Biochemical Pharmacology, Vol. 27, pp.145-147.
© Pergamon Press Ltd. 1979. Printed in Great Britain.

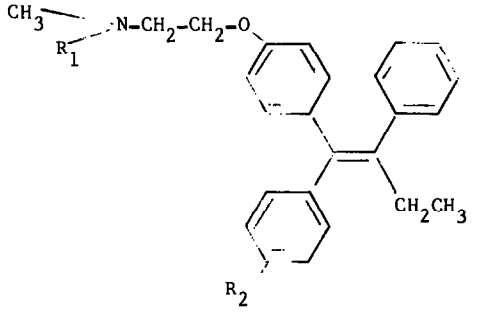
The Metabolism of Tamoxifen in Humans

H.K. Adam, E.J. Douglas and J.V. Kemp.

Safety of Medicines Dept.,
Imperial Chemical Industries Ltd.,
Pharmaceuticals Division,
Alderley Park, Macclesfield.
Cheshire.

(*Received* 13 *September* 1978; *accepted* 6 *October* 1978)

Recent work in our laboratories has been directed towards developing an assay procedure for the antioestrogenic agent tamoxifen (I) [Nolvadex*] which is being used in the treatment of breast cancer. The method is based on solvent extraction of the drug from serum at pH7 followed by separation from background materials on thin-layers. Irradiation of the tlc plate, after development, with U.V. light converts compounds containing the triphenyl ethylene skeleton to highly fluorescent phenanthrenes which can be quantified by densitometry in situ.[1] During the development of the assay procedure it was established that none of the metabolites previously identified by Fromson [2,3] interfered with quantification of the parent drug.



I $R_1 = CH_3$ $R_2 = H$

II $R_1 = CH_3$ $R_2 = OH$

III $R_1 = H$ $R_2 = H$

In his studies with ^{14}C tamoxifen (20mg single dose) Fromson claimed that human serum contained unchanged drug (I), 4-hydroxy tamoxifen (II) and very polar material. In our work we have confirmed the presence of parent compound and a metabolite. However this latter compound did not appear to correspond to the hydroxy compound(II.)

*Nolvadex is a trademark, property of Imperial Chemical Industries Ltd.,

Confirmation that the new metabolite and(II)were different compounds was provided as follows:

a) When a serum sample from a subject receiving tamoxifen was spiked with authentic(II,) extracted and chromatographed, two distinct spots were visible in the metabolite region (Rf 0.36 and 0.32 for the unknown and(II) respectively).

b) Parallel experiments were carried out using serum from subjects receiving tamoxifen and control serum spiked with(II.) When both sera were made alkaline by addition of 1ml of 4M NaOH before extraction, tlc evaluation revealed the presence of the unknown in the extract from the patients serum but no(II)was recovered from the control.

c) Serum from a subject receiving tamoxifen and a control serum spiked with (II) were extracted at pH7. Treatment of these extracts with ethereal diazomethane before tlc caused a shift in Rf of(II)but not of the unknown metabolite.

The major route of metabolism of the tricyclic antidepressants imipramine and amitriptyline in man is N-desmethylation. Because of the similarity in side chain between these compounds and tamoxifen it was postulated that a similar transformation could occur with the latter drug. A sample of desmethyl tamoxifen (III) was synthesised and subjected to the tests detailed above. Its behaviour was identical to the unknown in that it could not be resolved from it on tlc, it was extractable from alkaline serum and showed no changed in Rf after treatment with ethereal diazomethane.

Final proof of the identity of the new metabolite was obtained from gas-chromatography/mass spectrometry. The fragmentation pattern of tamoxifen is dominated by the side chain with the base peak at m/e 58. The pattern for the N-desmethyl derivative is similarly dominated by the side chain but the base peak in this case is at m/e 44. GC/MS examination of a serum extract (pH7) from a subject on tamoxifen confirmed that(III)was present in the serum in appreciable amounts whereas(II)could not be detected.

This work has clearly demonstrated that the major free metabolite of tamoxifen in human serum is N-desmethyl tamoxifen (III) and not, as previously claimed, the 4-hydroxy derivative (II). This finding has been confirmed in serum from healthy subjects and patients after single oral doses and from patients receiving chronic tamoxifen therapy. The present conclusion is based on examination of serum from subjects receiving tamoxifen. They do not, as yet, negate the finding of Fromson of the hydroxy compound in hydrolysed faecal extracts from such subjects[3]. However if (II) is present in human serum it must be at much lower concentrations than previously thought and hence its biological significance in terms of contributing to the antioestrogenic activity of tamoxifen during therapy[4] must now be open to question.

Acknowledgements

The authors wish to thank Dr. D.N. Richardson for N-desmethyl tamoxifen and Mr.J.T.Webster for GC/MS determinations.

References

1. M.A. Gay, H.K. Adam and R.H. Moore to be published.

2. J.M.Fromson, S. Pearson and S. Bramah, Xenobiotica 1973, 3, 693.

3. Idem p.711

4. V.C. Jordan, M.M. Collins, L. Rawsby and G. Prestwich, J. Endocrinol. 1977, 75, 305.