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# Methionine Sulfoximine Intensifies Cancer Anorexia[1]

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CHANCE, W. T., F.-S. ZHANG AND J. E. FISCHER. *Methionine sulfoximine intensifies cancer anorexia.* PHARMACOL BIOCHEM BEHAV **39**(1) 115–118, 1991.—Consistent anorexia was first observed 33 days after inoculating Fischer 344 rats with methylcholanthrene-induced sarcoma. Daily treatment of a similar group of rats with the glutamine synthetase inhibitor, methionine sulfoximine, elicited significant reductions of feeding by day 29 at a dose that had no effect on nontumor-bearing rats. Blood concentrations of ammonia were elevated in both groups of tumor-bearing rats and brain ammonia level was increased in the methionine sulfoximine-treated tumor-bearing rats. Forebrain concentrations of tyrosine, tryptophan, DOPAC and 5-HIAA were elevated in both groups of tumor-bearing rats. Since ammonia is detoxified through the glutamine synthetase reaction, these results suggest that blood and brain ammonia concentrations are more important than the neurochemical consequences of ammonia detoxification for the etiology of cancer anorexia.

Ammonia Anorexia Cancer Nutrition Neurotransmitters Dopamine Serotonin Amino acids

THE development of anorexia and cachexia present very severe problems in the therapy of cancer. In addition to limiting the aggressive use of available therapies (9), poor nutritional status is inversely related to therapeutic outcome (10,12). The seriousness of this problem is demonstrated by the observation that about 75% of the cancer patients will eventually become anorectic (17). In addition, cachexia has been reported to be a major cause of mortality in cancer patients (25).

Although acceptable animal models of cancer anorexia/cachexia have been available for nearly 40 years (17), no specific cause of the anorexia has been identified. Recent research (19) demonstrating the transfer of anorexia to parabiotic rats that shared a portion of their blood supply with anorectic tumor-bearing (TB) rats, suggests that some anorexigenic factor circulates in the blood. Several hypotheses of such a factor have been advanced including toxic hormones (18), peptides (24) or cachectin (16), however, elevated levels of these have not been demonstrated consistently in anorectic TB rats.

We have demonstrated (3) that blood ammonia concentrations are nearly doubled at the onset of anorexia in Fischer 344 rats bearing methylcholanthrene-induced (MCA) sarcomas and that blood levels of ammonia continue to increase as the tumor grows and as anorexia worsens. In addition, infusing ammonium salts IV into normal rats elicits anorexia at blood ammonia concentrations similar to those observed in anorectic TB rats (3). The generality of these observations was suggested by our recent report of hyperammonemia in Buffalo rats bearing Morris hepatomas (8). Return of normal feeding rapidly follows resection of experimental tumors (2, 17, 19) and blood ammonia concentrations are normalized within 24 hours of tumor resection (2).

Therefore, hyperammonemia appears to be of etiological importance in the development of experimental cancer-induced anorexia. In the present study this hypothesis was investigated using the inhibitor of glutamine synthetase, methionine sulfoximine [(14); MSO]. Since ammonia is detoxified through the glutamine synthetase reaction, we hypothesized that the anorexia would be worsened by MSO if hyperammonemia was the primary cause of the anorexia. Conversely, if the metabolic consequences of ammonia detoxification in the brain, such as elevated neutral amino acid level (3) and increased amine neurotransmitter metabolism (3), were responsible for experimental cancer anorexia, MSO treatment should decrease the intensity of the anorexia.

## METHOD

Thirty male, Fischer 344 rats (200–250 g) were purchased from Charles River Laboratories (Wilmington, MA) and acclimated to the laboratory environment for one week. These rats were housed individually with ad lib access to Purina rat chow and water. Following anesthetization with halothane, 18 of these animals were inoculated, using a 4 mm diameter trocar, with approximately 50 mg fresh MCA sarcoma tissue, taken from a donor rat in our colony. An additional 12 rats were sham-inoculated using an empty trocar.

