Rational Design and Molecular Effects of a New Topoisomerase II Inhibitor, Azatoxin

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ABSTRACT

Azatoxin [NSC 640737-M; 5R,11aS-1H,6H,3-one-5,4,11,11a-tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl) oxazolo (3′,4′:1,6)pyrido (3,4-b)indole] was rationally designed from a model for the pharmacophore of drugs with topoisomerase II inhibition activity. This pharmacophore has at least 2 domains: a quasiplanar polycyclic ring system proposed to bind between the DNA base pairs and a pendant substituent proposed to interact with the enzyme and/or to the DNA grooves. The present study shows that, in cell free systems, azatoxin induces a large number of double strand-breaks in linear Simian virus 40 and human c-myc DNA. These breaks yield cleavage patterns that are different from those of well established topoisomerase II inhibitors (epipodophyllotoxins, amsacrine, mitoxantrone). Azatoxin also inhibits the catalytic activity of purified topoisomerase II, and is a nonintercalator. The structure-activity relationship of 3 isomers and 6 derivatives of azatoxin shows a stringent stereochemical requirement for activity. The effects of azatoxin pendant ring substitution on topoisomerase II mediated DNA cleavage activity were similar to the relationship observed for etoposide.

INTRODUCTION

Eukaryotic DNA top 2 inhibitors are among the most active anticancer agents. They are anthracyclines [Adriamycin, daunorubicin, 4-demethoxydaunorubicin (idarubicin), 4′-epiadriamycin (epirubicin)], demethylepipodophyllotoxins (VP-16 and VM-26), anthraccenediones (mitoxantrone), acridines (m-AMSA), and ellipticines (2-methyl-9-hydroxyellipticinium) (1, 2). More recently, new inhibitors have been identified. Some act as out stabilizing cleavable complexes [merbarone (8), fostriecin complexes (amonafide (3), genistein (4, 5), saintopin (6), and terpenoids (7)], while others inhibit top 2 catalytic activity without stabilizing cleavable complexes [merbaron (8), fosfocin (9), dioxyperazin derivatives (10), and intercalators at high concentrations (11, 12)]. The topoisomerase II inhibiting activity of these drugs was found after they had been identified for their antitumor properties.

In efforts to discover new topoisomerase II inhibitors with increased efficiency, a series of well established top 2 inhibitors was examined by molecular modeling. Despite the apparent chemical diversity of these inhibitors, a composite model for the top 2 inhibitor pharmacophore was proposed (13). On the basis of this model and through the use of molecular modeling, azatoxin was designed as an analogue hybrid between VP-16 aglycone and ellipticine (Fig. 1). The present report describes the effects of azatoxin on cell free systems using purified top 2. A structure-activity relationship is also provided.

MATERIALS AND METHODS

Chemicals and Enzymes

Azatoxin and its derivatives, as well as the DMDP and DMEP, were synthesized at the Department of Chemistry, University of Virginia, Charlottesville, VA. Compounds were synthesized by the method of Tomioka et al. (14). All products were isolated as pure compounds and exhibited satisfactory analytical and spectral data. Full details will be published in a future article. m-AMSA and mitoxantrone were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. VP-16 and VM-26 were obtained from Bristol-Myers Co., Wallingford, CT. Drug stock solutions were made in dimethyl sulfoxide at 10 mM. Further dilutions were made in distilled water.

SV40 and c-myc human DNA insert in pBR322, restriction endonucleases, DNA topoisomerase I, T4 polynucleotide kinase, calf intestine phosphatase, and polyacrylamide/bis were purchased from Bethesda Research Laboratories (Gaithersburg, MD), from the American Type Culture Collection (Rockville, MD), or from New England Biolabs (Beverly, MA). [γ-32P]ATP was purchased from New England Nuclear Research Products (Boston, MA).

