced N intake. Tumor-bearing rats treated with 5-FU showed significantly poorer N balance during the second period after drug injection than healthy rats treated with the same drug. Thereafter, they showed elevated N balance, tending to restore lost N. Rats treated with CDDP showed the most negative N balance of all the treated groups. This was due to a large decrease in N intake, as well as a transient decrease in N digestibility, and did not substantially improve over the study period. Tumor-bearing rats developed severe diarrhea in the third period after CDDP treatment, and it was therefore not possible to determine N balance in these animals.

MTX injection was also associated with reduced N balance. Loss of intake was a primary factor in reduced N

Table 2A. Nitrogen Balance Following Drug Injection in Healthy and Tumor-Bearing Rats

	Fecal N (mg N/2 d)		Urinary N (mg N/2 d)		N Balance (mg N/2 d)	
Period	Healthy	Tumor	Healthy	Tumor	Healthy	Tumor
Prechemotherapy						
(n = 32)‡	159	140¶	505	326¶	279	262
SEM	7		13		12	
Postchemotherapy						
CYP (n = 8)						
Period 1	102†	87†	421†	360*	131†	139†
Period 2	141†	103*†	481†	341*	242	230
Period 3	169	133*	476†	304*	227	353*†
SEM	10		20		26	
5-FU (n = 8)						
Period 1	112†	115	304†	343	235	208
Period 2	152	91*†	368†	269*	273	159*†
Period 3	178	139*	415†	312*	299	369†
SEM	10		24		31	
CDDP (n = 8)						
Period 1	86†	71†	275†	288	−13†	32†
Period 2	72†	37*†	221†	111*†	27†	2†
Period 3	69†	—	200†	66*†	65†	—
SEM	12		30		30	
MTX (n = 8)						
Period 1	118	87†	342†	341	120†	186
Period 2	83†	47†	174†	266*	153†	−66*†
Period 3	149	104*	441	259*	256	302
SEM	14		23		27	

NOTE. See Table 2B for definition of superscripts and treatment description.

Table 2B. Nitrogen Balance Following Drug Injection in Healthy and Tumor-Bearing Rats

	Probability		
Drug	Period	Disease	Disease · Period
N balance			
CYP	<.01	NS	<.05
5-FU	<.05	NS	<.05
CDDP	<.01	NS	NS
MTX	<.01	NS	<.01
Fecal N			
CYP	<.01	<.01	NS
5-FU	<.01	<.01	<.01
CDDP	<.01	<.05	NS
MTX	<.01	<.01	NS
Urinary N			
CYP	NS	<.01	<.01
5-FU	<.01	<.01	<.01
CDDP	<.01	<.01	NS
MTX	<.01	<.01	<.01

NOTE. Healthy rats (n = 32) and rats bearing MH7777 (n = 32) were studied for 48 hours before administration of chemotherapy. Data were tested for effects of period after drug treatment, disease (tumor-bearing *v* healthy), and disease · period interactions using ANOVA; group means and pooled SEM are presented. Since disease · period interactions were present, differences between individual means within each drug were assessed using *t* tests.

*Different from healthy rats for the same period and drug treatment ($P < .05$).

†Different from prechemotherapy period for the same group of animals ($P < .05$).

‡Pooled data for all healthy and tumor-bearing rats before drug treatments.

¶Different from healthy rats ($P < .05$).