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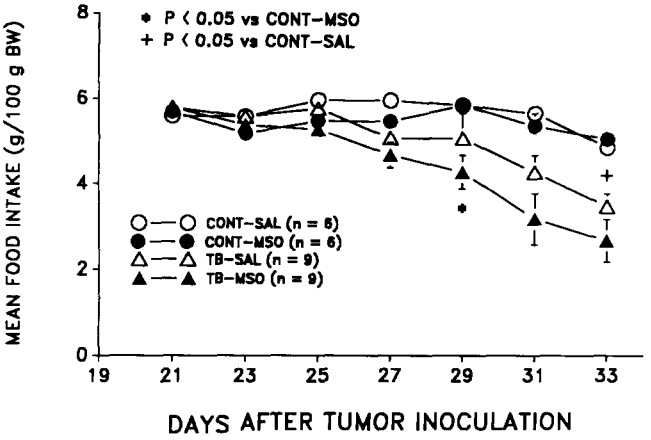


FIG. 1. Mean (±SEM) daily food intake by tumor-bearing (TB) and control (CONT) rats treated with saline (SAL) or 2.5 mg/kg/day methionine sulfoxamine (MSO) for 14 days, beginning 19 days after tumor inoculation. Analysis of variance revealed significant (*$p<0.05$) anorexia in the TB-MSO rats beginning with day 29, while the TB-SAL rats did not exhibit consistent anorexia (+$p<0.05$) until day 33.

Six non-TB (CONT) and 9 TB rats were treated (SC) daily with 2.5 mg/kg MSO, beginning 19 days after tumor inoculation, while the remaining rats received injections of normal saline (SAL). This dose of MSO was chosen from pilot investigations as a dose that would have minimal effects on food intake by control rats. Food intake and body weight were monitored daily for the next 14 days (day 33), at which time the rats were euthanized by decapitation for biochemical analyses. Immediately following decapitation the rats' heads were immersed in liquid nitrogen for several minutes and stored at −80°C until brain tissue was extracted. Tumors were excised from the carcass and weighed. A block of forebrain weighing approximately 200 mg and containing striatal, accumbens and septal tissue was cut from the frozen head at −20°C and processed for amine neurotransmitters and metabolites using HPLC-EC according to our previously published methods (3). Brain ammonia concentrations were determined using the glutamate dehydrogenase reaction (Sigma Chem. Co. Kit No. 170-10) on samples of the cerebral cortex following homogenization in 2 ml of 0.5 N $HClO_4$. Plasma samples were retained for similar determinations of ammonia levels and free amino acid profile employing a Beckman 121 MB automated amino acid analyzer as reported previously (3).

These results were analyzed statistically using analysis of variance techniques, with individual means being compared post hoc by Scheffe's multiple range test. Food intake was analyzed by a repeated measures ANOVA conducted for days 25 through 33 as well as by separate ANOVA tests conducted each day. All procedures in this report conform to NIH guidelines and were approved by the University of Cincinnati Animal Care Committee.

TABLE 1

MEAN (± SEM) PLASMA (nmol/ml) GLUTAMINE AND AMMONIA AND BRAIN (nmol/g) AMMONIA CONCENTRATIONS OF CONTROL (CONT) AND TUMOR-BEARING (TB) RATSW TREATED DAILY WITH NORMAL SALINE (SAL) OR 2.5 mg/kg METHIONINE SULFOXAMINE (MSO) FOR 33 DAYS

| Group | N | Plasma Glutamine | Plasma Ammonia | Brain Ammonia |
|---|---|---|---|---|
| CONT-SAL | 6 | 842 ± 53 | 78 ± 4 | 623 ± 37 |
| CONT-MSO | 6 | 593 ± 42* | 122 ± 14* | 684 ± 30 |
| TB-SAL | 9 | 650 ± 48* | 186 ± 24* | 680 ± 29 |
| TB-MSO | 9 | 475 ± 51*‡ | 177 ± 15*† | 840 ± 52*‡ |

*$p<0.05$ vs. C-SAL; †$p<0.05$ vs. C-MSO; ‡$p<0.05$ vs. TB-SAL.

## RESULTS

As presented in Fig. 1, treatment of TB rats with MSO reduced food intake. Although there was no significant difference between TB-MSO and TB-SAL rats, TB-MSO rats ate significantly ($p<0.05$) less food than did CONT-MSO rats beginning with day 29. A similar degree of anorexia was not observed in the TB-SAL rats until posttumor inoculation day 31, with the significant ($p<0.05$) difference between TB and CONT rats being lost on day 32. There was no significant difference between CONT-MSO rats and CONT-SAL on any of the treatment days. None of the groups differed in body weight, with group means falling between 264±7 g (CONT-MSO) and 270±8 g (TB-SAL) at the conclusion of the study. There was no difference in tumor weights between the MSO and SAL groups (45±3 g vs. 43±5 g, respectively) at sacrifice.

