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Acute tamoxifen treatment increases nitric oxide level but not total antioxidant capacity and adenosine deaminase activity in the plasma of rabbits

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Abstract. – Tamoxifen is a synthetic non steroidal anti-estrogen used to treat patients with breast cancer and healthy subjects with high risk of breast cancer. It was aimed to study the short term effects of tamoxifen on the plasma total antioxidant capacity (TAC), nitric oxide (NO) and the adenosine deaminase activity (ADA) in healthy rabbits. Sixteen healthy New Zealand rabbits were allocated to 2 groups including controls and tamoxifen treated animals. Controls received a single application of 0.9% saline via oral route while the treated rabbits received orally tamoxifen (dissolved in 0.9% saline, at a dose of 5 mg/kg). Blood samples were collected at 6 and 24 hours following the treatments. Plasma TAC and ADA were not affected by Tamoxifen treatment. However, NO level in tamoxifen treated group was increased at 24 hours following tamoxifen treatment as compared to controls.

In conclusion, acute tamoxifen treatment may not affect the antioxidant status and cellular immunity, as evidenced by unaltered TAC and ADA. However, NO level was increased as early as 24 hours following tamoxifen treatment.

Key Words:

Adenosine deaminase, Nitric oxide, Tamoxifen, Total antioxidant capacity.

Introduction

Tamoxifen (TAM) is a synthetic nonsteroidal antiestrogen agent used clinically in the chemotherapy of human breast cancer. It is the early representative of selective estrogen receptor modulators[1]. The therapeutic activity is considered to be due to its antiestrogenic effect on estrogen receptors leading to antiproliferative action on mammary glands. TAM shows antiestrogenic effect on mammary tissue, but it has an estrogenic activity on the serum lipids, bone and endometrium. Due to its estrogenic effect on endometrium, tamoxifen increases the risk of the endometrial hyperplasia[2]. In addition to breast cancers, the selective estrogen receptors have been increasingly utilized in healthy subjects with an high risk of breast cancer or in the prevention of recurrence[3,4]. Several studies have demonstrated that the antitumor action of TAM may not be solely depend on its antiestrogenic effect. There are reports indicating that TAM activity could also be associated with several other mechanisms including antioxidant, antinitrosative effects, radical scavenging activity[5-7], induction of antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)[8], induction of apoptosis[9], inhibition of protein kinase C[10]. Further, TAM could alter the formation of nitric oxide (NO) which is produced enzymatically from L-arginine by nitric oxide synthase (NOS). However, results regarding the NO changes are controvertial. For example, TAM was reported to inhibit the NOS activity and the nitrotyrosine formation in brain ischemia-reperfusion injury[11], while it induces the NOS activity and the NO synthesis *in vitro*[12]. Tamoxifen was also reported to alter some immune parameters such as the prevention of neutrophil infiltration and the hydrogen peroxide formation by the human neutrophils[13].

Adenosine deaminase (ADA) is a purine catabolic enzyme involved in the lymphocyte prolif-

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eration, maturation and differentiation. It is an indicator of T cell activation and may be used to evaluate the cellular immune response[14]. ADA is also known to play an important role in the ischemia-reperfusion induced oxidative stress[15,16]. Furthermore, total antioxidant capacity is used to evaluate the cumulative antioxidants in plasma and it has been inscreasingly utilized to assess the antioxidant status in many pathological conditions[17].

In this study, it is aimed to investigate alterations in blood TAC, NO and ADA activity which is marker for altered immune response and play an important role in oxidative damage in response to TAM treatment in healthy rabbits.

Materials and Methods

Experimental Animals

Study was conducted in New Zealand rabbits (2100-2500 g). Animals were provided by the Central Animal House Facility of Kafkas University, Kars, Turkey. Feed and water are provided *ad libitum*. Sixteen animals were divided into 2 groups as control (group C) and TAM-treated group (group TAM). Each group consisted of 8 animals and treated as follows: animals in group C received a single oral administration of 0.9% saline. Rabbits in group TAM were treated with a single oral administration of tamoxifen citrate (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) dissolved in 0.9% saline at a dose of 5 mg/kg body weight. Blood samples were collected from vena auricularis into heparinized tubes at timed intervals at 6 and 24 hours after drug administration. Collected blood samples were centrifuged at 3000 rpm for 10 minutes to obtain plasmas. Then, the plasma samples were stored at –50ºC until the day of assay.

Biochemical Analysis

Determination of NO Level

NO levels in plasma were measured by the method of Miranda et al.[18]. The samples were deproteinized with 10% zinc sulphate, and total NO concentrations (via NO metabolites: nitrate and nitrite) were determined colorimetrically by the acidic Griess reaction (via reaction involving reduction of nitrate to nitrite by vanadium (III) chloride).

Determination of Total Antioxidant Capacity

Antioxidants in the sample reduce dark blue-green colored 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog[19].

Determination of ADA Activity

ADA activity in plasma was determined at 37ºC according to the method of Giusti and Galanti[20] based on the Bertholet reaction, formation of coloured indophenol complex from ammonia liberated from adenosine, and quantified colorimetrically with spectrophotometer (UV-1201, Shimadzu, Japan). One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay condition. Results were expressed as international unit of enzyme activity.

Statistical Analysis

The data for biochemical parameters were analyzed by ANOVA which is followed by post hoc Tukey test. All data were presented as mean ± SE using SPSS Windows 10.0. Values were considered statistically significant if p value was less than $p<0.05$.

Results

Plasma TAC, NO levels and ADA activity at 6 and 24 hours following the treatments were presented in Table I. Plasma TAC and ADA activity were not statistically different between control and TAM group at 6 and 24 hours. While blood NO level was found to be significantly higher in TAM treated group than in control at 24 hours, no difference in NO level was observed between control and TAM treated group at 6 hour following the treatments.

