Tr. J. of Medical Sciences
28 (1998) 591-594

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Distribution of Tamoxifen in Rat Tissues During Steady-State Treatment

Received: April 08, 1996

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Abstract: Tamoxifen citrate (TAM), a nonsteroidal antiestrogen, is a drug of choice in the endocrine treatment for all stages of breast cancer in both pre and postmenopausal patients. The tissue distrubution of TAM alone and combined with medroxyprogesterone acetate (MPA) following long-term p.o. administration in immature rats is investigated in the present study. The highest consentrations of TAM has been found in the hypophysis in both groups. TAM consentrations were effected by MPA treatment in the liver and kidneys.

Introduction

Tamoxifen citrate is a firts-line endocrine treatment for estrogen receptor positive breast cancer patients (1). TAM has also been considered as a chemopreventive agent, for the primary prevention of breast cancer in high risk women (2). Although the mode of action is not clear yet, TAM acts as an estrogen antagonist in the presence of high endogenous estrogen levels and it may also act as a weak estrogen agonist when the endogenous estrogen levels are low (3). TAM is metabolized in the liver and several metabolites have been detected in serum (4). In serum, 98% of the tamoxifen is bound to albumin and the distribution volume of the drug is high (50-60 liters/kg) (5). The high volume distribution of TAM suggests the extensive distribution of the drug into the pheripheral tissues and the presence of a small amount in serum. Tissue distribution of the TAM and its metabolites have been reported both in rats and human following p.o or i.v. administrations (6-9). Since the tumorostatic effect of TAM is directly related to its concentration in tumor cells in rats, the tissue distribution of TAM is important (10).

Since MPA is known to prevent estrogen induced changes in endometrium, MPA is commonly administered together with TAM (I1).

The present study was designed to evaluate the tissue distribution of TAM in immature rats following long-term p.o. administration.

Materials and Methods

Animals

Sixteen immature (21 days) Female Wistar rats were purchased from Medical Research Institute of Istanbul University and divided into 2 groups randomly. All animals were maintained on a 12h light- 12h dark cycle. Water and pelleted food were supplied ad libitum.

Drugs

TAM (ICI Pharmaceuticals, Wilmington; DE) was dissolved in 2.5 ml tap water to a final concentration of 0.5 ng/µL. Medroxyprogesterone acetate (Upjohn, Kalamazoo, MI) was dissolved in 2,5 ml tap water to a final concentraiton of 12 mg/ml. The rats received oral TAM (0.5ng/kg) or TAM-MPA (0.3 mg/kg) solution by a micropipet daily.

Experimental Protocol

The immature female rats were randomly divided into 2 groups; then they were administered TAM or TAM-MPA for 30 days according to the following protocol:

Group 1 (TAM, n=8), oral TAM (0.5 ng/kg) treatment was commenced daily.

Group 2 (TAM-MPA, n=8), oral TAM (0.5 ng/kg) and oral medroxyprogesteron acetat (03 mg/kg) treatment was commenced daily.

Following 30th day of treatment, all animals were sacrificed by over dose penthotal (Nembutal Sodium, Abbott) injection intraperitoneally 3 hours following the labelled TAM administration. Blood samples were

