

Can Homeopathic Treatment Slow Prostate Cancer Growth?

Wayne B. Jonas, MD, Jaya P. Gaddipati, PhD, N. V. Rajeshkumar, PhD, Anuj Sharma, MS, Rajesh L. Thangapazham, MS, Jim Warren, MS, Anoop K. Singh, PhD, John A. Ives, PhD, Cara Olsen, MS, MPH, Steven R. Mog, DVM, and Radha K. Maheshwari, PhD

Background: Homeopathy is a complementary medicine widely used around the world. Despite extensive use of homeopathy for cancer and other serious conditions with reported success, clinical and laboratory research has been equivocal, and no rigorous research has been done on cancer. In 1999, the US National Cancer Institute evaluated the effects of homeopathic treatment of cancer from a clinic in India and has released a request for protocols to conduct further research into this treatment. Therefore, the authors conducted a series of carefully controlled laboratory studies evaluating the effects of commonly used homeopathic remedies in cell and animal models of prostate cancer. **Study Design:** One hundred male Copenhagen rats were randomly assigned to either treatment or control groups after inoculation with prostate tumor cells. **Methods:** Prostate tumor cells DU-145, LNCaP, and MAT-LyLu were exposed to 5 homeopathic remedies. Male Copenhagen rats were injected with MAT-LyLu cells and exposed to the same homeopathic remedies for 5 weeks. **In vitro** outcomes included tumor cell viability and apoptosis gene expression. **In vivo** outcomes included tumor incidence, volume, weight, total mortality, proliferating cell nuclear antigen (PCNA) expression, apoptotic cell death (terminal deoxynucleotidyl transferase mediated d-uridine triphosphate nick end labeling), and gene expression (rAPO-multiprobe). **Results:** There were no effects on cell viability or gene expression in 3 prostate cell lines with any remedies at any exposure time. There was a 23% reduction in tumor incidence ($P < .0001$), and for animals with tumors, there was a 38% reduction in tumor volume in homeopathy-treated animals versus controls ($P < .02$). At time of killing, experimental animals with tumors had a 13% lower average tumor weight ($P < .05$). Tumors in these treated animals showed a 19% increase in apoptotic cell death ($P < .05$) and reduced PCNA-positive cells. **Conclusions:** The findings indicate that selected homeopathic remedies for the present study have no direct cellular anticancer effects but appear to significantly slow the progression of cancer and reduce cancer incidence and mortality in Copenhagen rats injected with MAT-LyLu prostate cancer cells.

Keywords: homeopathy; prostate; cancer; rat; CAM; complementary medicine

Homeopathy is a 200-year-old therapeutic system that uses small doses of substances selected by matching a patient's symptoms with symptoms produced by these substances in healthy individuals. Given to patients with the appropriate symptoms, these medicines are thought to stimulate autoregulatory and self-healing processes.¹ Homeopathic medicines are prepared by serial dilution and shaking. The medicines are often used at extremely high dilutions, an approach that generates considerable controversy and leaves most scientists with serious doubts about the plausibility of any positive reports on its effects.²

Regardless, homeopathy is widely used around the world, especially in England, France, Germany, India, and South America, with increasing use in the United States.^{3,4} In India, there are a large number of clinics using homeopathy, and practitioners claim success in treating serious diseases such as cancer.⁵ While there have been a number of randomized, placebo-controlled clinical trials of homeopathy, most are poorly designed, and the high-quality studies report mixed results, meaning some positive and some negative reports.^{6,7}

There have been no high-quality studies of homeopathy on the treatment of cancer, despite its widespread use for this condition.⁸ Homeopathy is a complementary and alternative medicine used extensively around the world for the treatment of cancer. In 1999, the US National Cancer Institute conducted a best-case series evaluation of a homeopathic cancer clinic in India and concluded that there was sufficient evidence of possible efficacy to warrant further research; they recently issued a request for proposals

WBJ and JAI are at the Samuelli Institute, Alexandria, Virginia. JPG, NVR, AS, RLT, JW, and RKM are in the Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland. CO is at the Biostatistics Consulting Center, Uniformed Services University of the Health Sciences, Bethesda, Maryland. SRM is at the Armed Forces Radiobiology Research Institute, Bethesda, Maryland.

Correspondence: Wayne B. Jonas, Samuelli Institute, 1700 Diagonal Road, Suite 400, Alexandria, VA 22314.