HL-60 nuclear extracts were prepared as described previously (11). DNA topoisomerase II was purified from mouse leukemia L1210 cell nuclei as described previously, and was stored at −70°C in 40% (v/v) glycerol, 0.35 M NaCl, 5 mM MgCl2, 1 mM EGTA, 1 mM KH2PO4, 0.2 mM dithiothreitol, and 0.1 mM phenylmethyleneanisulfonate fluoride, pH 6.4. The purified enzyme yielded a single Mr, 170,000 band after silver staining of SDS-polyacrylamide gels (15, 16).

Preparation of End-labeled DNA Fragments

DNA fragments were 5′-end-labeled as described previously (17, 18). Briefly, native DNA was first linearized with a restriction enzyme, then the 5′-DNA termini were dephosphorylated with calf alkaline phosphatase and labeled with [γ-32P]ATP using T4 polynucleotide kinase. For double-strand breaks, assays using HL-60 nuclear extract SV40 DNA was digested with Bcll endonuclease and labeled at both DNA termini. For sequencing experiments, SV40 and c-myc DNA were first 5′-end labeled at the XhoI and XbaI restriction sites, respectively.

Then, in order to generate uniquely 5′-end-labeled fragments, labeled DNA were subjected to a second enzyme digestion, Pf11I plus HindIII for c-myc DNA. The resulting DNA fragments were separated by agarose gel electrophoresis and isolated by electroelution. Purifications by phenol-chloroform extraction and ethanol precipitation were included between each step and at the end of the labeling procedures (19, 20).

Topoisomerase II-induced DNA Cleavage Reactions

DNA fragments were equilibrated with or without drug in 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM ATP, and 15 μg/ml bovine serum albumin for 5 min before addition of purified topoisomerase II (40–70 ng) or HL-60 nuclear extract (2 μl corresponding to approximately the extract from 106 nuclei) in 20 μl final reaction volume. Reactions were stopped by adding SDS to a final

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3 The abbreviations used are: top 2, DNA topoisomerase II; azatoxin (NSC 640737-M; 5R,11aS-1H,6H,3-one-5,4,11,11a-tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl) oxazolo (3′,4′:1,6)pyrido (3,4-b)indole; SV40, Simian virus 40; VM-26, teniposide; VP-16, etoposide; m-AMSA, amsacrine; SDS, sodium dodecyl sulfate; DMDP, 4′-demethyl-4-desoxypodophyllotoxin; DMEP, 4′-demethylepipodophyllotoxin.
TOPOISOMERASE II INHIBITION BY AZATOXIN

Fig. 1. Azatoxin chemical structure (left) and stereochemical superimposition of DM DP (---) and azatoxin (---) (right). Structures were energy minimized using the Alchemy II sofware.

concentration of 1% and proteinase K to 400 µg/ml, followed by incubation 1 h at 42°C.

For agarose gel analysis, 3 µl (10×) loading buffer (0.3% bromophenol blue, 16% Ficoll, 10 mM Na2HPO4) was added to each sample, which was then heated at 65°C for 1–2 min before loading into an agarose gel made in (1×) 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8 (17, 18, 21). Azatoxin gel electrophoresis was at 2 V/cm overnight.

The quantification of drug-induced DNA double-strand breaks in the presence of HL-60 nuclear extract was done as follows. Radioactive gels were counted in a betascope 603-blot analyzer. Then, for each lane, radioactivity was measured in the DNA cleavage products (C) (size between 600 and 5243 base pairs), and in the total DNA present in the lane with a size superior to 600 base pairs (T). Drug induced cleavage was expressed as:

\[
\% \text{ DNA cleaved} = 100 \times \frac{C/T - C_0/T_0}{1 - C_0/T_0}
\]

where \(C_0\) and \(T_0\) are the counts for cleaved and total DNA, in presence of nuclear extract without drug, respectively.

For DNA sequence analysis, samples were precipitated with ethanol and resuspended in 2.5 µl loading buffer (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). Samples were heated to 90°C and immediately loaded into DNA sequencing gels (6% polyacrylamide; 19:1, acrylamide:bis) containing 7 M urea in (1x) 89 mM Tris, 89 mM boric acid, 2 mM EDTA, (pH 8) buffer. Electrophoresis was at 2500 V (60 W) for 4 h. Gels were dried on 3-MM paper sheets and autoradiographed with Kodak XAR-2 film (19).

