Middle-East Journal of Scientific Research 9 (1): 110-114, 2011
ISSN 1990-9233

Toxic Effects of Tamoxifen and the Protective Role of Silymarin and Zizyphus

[1]S. Al-Jassabi, [2]Ali Saad and [3]A. Al-Omari

[1]Faculty of Medicine, Masterskill University, JB Malaysia
[2]Faculty of Pharmacy, Jordanian University of Science and Technology/Jordan
[3]Faculty of Health Sciences, Taif University, KSA

Abstract: Tamoxifen (TAM), a triphenylethylene derivative, remains a frontline treatment for hormone-responsive breast cancer despite its use being associated with elevated risk of developing endomaterial carcinoma. The aim of this study was to investigate the toxicity of this drug by measuring the ability of TAM to induce oxidative DNA damage through the formation of 8-hydroxydeoxyguanosine (8-OH-dG) using HPLC with electrochemical detection. TAM initiates reactive oxygen species (ROS) formation followed by damage to DNA and other cellular compartments. Silymarin and zizyphus are free radical scavengers, markedly inhibited the formation of 8-OH-dG. Results showed that silymarin or zizyphus reduced the toxicity of TAM by 5 fold and 4 fold respectively. These findings are consistent with conclusion that silymarin or zizyphus has highly protective power against TAM toxicity and the protective action relates, at least in part to its direct free radical scavenging ability.

Key words: Tamoxifen · Silymarin · Zizyphus · 8-hydroxydeoxyguanosin · DNA damage

INTRODUCTION

Tamoxifen (TAM), a triphenylethylene derivative, is a selective estrogen receptor modulator (SERM) [1-2] that has become the treatment of choice for women diagnosed with all stages of hormone-responsive breast cancer [3-4]. The hepatocarcinogenicity of TAM to the rat has been demonstrated in numerous studies [5-9] and malignant transformation in human endometrium [10-14]. It was suggested that TAM is initially metabolized in the liver with subsequent accumulation of some metabolites such as 4-hydroxytamoxifen, 4-hydroxy-*N*-desmethyltamoxifen and *N*-desdimethyltamoxifen in various tissues [15]. Clinical data suggest that tamoxifen can cause changes in the endometrium ranging from reversible thickening of the lining, via dysplasia and glandular hyperplasia, to the formation of polyps and ultimately full-blown carcinosarcomas [16-18]. The mechanisms involved are unclear; endomaterial tumors are generally believed to rise via proliferative processes, but the endometrium does express a range of cytochrome P450 isozymes [19-20] which have the capacity to activate genotoxic carcinogen [21]. It has been shown by other investigators that tamoxifen can cause or exacerbate hepatic dysfunction [22-23].

There are unquestionable benefits in the use of this drug for the treatment of women with breast cancer. Its use as a chemopreventive agent in healthy women is not clear-cut [24]. Epidemiological evidence from women with breast cancer who have been treated with TAM suggests long term administration may result in a small increase in the incidence of endometrial [25-26] or GI tract tumors [27].

To assess the possible risk factors to women, the mechanism of liver tumor development in rats given tamoxifen has received particular attention [27]. There is no doubt that tamoxifen damages liver DNA, resulting in adduct formation [28]. Tamoxifen has also been shown to bind covalently to the liver DNA [29]. Proposed tamoxifen DNA adducts include the bridge epoxide (cyclic ether) [28] and an adduct formed from hydroxyethyl tamoxifen [30]. The increase in cell proliferation in the liver could be due to an oestrogenic effect of tamoxifen in the liver; however, there is also the possibility that tamoxifen can cause toxicity in the liver resulting in cell death [31].

Recent evidence suggested that generation of reactive oxygen species (ROS) and oxidative stress also played a role in the TAM toxicity [32]. Recently, a number of natural antioxidants have been evaluated for their chemoprotective effects in various pathological states

Corresponding Author: S. Al-Jassabi, Faculty of Medicine, Masterskill University, JB Malaysia.

[33]. Silymarin is a flavonoid complex consisting of silybin, which are the most active component, silydianin and silychristin [34]. Zizyphus has a common name "Nabka"[35], Arab used it to maintain a healthy lifestyle and used for soothing properties [36].

The aim of this study was to investigate the toxicity of TAM and the role of silymarin or zizyphus in the protection against TAM –induced liver oxidative damage in Balb/c mice.

MATERIALS AND METHODS

Chemicals: All chemicals used in this study were of analytical grade and purchased from Sigma Co.USA.