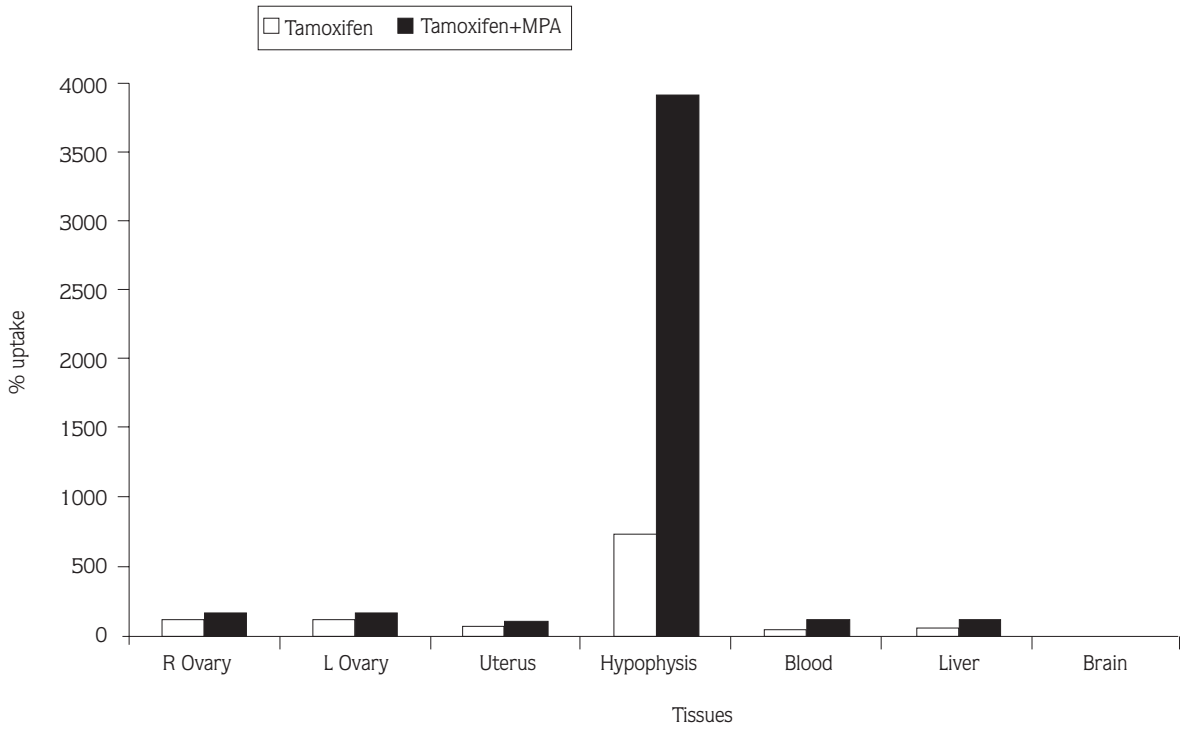

Figure 1. Mean tissue percentage uptake of TAM both in TAM and TAM+MPA treated rats.

obtained by cardiac puncture immediately after the sacrification of the rats. The brain, hypophysis, lung, spleen, liver, right and left kidneys, right and left adrenals, right and left ovaries, heart, thymus and bicorniate uteri were collected from each animal by laparatomy and craniotomy.

Tamoxifen Analysis

Radiopharmaceutical: 99mTc-Tamoxifen citrate was prepared according to the following procedure: 10 mg tamoxifen citrate tablet was dissolved in 2 mL distilled water in a glass vial. (Nolvadex ® Zeneca. Co., England.) Nolvadex ® (tamoxifen citrate) Tablets, a nonsteroidal antiestrogen, are for oral administration and contain 15.2 mg of tamoxifen citrate, (equivalent to 10 mg tamoxifen). In addition, each tablet contains as inactive ingredients: carboxymethylcellulose calcium, magnesium stearate, mannitol and strach.) The pH was adjusted to 5 with 0.5 NaOH. 0.2 mL SnCl2. 2H20 aq. solution (1 mg/mL) was added and mixed well. The mixture was passed through a 0.22 µm Millipore membrane filter into a sterile vial. 2 mL generator (Amersham International, Amersham, U.K.) eluate containing 370-555 MBq of 99mTc- pertechnetate was added and incubated at room temperature (R.T.) for 10 min. The labeling efficency was determined by impregnated thin layer chromatography, using ready plates ofITLC-SG (Gelman ınstrument Co., Ann Arbor, Mich., U.S.A.) 15 min after 99mTc-Tamoxifen citrate preparation. The sheets were put into 1 X 10 cm strips. Ten µL samples were applied at a point 1 cm from one end. The strips were developed in acetone or physiological saline. The solvents were allowed to reach 8 cm from the origin. The strips were cut into halves and the radioactivity in each segment was determined in a γ well-type counter (Model: DPC., Los Angeles, USA). Pertechnetate moved with the solvent front and hydrolyzedreduced (H-R) 99mTc remained at the point of application in both solvents. 99mTc-Tamoxifen citrate remained at the origin in acetone, but moved with the solvent front in saline. The labeling efficency was determined by subtracting the sum of the amounts that migrated in acetone and that which remained at the origin in saline from %96. The stability of 99mTc-Tamoxifen citrate was tested at 0.5, 1, 3, and 24 h after storage at R.T. using the same method. The organs were weighed and counted from 1/1000 dilution of the solution. The percentage uptake of each organ and % administered dose/tissue werecalculated.