RESULTS

Induction of DNA Double-Strand Breaks by Azatoxin in Presence of HL-60 Nuclear Extract. Drug-induced DNA double-strand breaks were first measured in SV40 DNA in the presence of HL-60 nuclear extracts (Fig. 2). SV40 DNA was chosen because: (a) it is a natural substrate of top 2 (19, 21); and

DNA Relaxation Assays

Inhibition of Topoisomerase II Catalytic Activity. Topoisomerase reactions were performed with 0.4 µg native SV40 DNA in 30 µl cleavage buffer (see above) for 30 min at 37°C. Reactions were stopped by adding SDS to a final concentration of 1% and proteinase K to 400 µg/ml, followed by incubation 1 h at 42°C (17). Azatoxin gel electrophoresis was performed as described below.

DNA Unwinding Assay. The DNA was either first relaxed by topoisomerase I (20 units) for 15 min and then drugs were added. Alternatively, the drugs and DNA were first mixed for 5 min and then topoisomerase I was added (22). These steps took place at 37°C. DNA-drug-topoisomerase I mixtures were incubated for an additional 30 min, and then stopped as described above. Samples were then submitted to agarose gel electrophoresis in 1% gels made in Tris-acetate-EDTA buffer (40 mM Tris-acetate, pH 7.6, 10 mM Na2-EDTA). Gels were run at 2 V/cm overnight, washed in H2O, and then stained with 1 mM ethidium bromide for 45 min. After an additional 45 min destaining in 1 mM Mg2SO4, the DNA was visualized under UV light and photographed with a Polaroid type 57 film.

Fig. 2. DNA double-strand breaks induced by azatoxin in the presence of HL-60 nuclear extract. 32P-end labeled SV40 DNA was reacted with increasing azatoxin concentrations and nuclear extract for 30 min at 37°C. Reactions were stopped by adding SDS and proteinase K (final concentrations, 1% and 0.4 mg/ml, respectively), and DNA fragments were separated in a 1% agarose gel. Left panel, autoradiography. Lane A, size markers; Lane B, control SV40 DNA (size, 5243 base pairs). Lanes C-K, HL-60 nuclear extract was present with the following azatoxin concentrations: 0, 10, 20, 30, 50, 100, 200, 300, and 500 µM, respectively. Right panel, quantification of cleavage as a function of the logarithm of azatoxin concentration. Points obtained from the gel on the left are identified by the name of the corresponding lane.

Fig. 4. DNA double-strand breaks induced by azatoxin in the presence of HL-60 nuclear extract. 32P-end labeled SV40 DNA was reacted with increasing azatoxin concentrations and nuclear extract for 30 min at 37°C. Reactions were stopped by adding SDS and proteinase K (final concentrations, 1% and 0.4 mg/ml, respectively), and DNA fragments were separated in a 1% agarose gel. Left panel, autoradiography. Lane A, size markers; Lane B, control SV40 DNA (size, 5243 base pairs). Lanes C-K, HL-60 nuclear extract was present with the following azatoxin concentrations: 0, 10, 20, 30, 50, 100, 200, 300, and 500 µM, respectively. Right panel, quantification of cleavage as a function of the logarithm of azatoxin concentration. Points obtained from the gel on the left are identified by the name of the corresponding lane.
TOPOISOMERASE II INHIBITION BY AZATOXIN

It is cleaved strongly at many sites by top 2 in the presence of inhibitors (18, 19). The smallest azatoxin concentration that produced detectable cleavage was 5-10 μM. Above 10 μM, cleavage occurred at many sites (Fig. 2, left panel) and was proportional to the logarithm of azatoxin concentration (Fig. 2, right panel). The potency of azatoxin was comparable to that of VP-16 (data not shown), and as in the case of VP-16, azatoxin-induced DNA cleavage was not suppressed at high drug concentrations (up to 1 mM), consistent with the possibility that azatoxin does not intercalate into DNA (see below).

Sequencing of Topoisomerase II Cleavage Sites by Azatoxin.