balance, but this was countered in part by reduced N losses, and MTX-treated healthy animals maintained positive N balance throughout the study. In healthy rats, urinary N excretion significantly decreased (by up to −65% *v* pretreatment values) when food intake decreased after MTX injection (Table 2). By contrast, relatively elevated urinary N excretion in tumor-bearing rats treated with MTX would appear to be related to poorer N balance in this group. Urinary N excretion did not decrease significantly from prechemotherapy levels after MTX injection (Table 2). It appeared that although healthy animals had decreased N intake after chemotherapy, they were in some measure capable of compensating for this deficit by reductions of urinary N losses; by contrast, tumor-bearing animals treated with MTX did not similarly reduce urinary N when intake decreased. This difference is clarified in Fig 4, in which urinary N excretion is plotted against N absorbed (N intake − fecal N) for each period (period O prior to chemotherapy; periods 1 to 3 postchemotherapy). After MTX injection, healthy rats showed a decrease in urinary N excretion as their N absorbed declined, in a nearly linear fashion so that N balance remained near pretreatment values. During the last period, both parameters returned to the pretreatment level. Following MTX injection, tumor-bearing rats showed only a slight reduction in N excretion, while N absorbed decreased markedly. During the last period, the increase in N absorbed was not associated with a proportional increase of urinary N excretion, thus leading to rapid improvements in N balance.

Organ Weights and N Content

Animals were killed after 6 days of N balance study, when different groups tended to be recovering (CYP, 5-FU, and MTX) or were still losing weight (CDDP) (Fig 2). At the muscle level, no effect of chemotherapy was observed in healthy treated rats at the end of study except in the CDDP group, in which EDL weight was significantly reduced by 9.3% as compared with that in untreated rats (data not shown). In tumor-bearing rats treated with CDDP, EPI and EDL weights were significantly decreased by 24% and 10.6%, respectively, as compared with untreated tumor-bearing rats. A reduction in N mass of EPI muscle was noted in tumor-bearing rats treated with CDDP only (−19%, $P < .05$). Liver weight was significantly decreased by 16.8% in healthy rats treated with CDDP, but was not different among other groups (Fig 5A). Chemotherapy induced no change in liver weights in tumor-bearing rats. None of the drugs studied had an effect on liver N mass (data not shown). Small intestine weight (Fig 5B) and protein mass (data not shown) were significantly increased in healthy rats treated with CYP (+21%), 5-FU (+26%), and MTX (+44%) as compared with untreated rats. No changes were observed in the CDDP group. Small intestine weight (Fig 5B) and N mass (data not shown) were significantly increased in tumor-bearing rats receiving MTX only.

DISCUSSION

Chemotherapeutic agents are widely used in the treatment of human cancers.[9] Their negative effects on host

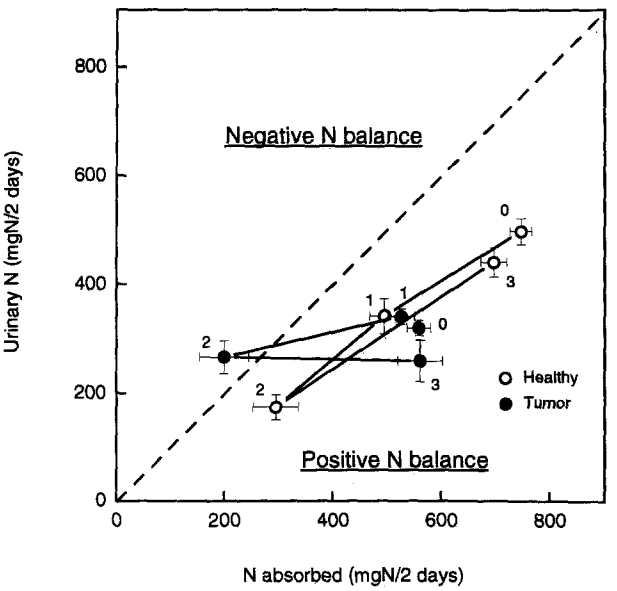

Fig 4. Time course of N absorbed and urinary N following treatment with MTX.

