ONCOLYTIC ACTION OF ZAMZAM WATER ON AZOXYONETHONE (AOM) INDUCED COLON TUMORS IN RATS

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ABSTRACT

Introduction: Proximal colon tumor is one of tumor which depend on diet, we induced proximal colon tumor by injection of azoxymethane AOM in rat due to specific nature of zamzam water and its chemical composition we use zamzam water intake to test the hypothesis that zamzam water had an oncolytic action.

Material and method: we induced colon tumor in 50 rats by injection of Azoxymethane (AOM), zamzam water rats were given 250-500 c.c for one month subjected to gene expression by RNA processing, microarray procedure Bioinformatics, RTPCR, serum somatostatin level was detected by (ELISA). All data subjected to statistical analysis.

Results: After zamzam water intake statistically significant decrease in the size of tumor P<005, and increase in lymphocyte P<0.05, zamzam water cause upregualtion of gene which stimulate reduction of tumor and down regulation of gene which increase in size in the tumor and its spread.

Conclusion: the oncolytic action of zamzam water has been born due to its effect on immunoneuroendocrinal growth systems of the body.

Keywords: Zamzam water, Azoxymethane, Colon tumor, Gene, Somatostatin, growth factor.

INTRODUCTION

Zamzam water is unique in its natural characteristics; zamzam water has special optical parameters that are different from those of bottled drinking and distilled water. The aim of this work is to explore the oncolytic action of zamzam water i.e. it's ability to reduce the size of tumor and its spread [1,2,3].

We choose rat proximal colon because this type and site is mostly affected by diet[4,5,6]. Colorectal cancer (CRC) is the third most common cancer. Accumulating evidence suggests that diet is an important environmental factor in the etiology of CRC[4].

MATERIAL AND METHODS

50 rats were enrolled in the study, induction of colon tumor was carried out by injection of Azoxymethane (AOM), once the establishment of tumor occurred, zamzam water intake 250-500c.c daily, for one month a biopsy was taken before zamzam intake and after, genes expression were carried by the following procedure. RNA processing, microarray procedure bioinformatics, quantitiative real time RT PCR[7].

Serum somatostatin level before and after zamzam water intake were determined by enzyme linked immunoabsorbant assay (ELISA)[8].

Data analysis:

Difference between the size of the tumor, lymphocyte in the tumor, were analyzed by two tailed t test, chi square test was used for comparison a difference of < 0.05 was considered significant.

RESULTS

We induce proximal colon cancer tumor in rats by azoxymethane (AOM)[9], feeding the rat with zamzam water 500 c.c daily for one month, a biopsy was taken before zamzam water and after the results were summarized in the following tables.

The down regulated genes are related to cell adhesion, cell cycle growths immunity, metabolism, ligand binding/carrier signal transduction, stress response apoptosis, structure protein.

The upregulated genes related to cell adhesion, cell cycle control, immunity ligand carrier, metabolism, signal transduction, stress response structure protein, transcription factor.

Table (1): Rats fed with zamzam water down regulated genes

Gene Name	Decrease change
3-methylcholanthrene-inducible UDP-glucuronosyltransferase	-7
A2b-adenosine receptor	-0.8
Aldehyde dehydrogenase	-8.56
Angiotensin/vasopressin receptor (AII/AVP)	-0.3
Apolipoprotein B mRNA editing protein	-0.34
Beta defensin-1	-24.78
Brain glucose-transporter protein	-0.97
Brain serine protease bsp1	-2.42
Cadherin 17	-2.8
Carboxylesterase precursor	-3.29
Cathepsin S	-0.62
Cation transporter	-1.62
Chemokine CX3C	-3.04
Claudin 3	-4.68
Cyclin D1	-0
Cytochrome P450 4F4	-4.43
Cytochrome P450 monooxygenase	-0.82
Cytokeratin 21	-2.96
Cytokeratin-8	-0.71
D-amino-acid oxidase	-11.69
Fucosyltransferase 2	-5.97
Glutathione S-transferase	-3.17
Guanylate cyclase activator 2A	-2.18
Keratin 19	-0.69
Kruppel-like factor 4 (gut)	-1.08
Liver fatty acid binding protein	-0.62
Methionine adenosyltransferase II, alpha	-0.91
Mitochondrial dicarboxylate carrier	-1.55
Muc3	-9.07
Mucin-like protein	-9.97
Na_H_Exchanger	-7.81
Neu oncogene	-0.61
Peptide tyrosine-tyrosine (YY)	-2.56
Peroxiredoxin 6	-1.55
Phenylalanine hydroxylase	-5.43
Phospholipase D	-0.71
Plasmolipin	-5.2
Polymeric immunoglobulin receptor	-0.93
Prolactin receptor	-1.26
Prostaglandin D synthetase	-41.11
Protein kinase C delta	-0.48
Protein tyrosine phosphatase	-0.64
Tumor-associated calcium signal transducer 1	-7.37
Type II Hexokinase	-0.7