Induction of top 2 cleavage by azatoxin was tested directly by using purified murine leukemia top 2. Since the SV40 nuclear matrix associated region has been shown to be preferentially cleaved by top 2 (19, 21), this region was chosen for analysis (Fig. 3). Sites of cleavage were also determined by DNA sequencing in the 5'-flank of c-myc first intron (Figs. 4 and 5). Azatoxin induced many cleavage sites both in the SV40 (Fig. 3, Lane 5) and the c-myc DNA fragments (Fig. 4, Lane 3; and Fig. 5, Lanes 4–5). In general, azatoxin induced more cleavage sites than mitoxantrone (Fig. 3, Lane 10; and Fig. 5, Lane 3), m-AMSA (Fig. 3, Lane 4), VM-26 (Fig. 3, Lane 3; Fig. 4, Lane 6), or VP-16 (Fig. 5, Lane 6). The active concentrations of azatoxin were comparable to those of VP-16 and higher than those of VM-26, mitoxantrone, and m-AMSA.

Azatoxin was also compared to the epipodophyllotoxin derivatives whose structures are quite similar (Fig. 6). DMDP, whose structure is most similar to azatoxin, was less potent than azatoxin, and its cleavage pattern exhibited only minor differences from that of azatoxin (Fig. 6; in Fig. 5, compare Lanes 5 and 8). The 0-4-hydroxy derivative of DMDP, DMEP, was less potent than azatoxin and VP-16 (Fig. 6; in Fig. 5, compare Lanes 6 and 7). Interestingly, the cleavage pattern of DMEP showed some local differences when compared to VP-16, indicating that the VP-16 sugar affects the DNA sequence selectivity of top 2 inhibition by 4'-demethylepipodophyllotoxin derivatives.

Taken together, these results show that azatoxin is an active top 2 inhibitor with a unique DNA cleavage pattern.

Effects of Azatoxin Structural Modifications on Topoisomerase II Inhibition. Three isomers and 6 azatoxin derivatives (see Fig. 6 for structures) were tested for the drug-induced
topoisomerase II inhibition by azatoxin

Azatoxin Isomers. Three azatoxin isomers (Fig. 6, azatoxins 8–10) were shown to be inactive top 2 inhibitors in DNA cleavage assays (Fig. 4, Lanes 1, 2, and 4). The finding that the 2 diastereoisomers (diastereoisomers 9 and 10) were inactive demonstrates that a strict stereochemical relationship between the polycyclic ring system and the pendant aromatic ring must exist for activity, as defined by our pharmacophore model. The top 2 inactivity of isoazatoxin (compound 8) is a surprising result and indicates the great sensitivity of the binding site for these agents to minor structural modification. Azatoxin and isoazatoxin (compound 8) differ only in the orientation of the tetrahydrooxazolopyrido ring fusion into the indole ring; this change in orientation imparts: (a) only a subtle differential "curve" to the tetracyclic nucleus of the molecule, without altering the spatial relationship between the indole and phenyl ring systems; and (b) a change in orientation of the nitrogen indole. These results indicate that top 2 inhibition by azatoxin is highly sensitive to structural alteration.

Azatoxin Derivatives. Six azatoxin derivatives were also tested for top 2 inhibition (Fig. 6). The experimental results for these compounds are only shown in a tabulated form (Fig. 6). Two of them are modified on the polycyclic ring system and the 4 others are modified on the pendant ring.

Hydroxylation at position 11 (R4) of the azatoxin polycyclic ring system yields a compound (compound 6) structurally quite similar to DMEP but without strong top 2 activity. This observation is consistent with the results obtained in the 4’-demethylpodophyllotoxin series since DMEP, which is hydroxylated at the R4 position of DMDP, is less active than DMDP (Fig. 6).

Compound 7, which differs from azatoxin by its polycyclic ring system (Fig. 6), is also inactive. Therefore, the structure of the polycyclic ring system is critical for azatoxin activity.