DOI: 10.1177/1534735406294225

to further study this treatment.⁹ Therefore, we decided to investigate the effects of selected homeopathic drugs in cell and animal models of cancer. While this may not exactly mimic the use of these remedies in clinical practice, it does allow for more precise controls to rule out artifact, bias, and placebo effects and to examine potential mechanisms of action.

We selected prostate cancer as a test model. Despite major advances in early diagnosis and improved treatment methods, the American Cancer Society estimates that about 232 090 men in the United States will be diagnosed to have prostate cancer and 30 350 will die from the disease in 2005.¹⁰ There are several homeopathic remedies reported as useful for prostate cancer in the homeopathic literature and from cancer clinics. The main ones are (1) *Conium maculatum*, (2) *Sabal serrulata*, (3) *Thuja occidentalis*, (4) *Zincum metallicum*, (5) *Lycopodium*, (6) *Carcinosin*, (7) *Colchicum*, and (8) *Hydrangea*.¹¹ Of these, we tested the 4 drugs most frequently prescribed for the treatment of prostate cancer, namely, *C maculatum* (1000 c), *S serrulata* (200 c), *T occidentalis* (1000 c), and *Carcinosin* (1000 c). (The number followed by “c” in the parentheses indicates the number of centesimal [c] dilutions.) A final concentration is not an appropriate way for describing homeopathic remedies since the process results in a potency, not a concentration. Thus, 1000 c means the starting substance was diluted 99:1 stepwise 1000 times with vigorous shaking of the diluting chamber between each dilution step.

The goals of this study were to (1) systematically investigate homeopathic treatment using these remedies in vitro on prostate cancer cells lines DU 145, LNCaP, and MAT-LyLu; (2) conduct a sufficiently powered, blinded, controlled study of the in vivo effects of these drugs on tumor growth and progression in a Copenhagen allograph rat model using MAT-LyLu prostate cancer cells; and (3) explore possible mechanisms of action if cancer-modulating effects were observed.

Methods

Cell lines: prostate cancer cell lines. MAT-LyLu, LNCaP, and DU 145 cells were obtained from American Type Culture Collection (Manassas, Va). MAT-LyLu cells were maintained in RPMI 1640 medium supplemented with 250 nM dexamethasone and fetal bovine serum in humidified incubator (5% CO₂; 37°C).

Homeopathic medicines. Homeopathic remedies *T occidentalis* (1000 c), *C maculatum* (1000 c), and *S serrulata* (200 c) were purchased from Boiron Inc (Newton Square, Pa). For *Carcinosin* preparation, MAT-LyLu cells grown to 80% confluence were trypsinized and washed twice with cold phosphate-buffered saline

(PBS). The cells were resuspended in 75% ethanol and passed through a 26-gauge syringe needle 10 to 15 times. A 0.1-mL suspension was diluted with 9.9 mL of 75% ethanol. Subsequent dilutions to 1000 c were done in water by Washington Homeopathic Products Inc (Bethesda, Md). Controls were diluted and shaken in tap water.

Animals and treatment. The in vivo studies were performed on 4- to 5-week-old Copenhagen rats (Charles River Laboratories) at the Department of Pathology, Uniformed Services University of the Health Sciences (USUHS). The animal protocol was approved by Institutional Animal Care and Use Committee, USUHS. Animals were inoculated with 10 000 MAT-LyLu cells in 100 µL PBS by intradermal injection. From the second day of cell inoculation, animals were given 100 µL of homeopathic remedy or control water by oral gavage once daily. Control water was prepared by serially diluting and shaking distilled water in a manner similar to the experimental preparations. All animals were housed in individual cages and had ad libitum access to food and water. Body weight was determined weekly.

Tumor and histopathologic assessment. Tumors were measured under blind conditions every fourth day with calipers, and tumor volume was calculated using the formula for volume (V), $0.5236 \times a \times b \times c$, where a, b, and c are the 3 radii. Animals killed at the end of the experiment had blood collected and centrifuged. Tumor and lungs were removed, weighed, and used for histology studies. Lungs were inspected under blind conditions for visible morphological changes. All tissues collected were fixed in neutral buffered 10% formalin solution. Lungs were inflated with formalin in situ before fixing. Tissue was embedded in paraffin, and 5-µm-thick sections were stained with hematoxylin eosin. All histological examinations were carried out blind to group assignment by a pathologist (S.R.M.) and captured using a Nikon Eclipse E400 microscope with a Nikon digital camera (DXM1200).

