Cytotoxicity of the Urine of Different Camel Breeds on the Proliferation of Lung Cancer Cells, A549

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Abstract

Objective: Cancer is a disease characterized by uncontrolled cellular proliferation and differentiation. Nearly all conventional cancer treatments have undesirable negative impacts, and safer chemotherapeutics would be advantageous. Consequently, the goal of current study was to evaluate and compare the effects of urine derived from two different camel breeds on proliferation of cultured human cancer cells. Human lung adenocarcinoma cells (A549) were cultured in the presence or absence of varied dilutions of urine obtained from two different camel breeds (Magateer and Majaheem). Within breeds, we compared the effects of sex and age of donor camels on urine cytotoxicity to A549 cells. After 48 hrs, surviving A549 cells were enumerated using the sulfarhodamine assay. A549 cell survival was lower using urine from Magateer versus Majaheem camels (84.8% versus 94.2% of starting cell number, respectively; n=20 for both groups, $p<0.001$). When evaluating the effect of camel age, urine from older Magateer camels was significantly more effective in inhibiting A549 proliferation than was urine from younger camels of this breed. An age-related effect was not observed for Majaheem camels. When comparing sex-effects on camel urine inhibition of A549 proliferation (n=10 in each group), we observed a trend towards more A549 inhibition using female versus male urine, in both camel breeds; however, this difference did not reach statistical significance. The present study confirms previous studies that showed that camel urine can inhibit the growth of cancer cells. It also provides the first evidence that there are slight differences in the cancer cell growth-inhibitory effect of camel urine depending on the camel breed, age, and, possibly, sex.

Keywords: Camel breeds, Urine, Cancer cells, Cytotoxicity.

1. Introduction

Cancer is a disease characterized by uncontrolled cellular proliferation and differentiation. Nowadays, cancer is a very common disease with a high annual incidence rate (Parkin, et al ; 1999]. Ferlay et al. (2000) reported that worldwide more than 5 million people are diagnosed with cancer and more than 3.5 million people die from cancer each year. Managing human malignancies still constitutes a major challenge for contemporary medicine (Coufal et al., 2007 and Widodo et al., 2007). Although with progress in understanding cancer biology, many new antineoplastic therapies have been developed that rely primarily on surgery, chemotherapy, radiotherapy, hormone therapy, and immunotherapeutic approaches (Khorshid et al., 2010). However, all available therapies are still far from ideal, in which treatment would selectively kill the malignant cells while sparing healthy tissues and vital organ function (Grever and Charbner, 1997 and Moshref, 2007). chemotherapy resulted in an overall increase in the survival rate and longevity of patients with life-threatening tumors, On the other hand also mean increased exposure to toxic substances and harmful effects on different tissues (Maino, et al.,2000).

Natural products play an important role in our healthcare system (Pezzuto, 1997 and Schwartsmann, 2000). They offer a valuable source of potent compounds with a wide variety of biological activities and novel chemical structures, many of which might be important for novel drug development (Vuorela, et al., 2004). Animal studies have shown that green tea is a potent inhibitor of lung tumor development (Zhang et al., 2000). PM 701 is another natural product readily available, cheap, and non-toxic (Khorshid, 2008). PM 701 was proven to be an anticancer substrate (Khorshid et al., 2005, 2008, Moshref et al., 2006 and El-Shahawy et al., 2010), and was found to be effective in limiting the metastatic spread of leukemia cells in an animal model (Moshref et al., 2006). PM 701 is considered safe as a potential anti-cancer agent, and exerts negligible effects on vital organs (Khorshid, 2009).

Camel urine, also a natural product, has been used traditionally in the treatment of many diseases in Arabic countries. Drinking camel urine was shown to be effective in treating numerous cancer cases (Alhaider et al.,

2011). Moreover, according to Saudi Gazette.com, Dr. F.A. Khorshid has a potential cure for cancer based on camel urine. After 8 years of research she has announced that nano-particles in camel urine can be used to fight cancer. Moreover, The Saudi Center for Medical Research added that there is a tendency to start in the production of a medical capsule containing camel's urine for use in the treatment of cancer. In the same respect, Alhaider et al. (2011) examined the ability of three different camel urine samples (virgin, lactating, and pregnant sources) to modulate a well-known cancer-activating enzyme, cytochrome P 450 1a1 (Cyp 1a1) in the murine hepatoma Hepa 1c1c7 cell line. They found that all types of camel urine, but not bovine urine, differentially inhibited the induction of Cyp 1a1 expression by TCDD, a potent Cyp 1a1 inducer and a known carcinogen. Virgin camel urine showed the highest degree of Cyp 1a1 inhibition, followed by lactating and pregnant camel urine.

Khorshid (2001) stated that in vitro approaches are the best way to initially evaluate the effect of novel biological compounds, utilizing growing mammalian cells in tissue culture. Consequently, the main goals of current study were to: 1) evaluate the inhibitory effect of urine obtained from two different camel breeds on the growth of lung cancer cells (A549),in vitro; and 2) study whether urine's effect is changed according to differences in the camel's breed, age, or sex.