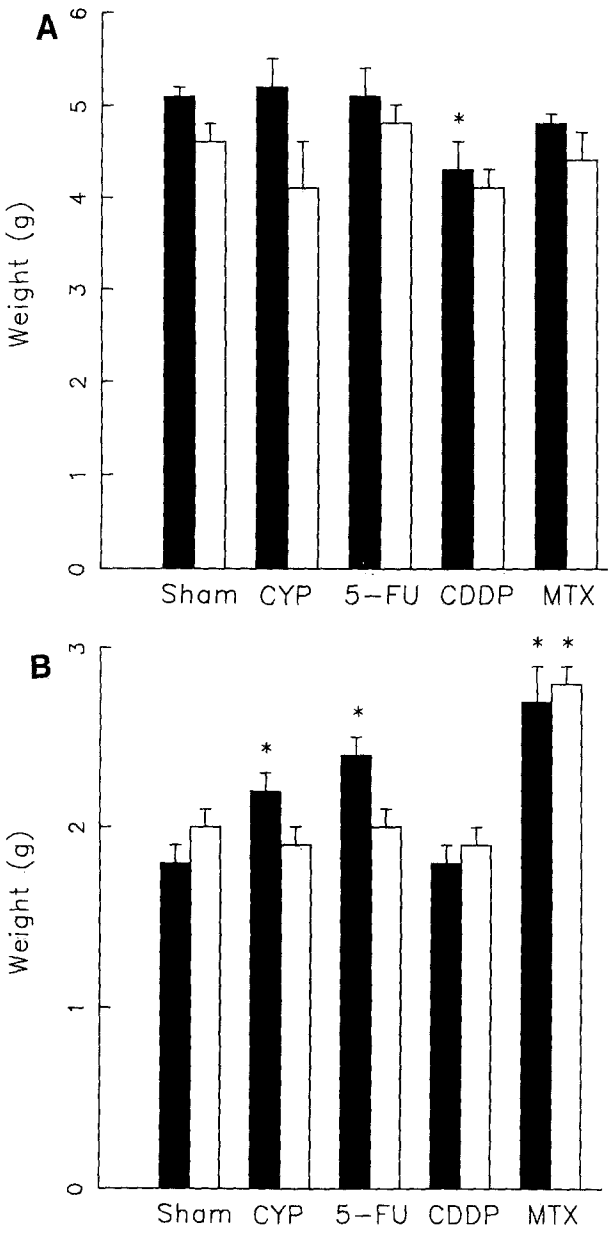

Fig 5. Effects of chemotherapy on liver and small intestine weight in healthy and tumor-bearing rats. Rats were sham-implanted (■; n = 40) or implanted subcutaneously with MH7777 (□; n = 40). (A) Liver; (B) small intestine. *Different from sham-treated rats ($P < .05$).

protein metabolism have been suspected from clinical data,[17-19] as well as a few experimental studies.[7,8] However, this report is the first descriptive and systematic study of the effects of chemotherapy on host N metabolism. Using a single high-dose injection of each drug, we showed distinct treatment effects and treatment-disease interactions. These results provide a basis for the understanding of factors involved in the development of cancer cachexia, in which aggressive treatments such as chemotherapy might play a key role.

Progressive growth of MH7777 is associated with profound alterations of host protein metabolism when the tumor reaches a substantial mass (> 10% of body weight).[4] After 7 days of growth when the tumor first became palpable (0.18% of body weight), MH7777 was responsible for moderate anorexia and body weight loss. Treatment was initiated at this time in the current study to mimic the clinical situation. Since the time course of changes following chemotherapy was unknown, we selected a 6-day study period and continuous measurements of N balance. We used four drugs widely used in treatment of human malignant diseases[9] that have shown activity against experimental tumors.[11,13,14,20-22] An IP route of administration was selected since it is frequently used in animals receiving single-dose chemotherapy[11,23-25] and allows for rapid absorption. High doses were selected according to the concept that chemotherapeutic agents be used at their maximum tolerable dose to kill the highest number of tumor cells and therefore prevent tumor recurrence and metastasis.[6] Doses of CYP and CDDP corresponded to approximately 50% of the 50% lethal dose (LD50) for rats (by IV or IP route).[9] For MTX, we used a dose approximately two times higher than the reported LD50,[9] based on many studies in which higher doses from 20 mg/kg/d[23,24] to 30 mg/kg/d[13,22] were used without mortality. In the case of 5-FU, we selected a dose that was approximately 20% of LD50,[9] since this agent is highly toxic for the gastrointestinal tract in rats with bleeding, diarrhea, and anorexia[5,9]; this can still be considered a high dose.[25,26] It is difficult to directly compare these doses with those used clinically, because of the relatively elevated metabolism of the rat.