Proliferating cell nuclear antigen analysis. We evaluated the percentage of proliferating cells in tumor tissue by immunohistochemical localization of proliferating cell nuclear antigen (PCNA) in tumor sections. Tumor sections were deparaffinized using citrisolv and rehydrated in a graded ethanol series with a final PBS wash. Antigen retrieval was done in 10 mM citrate buffer (pH 6.0) at 95°C for 20 minutes and cooled gradually. Endogenous peroxidase activity was blocked by immersing the sections in 3% H₂O₂ in PBS, followed by 2 PBS changes. The sections were then treated with normal horse serum (R.T.U Vectastain 7800) followed

by a change in PBS and then incubated with mouse monoclonal anti-PCNA antibody (sc-56; Santa Cruz Biotechnology Inc, Santa Cruz, Calif) overnight at 4°C. Following a wash with PBS, sections were incubated with biotinylated secondary antibody (Vector RTU SK 7800) for 10 minutes at room temperature, followed by conjugated horseradish peroxidase streptavidin complex for 5 minutes, DAB substrate for peroxidase (vector sk4100) for 7 minutes, and then a final PBS wash. Sections were counterstained with hematoxylin and rinsed in water, then dehydrated with alcohol, washed with xylene, and mounted. Finally, proliferating cells were quantified by counting PCNA-positive cells and total number of cells at 10 arbitrarily selected fields at $\times 600$.

Western blot. PCNA protein was detected by Western blotting using primary and secondary antibodies (Santa Cruz Biotechnology Inc, Santa Cruz, Calif) following a protocol previously described.¹² Immunoreactive bands were visualized by enhanced chemiluminescence, blots were scanned, and optical densities were quantitated with NIH image 1.62 software.

In situ apoptosis detection by terminal deoxynucleotidyl transferase mediated d-uridine triphosphate nick end labeling staining. Formalin-fixed and paraffin-embedded sections of all tumor samples used for PCNA staining were used to identify apoptotic cells by the terminal deoxynucleotidyl transferase (TdT) mediated d-uridine triphosphate nick end labeling (TUNEL) technique using an apoptag peroxidase in situ apoptosis detection kit from Chemicon International (S7100; Billerica, Mass). This method is based on the specific binding of TdT to the 3-OH end of DNA, ensuring synthesis of a polydeoxynucleotide polymer. Briefly, sections were digested using proteinase K, and endogenous peroxidase activity was blocked using 3% H₂O₂ in PBS. Sections were then placed in equilibration buffer and incubated with TdT enzyme in a humidifying chamber at 37°C for 1 hour and terminated with stop/wash buffer. The apoptotic nuclei were stained by direct immuno peroxidase detection of digoxigenin-labeled DNA in test sections. Apoptotic cells were quantified by counting TUNEL-positive cells and total number of cells at 10 arbitrarily selected fields at $\times 600$.

Statistical analysis. Tumor-free and overall survival days were compared using Kaplan-Meier plots and a log rank test. Mean weekly body weight and geometric mean tumor volume (every 4 days) were compared using repeated-measures analysis of variance, followed by Bonferroni-adjusted post hoc comparisons where appropriate. Average tumor weight at

time of killing was compared using a 2-sided Student *t* test for independent samples, and the number of animals with visible lung nodules was compared using the Fisher exact test. *P* values less than .05 were considered statistically significant.

In vitro tests. Homeopathic medicines were applied in different sets of plates by adding 100 μ L 30 c, 200 c, and 1000 c concentrations for *T occidentalis*, *C maculatum*, and *S serrulata* and 1000 c for MAT-LyLu *Carcinosin* for prostate cells. Cells in untreated wells served as controls. Cells were allowed to grow for 24, 48, 72, and 96 hours. We then evaluated cell viability with the MTT assay and for apoptosis gene expression using an rAPO-1 multiprobe. This probe contains Fas antigen, bclXS, bclXL, FasL, caspase-1, caspase-2, caspase-3, and GAPDH.

