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VI.4 Identification of Tamoxifen-DNA Adducts

E.A. Martin,[1] I.N.H. White,[1] K.W. Turteltau[2] and L.L. Smith[1]

[1]MRC Toxicology Unit, Leicester, U.K.; and [2]Lawrence Livermore National Laboratory, University of California, California, U.S.A.

We have developed a high performance liquid chromatography (HPLC) technique which separates tamoxifen-DNA adducts in rat liver into 12 peaks. Our results suggest that α-hydroxylation is the major route of activation in rat liver, whereas 4-hydroxylation represents a minor pathway. In humans, adducts are absent or at low levels making detection a problem. Using HPLC, no tamoxifen adducts have been detected in human uterus and to improve sensitivity we are using [^{14}C]tamoxifen and accelerator mass spectrometry to detect adducts. Before surgery, patients take a capsule containing [^{14}C]tamoxifen and the DNA in excised tissues is analysed for [^{14}C], to assess the irreversible binding. © **1998 Elsevier Science Ltd. All rights reserved.**

IN TAMOXIFEN-treated rats, large numbers of different adducts are formed in the liver as detected by ^{32}P-postlabelling followed by thin layer chromatography (TLC). One major radioactive spot is seen, which is composed of several individual products, together with a few minor components [1]. Because of this, identification and characterisation of adducts is complex. We have developed a novel HPLC system which gives improved resolution of tamoxifen DNA adducts and gives important information on the origin of adducts. Hepatic DNA from humans given tamoxifen therapeutically failed to show ^{32}P-postlabelled adducts using TLC [2]. If tamoxifen forms DNA adducts in human tissues, they are produced at low levels. To improve sensitivity we have developed the highly sensitive technique of accelerator mass spectrometry (AMS).

The HPLC procedure includes an initial solid phase extraction (SPE) step which removes excess radioactivity due to ^{32}P-ATP (> 85%). SPE uniformly reduces the size of the peaks by approximately 14%, allowing its use except when adduct levels are very low. The limit of detection is one adduct per 10^9 normal nucleotides (compared with 0.5 per 10^{10} for TLC). However, HPLC can separate ^{32}P-labelled tamoxifen treated rat liver DNA into at least 12 peaks, including two major peaks jointly accounting for 70% of total adducts formed *in vivo*. By TLC, most of the peaks elute with the major ^{32}P-labelled product observed *in vivo*, confirming that this spot is composed of a number of different co-eluting adducts.

The pattern and retention time of ^{32}P-postlabelled adducts in α-hydroxytamoxifen-treated rat livers was similar to the spectrum of DNA adducts resolved after tamoxifen itself. When α-acetoxytamoxifen is reacted with DNA *in vitro*, only adducts directly attributed to α-hydroxylation are expected. Separation of these adducts revealed six peaks, including the two major peaks seen in rats. *In vitro*, tamoxifen produces three guanosine and one adenosine adduct [3]. In our HPLC system the geometric or stereoisomers of the deoxyguanosine adducts are not resolved, but elute together in one of the major peaks. ^{32}P-Postlabelling and HPLC analysis of DNA reacted with enzymatically activated 4-hydroxytamoxifen *in vitro*, gives a more polar adduct peak seen at trace levels in rat liver. Results suggest that the majority of adducts formed in rat liver by tamoxifen are produced via the α-hydroxylation pathway. Activation via 4-hydroxytamoxifen represents a minor route, while the role of 3,4-epoxides has not been defined. In rats given tamoxifen or α-hydroxytamoxifen or in humans given tamoxifen therapeutically, no ^{32}P-postlabelled adducts have been detected in uterine DNA either by TLC [4] or HPLC, to date. To increase the detection limits for tamoxifen-DNA adducts in humans we have utilised the highly sensitive technique of AMS for the detection of [^{14}C]isotope [5]. This gives specific information in that it shows covalent interaction of [^{14}C]molecules with DNA. AMS is at least an order of magnitude more sensitive than ^{32}P-postlabelling. Using AMS we have shown that [^{14}C]tamoxifen binds to rat liver DNA following administration at low levels equivalent to the human therapeutic dose. We have shown irreversible binding of [^{14}C]tamoxifen to DNA in extrahepatic organs including the uterus at levels below detection using ^{32}P-postlabelling. Currently a human study is underway investigating whether tamoxifen forms adducts in human tissues. Prior to surgery, female volunteers take a [^{14}C]tamoxifen capsule (20 mg, 0.37 MBq).

Correspondence to E.A. Martin.

Breast, uterine or colon tissue is removed, and DNA extracted analysed for [^{14}C]. Initial data indicate that although tamoxifen is reaching the uterine tissue, no DNA adducts can be detected. A further study with an increased specific activity is being undertaken.

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VI.5 Tamoxifen-DNA Adducts in Breast Cancer Patients

K. Hemminki and H. Rajaniemi

Center for Nutrition and Toxicology, Karolinska Institute, Huddinge, Sweden

TAMOXIFEN IS an important anticancer agent used in a long-term adjuvant therapy of breast cancer. A side-effect of treatment is the risk of secondary cancer in uterine endometrium. An estimate of 10% (100 patients/year) of endometrial cancer is diagnosed in Sweden in patients who have received tamoxifen earlier in their life. We have recently developed a ^{32}P-postlabelling method, applying high-performance liquid chromatography (HPLC) and radioactivity detection for a sensitive and reproducible measurement of tamoxifen adducts in humans [1]. Using the method we demonstrated DNA adducts of tamoxifen in total white blood cell and endometrial cell DNA in blinded studies [2, 3]. The measured levels of adducts were 5/10^9 nucleotides in white blood cells and one-half in endometrial DNA. There have been further methods development and further analysis from other human and animal tissues. Additionally, attempts have been made to identify specific adducts with the help of standard compounds.

Correspondence to K. Hemminki.

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VI.6 Detection of DNA Adducts in the Human Endometrium: a Lack of Evidence

P.L. Carmichael,[1] S. Sardar,[1] P. Neven,[2] I. Van Hoof,[2] A. Ugwumadu,[3] T. Bourne,[3] E. Tomás,[4] P. Hellberg,[5] A.J. Hewer[6] and D.H. Phillips[6]

[1]Imperial College School of Medicine at St Mary's, Division of Biomedical Sciences, Molecular Toxicology, London, U.K.; [2]Kliniek St. Jan, Brussels, Belgium; [3]St George's Hospital, Tooting, SW17, U.K.; [4]Oulu University Hospital, 90220 Oulu, Finland; [5]Sahlgrenska Hospital, S-41345 Gothenburg, Sweden; and [6]The Institute of Cancer Research, Haddow Laboratories, Surrey, U.K.

Correspondence to P.L. Carmichael.