As presented in Table 1, plasma ammonia concentrations were elevated significantly in both groups of TB rats and in CONT-MSO rats. Brain levels of ammonia were also increased ($p<0.05$) in the TB-MSO rats as compared to the TB-SAL group or either control group. Plasma glutamine concentrations were decreased ($p<0.05$) in the CONT-MSO rats as well as in both TB-SAL and TB-MSO groups, which were significantly ($p<0.05$) from each other (Table 1).

Alterations in amine neurotransmitter metabolism are presented in Table 2. Both groups of TB rats exhibited elevations in brain concentrations of TYR, TRP, DOPAC and 5-HIAA suggesting increased synthesis and metabolism of DA and 5-HT. MSO treatment had no significant effect on neurotransmitter metabolism in TB or control rats.

## DISCUSSION

These results continue to suggest that hyperammonemia is of primary importance in the etiology of experimental cancer anorexia. Thus TB rats treated with MSO exhibited significantly reduced feeding 4 days before the appearance of a similar degree of anorexia in a group of saline-treated TB rats. Since tumor weight and body weight were not significantly different between the TB-MSO and TB-SAL groups, the difference in anorexia could not be due to these factors. The increase in brain ammonia concentration in the TB-MSO rats is consistent with the hypothesis of hyperammonemia causing experimental cancer anorexia.

As indicated in reports concerning the etiology of hepatic encephalopathy (23), ammonia is a toxic substance to the central nervous system. Although high blood concentrations of ammonia have been suggested (15) to alter blood-brain barrier permeability to large neutral amino acids (LNAA: TYR, TRP, phenylalanine, methionine, threonine, valine, leucine, isoleucine and histidine), other reports suggest that LNAA changes are secondary to increased brain glutamine levels (13,14). Thus ammonia is detoxified in brain and other organs by the glutaminesynthetase reaction to form glutamine. Consequently,

TABLE 2

MEAN (± SEM) CONCENTRATIONS OF NEUROTRANSMITTERS, NOREPINEPHRINE (NE), DOPAMINE (DA) AND SEROTONIN (5-HT), PRECURSORS, TYROSINE (TYR) AND TRYPTOPHAN (TRP), AND METABOLITES, 3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC), HOMOVANILLIC ACID (HVA) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN THE FOREBRAIN OF CONTROL (C) AND TUMOR-BEARING (TB) RATS TREATED DAILY WITH SALINE (SAL) OR METHIONINE SULFOXAMINE (MSO) FOR 16 DAYS

| Group | TYR (μg/g) | DA (ng/g) | DOPAC (ng/g) | HVA (ng/g) | NE (ng/g) | TRP (μg/g) | 5-HT (ng/g) | 5-HIAA (ng/g) |
|---|---|---|---|---|---|---|---|---|
| C-SAL | 12.4 ± 0.8 | 1954 ± 436 | 185 ± 28 | 88 ± 11 | 572 ± 82 | 2.1 ± 0.1 | 1134 ± 69 | 726 ± 80 |
| C-MSO | 12.0 ± 0.8 | 2144 ± 667 | 199 ± 46 | 88 ± 26 | 640 ± 67 | 2.1 ± 0.2 | 1192 ± 49 | 828 ± 53 |
| TB-SAL | 35.8 ± 2.9* | 2688 ± 300 | 334 ± 25* | 118 ± 11 | 705 ± 46 | 4.6 ± 0.4* | 1302 ± 24 | 1313 ± 183† |
| TB-MOS | 40.8 ± 4.1‡ | 3407 ± 435 | 398 ± 45‡ | 143 ± 22 | 602 ± 68 | 5.0 ± 0.4‡ | 1298 ± 29 | 1156 ± 79‡ |

*$p<0.01$; †$p<0.05$ vs. C-SAL; ‡$p<0.01$ vs. C-MSO.