Animals and Treatment: 60 Balb/c male mice (6-7 weeks old and around 28-30g weight each) were used in this study. Animals were obtained from the Animal House of University of Malaya and kept on standard laboratory diet and tab water ad libitum through the experiments. Five animals were housed stainless metal cages under 12:12h light-dark cycle and room temperature of 23-26°C. Mice were randomly assigned into 6 groups (10 mice each); control group (CON), mice of this group were received neither TAM nor any of antioxidants but only normal standard diet and water. Group 2 was silymarin control group (SC), mice were received silymarin extract supplementation (300 mg/kg) orally, daily for 4 weeks, prior to scarification. Group 3 was zizyphus control group (ZC), mice were received zizyphus extract supplementation (300 mg/kg) orally, daily for 4 weeks, prior to scarification. Group 4 was TAM group (TAMC), mice were supplemented with 20 mg/kg/day TAM orally, daily for 4 weeks prior to sacrification. 5th group was the experimental group (TAMS) mice were supplemented daily/ orally for 4 weeks with 300 mg/kg of silymarin as well as 20 mg/kg of TAM then sacrificed.

Group 6 was (TAMZ), mice were supplemented with 300 mg/ kg of zizyphus orally,daily and 20 mg /kg of TAM for 4 weeks then sacrificed.

Toxicological Studies: After scarification of all mice, blood was collected, serum isolated and frozen in -70°C for further studies. The livers were immediately removed and perfused with Hanks-buffered saline to remove excess blood. To assay 8-OH-dG the method described before [37] was used. The assessement of the liver toxicity was performed by using serum enzymes levels as a biomarkers; alanine transaminase (ALT) [38], glutamyl-oxaloacetic transaminase (SGOT) [39] and glutamyl-pyruvic transaminase (SGPT) [40]. Activitiesof antioxidant enzymes, Catalase (CAT) was determined using the method reported before [41], superoxide dismutase (SOD) using the method of Stief [42] and Glutathione peroxidase (GSHpx) using the method recommended by Leopold [43]. For the measurement of lipid peroxidation, the thiobarbituric acid (TBA), method described before [44-45] was used by measuring the amount of malondialdehyde (MDA) present in the liver homogenate. Statistical analysis: Results are expressed as mean± standard deviation. For comparison between groups, data were analyzed by one-way ANOVA; $P \leq 0.05$ was considered statistically significant.

RESULTS

The present results revealed non significant alterations in the body weights of mice of the various treated groups. TAM treated mine liver showed a little increase in the liver body mass index ratio due to massive intra-hepatic hemorrhage and pooling of blood in the liver, making the liver appearance darker in colour when compared with the other groups, which were all within the normal values.

Serum values of ALT, SGOT and SGPT were utilized to evaluate liver injury. TAM administration increased serum values of ALT (6-fold), SGOT (5-fold) and SGPT (about 9 fold) compared to those in CON mice, while pretreatment with silymarin or zizyphus significantly ($P \leq \alpha$ 0.05) inhibit the rise of these enzymes induced by TAM as shown in Table 1.

Antioxidant enzymes (CAT, SOD and GSHpx) activities were decreased significantly ($P \leq 0.05$) in mice treated with TAM only, while these activities were near the normal after pretreatment with silymarin or zizyphus.

MDA is a product of oxidative damage to lipids and in this study, the concentration of MDA in liver homogenate is considered as a biomarker of TAM toxicity. Results in Table 1 shows that liver homogenate of mice exposed to TAM contained higher levels of MDA (about 23 fold increase) when compared with CON values. These levels decreased significantly ($P \leq 0.05$) in liver homogenates of mice supplemented with silymarin or zyziphus.

There was a significant increase ($P \leq 0.05$) in the level of 8-OH-dG in TAM treated mice. Table 1 show that silymarin and zizyphus inhibited TAM -induced formation of 8-OH-dG.

Table 1: Summery of the results of the effects of silymarine and zizyphus on the Toxicity of TAM

	CON	SC	ZC	TAMC	TAMS	TAMZ
ALT (U/L)	60.77±0.443	56.66±0.661	58.12±0.614	428.22±3.112	97.56±1.370	107.66±1.226
LPO (MDA) µM	0.072±0.0.003	0.051±0.003	0.048±0.001	4.224±0.007	1.008±0.004	1.028±0.004
8-OH-dG/10^5 2dG	4±0.014	3±0.13	3±0.013	49±0.334	11±0.172	12±0.330
SGOT (U/L)	12.06±1.011	10.08±0.98	10.17±0.87	89.66±3.41	27.86±0.88	26.42±0.79
SGPT (U/L)	11.15±1.032	10.64±0.76	10.11±0.43	93.22±3.22	3.91±0.67	25.55±0.62
SOD (U/mg Protein)	68±2.970	71±3.033	73±3.110	18±1.002	41±2.012	43±1.881
CAT (U/min/ mg protein)	70±2.170	72±3.122	72±3.027	26±1.776	44±2.654	44±2.335
GSHpx (µmol/ Min/mg protein)	0.98±0.007	1.21±0.06	.16±0.043	0.22±0.002	0.41±0.008	0.47±0.003