Results

All tissue weights were similiar in both group. The highest mean percentage of TAM uptake was observed in

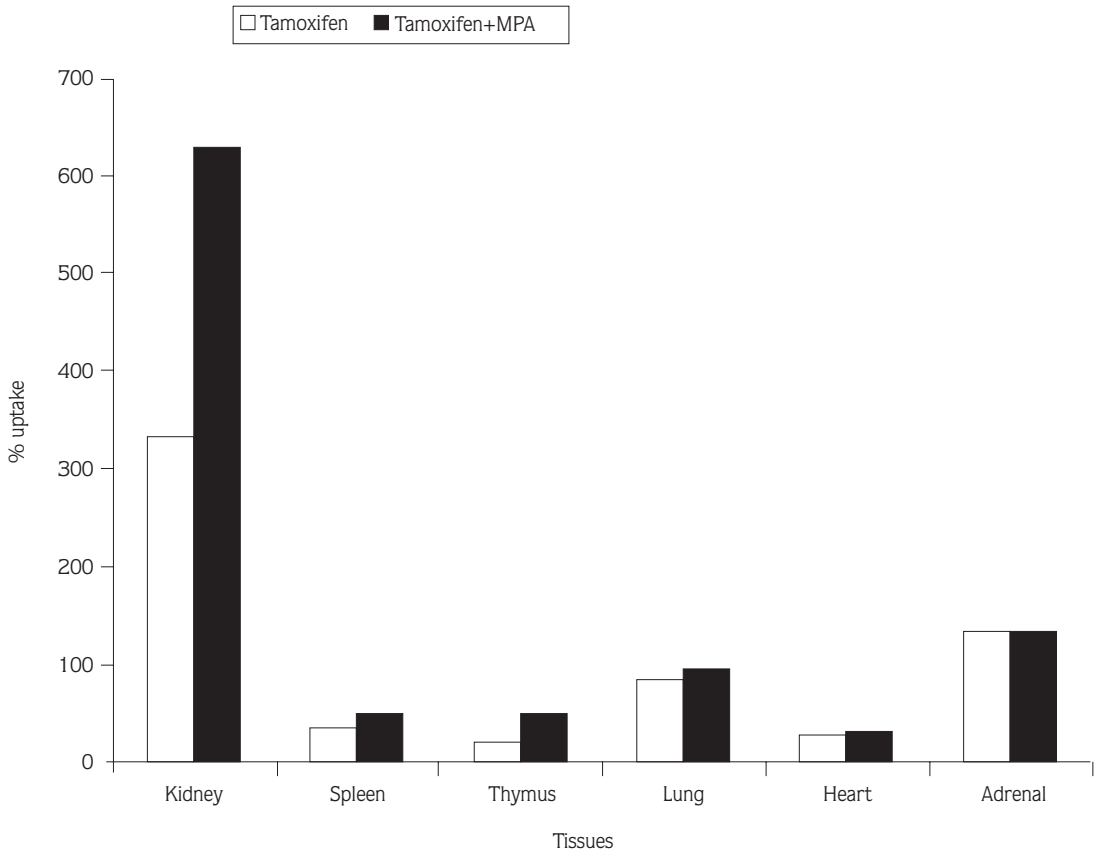

Figure 2. TAM uptake in kidneys, adrenals, thymus, heart, spleen and lung.

the hypophysis in both TAM and TAM-MPA treated groups ($p<0.05$) (Fig. 1, 2).