Table (2): Rats fed with zamzam water up-regulated genes

Gene Name	Fold Change
Alpha-actinin-2 associated LIM protein	1.74
Angiotensin receptor	1.75
Apoptosis	4.7
Aquaporin 1	4.4
cannabinoid receptor 1	1.17
Guanylyl cyclase A	2.18
IgG gamma heavy chain	1.21
Lipoprotein lipase	1.88
Neuroendrocrine protein	2.7
Neurofilament protein middle	1.97
Peripherin	1.82
Protein kinase C-binding protein Zeta1	2.14
Protein phosphatase inhibitor-1	1.6
Protein tyrosine phosphatase	1.47
Retinol-binding protein	1.17
Small inducible cytokine	2.70
snRNP-associated polypeptide	2.99
Somatostatin	4.12
Somatostatin-14	4.66
T-cell receptor beta chain	1.66

Table (3): Tumor size before taking zamzam water and after

Tumor size	Before zamzam water intake	After zamzam water intake	P value
mean±SD	3.1±0.1	1.5±0.15	<0.05

Table (4): Number of lymphocytes per ml of the tumor before and after zamzam water intake

Number of lymphocyte	Before zamzam water intake	After zamzam water intake	P value
Mean±SD	101±3.1	688±11.22	<0.05

Table (5): Serum somatostatin before and after zamzam water intake

Serum somatostatin	Before zamzam water intake	After zamzam water intake	P value
Mean±SD	3.3±0.5	18.2±1.5	<0.05

DISCUSSION

To study the oncolytic action of zamzam water we used experimental animal, acute injected with azoxymethane (AOM)[9] to induce colon tumor in rat when the tumor develop, a biopsy was taken from nontumor tissue to prepare microarray data for differentially expressed genes. We fed the rat with zamzam water 500c.c. daily for one month and again a biopsy was taken after zamzam water intake after killing the animal we demonstrated statistically significant diminished the size of tumor $P<0.05$. In table 5 statistically significant increase in serum somatostatin after feeding the rat with zamzam water (Table 3), somatostatin is a well known antiproliferative again[8] for colon[10] tumor cell, this hormone is also a negative regulator of angiogenesis[11], other explanation depend upon the biochemical nature of zamzam water it affect directly the populations of lymphocytes resident is the colon which in turn affect tumorigenesis meaning that zamzam water works by specific immune mechanisms causes decrease in the tumor size[12] (Table 3) on the molecular level by which zamzam water reduce the incidence of chemically induced colon tumor, the present study showed genes that are differentially expressed. We studied proximal colon not distal colon because it is well known that proximal colon is more affected by diet than distal colon[4] from Table (1) we find that oncolytic action of zamzam water is due to downregulation of oncogenic gene, in our work we found that zamzam water induce apoptosis and this evident in upregulation of genes which control apoptosis[13] (Table 2).

In our work we found downregulation of cyclin D_1[14,15,16] (Table 1). This cause regression of the tumor in our work we found downregualtion of aquaporin after zamzam water intake aquaporin are thought to be induced early in colon cancer and facilitate spread of tumor[18,19].

So on the molecular level it is event from Tables (1,2) that zamzam water act by stimulating downregulation of gene which facilitate tumor growth, spread, and formation and again upreguation of gene which benefit for the tissue.

CONCLUSION

Zamzam water is a miracle, it has oncolytic action through affection of endocrine-immunological and growth system of the body.

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