In vivo tests. We performed 2 pilot studies before conducting a fully powered, blind study (data not shown). The studies basically involved 16 rats to evaluate whether the sequential administration of the 4 selected drugs produced any in vivo effects on cancer growth. With this and all subsequent in vivo experiments, we used 4- to 5-week-old male Copenhagen rats injected intradermally on the dorsal side with 10 000 MAT-LyLu prostate cancer cells suspended in 100 μ L PBS. Inoculation of MAT-LyLu cells results in induction of tumors in 100% of untreated Copenhagen rats. Animals were then randomly distributed into homeopathically treated or control water-treated groups. The sequence of drugs in the homeopathically treated group was *T occidentalis* given the first and fourth day of the week, *C maculatum* given the second and fifth day of the week, *S serrulata* given the third and sixth days of the week, and *Carcinosin* given on the seventh day of the week. Control animals were given an identical volume (100 μ L) of control water each day of the week through the same route (oral gavage). This regimen was recommended by several of our homeopathic clinical consultants and was followed for 5 weeks. The pilot studies showed reduced tumor incidence, slower tumor growth, and reduced mortality in the homeopathically treated group. There were no significant differences in body weight between groups, and animals also showed no visible toxicity as measured by appetite and hair loss or lack of movement (data not shown). The pilot studies allowed us to calculate a sample size of 48 animals per group for a statistically definitive test with an estimated 80% power to detect a 25% difference in mortality at the *P* < .05 level.

We then conducted a large, double-blind, randomized experiment to replicate the pilot studies and evaluate tumor incidence, growth, metastasis, and animal

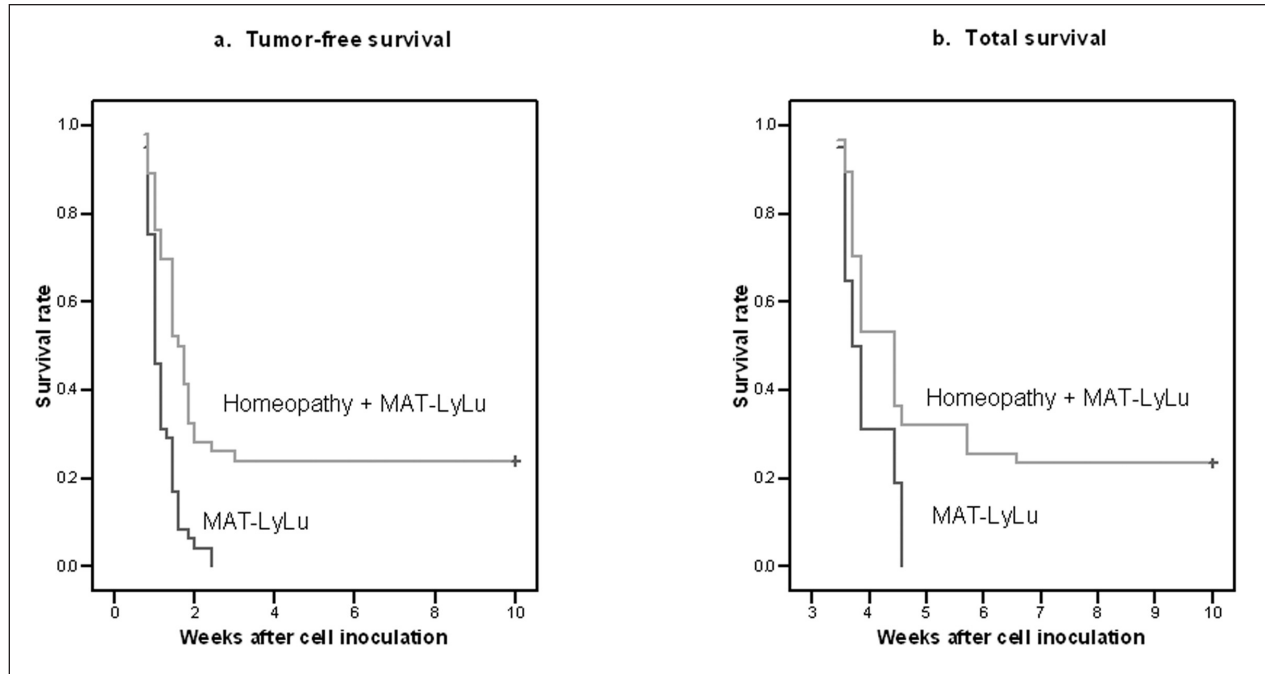


Figure 1 Kaplan-Meier analysis of (a) homeopathy treatment tumor-free survival and (b) water treatment (controls) survival of Copenhagen rats inoculated with MAT-LyLu cells. Homeopathic treatment resulted in a 23% decrease in tumor incidence ($P < .0001$).

mortality. One hundred animals were selected for either homeopathic or control water treatment using a random number table. All preparations were coded, and the codes were placed in a sealed envelope in a locked drawer by a third party not involved in the experiment. Data were sent to a statistician for blind and independent evaluation, with the code broken only after all evaluations were done.