DISCUSSION

TAM or triphenylene compounds undergo metabolic activation reactions, such as 4-hydroxylation, 3 hydroxylation, α-hydroxylation, *N*-demethylation, etc., in animal tissues [4-5], which raised the production of reactive oxygen species (ROS) [16]. Great attention now focused on the clinical usage of this drug because its several diverse effects, mainly idiosyncratic hepatotoxicity [18]. The level of serum ALT, SGOT and SGPT activities reflect damage to hepatocytes and indicated the increased cellular permeability [46] and are considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity [8]. Our results show that silymarin and zizyphus provide a hepatoprotective effect by reversing the changes produced by TAM.

Our results show that TAM increase lipid peroxidation with high level of malondialdehyde (MDA) as a main product of lipid break down, but silymarin and zizyphus has been proved by others [3, 7, 33, 46] to be a powerful antioxidants, reduce the formation of MDA likely via its ability to scavenge free radicals [47].

Peroxidation reaction of tamoxifen could yield metabolites reactive with DNA [28].

The generation of 8-OH-dG resulted from oxidative modification of DNA [31]. A strong correlation between higher amounts of 8-OH-dG and greater degree of oxidative stress, DNA strand break, or DNA damage has been reported [29]. As expected, the antioxidants silymarin and zizyphus reduced the level of 8-OH-dG.

H_2O_2 is a normal metabolite in the cell; its steady state concentrations range from 10^{-14} 10^{-9} [48]. Although H_2O_2 may not cause DNA damage under physiological conditions, it participates in the metal ion-catalyzed Haber-Weiss reaction and generates the highly reactive hydroxyl radical, which can target DNA resulting in oxidative DNA damage [30].

In this study, the reduction or prevention of oxidative hepatic injury caused by TAM metabolites has been proposed to be achieved through increasing host antioxidant defense system by supplementation with silymarin or zizyphus. The body has defense mechanisms of antioxidants to limit damage caused by different species of ROS. These protective mechanisms consist of enzymatic species, SOD, CAT and GSHpx and our results showed that the activities were effected by TAM, but supplementation of silymarin or zizyphus attenuated their activities to the about normal. SOD is considered as the first line of defense to scavenge superoxide anions generated in cytosolic and mitochondrial compartments of the cell [42]. CAT and GSHpx causes direct breakdown of hydrogen peroxide to oxygen and water [43]. Thus, our results indicate that TAM treatment disrupted the antioxidant defense mechanism of the liver cells causing oxidative damage.

It was concluded that silymarin and zizyphus, widely used for food purposis especially in Asia, act as a very potent antioxidant against the toxicity of TAM an anti breast cancer drug widely used all over the world for treating this type of cancer.

REFERENCES

1. Singh, M.N., H.F. Stringfellow, E. Paraskevaidis, P.L. Hirsch and F.L. Martin, 2007. Tamoxifen: Important considerations of a multi-functional compound with organ-specific properties. Cancer Treatment Review, 33: 91-100.
2. Cuzick, J., T. Powles and U. Veronesi, 2003. Overview of the main outcomes in breast cancer prevention trails. Lancet, 36: 296-300.
3. Bradbury, B.D., T.L. Lash, J.A. Kaye and S.S. Jick, 2005. Tamoxifen-treated breast carcinoma patients and the risk of acute myocardial infarction and newly -diagnosed angina. Cancer, 103: 1114-1121.

