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Oral dosing of Recombinant Methioninase Is Associated With a 70% Drop in PSA in a Patient With Bone-metastatic Prostate Cancer and 50% Reduction in Circulating Methionine in a High-stage Ovarian Cancer Patient

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Abstract. *Background/Aim: Methionine addiction is a general and fundamental hallmark of cancer. Methionine addiction can be targeted by methionine restriction (MR). Our laboratory has studied methionine addiction in cancer and MR for almost 50 years. The present study describes oral recombinant methioninase (o-rMETase), as a supplement, to induce MR in cancer patients. Patients and Methods: One patient, a 67-year-old female with high-stage ovarian cancer took o-rMETase twice a day at 250 units per dose for approximately one month. A second patient, a 76-year-old male with bone-metastatic prostate cancer, took o-rMETase twice a day at 250 units per dose during three months. Results: The first patient's circulating methionine levels decreased 50% within 4 hours of taking 250 units of o-rMETase. The second patient's PSA dropped approximately 70% over 3 months. During this time the patient's hemoglobin increased. Conclusion: o-rMETase has no side effects and is potentially efficacious. Future studies involving larger cohorts of patients with high-stage cancer are required to determine if o-rMETase, as a supplement, can increase survival and improve the quality of life.*

Methionine addiction is a general and fundamental hallmark of cancer (1, 2). Methionine restriction (MR) selectively arrests cancer cells in the S/G_2-phase of the cell cycle and kills clonogenic cells which are equivalent to tumor-initiating cells and renders cancer cells sensitive to cell-cycle-specific drugs (3-6). Methionine addiction is due to cancer cells having an increased overall rate of transmethylation compared to normal cells that leads to methionine overuse, thereby depleting cellular pools of S-adenosylmethionine (7, 8). Overuse of methionine by cancer cells is called the "Hoffman effect" (9), a possibly stronger effect than the Warburg effect of overuse of glucose by cancer cells (10). DNA hypomethylation in human cancer, discovered in our laboratory (11-13), is probably the result of elevated (7) and altered transmethylation occurring in cancer cells (14). DNA hypomethylation leads to aneuploidy and subsequent cancer (15). Other fundamental characteristics of cancer are linked to methionine addiction (16).

We have used L-methioninedeamino-mercaptomethane-lyase (methioninase) for methionine restriction (MR) (17). The methioninase gene from *P. putida* has been cloned and expressed in *Escherichia coli* and is termed recombinant methioninase (rMETase) (17).

Prostate cancer causes more cancer deaths in North American men except for lung cancer (18). There has been improvement in the early detection of prostate cancer due to the measurement of circulating prostate-specific antigen (PSA) and sophisticated imaging techniques (19). However, metastatic prostate cancer remains recalcitrant (19). Androgen-deprivation therapy was first-line therapy from 1941 until 2015, when clinical trials showed that androgen-deprivation therapy combined with docetaxel improved survival (20, 21). However, survival of bone-metastatic prostate cancer patients is still low (18) and in need of improved therapy and a possible candidate is MR by methioninase.

Recombinant methioninase (rMETase) is effective against human cancer cell lines (lung, colon, kidney, melanoma, brain, and prostate) *in vitro*. rMETase had a mean IC_{50} for cancer cells 10 times lower than that for normal cell lines (22).

rMETase has arrested all cancer types tested in mouse models including patient-derived orthotopic xenograft (PDOX) models (23-49).

Oral administration of rMETase (o-rMETase) is highly effective on recalcitrant sarcoma, pancreatic cancer, colon

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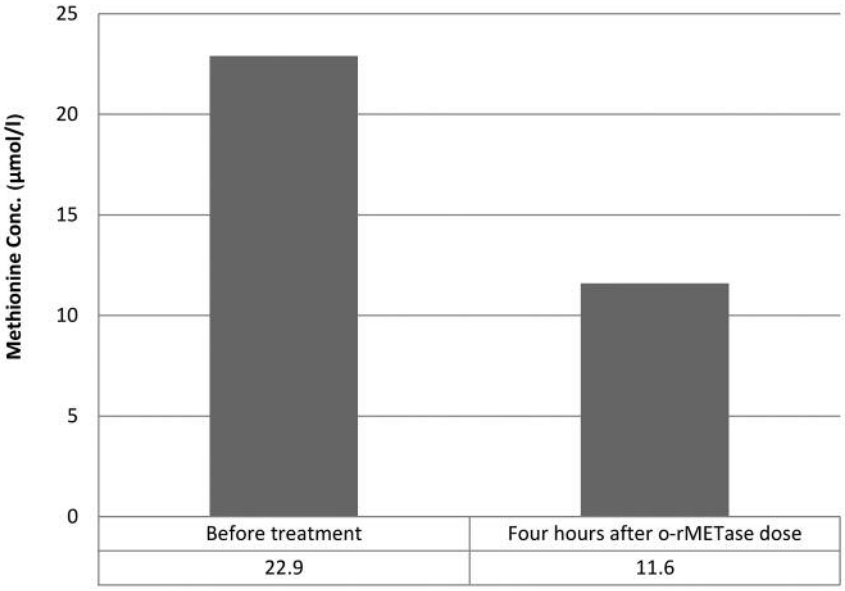

