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Curcumin's Effects on Sialic Acid Level and Sialidase Activity in Ehrlich Ascites Tumor Bearing Mice

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OZEN, N., USLU, E., OZEN, M., AYDIN, S., ALTUG, T., BELCE, A. and KOKOGLU, E. *Curcumin's Effects on Sialic Acid Level and Sialidase Activity in Ehrlich Ascites Tumor Bearing Mice.* Tohuku J. Exp. Med., 2002, **197** (4), 221-227 —— In this study, we determined curcumin's anticancer and chemopreventive effects in mice bearing Ehrlich ascites tumor by evaluation of cancer biomarkers, sialic acid level and sialidase activity. Both plasma sialic acid level and sialidase activity increased significantly in the mice group with Ehrlich ascites tumor. When the tumor groups fed with curcumin and fed with sesame oil were compared, sialic acid level and sialidase activity in ascites fluid significantly reduced in the group fed with curcumin in addition to the increases of plasma sialic acid level and sialidase activity. The tumor group fed with curcumin lived twice longer than the one fed with sesame oil. Curcumin as a phenolic compound decreased all these parameters in Ehrlich ascites tumors and lengthened survival by 88% in the mice with tumor. We concluded that curcumin has anticancer activity. ——
Curcumin; sialic acid; sialidase; Ehrlich ascites tumor
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Curcumin has been widely used as a spice and coloring agent in foods, presented in the root of plant *Curcuma longa*. Substantial scientific researchs on the medicinal properties of curcumin have been very well documented. Many studies have shown that curcumin had strong antioxidant, antiinflammatory and anticancer properties. Although the exact mechanisms of the molecule are yet to be elucidated, it is thought that its activity is mainly due to its conjugated diene moiety, and partly to the adjacent phenolic group. Curcumin inhibits

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carcinogenesis in various tissues, including skin, mammary colorectal, duodenal and forestomach cancers (Azuine and Bhide 1992; Huang et al. 1994; Pereira et al. 1996).

Ehrlich ascites tumor (EAT) is an undifferentiated malignancy, also known as a mouce specific tumor, and is widely used in experimental neoplastic studies. Its preference as an experimental malignancy depends on its high chance of tumor development even when subsequent passages are done. Since the passed malign cells divide rapidly and alter the host's hemodynamic balance, these subjects are lost instantly. Once the tumor is inoculated to mice, its swift penetration to tissues and high rate of tumor development are mainly based on the absence of "tumor associated transplantation antigens (TATA)" (Lettre et al. 1972).

Sialic acids are one of the most important molecules of life, since they occupy the terminal position on macromolecules, i.e., glycoproteins and glycolipids, that constitute cell surface and are involved in many biological and pathological phenomena. Neoplastic cells often have increased sialic acid concentrations on their surface (Schauer 1982). Sialoglycoproteins are shed or secreted by tumor cells which increase their concentration in blood (Erbil et al. 1985; Narayanan 1994). Sialidases are most important enzyme group for hydrolytic release of α-glycosidically bound sialyl residues.

In the present study, we aimed to search curcumin's anticancer and chemopreventive effects in mice bearing EAT, by evaluating cancer biomarkers, sialic acid level and sialidase activity.

Materials and Methods

All animal experiments were performed according to the standards in "The UFAW Handbook on the Care and Management of Laboratory Animals" (Poole 1999). Forty five Balb/C male young mice were obtained from Istanbul University Cerrahpaşa Medical Faculty, Experimental Animal Breeding and Research Laboratory. The animals (weighing about 20 ± 2 g) were equally divided into three groups each containing 15 mice.

Group 1. Intraperitoneal EAT cell lines were passed to mice. They were fed with curcumin, 75 mg/kg/day, suspended in sesame oil by gastric lavage.

Group 2. Mice in this group were again undergone EAT passage, but were fed with sesame oil only.