Discussion

Tamoxifen was reported to have antioxidant and radical scavenging effects[6,7]. It undergoes he-

Table I. *Plasma* total antioxidant capacity (TAC), nitric oxide (NO) and adenosine deaminase (ADA) activity of healthy rabbits in the treatment group (n=8).

Parameters	Time	Groups	
		Control	*Tamoxifen*
TAC (mmolTrolox equivalent/L)	6	0.322 ± 0.03[a]	0.373 ± 0.03[a]
	24	0.319 ± 0.03[a]	0.367 ± 0.02[a]
NO (µmol/L)	6	6.87 ± 1.75[a]	8.94 ± 0.93[a]
	24	6.47 ± 1.98[a]	10.90 ± 1.40[b]
ADA (U/L)	6	5.42 ± 0.28[a]	6.09 ± 0.32[a]
	24	6.72 ± 0.55[a]	6.17 ± 0.76[a]

Values with different superscripts in the same row are significantly different at $p<0.05$.

patic metabolism giving rise to 2 metabolites namely 4-hydroxytamoxifen and N-desmethyltamoxifen. Tamoxifen and its metabolites were shown to prevent lipid peroxidation induced by Fe (III) and ascorbic acid system in vitro[8,21]. In addition to the antilipid peroxidative action, it was reported that TAM increases the components of the enzymatic and non-enzymatic antioxidant system in humans with breast cancer following 3 and 6 months treatment. Serum glutathione and erythrocyte CAT, SOD, GSH-Px and glutathione-S-transferase enzyme activities were elevated in response to the TAM treatment at 3 and 6 weeks[8]. Tamoxifen was also reported to inhibit the hydroxyl radical generation induced by the phenelzine and 1-methylhy-4-phenylpyridine in the rat striatum[5,22]. Measuring TAC provides a broad spectrum of antioxidant activity of measurable antioxidants in response to the reactive oxygen and nitrogen species including endogenous and exogenous antioxidant molecules[17]. In the present study, plasma TAC was not altered in a short term treatment with TAM. Although it was shown that TAM is capable of inducing antioxidant system, the unaffected TAC could be due to the short term treatment. The increases in the enzymatic and non-enzymatic antioxidant system could take longer times with respect to the duration of TAM treatment. However, the results of this study imply that TAM has no effect on the TAC in a short term trial. Furthermore, it is known that TAM could also act as an oxidant leading to lipid peroxidation in the liver during the biotransformation of parent molecule. Tabassum et al.[23] reported that the TAM treatment resulted in an increased lipid peroxidation and an inhibition of antioxidant enzymes in the liver of mice. It is possible that the unaltered TAC in the current study may also be associated with the dual effect of TAM including both oxidant and antioxidant effects which may counterbalance the oxidant and antioxidant state as well as the elements of the antioxidant system.

In the present research, no alteration was observed in adenosine deaminase activity in response to acute effect of tamoxifen compared to control at 6 and 24 hours. ADA is an important enzyme in the purine metabolism and serves in the catalytic deamination of adenosine to inosine and ammonia. It is required for the lymphocyte proliferation and differentiation. ADA is measured to assess the cell mediated immunity in several diseases[24]. Elevation in the ADA activity is attributed to the stimulated cell mediated immune response and could also be used as a marker of activated neutrophil functions during the oxidative stress[25]. The results show that the acute TAM treatment has no effect on the cell mediated immune response which is one of the important components utilized in the treatment of cancer. Furthermore, it was reported that the ADA could also be increased in the ischemia-reperfusion induced oxidative stress. Raised ADA activity results in the adenosine depletion leading to the increased free radical production since adenosine inhibits the ROS formation[15,16]. The unaltered ADA activity in the current study correlates with unaffected TAC. Therefore, the results of the antioxidant status are in accordance with the results of the ADA activity in response to a short term TAM treatment.

Nitric oxide is a biologically active molecule with diverse effects on many physiological and pathological processes. It plays some important roles in carcinogenesis including cancer initiation, promotion, metastasis and angiogenesis[26]. However, role of NO in these processes is somewhat controversial. Some of the reports indicate

that increased production of NO is protective against tumorogenesis but some others claim opposite[27]. With respect to the effect of TAM on NO production, it was reported that TAM treatment prevented the transformation of C3H 10T1/2 murine fibroblast cell line and increased the NO production via induction of (inducible nitric oxide) iNOS at concentrations blocking the cell transformation. Therefore, the increased NO production by TAM could alter some cellular processes involved in the anti-tumor action of TAM[12]. In this study, TAM treatment increased blood NO level at 24 hours but no alteration was observed at 6 hours compared to controls. In a long term study, TAM resulted in non-significant increase in the plasma of patients with breast cancer following 3 months of treatment[28]. On the other hand, Osuka et al.[11] reported that TAM is a potent inhibitor of the NOS activity which was shown by an inhibition of the nitrotyrosine production in the cerebral cortex of rat subjected to the ischemia-reperfusion injury. Nitrotyrosine is considered to be a footprint of peroxynitrite formed by the reaction of superoxide radical and NO. Indeed, NO may play a dual function depending on the environment where NO could be mutagenic and harmful on DNA in the presence of O_2. Yet it may be beneficial as an antioxidant molecule by reacting with superoxide radical which is a powerful free radical considering the various physiological role of NO. It was suggested that the modulation of NO production by TAM could possibly be involved in its antitumor action[12].

In conclusion, plasma TAC and ADA activity in acute treatment of TAM were found to be unaltered in healthy rabbits indicating that a short term TAM treatment may not affect the antioxidant status and cellular immunity. However, TAM could alter the blood NO level as early as 24 hours following the treatment.

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