But the TAM uptake was 5-fold higher to the TAM-MPA group compared with the TAM treated group. The mean percentage of TAM uptake were 3907.70255 in the TAM-MPA and 738.70245 in the TAM treated group. TAM uptake was detected 2, 4, 2, 9, 2-fold higher in the liver, ovaries, lung, kidneys and uterus then that serum in the TAM treated group respectively. In contrast, TAM uptake was lower in the lung, liver and uterus than the serum, but the renal uptake was still 5-fold higher when compared with the serum in the TAM-MPA treated group. The highest levels were observed in the kidneys (331.065, TAM treated; 627.0121 TAM-MPA) and the ovaries (128.9015, TAM treated; 159.5044, TAM-MPA) respectively after the levels in the hypophysis in both groups. Mean percentage of TAM uptake was significantly high in heart, serum, liver and kidneys in TAM-MPA group compared with TAM treated group ($p<0.05$). TAM concentration in the uterus was higher in TAM-MPA group, but this difference was not significant ($p>0.05$). Brain TAM concentrations were lower in both groups (609.016, TAM treated; 497.106, TAM-MPA. Tissue distrbution of TAM in TAM treated and TAM-MPA groups were similiar after long term oral TAM and TAM-MPA administration.

Discussion

TAM has been extensively used as a chemotherapeutic agent in the treatment of breast cancer patients (1, 2). Recently, TAM has been considered to be prescribed for the primary prevention of the breast cancer in high risk women (2, 3). The tumorostatic effect of TAM has been directly related to the tissue concentrations of the drug in rats (4). Estrogenic effects of TAM have also been reported to breast cancer patients (12-14). Since MPA is known to prevent the estrogen induced changes in the endometrium, TAM and MPA may be used together in breast cancer patients. On the other hand, tumorostatic effect of TAM is not similiar in different tissues (15). The results of these numerous report have been suggested the differences in the tissue distribution of TAM (6, 7, 8, 9).

The concentrations of TAM was detected 2-9-Fold higher in some organs, such as the liver, ovaries, lung and kidneys, than the serum levels. Similiar results have been reported following long term TAM administration both in human and animals (7, 8). Tissue accumulation of TAM may be related to the presence of the antiestrogenic-binding-sites (AEBS) in the tissue. Liver has been reported to have high levels of AERS (15). In the present study, TAM uptake was found to be high in the liver, furthermore, TAM uptake was high in TAM-MPA group compared with TAM treated group. Although the factors which are associated with AEBS changes in the liver are not clear yet, the higher level of TAM uptake may be the result of MPA treatment in combination with TAM.

In addition large amount of TAM was detected in the lung in both groups; this retansion may be due to the interaction between the phospholipids and the TAM (16). High levels of TAM was recovered from the kidneys and the TAM level was higher in TAM-DPA group than in the TAM treated group. The highest TAM uptake was obsereved in the hypophysis in the both groups, in contrast the brain has a minimal TAM concentrations. The low TAM conceatrations in the brain has been reported in a previous study (6). These results have suggested that TAM crossed the blood-brain barier. In human, equal amount of TAM and its metabolites were found in both normal brain tissue and the cerebral metastasis (17). TAM uptake was not affected from MPA treatment in the ovaries and the uteri.

As a conclusion, MPA treatment can alter the tissue distribution of TAM and the reason of high concentrations of TAM in the kidneys and the hypophysis needs to be explaned by further experiments.

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