Group 3. Mice forming control group were injected 0.5 cc physiological saline intraperitoneally and fed with sesame oil only.

All mice in this study were fed with tap water and mouse fodder consisting 21% protein daily (Eris Fodder Company, Tekirdag, Turkey). Regularly the cage sanitary, temperature and humidity were checked daily.

Tumor cell line

Frozen EAT, stored at -86°C, has been solved in warm water bath and cell lines are transferred to a Thoma slide to count. They are diluted with physiological saline and passed to Group 1 and Group 2 mice intraperitoneally, each 0.2 cc possessing 1.2×10^6 cells on average.

Simultaneously 0.5 cc physiological saline was injected to control group (Group 3), intraperitoneally. At the end of the test when animals begun to die, samples were withdrawn and animals were sacrificed by prolonged ether anesthesia. Samples were stored at -86°C until measurements.

Measurement of sialic acid and sialidase

Plasma and peritoneal fluid sialic acid concentrations were determined by Warren's thiobarbituric acid method (Warren 1959). Since free plasma sialic acid concentrations were low, all samples were first hydrolyzed with 0.1 N H_2SO_4 at 80°C for 60 minutes, so that subsequent determinations reflected bound sialic acid levels. Sialidase activity were studied by using a coupled enzyme assay described by Alon et al. (1991).

Statistical analysis

The results were compared by Student's t-test. All data were expressed as mean±S.D. Comparison between groups was made by using Student's t-test. A p-value less than 0.05 was assumed to be significant. Pearson's correlation analysis was used for correlation among the pairs. In statistical analysis SPSS released 10.0 program for Windows (SPSS Inc., Chicago, IL, USA) was used.

RESULTS

Plasma and peritoneal fluid sialic acid levels (SAL) and sialidase activity (SA) measurements were summarized in Tables 1 and 2. There was no statistical difference of plasma SAL and SA between three groups initially. Both of plasma SAL and SA increased significantly in the tumor groups respect to control. When comparing the tumor groups, not only both plasma SA and SAL, but also ascites fluid SAL and SA significantly reduced in the group fed by curcumin (Figs. 1-6). The tumor group fed by curcumin had lived twice longer (88%) than the one fed by sesame oil ($p<0.05$) (Table 2) (Fig. 7).

In both EAT groups, plasma SAL demonstrated a significantly positive correlation with plasma SA, peritoneal SAL and SA (Table 3), although all 4 parameters were slightly but statistically significantly lower in the mice fed by curcumin.

DISCUSSION

As EAT does not have TATA, the malign cells rapidly diffuse into certain tissues causing high rate of cancer development proving to be

TABLE 1. *Plasma sialic acid and sialidase levels in Ehrlich ascites tumors and controls*

Groups	Plasma Sialic Acid mg/100 ml			Plasma Sialidase U/liter		
	Pre	Post	p	Pre	Post	p
Group 1 EAT+curcumin ($n=15$)	70.48±9.71	78.20±9.69	<0.05	53.34±3.74	65.42±3.89	<0.05
Group 2 EAT+SO ($n=15$)	68.42±9.28	92.10±7.93	<0.05	53.40±3.30	80.78±9.15	<0.05
Group 3 Control ($n=15$)	68.32±9.94	64.72±8.56	>0.05	53.46±3.69	52.47±3.60	>0.05

EAT, Ehrlich ascites tumors; SO, Sesame oil; Pre, At the begining of test; Post, Before sacrification.

TABLE 2. *Ascites fluid sialic acid and sialidase levels and surveys in Ehrlich ascites tumors*

	Ascites Fluid		Survey days
	Sialic acid mg/g protein	Sialidase U/g protein	
Group 1 EAT+Curcumin	195.33±26.37	35.22±3.99	17.53±1.86
Group 2 EAT+SO	222.13±33.20	45.28±4.54	9.73±1.09
p	<0.05	<0.05	<0.05
Increase in life span			88%

EAT, Ehrlich ascites tumors; SO, Sesame oil.