4. McDonald, C.C., F.E. Alexander, B.W. Whyte, A.P. Forrest and H.J. Stewart, 1995. Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomized trail. Biochemical and Moleclar J., 311: 977-980.
5. Steiner, A.Z., M. Terplan and R.J. Paulson, 2005. Comparison of tamoxifen and clomiphene citrate for ovulation induction: a meta-analysis. Human Reproductive, 20: 1511-1515.
6. Neven, P. and I. Vergote, 2001. Tamoxifen, screening and new oestrogen receptor modulators. Best Practical Research of Clinical Obstetric Gynaecol., 15: 365-380.
7. Ismail, S.M., 1996. The effects of tamoxifen on the uterus. Current Opinion of Obstetric Gynocol., 8: 27-31.
8. Nevan, P. and I. Vergote, 2000. Guidelines for monitoring patients taking tamoxifen treatment. Drug. Safity, 22: 1-11.
9. Davis, W., S. Venitt and D.H. Phillips, 1998. The metabolic activation of tamoxifen and a-hydroxytamoxifen to DNA binding species in rat hepatocytes proceeds via sulphation. Carcinogenesis, 19: 861-866.
10. Lissoni, P., P. Vigano and M. Vaghi, 2005. A phase II study of tamoxifen in hormone-resistant metastatic prostate cancer: possible relation with prolactin secretion. Anticancer Res., 25: 3597-3599.
11. Sharma, M., D.E. Shubert, R.J. Rodabaugh, C.M. Vezina, D.P. Bofinger and J.R. Olson, 2003. Antioxidants inhibits tamoxifen-DNA adducts in endometrial explants culture. Biochemical & Biophysical Research Communication, 307: 157-164.
12. Jordan, V.C., 2006. Tamoxifen as a targeted therapy to treat and prevent breast cancer. British J. Pharmacol., 147: 269-276.
13. Stanley, L.A., P. Carthew, R. Davies, F. Higginson, E. Martin and J.A. Styles, 2001. Delayed effects of tamoxifenin hepatocarcinogenesis-resistant Fischer 344 rats as compared with susceptible strains. Cancer Letters, 171: 27-35.
14. Love, C.D. and J.M. Dixon, 2000. Thickened endometrium caused by tamoxifen returns to normal following tamoxifen cessation. Breast, 9: 156-159.
15. Carthew, P., B.M. Nolan, R.E. Edwards and L.L. Smith, 1996. The role of cell death and cell proliferation in the promotion of rat liver tumours by tamoxifen. Cancer Letters, 106: 163-169.
16. Rajaniemi, H., M. Koskinen, E. Mantyla and K. Hemminki, 1998. DNA binding of tamoxifen and its analogues: identification of the tamoxifen-DNA adducts in rat liver. Toxicological Letters, 102: 453-457.
17. King, C.M., 1995. Tamoxifen and the induction of cancer. Carcinogenesis, 16: 1449-1454.
18. Davis, W., S. Venitt and D.H. Phillips, 1998. The metabolic activation of tamoxifen and a-hydroxytamoxifen to DNA-binding species in tat hepatocytes proceeds via sulphation. Carcinogenesis, 19: 861-866.
19. Rosell, J., T. Formander, T. Hastschek and H. Limdaman, 2011. Time dependent effects of adjuvant tamoxifen therapy on cardiovascular disease. British J. Cancer, 104: 899-902.
20. Chang, Y., J.J. Lee, Wei W.F. and D.S. Chou, 2011. A novel role for tamoxifen in the inhibition of human platelets. Transplant Res., 157: 81-91.
21. Liu, Y., J. Suckate, J. Maskur, M. Magro and M. Solimena, 2010. Tamoxifen-independent recombination in the RIP-CreER mouse. Res. Oncol., 5: 33-44.
22. Massip, L., P. Caron, J.S. Lacovoni and D. Trouche, 2010. Deciphering the chromatin landscape induced around DNA Double Strand Breaks. Cell Cycle, 9: 2963-2972.
23. Ting, A.Y. and B. Petroff, 2010. Tamoxifen decreases ovarian follicular loss from experimental toxicant DMBA and chemotherapy agent cyclophosphamide and doxorubicin in the rat. Journal of Assistant Report of Genetics, 27: 591-597.
24. Cohen, I., 2004. Endometrial pathologies associated with postmenopausal tamoxifen treatment. Gynecologic Oncol., 94: 256-266.
25. Seoud, M.A., A. Shamseddine and A. Khalil, 1999. Tamoxifen and endometrial pathologies: a prospective study. Gynecologic Oncol., 75: 15-19.
26. Berliere, M., A. Charles and C. Galant, 1998. Uterine side effects of tamoxifen: a need for systemic pretreatment screening. Obstetric Gynecol., 191: 40-44.
27. Jordan, V.C. and J. Assikis, 1995. Endometrial carcinoma and tamoxifen: clearing up a controversy. Clinical Cancer Res., 1: 467-472.
28. Narod, S.A., T. Pal and T. Graham, 2001. Tamoxifen and risk of endometrial cancer. Lancet, 357: 65-67.