Pendant ring (Fig. 6, Y ring) modifications gave the following results. Monodemethoxylation (compound 2) reduced top 2 activity by a factor 5, while didemethoxylation (compound 3) abolished the top 2 activity. Position 4’ (Fig. 6, R2) was also crucial as methylation of the hydroxyl residue (compound 4) abolished top 2 inhibition. These results indicate that, as previously shown for demethyllepipodophyllotoxins (23, 24), which have the same pendant (Y) ring as azatoxin, the nature of the substituents on the pendant ring are essential for top 2 inhibition. Interestingly, compound 5, in which the azatoxin pendant ring had been replaced by that of AMSA, was inactive (Fig. 6).

Inhibition of Topoisomerase II Catalytic Activity by Azatoxin. Fig. 7 shows that azatoxin inhibits top 2-mediated relaxation of native SV40 DNA. At the same time, azatoxin

Fig. 6. Structure-activity relationship for azatoxin and demethylepipodophyllotoxin derivatives. Topoisomerase II-induced DNA cleavage is expressed as the potency of a given drug relative to azatoxin. Cleavage was determined as shown in Fig. 2.

Fig. 5. Sequencing of top 2 cleavage sites in the 5′-flank of the human c-myc first intron using purified top 2 (same fragment as in Fig. 4). Lane 1, enzyme without drug; Lane 3, + 0.5 μM mitoxantrone; Lane 4, + 100 μM azatoxin; Lane 5, + 200 μM azatoxin; Lane 6, + 500 μM VP-16; Lane 7, + 500 μM DMEP; Lane 8, + 500 μM DMDP; Lane 9, DNA without enzyme; Lane 2, purine sequencing reaction. The 2 panels correspond to the same gel that was cut in 2 pieces. Numbers to the right of each panel, genomic positions of cleavage sites.

Fig. 7. Inhibition of Topoisomerase II Catalytic Activity by Azatoxin.
produces a significant amount of linear DNA without significant increase of nicked DNA (Fig. 7, Lane 2).

**Azatoxin Does Not Unwind DNA.** The DNA unwinding assay using excess topoisomerase I and relaxed SV40 DNA was used to assess azatoxin intercalation (22). Fig. 8 shows that azatoxin did not induce detectable DNA unwinding even at drug concentrations as high as 1 μM (Fig. 8, Lane 5). This was also the case for the 2 azatoxin isomers, 8 and 10 (Fig. 8, Lanes 3 and 4), and for the demethylepipodophyllotoxins, DMDP and DMEP (Fig. 8, Lanes 1 and 2). In the same experiment, mitoxantrone induced DNA unwinding, indicating that topoisomerase I was still active following the 15-min preincubation with supercoiled DNA in the absence of drug (data not shown). Similar results were obtained with supercoiled DNA. Therefore, lack of unwinding by azatoxin strongly suggests that the drug does not intercalate into DNA.

**DISCUSSION**

The present study shows that azatoxin is a novel and very active top 2 inhibitor that does not intercalate into DNA. Azatoxin induces a large number of DNA double-strand breaks in linear and supercoiled SV40 DNA and in human c-myc DNA. These breaks yield unique cleavage patterns that are different from those of the well established top 2 inhibitors, VP-16 or VM-26, m-AMSA, and mitoxantrone. Azatoxin also inhibits the catalytic activity of purified top 2, and does not unwind relaxed or supercoiled DNA.