2. Materials and Methods

2.1. Study area:

The main part of this study was carried out at yebreen region located in the southern west of the eastern region at the periphery of The Rub' alkali (Empty Quarter) included in Kingdom of Saudi Arabia.

2.2. Animals:

This study was conducted on 40 camels from two different breeds (Magateer and Majaheem). Ten males and 10 females were selected from each breed. The males ranged between 1-8 years old, whereas the females ranged from 3 to 9 years old.

2.3. Urine sampling and storage:

Twenty milliters of urine were collected from each camel, kept in insulated boxes using freezing packs, and transferred to the laboratory (Tissue Culture Unit, King Fahd Medical Research Center (KFMRC), King Abdul Aziz University in Jeddah, Saudi Arabia).

2.4. Methods:

Human non-small-cell adenocarcinoma cells (A549) were obtained from the American Type Culture Collection (ATCC) and were stored in the cell bank of tissue culture laboratory, where cytotoxicity assays were also conducted, as pioneered by a research team working in the medical center (Khorshid et al.,2005; Khorshid and Alameri, 2011). Different concentrations of PM 701 were used (1.0, 2.5, 5.0, 7.5, and 10 □g/ml) and were added to A549 cell monolayers. The control group of A549 cells was not treated with PM 701 and is indicated as 0 concentration.

Cytotoxicity assays were performed using the method of Skehan et al. (1990). Cancer cells were suspended in DMEM medium and plated in 96-well plates (104 cells/well) for 24h in a 5% CO2 incubator adjusted at 37°C before treatment with PM701, to allow cell attachment to the bottom of the plate. Different concentrations of the test substance (0, 1, 2.5, 5, and 10 □g/ml) were then added to the cells monolayer. Triplicate wells were prepared for each individual concentration. Cell monolayers were incubated with PM701 for 48 h at 37°C and in atmosphere of 5% C02. After 48 h, cells were fixed using 50 µl/well trichloroacetic acid, refrigerated at 8°C for 1 hour, washed with distilled water, and then stained with Sulforhodamine B (SRB) (50 µl/well) for 30 min. Excess stain was washed with off with acetic acid and remaining attached stain was recovered with Tris EDTA buffer (100 µl/well). Color intensity was measured immediately in an ELISA reader at wavelength 570 nm. The relation between surviving cells and drug concentration was plotted to get the survival curve of each cell line after the specified period.

2.5. Statistical analysis

Statistical analysis of the data was performed with SPSS for Windows (Version 17.0.0). Data were calculated as follows: The different urine samples were collected from the two camel breeds from both sexes. Five concentrations of urine were tested from each individual camel (1, 2.5, 5, 7.5, 10), with 0 concentration used as controls. Each experimental concentration was added to six tissue culture wells containing cancer cells. Forty total urine samples were collected from each camel with their detected concentrations mentioned above, so 40 camels × 5 concentrations equals 200 urine samples. Urine specimens at the listed concentrations were directly applied to the six wells of cultured cancer cells, so the total wells assayed equaled 1200.

3.Results and Discussion:

3.1.Differences between two camel breeds:

Data shown in Table 1 revealed that, camel urine reduced lung cancer cells to 84.75% and 92.81%, in Magateer and Majaheem breeds, respectively, versus untreated controls (100%). Highly significant differences were noticed between treated and control cultures when comparing urine activity within each breed and between the different breeds (P=0.000 and 0.001, respectively). Magateer urine significantly reduced cancer cell numbers more than did Majaheem urine.

These results are in accordance with those of Alhaider et al. (2011) who reported that drinking camel urine has been used traditionally to treat numerous cases of cancer. The authors attributed this anticancer effect to the ability of camel urine to modulate the well-known cancer-activating enzyme, Cyp 1a1. They found that all types of camel urine differentially inhibited the induction of Cyp 1a1 gene expression by TCDD, the most potent Cyp 1a1 inducer and a known carcinogenic chemical. In the same respect, Eldor (1997) hypothesized that because some cancer cell antigens are transferred through urine, through oral autourotherapy, these antigens could be introduced to the immune system that might then create antibodies.

3.2.Camel age effects on cancer cell proliferation:

3.2.1. In the same strain*:*

Table 2 clarifies the effects of urine obtained from young and adult Magateer and Majaheem camels on the growth of lung cancer cells (A549) in vitro. Urine obtained from adult Magateer camels induced a highly significant reduction in A549 cell survival (P≤0.004) than that obtained from the same younger breed (81.538% versus 87.947%, respectively), while urine obtained from adult Majaheem breed induced a non-significant (P≤ 0.179) reduction in cancer cells when compared to younger camels of the same breed (93.486% versus 96.974%, respectively).

No available literature could be found regarding the influence of age on the anti-cancer effect of camel urine. However, Alhaider et al. (2011) studied the ability of three different camel urines (virgin, lactating and pregnant) to modulate the cancer-activating enzyme CyP 1a1. They found that virgin camel urine showed the highest degree of inhibition at the activity level, followed by lactating and pregnant camel urine.