29. Curtis, R.E., J.D. Boice and D.A. Shriner, 1996. Second cancers after adjuvant tamoxifen therapy for breast cancer. J. National Cancer Instute, 88: 832-834.
30. Clemons, M., S. Danson and A. Howell, 2002. Antitumour treatment, Tamoxifen. Cancer Treatment Review, 28: 165-180.
31. Jordan, V.C., 2003. Tamoxifen: a most unlikely pioneering medicine. Drug Discovery, 2: 205-213.
32. Jin, Y., Z. Desta, V. Stearns, V. Ward, T. Skaar, A.M. Storniolo and A. Araba, 2005. CYP2D6 genotype, antidepressant use and tamoxifen metabolism during adjuvant breast cancer treatment. J. National Cancer Institute, 97: 30-39.
33. Tabassum, H., S. Parvez, H. Rahman, B. Banerjee and S. Raisuddin, 2007. Nephrotoxicity and its prevention by taurine in tamoxifen induced oxidative stress in mice. Human& Expermental Toxicol., 26: 509-518.
34. Okubo, T., F. Nagai, S. Ozawa, K. Kano, S. Tomita and Itsu Kano, 1998. DNA cleavage and 8-hydroxydeoxyguanosine formation caused by tamoxifen derivatives in vitro. Cancer Lett., 122: 9-15.
35. Brauch, H., T.E. Murdter, M. Schwab and M. Eichelbaum, 2009. Pharmacogenomics of tamoxifen therapy. Clinical Chemistry, 55: 1770-1782.
36. Sestak, I., K.R. Edwards, J. Forbes and J. Cuzick, 2006. Influence of hormone replacement therapy on tomaxifen-induced vasomotor symtomes. J. Clinical Oncol., 24: 3991-3996.
37. Schroth, W., L. Antoniadou, P. Fritz, M. Schwab and U. Zanger, 2007. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. J. Clinical Oncol., 25: 5187-5193.
38. Teunissen, S.F., H. Rosing, A.H. Schinkel and J.H. Beijnen, 2010. Biochemical methods for determination of tamoxifen and its phase I metabolites. Analitical Chemistry Acta, 683: 21-37.
39. Singh, M.S., P.A. Francis and M. Michael, 2011. Tamoxifen, Cytochrome P450 genes and breast cancer clinical outcomes. The Breast, 20: 111-118.
40. Tabassum, H., H. Rehman, B.D. Banerjee, S. Raisuddin and S. Parvez, 2006. Attenuation of tamoxifen-induced hepatotoxicity by taurine in mice. Clinical Chemistry Acta, 370: 129-136.
41. Upadhyay, G., A. Kumar and M.P. Singh, 2007. Effect of silymarin on pyrogallol- and rifampicin-induced hepatotoxicity in mous. Europian J. Pharmacol., 565: 190-201.
42. Rolo, P.A., P.J. Moreno and A. Palmeira, 2003. Protection against post-ischemic mitochondrial injury in rat liver by silymarin or TUDC. Hepatological Res., 26: 217-224.
43. Tasduq, S., K. Peerzada, K. Koul and R. Johri, 2005. Biochemical manifestation of anti-tuberculosis drugs induced hepatotoxicity and the effect of silymarin. Hepatological Res., 31: 132-135.
44. Desai, P., S. Nallani and R. Sane, 2002. Induction of cytochrome *P*450 3A4 in primary human hepatocytes and activation of the human pregnane X receptor by tamoxifen and 4-hydroxytamoxifen. Drug Metabolism Disposal, 30: 608-612.
45. Karki, A., E. Mantyla, Y. Hirsimaki, S.Karlsson and S. Toikkanen, 2000. Comparison of the effects of tamoxifen and toremifene on rat hepatocarcinogenesis. Archive Toxicol., 74: 249-256.
46. El-Beshbishy, H., 2005. The effect of dimethyl dimethoxy biphenyl dicarboxylate (DDB) against tamoxifen-induced liver injury in rats: DDB use in curative or protective. Journal of Biochemistry and Molecular Biol., 38: 300-306.
47. Simeone, A.M., S. Ekmikciolu, E.M. Grimm and A.M. Tari, 2002. A novel mechanism by which retinamide inhibits breast cancer growth: the production of nitric oxide. Molecular Cancer Therapy, 1: 1009-1017.
48. Shahat, A.A., L. Pieters, N. Nazeif and N. Abdel-Azim, 2001. Chemical and biological investigations on *Zizyphus spina-christi* L. Phytotherapy Res., 15: 593-597.