Azatoxin is the first top 2 inhibitor that was rationally designed from a proposed model. This model emerged from the superimposition of anthracyclines, acidines, epipodophyllotoxins, ellipticines, and anthracenedione structures, which display the proposed common composite pharmacophore with 3 domains (13). The first and common domain to all known top 2 inhibitors is a quasiplanar polycyclic ring system that may intercalate into DNA or stack into top 2 cleavage sites (25, 26). While azatoxin does not intercalate into DNA, its planar polycyclic ring system may be essential for stacking into top 2 cleavage sites. The second domain is a pendant aromatic ring that may stabilize the drug molecule in the ternary complex by binding to the enzyme and/or the DNA grooves; this domain is represented by the azatoxin pendant ring (Fig. 6, Y ring), which is analogous to that of VP-16 (or VM-26) (Fig. 6). The third and least dominant domain is illustrated by the glycosidic residue appended to the multiring system (anthracyclines and VP-16 or VM-26). It is clear that in the case of demethylepipodophyllotoxins, this domain can accommodate a diversity of structural units, as illustrated by the work of Liu et al. (27) and Chang et al. (28). Furthermore, this domain is not absolutely required for top 2 activity, since the activity of DMEP is comparable to that of VP-16 (Fig. 6) (29). Also, no substituent is present in this hypothetical domain in azatoxin as well as in top 2 poisons such as m-AMSA and ellipticines. Azatoxin’s ability to trap top 2 cleavable complexes is a success of this model. This molecular modeling and synthetic approach may serve as an example for the discovery of other top 2 (and possibly top 1) inhibitors.

Because the azatoxin skeleton is synthetically accessible, a large number of derivatives have been prepared. The present structure-activity relationship is based on the comparison of 3 isomers and 6 derivatives of azatoxin (Fig. 6). The main conclusions are: (a) as predicted from the pharmacophore model, the diastereoisomers of azatoxin (compounds 9 and 10), which have different orientations of the polycyclic ring system and the pendant ring (ring Y), respectively, are inactive; (b) as the polycyclic ring systems are very different between top 2 inhibitors, the loss of activity for compounds 7 and 8 (iso-azatoxin) is interesting because it indicates that very specific interactions may take place at this level (compare azatoxin with its isomer on the indole group, compound 8; Fig. 4, Lane 4); (c) the structure-activity relationships of the pendant ring system are identical for azatoxin and demethylepipodophyllotoxins (Fig. 6, Y substituent) (23, 24, 29, 30). Therefore, a 4'-OH group (Fig. 6, R2) is essential for top 2 inhibition (compound 4 with a 4'-methoxy group is inactive), and methoxy groups in 3' and 5' (Fig. 6, R1 and R3) enhance the activity (compounds 2 and 3); (d) substitution of an AMSA pendant ring (compound 5) instead of that of demethylepipodophyllotoxin abolishes activity despite the fact that AMSA is a potent top 2 inhibitor. Thus, there must be an interdependence between the chemistry of the pendant ring and that of the polycyclic ring system.

**Fig. 7.** Inhibition of topoisomerase II-mediated DNA relaxation by azatoxin. Supercoiled SV40 DNA was reacted with purified top 2 with or without azatoxin for 30 min at 37°C. Reactions were stopped by adding SDS and proteinase K and run in a 1% agarose gel. Lane 1, migration positions of linear (L) and nicked circular (N) DNA; Lane 2, top 2 + azatoxin (200 μM); Lane 3, top 2 without drug; Lane 4, native supercoiled SV40 DNA (S).

**Fig. 8.** DNA unwinding assay in the presence of azatoxin and demethylepipodophyllotoxin derivatives. Native supercoiled SV40 DNA was first relaxed by excess topoisomerase I for 15 min, prior to drug addition. Reactions were carried out for an additional 30 min at 37°C, and were stopped by adding SDS and proteinase K prior to gel electrophoresis in 1% agarose. Lanes 1–4, compounds 8 and 10, DMEP, and DMDP at 1 μM; Lane 5, azatoxin at 1 μM; Lane 6, topoisomerase I alone; Lane 7, native supercoiled SV40 DNA. S, supercoiled DNA; R, relaxed DNA topoisomers.
Despite their similarities, azatoxin and demethylepipodophyllotoxins show some differences. First, by contrast to demethylepipodophyllotoxins, hydroxy substitution at position 11-2 (Fig. 6, R4) abolishes the top 2 activity of azatoxin, while it only decreases that of demethylepipodophyllotoxins (Fig. 6) (29, 30). In addition, an analogue of VP-16 that possesses a nitrogen instead of a carbon in position 4 (4-aza VP-16), although closely related in structure to azatoxin with 3 of the 5 rings identical, is inactive (31). Also, the cleavage patterns induced by different demethylepipodophyllotoxins are closely related but different from those of azatoxin. Thus, in spite of spatial homology (Fig. 1), these 2 nonintercalating topo 2 inhibitors probably interact with top 2-DNA complexes differently. Azatoxin induces the largest number of top 2 cleavage sites among top 2 inhibitors both in SV40 and c-myc DNA. Preliminary results indicate that azatoxin is also very active at inducing protein-linked DNA breaks in cells. Therefore, azatoxin may be useful for mapping top 2 sites in chromatin. Finally, investigation of the antitumor activity of azatoxin may provide information on the clinical value of extensive cleavage sites versus specific sites.