Results

No significant differences in cell viability or gene expression were seen on any cell line, with any drug, or at any exposure time (data not shown). Thus, these drugs have no direct cytotoxic or apoptotic effects on prostate cancer cell lines. Two animals from the homeopathy-treated group died on the first day after inoculation of unknown cause. The median time for the first appearance of tumor was 7 days in the water-treated group and 11 days in the homeopathy-treated group. The homeopathic group had a 23% decrease in tumor incidence, with tumors occurring in 36 of 47 animals. The tumor-free survival curves differed significantly (log rank statistic 22.64, $P < .0001$; Figure 1a). Animals that did not develop tumors remained disease free until termination of the experiment 10 weeks after cell inoculation. Median time to sacrifice was 26 days in the water-treated control group and 31 days in the homeopathy-treated group ($P = .0002$; Figure 1b).

A decrease in tumor burden was observed in animals that were treated with homeopathic medicines compared to control water-treated controls (Figure 2a). After 3 weeks of tumor initiation, a significant increase of tumor growth was observed. Homeopathic treatment inhibited tumor growth. Tumor-bearing animals receiving homeopathic remedies showed a 38% reduction in the mean tumor volume 30 days after inoculation compared to control water-treated animals ($P < .02$). At the time of killing, there was a 5.5 g (approximately 13%) lower average tumor weight for the homeopathy-treated group compared to the control water-treated group ($P < .05$; Figure 2b).

Among animals with primary tumors, there was a nonsignificant ($P = .693$) difference between the homeopathy-treated (5.7%) and control water-treated groups (10.4%) in visible lung metastases. Blinded histopathological examination of control water-treated animals showed numerous unencapsulated nodular accumulations of neoplastic cells that frequently surrounded small vessels or were adjacent to bronchovascular structures. The histologic features of lung tumor nodules in the homeopathy-treated animals with primary tumors were comparatively smaller.

To explore possible mechanisms of these effects, we evaluated the cell proliferation and apoptotic cell death in tumor tissues. There was a 6% reduction in PCNA-positive cells in the homeopathy-treated group

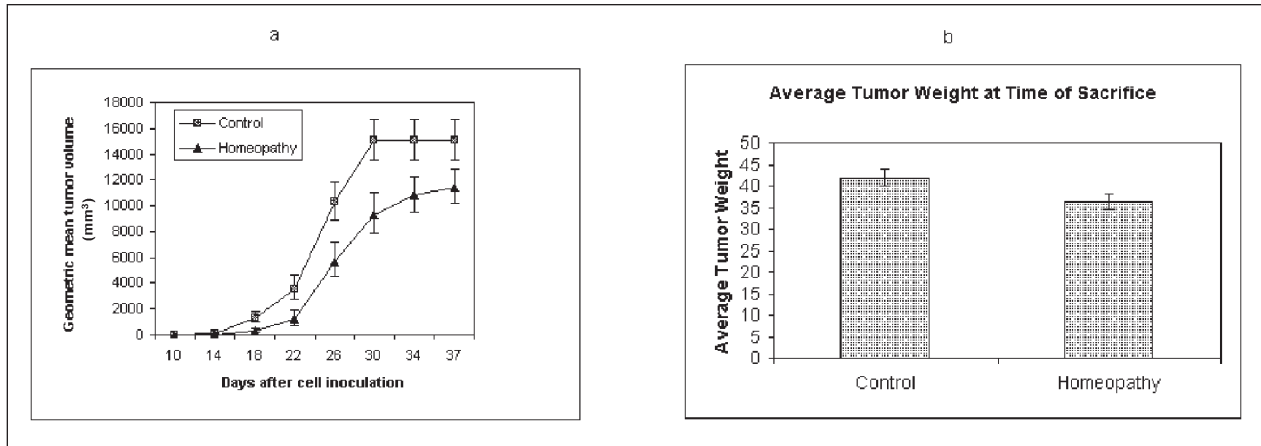


Figure 2 Effect of homeopathic treatment on tumor growth in Copenhagen rats inoculated with MAT-LyLu cells, (a) tumor volume and (b) tumor weight. (a) Tumor-bearing animals receiving homeopathic remedies showed a 38% reduction in the geometric mean tumor volume after 4 weeks of inoculation compared to control water-treated animals ($P < .02$). By day 30, all control animals had been killed. Day 34 and 37 control data represent day 30 data carried forward for comparison. (b) At the time of killing, there was a 5.5 g (approximately 13%) lower average tumor weight for the homeopathy-treated group compared to the control water-treated group ($P < .05$). Error bars represent 1 standard error of the mean.