3.2.2.Age effects between the different camel breeds*:*

Table 3 shows a comparison between the anti-cancer effect of urine obtained from the two young camel breeds as well as the anti-cancer effect of that obtained from the two adult camel breeds. The results revealed that urine from young Magateer camels induced a significant (P≤0.01) reduction in the growth of cancer cells versus that obtained from young Majaheem camels (87.947% versus 96.974%, respectively). In addition, urine obtained from adult Magateer camels induced a significant higher reduction (P=000) of cancer cells versus that obtained with adult Majaheem camels (81.536% versus 93.486%, respectively).

The reason for the variability in the anti-cancer efficacy of camel urine obtained from Magateer and Majaheen breeds is not yet known. Further study is needed to determine the specific differences in the urine constituents of each breed, to know which compound(s) is responsible for this variable effect.

3.2.3.. Sex affects camel urine-mediated cancer cell proliferation:

3.2.3.1 In the same breed*:*

Table 4 represents the effect of sex on the ability of camel urine to inhibit the growth of lung cancer cells in vitro. It appears that the sex of camels within the same breed did not significantly affect camel urine-inhibition of A549 cancer cell proliferation. However, urine of males induced a slight, though insignificant inhibition in cancer cell proliferation versus that of females of the same breed.

3.2.3.2. In the different breeds:

Table 5 shows a comparison between the anti-cancer effect of urine obtained from males and females of the two different camel breeds. Urine from male Magateer camels caused a significantly greater reduction in cancer cells when compared to that induced by urine of male Majaheem camels (86.568 versus 94.014, respectively; P=.000). Urine of female Magateer camels also induced a significantly greater reduction in cancer cells compared to that induced by urine of female Majaheem camels (82.935 versus 91.368; P=.000). Urine from male and female Magateer camels were more efficient in reducing lung cancer cell numbers compared with that observed using Majaheem camel urine.

5. Conclusion

The present study confirms the findings of previous studies that camel urine can inhibit the growth of cancer cells. It also provides the first evidence that there are differences in the cancer-inhibiting effect of camel urine depending on the camel breed, age, and sex.

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Table1: Effect of camel urine obtained from Magateer and Majaheem breeds on the growth of lung cancer cells in vitro.

Group	No.	Mean %	SD	Control	Test	Sig.	T.test	Sig.
Magateer	600	84.752	23.641	100.00	15.798	.000*	6.156	.001**
Majaheem	600	92.805	19.805	100.00	9.126	.000*		

. No: number of samples.

. Mean: percentage of the mean value of the number of living cancer cells.

. SD: Standard deviation

. Control: Tissue culture containing untreated cancer cells (100 cell).

. * Comparison between the same strain treated cancer cells and non-treated cancer cells (control).

. ** Comparison between two strains.

Table 2: Effect of urine obtained from young and adult Magateer and Majaheer breeds on the growth of lung cancer cells (A549) in vitro.

Group	No.	Mean %	SD	T.test	Sig.
Magateer (young)	150	87.947	16.592	2.911	.004*
Magateer (adult)	150	81.536	24.454		
Majaheem (young)	150	96.974	29.460	1.346	.179*
Majaheem (adult)	150	93.486	11.810		

. No: number of samples.

. Mean: percentage of the mean value of the number of living cancer cells.

. SD: Standard deviation.

. * : Comparison between young and adult at same strain.

Table 3: Comparison between the anti-cancer effect of urine obtained from the two young camel breeds as well as the anti-cancer effect of that obtained from the two adult camel breeds.

Group	No.	Mean %	SD	T.test	Sig.
Magateer (young)	150	87.947	16.592	3.499	.001*
Majaheem (young)	150	96.974	29.460		
Magateer (adult)	150	81.536	24.454	5.476	.000*
Majaheem (adult)	150	93.486	11.810		

. No: number of samples.

. Mean: percentage of the mean value of the number of living cancer cells.

. SD: Standard deviation

. * Comparison between the two strains.

Table 4: Effect of sex on the ability of camel urine to inhibit growth of lung cancer cells in vitro.

Sex	No.	Mean %	SD	T.test	Sig.
Male Magateer	300	86.568	15.288	1.886	.060*
Female Magateer	300	82.935	29.653		
Male Majaheem	300	94.014	23.369	1.595	.111*
Female Majaheem	300	91.368	15.867		

- No: number of samples.

- Mean: percentage of the mean value of the number of living cancer cells.

- SD: Standard deviation

- *Comparison between the males and females within each breed.

Table 5: In vitro comparison between the anti-cancer effects of urine obtained from females and males in the two different camel breeds (Magateer and Majaheer).

Sex	No.	Mean %	SD	T.test	Sig.
Male Magateer	300	86.568	15.288	4.543	.000*
Male Majaheem	300	94.014	23.369		
Female Magateer	300	82.935	29.653	4.343	.000*
Female Majaheem	300	91.368	15.867		

. No: number of samples.

. Mean: percentage of the mean value of the number of living cancer cells.

. SD: Standard deviation

. * Comparison between the two strains.