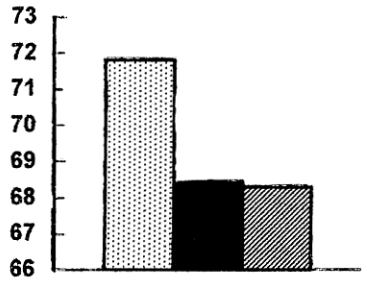


Fig. 1. At the begining of the test plasma sialic acid levels.
▨, EAT+Curcumin; ■, EAT+Oil; ▨, Control.

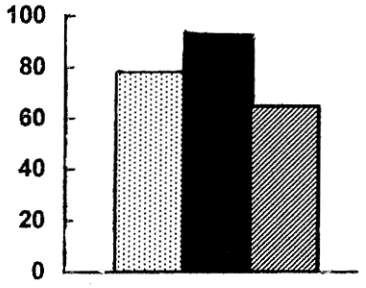


Fig. 2. Before sacrification plasma sialic acid levels.
▨, EAT+Curcumin; ■, EAT+Oil; ▨, Control.

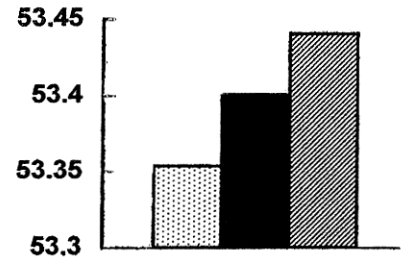


Fig. 3. At the begining of the test plasma sialidase activity.
▨, EAT+Curcumin; ■, EAT+Oil; ▨, Control.

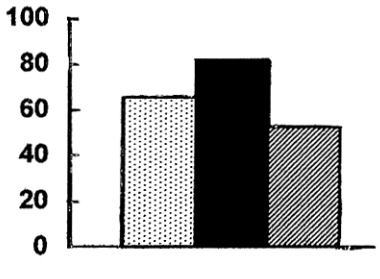


Fig. 4. Before sacrification plasma sialidase activity.
▨, EAT+Curcumin; ■, EAT+Oil; ▨, Control.

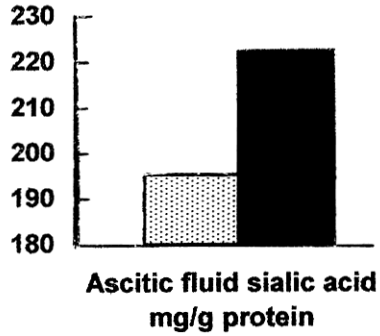


Fig. 5. Before sacrification ascitic fluid sialic acid levels.
▨, EAT+Curcumin; ■, EAT+Oil.

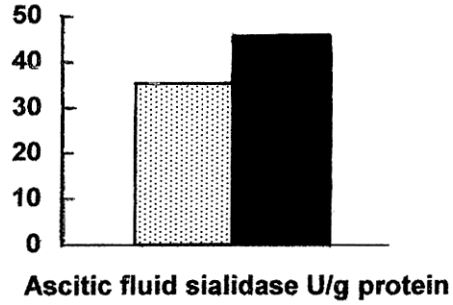


Fig. 6. Before sacrification ascitic fluid sialidase activity.
▨, EAT+Curcumin; ■, EAT+Oil.

high-potent malignancy. Since the tumor originated from effusions in abdominal cavity, ascites fluid is the most common complication of this pathology (Kaleoglu and Isli 1977).

Ascites fluid accumulates 4–6 days after passage of tumoral cells and it lasts with mouse loss in 7–12 days. In our study mice fed by sesame oil lived 9 days, whereas the ones fed by curcumin survived 17.5 days, doubling their



Fig. 7. Surveys of Ehrlich ascites tumor bearing mice.
▨, EAT+Curcumin; ■, EAT+Oil.