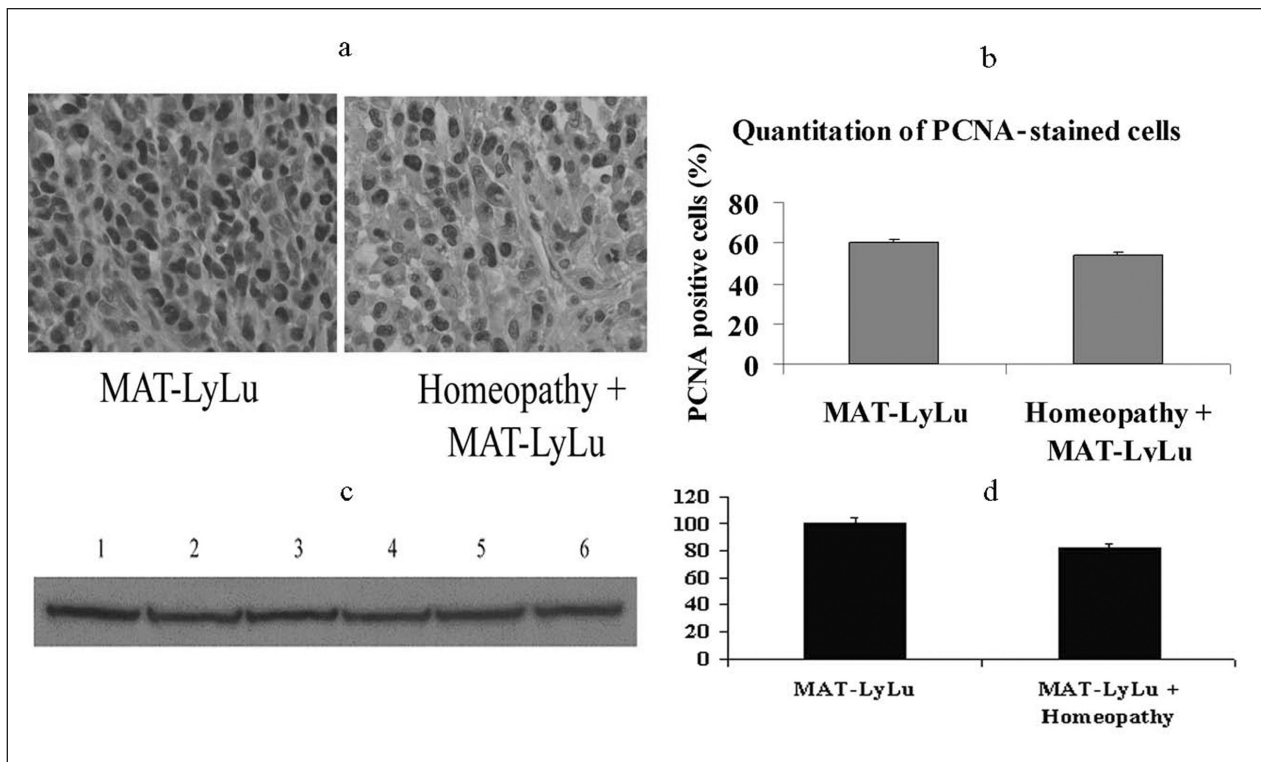


Figure 3 Proliferating cell nuclear antigen (PCNA) assay of tumor tissues from homeopathy-treated untreated animals. (A) Representative picture for histologic detection of cellular proliferation in tumors. Tumor tissue sections were stained by antibody against PCNA. (b) Quantification of PCNA-positive cells expressed as a percentage of total cells in tumors of homeopathy-treated and water-treated animals. Homeopathy treatment showed a reduction in PCNA-positive cells compared to untreated tumors ($P < .05$). Values are mean \pm SEM of 4 animals. (c) Analysis of PCNA in cell extracts of tumors. Western blot analysis using PCNA antibody for whole-cell extracts obtained from tumors 1 to 3 (control tumors) and 4 to 6 (homeopathy-treated tumors). (d) Quantification of PCNA protein expression. Densitometric analysis indicates an 18% reduction in homeopathy-treated tumors compared to control animals. Values are mean \pm SEM of 3 animals ($P < .05$).