Figure 1. *Circulating methionine levels (µM) four hours after the ovarian cancer patient took 250 units o-rMETase on day 15. Methionine was measured as described in Materials and Methods.*

cancer, and melanoma in PDOX models (24, 25, 26, 27, 31, 32, 33, 39, 40, 46).

Previous primate safety studies of PEGylated r-METase in macaque monkeys showed that *i.v.* administration of PEGylated rMETase depleted plasma methionine to <5 µmol/l with transient anemia as the only side effect. Repeated treatment did not result in anaphylaxis (50).

A pilot phase I clinical trial was carried out to determine i.v. rMETase toxicity, and the extent of methionine depletion in high-stage cancer patients. Circulating methionine was lowered to 0.1 µM by methioninase without toxicity (51, 52).

In the present study, oral recombinant methioninase (o-rMETase) was taken as a supplement by a 76-year-old male with bone-metastatic prostate cancer with a PSA of over 2000 and a 67-year-old female with high-stage ovarian cancer.

Materials and Methods

Oral rMETase production and formulation. rMETase was produced by fermentation of recombinant *E. coli*. and purified using column chromatography with DEAE-Sepharose FF, Sephacryl S-200HR, and ActiCleanEtox, resulting in 98% purity with low endotoxin and high yield (17). Pure methioninase was dissolved in PBS or water at 5 mg/ml, which comprised one dose for oral administration after breakfast and dinner.

Methionine measurement. Methionine in plasma was derived with o-phthalaldehyde (OPA), and separated by HPLC using a Supelcosil LC-18-DB column (25 cm × 4.6 mm), and eluted with tetrahydrofuran/methanol/0.1 M sodium acetate, pH 7.0, v/v/v=5/95/900 and methanol. Detection was by fluorescence (53).

Prostate specific antigen (PSA). PSA was measured with the Abbott Architect; i2000 chemiluminescence immunoassay.

The ARCHITECT Total PSA assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of total PSA.

Results

A patient with high-stage ovarian cancer took o-rMETase twice a day at a dose of 250 units. After approximately one month of administration, the patient had no side effects. Within four hours of a dose of o-rMETase the patient's circulating methionine decreased 50% (Figure 1).

A second patient with bone-metastatic prostate cancer and a PSA of over 2000 took o-rMETase twice a day at a dose of 250 units for three months, thus far. During this time the PSA levels decreased approximately 70% (Figure 2A, B). The patient's hemoglobin levels increased from 7.4 to 8.7 g/dl during this time (Figure 3). No side effects were observed.

Discussion

Methionine addiction is a general and fundamental hallmark of cancer (1, 2). It has been studied for over 60 years, since Sugimura (54) observed that MR diets reduced significantly the growth of tumors growing in rats. In the early and mid-seventies, cell culture experiments showed that cancer cells had an elevated requirement for methionine (55, 56). It was thought that cancer cells were simple auxotrophs that could