A high therapeutic index was obtained in the MTX group, with a 100% tumor response without major clinical signs of drug toxicity. A good response was also obtained with CDDP, but at the dose of 5 mg/kg this treatment induced severe diarrhea in tumor-bearing animals. Toxicity of chemotherapeutic agents against cell populations of the gastrointestinal tract is well established[5,28-32] and is dose-dependent.[30] For example, a nearly 50% increase in intestinal permeability is noted 72 hours after subcutaneous administration of MTX (25 mg/kg).[29] Small-intestinal morphological alterations, including decreased crypt mitotic rate and villus height, width, and contour length, have been observed following MTX administration[28] or polychemotherapy with vincristine, 5-FU, and CDDP.[30] Interestingly, drug injection did not appear to interfere with the ability to absorb dietary N in the absence of diarrhea, and the apparent digestibility of N was not affected by the treatments.

This study provides a precise description of the time course (over 6 days) of several parameters of N metabolism following chemotherapy. All four agents induced anorexia, body weight loss, and poor N balance; however, these changes were drug-specific with a distinct temporal sequence. Even at the high doses used here, CYP and 5-FU had little impact on N metabolism. By contrast, MTX and CDDP induced profound anorexia, significant body wasting, and negative N balance. The presence of a characteristic response to each drug suggests the need for further study

to describe the effects of dose, repeated treatment, and polychemotherapy (multidrug) on host metabolism. It is noteworthy that MTX and CDDP induced negative effects on N balance but were also the drugs exhibiting significant antitumor activity. The relationship between severity of toxic and/or negative effects on the treated host and effectiveness of the treatment itself remains to be explained. Elimination of the drugs used here and of their metabolites is mainly urinary and complete within 6 days, except for CDDP.[9] Calculated N content in those agents varies from 10.5% (CDDP) to 24.7% (MTX), so that their relative contribution to N excreted in the urine (0.5 to 25 mg N over the study) was small compared with that of endogenous origin. It may also be calculated that the amount of N contained in tumor cells killed by these drugs was negligible compared with other sources of urinary N; however, an immune response to these dead cells may be an underlying cause of poor N balance in treated animals. Alterations of host protein metabolism in various organs (eg, reduced protein synthesis and/or increased protein breakdown) may also have contributed to increase the N losses in urine observed in treated, tumor-bearing rats. Specific time points (eg, day 3 to 4 after MTX injection) when rats showed a marked inability to retain N would be excellent end points to study the effects of chemotherapy at the tissue level.

The importance of chemotherapy-induced anorexia in poor N balance has been shown in healthy[8] and tumor-bearing rats.[7] In cancer patients receiving chemotherapy, the role of anorexia in body wasting is supported by clinical data: daily N and energy intake were reduced by 50% in cancer patients with weight loss as compared with those with stable weight.[18] In healthy rats receiving daily 5-FU at the dose of 17.5 mg/kg for 7 consecutive days, N balance became negative as oral intake progressively declined.[8] When N intake was maintained at normal levels by IV hyperalimentation, daily positive N balance was achieved; however, cumulative N balance remained lower than in untreated rats.[8] Similar results have been noted in cancer patients receiving chemotherapy and constant N intake through parenteral nutrition.[17] In our study, the degree of weight loss was clearly related to the degree of anorexia: a body weight loss greater than 10% of initial body weight was only observed in rats that reduced their food intake by greater than 50% and for more than 48 hours (eg, in MTX and CDDP). Healthy treated rats were able in some extent to compensate for reduced N intake through a proportional reduction in N excretion, so that N balance remained positive. Reduced fecal N excretion contributes to the N-sparing process following chemotherapy; similar results have been observed in cancer patients receiving polychemotherapy.[18] Treatment-induced anorexia and body weight loss were observed for each drug tested, suggesting that the anorexic effect of chemotherapy might play a key role in the development of cancer cachexia.