compared to the water-treated group (Figure 3a, 3b). Western blot analysis also indicated a similar reduction in PCNA expression in homeopathy-treated tumors

compared to controls (Figure 3c, 3d). Apoptotic cell death was determined by TUNEL assay. Homeopathy-treated animals that grew tumors showed a 19%

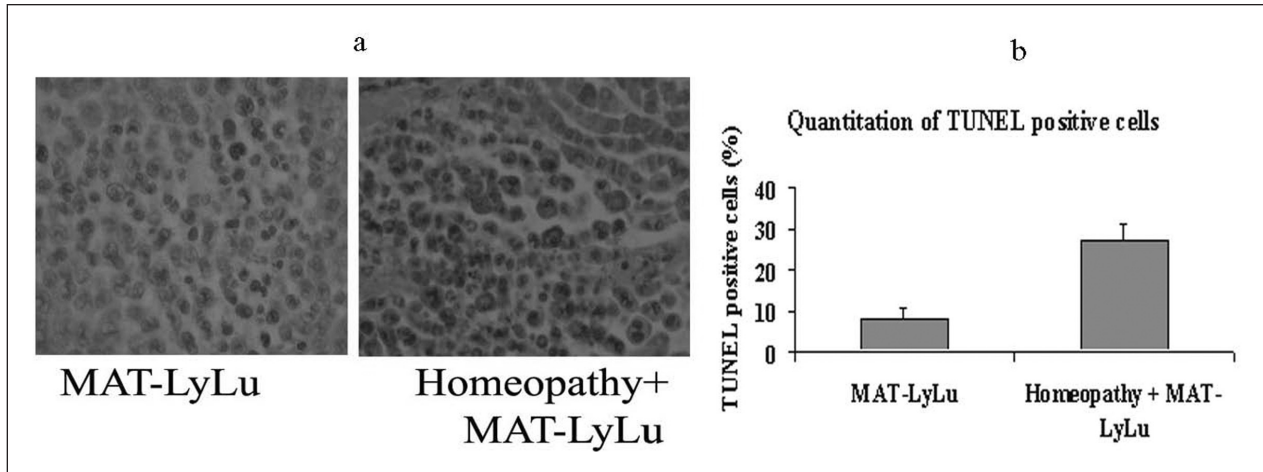


Figure 4 Terminal deoxynucleotidyl transferase mediated d-uridine triphosphate nick end labeling (TUNEL) assay of tumor tissues from homeopathy-treated versus water-treated animals. (a) Representative picture of histologic detection of apoptosis in homeopathy-treated tumors. Tumor tissue sections were stained by TUNEL. (b) Quantification of TUNEL-positive cells expressed as a percentage of total cells. Homeopathy treatment showed an approximately 19% increase in TUNEL-positive cells. Values are mean \pm SEM of 4 animals ($P < .05$).

increase in apoptotic positive nuclei as compared to water-treated controls ($P < .05$; Figure 4a, 4b).

Discussion

We conducted a series of carefully controlled laboratory studies evaluating the effects of 4 widely used homeopathic remedies for the treatment of prostate cancer (*T occidentalis*, *C maculatum*, *S serrulata*, and *Carcinosin*). None of these homeopathic remedies at any dose or exposure time were cytotoxic in vitro. However, a significant difference in survival, tumor incidence, tumor volume, and tumor weight was observed in the homeopathically treated animals compared to control animals treated with control water alone. There was no significant difference in lung metastasis or body weight between groups of animals. There was a small but significant difference in tumor proliferation and apoptosis among the homeopathically treated animals compared to controls. These data indicate that homeopathic remedies have no direct cellular anticancer effect but significantly slow the progression of cancer and reduce cancer incidence and mortality in Copenhagen rats injected with MAT-LyLu prostate cancer cells.