TABLE 3. *Correlation of plasma and ascitic fluid sialic acid levels and sialidase activity in curcumin and sesame oil given mices*

	Ascitic fluid sialic acid	Plasma sialidase	Ascitic fluid sialidase
Plasma sialic acid	r: 0.879	r: 0.619	r: 0.723
In curcumin fed	p: 0.000	p: 0.034	p: 0.002
Plasma sialic acid	r: 0.741	r: 0.581	r: 0.619
In sesame oil fed	p: 0.002	p: 0.04	p: 0.014

lifetime on average. In Menon's study, a group of mice bearing experimentally developed lung cancer has been fed with curcumin for 21 days, and it has been shown that curcumin has lowered metastatic foci about 89% compared to the mice with normal diet. These resultes were in in concordance that curcumin had strongest antimetastatic activity among the other polyphenolic compounds (Menon et al. 1995). In our study we found 88% lengthening of survival in the mice fed by curcumin.

Hepatic failure and tumoral pathologies are the most common causes of ascites fluid. Differentiation of malignancy originating ascites has paramount importance in clinical follow-up of cancer patients. Increased SAL has 82% diagnostic specificity in evaluated malignancy originated ascites fluid, concluding its reliability as a tumor marker (Colli et al. 1989). In this study, we also determined increased peritoneal SAL in both tumor groups. There was a positive correlation between increased plasma and peritoneal SAL in these groups, hence indicating tumoral pathology.

Sialic acids are terminal components of oligosaccharides, and donate important biochemical properties to the molecules of their glycoprotein structure. High sialic acid concentrations in various type tumoral cell surfaces are most probably related to their preventive activity versus immune system recognition and also enabling distant metastasizing capacity.

Increasing sialic acid concentrations in tumor tissue lead stickiness of cells, thereby complicating by tumor embolization. Sialic acids facilitate tumoral cell attachment to vascular endothelium and platelet aggregation as a result (Yogeeswaran and Salk 1981; Schauer 1985). Sialyltransferase and sialidase enzymes participating not only in sialyzation but also in sialic acid metabolism have been altered in various certain cancer types (Mitagi et al. 1990; Sonmez et al. 1999). Therefore serum SAL and SA have widely gained acceptance as markers for tumoral possession and metastatic capacity (Hogan-Ryan et al. 1980; Kokoglu et al. 1989).

In our study, plasma SAL and SA in both tumor groups increased significantly compared to control group. Sialic acid enters the circulation by either shedding or cell lysis with sialidase activity. Increased sialidase activity is responsible for high serum SAL by cleaving the molecules from the cell surface. Hence, we determined significant correlation between plasma and peritoneal SA and SAL in both tumor groups. This results correlate prolongation of survival. After curcumin treatment, decreased plasma sialic acid levels and sialidase activity indicate that curcumin has preventive effect to cancer.

The mechanism of the antimetastatic activity of polyphenols is not known clearly. Polyphenols produce hydrogen bounds which stabi-

lize the basement membrane proteins and make them less susceptible to proteolitic degradation (Menon et al. 1995).

Activities of enzymes participating DNA synthesis are rapidly increase as soon as S-phase starts. This is exactly where curcumin interferes with cell division in tumor development thus blocking or slowing down DNA synthesis. After 12 hours incubation of endothelial cells with curcumin, 75% decrease in thymidilate kinase activity has been shown recently (Singh et al. 1996).

In the group fed by curcumin, plasma and peritoneal SAL and SA were significantly lower than sesame oil group. But positive correlation between plasma and peritoneal SAL was still preserved. These findings support the chemopreventive action of curcumin in the mice bearing EAT.

In conclusion, plasma and peritoneal SAL and SA are increased in the mice bearing EAT, thus pointing out malignancy. Curcumin as a phenolic compound decreases all these parameters in the mice with EAT and lengthens survival by 88%, concluding its anticancer activity.

Acknowledgments

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