brain concentrations of glutamine are more than doubled in MCA sarcoma-bearing and in ammonia-infused rats (3,14). The excess glutamine is cleared from the brain by the same carrier system that facilitates the influx of LNAA (13,20). Thus brain concentrations of TYR, TRP, methionine, phenylalanine, threonine and histidine were also elevated in TB and ammonia-infused rats (3). Brain levels of the branched-chain amino acids (valine, leucine and isoleucine) were not elevated in TB rats, probably due to their decreased plasma concentrations. Since TRP and TYR are respective precursors for the neurotransmitters, 5-HT and DA, elevations of these amino acids may also increase turnover of 5-HT (11) and DA (7). Anorectic TB rats and ammonia-infused rats exhibited elevated metabolism of both 5-HT and DA (3). Therefore, a cascading series of neurochemical alterations appear to be set in motion by the hyperammonemia of TB rats. Any neurochemical aberration within this series of changes may be important for the etiology of experimental cancer anorexia. These results, however, appear to favor ammonia as the primary cause of experimental cancer anorexia.

Similar conclusions have been drawn from additional experiments demonstrating hyperammonemia and anorexia in Buffalo rats bearing Morris hepatomas (8). In this experiment (8), minimal alterations in neurotransmitter metabolism were observed. In addition, unpublished experiments in our laboratory employing DA and 5-HT receptor blockers and depletors as possible treatments for cancer anorexia have yet to produce positive results. Therefore, ammonia may cause anorexia without significantly affecting neurotransmitter metabolism. In addition, the peripheral effects of ammonia may also influence feeding behavior, since there is evidence of an effect of ammonia on hepatic glucoreceptors (21).

Previous experiments investigating the development of anorexia as the tumor grows (5) or anorexia following the infusion of ammonium salts into non-TB rats (3) suggest a threshold for anorexia of approximately twice the normal blood concentration of ammonia. Therefore, although the CONT-MSO rats exhibited elevated plasma ammonia levels, significant anorexia was not observed in these rats. Pilot investigations employing larger doses of MSO, however, indicated that even 5 mg/kg MSO was sufficient to reduce food intake in non-TB rats.

Since MSO blocks the glutamine synthetase reaction, the detoxification of ammonia and formation of glutamine should be reduced. That these effects occurred was supported by the increase in plasma ammonia in control rats treated with MSO and by the increase in brain ammonia level in TB rats treated with MSO. Alterations in plasma glutamine concentrations also support this action of MSO, with both groups of MSO-treated rats exhibiting lower glutamine levels than their respective controls. Saline-treated TB rats also exhibited significantly decreased plasma glutamine concentrations due to the tumor's utilization of this amino acid (4), while brain concentrations of glutamine were greatly elevated due to cerebral detoxification of ammonia through the glutamine synthetase reaction (3). Analysis of neurotransmitter alterations, however, did not reveal the expected MSO-specific alterations, such as decreases in TYR, TRP, DOPAC or 5-HIAA. Perhaps the dose of MSO was too low or the hyperammonemia too minimal for these alterations to be expressed. The previously observed (3) increases in DA and 5-HT neurotransmission, however, were exhibited in the TB rats.

The source of this hyperammonemia is apparently the tumor tissue itself. Measurement of ammonia concentrations in venous blood draining MCA sarcomas revealed ammonia levels that were several fold greater than those in arterial blood (5). In addition, other experimental tumors have been reported to release ammonia both in vitro (1) and in vivo (1,22). We have also observed hyperammonemia in Buffalo rats transplanted with Morris hepatomas (8). The hyperammonemia apparently results from the tumor's metabolism of glutamine through the glutamate synthetase reaction to form glutamate and ammonia as well as by other pathways in the synthesis of RNA (26). That glutamine is an essential amino acid for tumor growth has been demonstrated the effectiveness of the glutamine antimetabolite, acivicin, in stopping tumor growth (4,26). Therefore, reducing the growth of the tumor mass or removing it entirely should reduce blood ammonia concentration and lead to the return of normal feeding. Although tumor removal has been shown to enhance feeding (6,19) and reduce blood ammonia level (2), reduction of tumor growth, at least by chemotherapy or radiation, is usually associated with continued anorexia, perhaps due to the direct toxic effects of the treatment. Future studies should be directed toward enhancing the host metabolism of ammonia in addition to reducing the growth of neoplasms.

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