To our knowledge, these studies are the first to systematically evaluate possible anticancer effects of homeopathy on prostate cancer in the laboratory. Despite the implausibility of homeopathy, millions of individuals around the world and hundreds of practitioners claim it is of value, including for cancer.¹³ The National Cancer Institute has reported preliminary evidence of efficacy in a series of cases from an Indian clinic.⁹ In addition, there have been periodic reports of cancer-modulating effects from homeopathy in the

laboratory including on phenobarbital-induced liver cancer,¹⁴ hepatocarcinoma,¹⁵ glioblastoma,⁵ and breast cancer.¹⁶ However, this is the first systematic research investigating homeopathy for prostate cancer and the first to be done with a large number of animals using a blinded, randomized, placebo-controlled design after model and dose development in pilot studies. We believe this approach may have promise for prostate cancer treatment and for evaluation of homeopathic claims from clinical practice.

Conclusions

Given the lack of mechanism to explain our findings, any interpretation of these data should be done cautiously. However, we employed a careful, systematic, and blinded approach (pilot tests; sufficiently powered, blinded study; confirmatory histopathology; PCNA and TUNEL assays) and therefore felt the information should be published so others can attempt independent replications. Although caution is always advisable when interpreting animal studies, these findings may be viewed as supporting evidence of anecdotal clinical claims that homeopathy is effective in cancer. Additional research is in process.

Acknowledgments

The opinions or assertions contained herein are the private views of the authors and should not be construed as official or necessarily reflecting the views of the Samueli Institute, the Uniformed Services University of the Health Sciences, or the Department of Defense. The authors are thankful for the excellent technical assistance of Brant Freed and assistance with the manuscript by Cindy C. Crawford. We

also want to thank Christine Goertz for her helpful comments and editorial review. This work was supported by a grant from the Samueli Institute for Information Biology.

References

1. Bellavite P, Conforti A, Piasere V, Ortolani R. Immunology and homeopathy 1. Historical background. *Evid Based Complement Alternat Med.* 2005;2:441-452.
2. Maddox J, Randi J, Stewart W. High-dilution experiments a delusion. *Nature.* 1988;334:287-291.
3. Thompson E, Kassab S. Homeopathy in cancer care. *Br Homeopath J.* 2000;89:61-62.
4. Eisenberg D, Davis R, Ettner S, et al. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA.* 1998;280:1569-1575.
5. Pathak S, Multani A, Banerji P, Banerji P. Ruta 6 selectively induces cell death in brain cancer cells but proliferation in normal peripheral blood lymphocytes: a novel treatment for human brain cancer. *Int J Oncol.* 2003;23:975-982.
6. Jonas W, Kaptchuk T, Linde K. Critical overview of homeopathy. *Ann Intern Med.* 2003;138:393-399.
7. Shang A, Huwiler-Muntener K, Nartey L, et al. Are the clinical effects of homeopathy placebo effects? Comparative study of placebo-controlled trials of homeopathy and allopathy. *Lancet.* 2005;366:726-732.
8. Paterson I. Homeopathy: what is it and is it of value in the care of patients with cancer? *Clin Oncol.* 2002;14:250-253.
9. National Cancer Institute. Electronic request for proposals number NO2-CO-47039-48. Practice outcomes monitoring and evaluation system (POMES) at a homeopathic clinic in Calcutta, India. 2004. Available at: <http://rcb.cancer.gov/rcb-internet/appl/rfp/47039/toc.pdf>.
10. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. *Cancer J Clin.* 2005;55:10-30.
11. Ramakrishnan A, Coulter C. *A Homeopathic Approach to Cancer.* St. Louis, Mo: Quality Medical Publishing; 2001.
12. Gaddipati J, Sundar S, Calemine J, et al. Differential regulation of cytokines and transcription factors in liver by curcumin following hemorrhage/resuscitation. *Shock.* 2003;17:150-156.
13. Montfort H. A new homeopathic approach to neoplastic diseases: from cell destruction to carcinogen-induced apoptosis. *Br Homeopath J.* 2000;89:78-83.
14. Roberfroid M. Action of Hahnemannian potencies upon artificially produced cancer in animals. In: Boiron J, Abecassis J, Belon P, eds. *Aspects of Research in Homoeopathy.* Lyon, France: Borion; 1983:11-18.
15. Biswas S, Kuda-Bukhsh A. Effect of homeopathic drug, helidonium, in amelioration of p-DAB induced hepatocarcinogenesis in mice. *BMC Complement Altern Med.* 2002;2:1-12.
16. Maliekal T. Antineoplastic effects of 4 homeopathic medicines. *Br Homeopath J.* 1997;86:90-91.