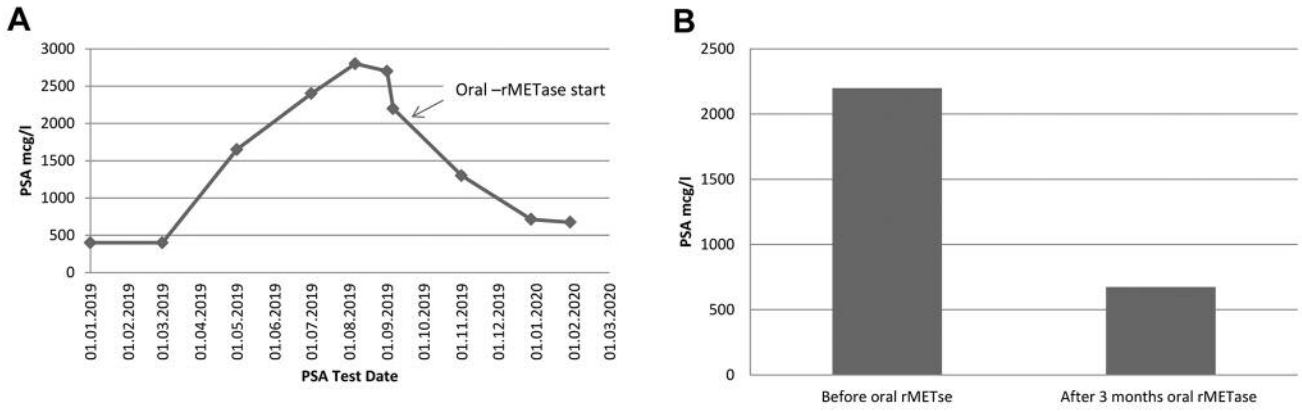

Figure 2. *PSA levels. A). PSA levels in the prostate cancer patient were measured at the indicated times and o-rMETase was started at the indicated time, twice a day at 250 units per dose: B). Summary bar graph of PSA levels at the start of o-rMETase use and after three months.*

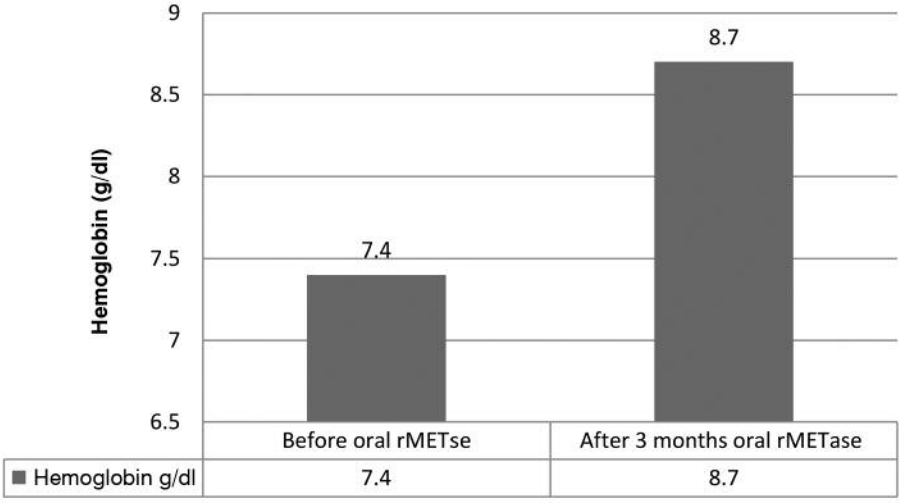

Figure 3. *Hemoglobin levels in the prostate cancer patient before and after taking o-rMETase for 3 months.*

not synthesize methionine from homocysteine. However, we showed that cancer cells synthesize as much methionine or more than normal cells (2, 8). It became clear that cancer cells were addicted to methionine, which we showed was due to excess transmethylation (7).

Recently papers have come out claiming novelty regarding methionine addiction (57, 58), about which we published long ago (1-8, 59-62).

The first methioninase was isolated by Kreis from Clostridium (63). A more stable methioninase was subsequently isolated in Japan by Tanaka *et al*. (64) from Pseudomonas putida, which we later cloned and developed into a very effective anticancer agent (17). Many studies have demonstrated that rMETase is efficacious in cancer cells *in vitro*, cell-line tumors growing in mice, and PDOX models (23-49). However, it was difficult to develop an injectable rMETase into a drug since it is a bacterial protein that elicited strong immune reactions in monkeys, which could be partially overcome by PEGyletion (50).

A major breakthrough occurred in 2017 when we observed that rMETase (31), despite being a very large protein, could be administered very effectively orally (24, 25, 26, 27, 32, 33, 39, 40, 46). o-rMETase restricts methionine in the gut, and thereby lowers the levels of methionine in the bloodstream (33). o-rMETase itself did not enter the bloodstream making it a far safer agent than injectable methioninase. Therefore, our strategy was to develop methioninase as an oral supplement.

In the present studies, we showed that oral methioninase greatly reduced circulating methionine in a human subject with high-stage ovarian cancer (Figure 1). Therefore, we then tested o-rMETase on an advanced bone-metastatic prostate cancer patient with an extremely high PSA. The patient received o-rMETase for three months without any side effects and his PSA dropped approximately by 70% (Figure 2). The patient's overall performance was improved, with increased hemoglobin levels (Figure 3) and the patient was feeling much better during the time he was taking oral methioninase. After the patient started to take oral methioninase, he also began oral dexamethasone (65), which may have helped to lower PSA levels. We will recruit additional patients with high-stage cancer for the testing of oral methianinase as a supplement.

Conflicts of Interest

The Authors report no conflicts of interest related to this study.

Authors' Contributions

Qinghong Han and Yuying Tan performed all experiments including the production of methioninase. Robert M. Hoffman and Qinghong Han wrote and revised the paper.

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