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REFERENCES

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Arizona Cancer Center Fifth International Workshop on Chromosomes in Solid Tumors, January 10–12, 1993, Doubletree Inn, Tucson, AZ. Contact: Nancy Rzewuski, Arizona Cancer Center, 1515 North Campbell Avenue, Tucson, AZ 85724. Telephone: (602) 626-6044; FAX: (602) 626-2284.

Basal and Squamous Cell Head and Neck Skin Cancer—Partners in Excellence Series, February 6, 1993, M. D. Anderson Cancer Center, Houston, TX. Contact: Carol Harreld, Conference Services, Box 131, 1515 Holcombe Boulevard, Houston, TX 77030-4095. Telephone: (713) 792-2222; FAX: (713) 794-1724.

Seventh International Conference on the Adjuvant Therapy of Cancer, March 10–13, 1993, Tucson Convention Center, Tucson, AZ. Abstract deadline: December 1, 1992. Contact: Nancy Rzewuski, Arizona Cancer Center, University of Arizona College of Medicine, 1515 N. Campbell Avenue, Room 2933, Tucson, AZ 85724. Telephone: (602) 626-2276; FAX: (602) 626-2284.

Women's Health and Breast Cancer—A Continuing Education Course, March 13, 1993, University of California, Davis, Medical Center, Sacramento, CA. Credits: 6 hours Category 1 AMA/CMA. Contact: Office of Continuing Medical Education, University of California, Davis, Medical Center, 2701 Stockton Boulevard, Sacramento, CA 95817. Telephone: (916) 734-5390; FAX: (916) 736-0188.

Second Consensus Development Conference on the Treatment of Radiation Injuries: Marrow Aplasia, Sepsis, Gastrointestinal Injury, and Radiation Accidents, April 14–17, 1993, Bethesda, MD. Contact: Dr. Doris Browne, Armed Forces Radiobiology Research Institute/MRA, 8901 Wisconsin Avenue, Bethesda, MD 20889-5603. Telephone: (301) 295-0316; FAX: (301) 295-5673.

Critical Issues in Tumor Microcirculation, Angiogenesis, and Metastasis: Biological Significance and Clinical Relevance—A Continuing Education Course, June 7–11, 1993, Harvard Medical School, Boston, MA. Credits: 22 hours Category 1 AMA. Contact: Dr. Norman Shostak, Department of Continuing Education, Harvard Medical School, 641 Huntington Avenue, Boston, MA 02115. Telephone: (617) 432-1525; FAX: (617) 432-1562.


Errata

Two errors have been found in the article by Toyokuni et al., entitled “Combined Histochemical and Biochemical Analysis of Sex Hormone Dependence of Ferric Nitrilotriacetate-induced Renal Lipid Peroxidation in ddY Mice,” which appeared in the September 1, 1990 issue of Cancer Research (pp. 5574–5580). In Table 1, the column labeled “Estriol” under the “Female” heading should have been labeled “Testosterone.” In addition, in the legend to Fig. 3, the word “patchy” should be omitted from the expression “diffuse patchy Schiff-positive areas.”

Coauthor Timothy J. Cuzi's name was misspelled as Timothy J. Cuzi in the article by Leteurtre et al., titled “Rational Design and Molecular Effects of a New Topoisomerase II Inhibitor, Azatoxin,” which appeared in the August 15, 1992 issue of Cancer Research (pp. 4478–4483).
Rational Design and Molecular Effects of a New Topoisomerase II Inhibitor, Azatoxin

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