Except for rats treated with CDDP, the postinjection catabolic phase was followed by an anabolic phase characterized by a progressive return to normal food intake, body weight gain, and positive N balance. At the time when animals were killed, chemotherapy had no negative influence on tissue mass and N content, including liver and skeletal muscle; gut mass and N content were even increased at this time, especially in MTX-treated animals. Small intestine is characterized by a remarkably rapid anabolic process after chemotherapy. Following a single dose of CDDP in mice (8 mg/kg), the jejunal epithelium undergoes a temporary interruption of cell proliferation followed by a hyperplasia at 7 days posttreatment.[32] It is noteworthy that while CDDP induced wasting in skeletal muscle and liver, small intestine mass and N content were not decreased by this treatment at day 6 postinjection.

Our results show that alterations of N metabolism following chemotherapy are drug-specific, but more importantly confirm that they depend on animal status (tumor-bearing *v* healthy). A single prior study suggested that the impact of chemotherapy on host N metabolism might be different between tumor-bearing rats and healthy animals.[7] A single IV injection of 4-epidoxorubicin decreased cumulative N balance over 5 days by 55% in rats bearing Walker 256 carcinosarcoma, but not in healthy treated rats.[7] In the present study, effects of all chemotherapeutic agents were more pronounced in tumor-bearing than in healthy animals, both in terms of toxicity (diarrhea) and influence on N balance, even though the tumor comprised a small proportion ($<0.2\%$) of host body weight. For example, treatment with CDDP in healthy rats had minimal effects on N digestibility and a low incidence of mild diarrhea, but resulted in severe diarrhea in tumor-bearing animals. MH7777-bearing rats treated with MTX all went into negative N balance, while all healthy animals treated with the same dose of MTX remained in positive balance. A transient increase in the proportion of N lost in the urine was responsible for negative N balance in tumor-bearing animals, although the metabolic source of this N remains to be determined. Finally, the time required to achieve full recovery of pretreatment body weight or cumulative N balance was longer in tumor-bearing than in healthy rats. The fact that food intake did not return to normal levels in treated tumor-bearing rats has been described previously in cancer patients.[19] The reason(s) for these differences in the severity of wasting, anorexia, and poor N balance between healthy and tumor-bearing animals remains to be clarified. Diminished plasma clearance of MTX has been observed in malnourished rats[33,34] and in tumor-bearing rats.[35] In malnourished rats receiving 5-FU, higher toxicity as compared with well-nourished rats (diarrhea, weight loss, and mortality) was attributed to a decrease in hepatic activity of the rate-limiting enzyme in the degradation of 5-FU.[36]

There has been great interest in the possible applications of nutritional support in cachexic cancer patients. Based on the results presented here, it may be hypothesized that the anabolic phase after chemotherapy may be an appropriate time for the effective utilization of enteral feeds designed to preserve N in tumor-bearing subjects. A recent report[37] showed that glutamine-supplemented total parenteral nutrition improved protein synthesis in skeletal muscle and

suppressed protein degradation without influencing tumor growth in rats bearing an ascites hepatoma and treated with mitomycin C. At the same time, mitomycin C severely inhibited protein synthesis in liver, jejunum, and colon. It remains to be clarified whether such specific nutritional support would interact similarly in animals bearing different tumors and treated with different chemotherapeutic agents.

In an experimental situation comparable to that of human cancer (low tumor burden, moderate anorexia, and weight loss), we have shown that chemotherapy and the tumor interact to promote poor N balance and wasting in the host. We conclude that further studies of the mechanisms and therapy for cancer-associated cachexia must take into consideration the effects of both disease and treatment. Experimental models using untreated animals bearing large tumors or healthy animals treated with chemotherapy alone[23,27] may be of limited clinical relevance.

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