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Short communication

Antibodies to 4-hydroxyandrostenedione – a new anti-breast cancer agent suitable for use in a radioimmunoassay

J. Khubieh, G. W. Aherne, and J. Chakraborty

Division of Clinical Biochemistry, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH UK

Summary. A specific antiserum suitable for radioimmunoassay (RIA) of 4-hydroxy-4-androstene-3,17 dione (4-OHA) has been developed. Sheep antiserum was raised by injecting two different conjugates prepared by coupling 4-OHA to ovalbumin. Antisera obtained from a sheep immunised with 4-hydroxy-testosterone-17-hemisuccinate ovalbumin conjugate were of higher titre and more specific than antisera obtained from sheep immunised with 4-hydroxyandrostenedione-7α-carboxyethylthioether. The antiserum bound 50% of 20 picograms of [6,7-^{3}H]-4-OHA at an initial dilution of 1:270. The most relevant steroids, androstenedione (AD) and testosterone (T) were tested and showed cross reactivity of 2.7% and 5.1% respectively. The lower limit of detection was 4.5 pg/tube. Antisera from this animal will prove useful as the basis of a sensitive and specific RIA for clinical pharmacokinetic studies of 4-OHA.

Introduction

4-Hydroxyandrostenedione (4-OHA) is a potent and selective inhibitor of the enzyme complex aromatase (oestrogen synthetase). It has important applications as a research tool for investigating oestrogen-mediated processes [2, 6], and also great potential clinical value for controlling tumours which are oestrogen-dependent [8]. A phase II study of the drug has been published [4] recently, which confirms the biological activity of 4-OHA in advanced post-menopausal breast cancer. Plasma levels of 4-OHA were measured in this study using a radioimmunoassay (RIA) for androstenedione after chromatographic separation of 4-OHA from androstenedione and utilizing antibodies with a 25% cross-reactivity with 4-OHA. Up to date no reports of the production of specific antisera to 4-OHA have been published. We report here the production of specific antibodies to 4-OHA in sheep.

Materials and methods

4-Hydroxyandrostenedione was kindly supplied by Ciba-Geigy, Basel, Switzerland. Other steroids were purchased from Sigma Chemicals Ltd. Two ovalbumin immunogens were prepared and injected into animals. 4-OH testosterone-17-hemisuccinate (4-OHTHS) was prepared from testosterone-17-hemisuccinate by hydroxylation at C-4 [1] and conjugated to ovalbumin using the mixed anhydride method described by Erlanger et al. [3]. For the second immunogen, androstenedione was converted to 4-hydroxyandrosta-4,6-diene-3,17-dione [7], and then to 4-hydroxyandrostenedione-7α-carboxyethylthioether (4-OHA-7αCET) [5] before conjugating to ovalbumin. Each conjugate was injected into two sheep using a conventional immunisation schedule. [6,7-^{3}H]-4-OHA with specific activity of 15.6 Ci mmol^{-1} was prepared [7] and used, after purification on a silica gel chromatography column, to assess the binding capacity of the antisera. Standard RIA procedures were employed. Tritium-labelled 4-OHA (20 pg) was incubated for 1 h at room temperature with dilutions of antisera. The diluent buffer was 0.05 *M* phosphate saline, pH 7.2, containing 0.1% sodium azide and 0.1% gelatin. Separation of the free and antibody-bound fractions of the tracer was achieved with 2.5% dextran-coated charcoal.

Results

Antisera obtained from a sheep (HP/S/1513) immunised with 4-OHTHS ovalbumin conjugate were of higher titre (1:270) and more specific than those obtained with 4-OHA-7α-CET-ovalbumin. A calibration curve (Fig. 1) was produced using this antiserum and 100-μl aliquots of standard 4-OHA (50 pg to 10 ng/ml). The theoretical sensitivity of the assay was 45 pg/ml, and the within-assay coefficient of variation, 5.1%, at a standard concentration of 200 pg/ml. Initial studies have shown that the antibodies from HP/S/1513 cross-reacted with androstenedione by 2.7% and with testosterone by 5.1%.

Discussion

An antiserum (HP/S/1513) suitable for use in an RIA for the measurement of 4-OHA was obtained 10 weeks after the priming injection of 4-OHTHS-ovalbumin. As might have been predicted [5], its specificity towards closely related steroids was higher than that of antisera produced to 4-OHA-7αCET. The sensitivity of the curve obtained is sufficiently high to allow the measurement of the plasma 4-OHA concentrations expected (0.7–23.2 ng/ml) [4] during clinical use. Further plasma samples resulting from HP/S/1513 obtained following a second immunization are

Offprint requests to: J. Khubieh

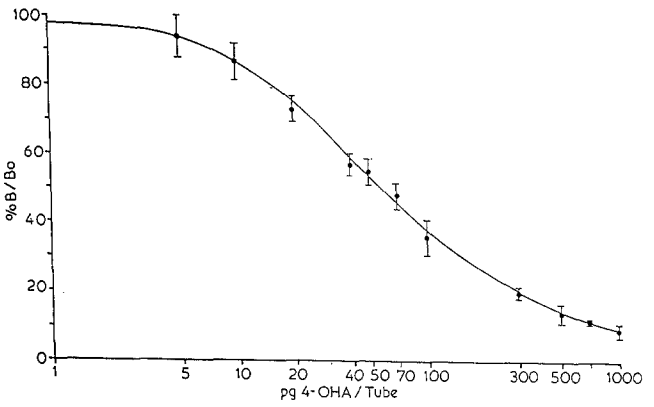

Fig. 1. The mean (±SD) of five separate 4-OHA standard curves; 20 pg ^{3}H-4-OHA/tube; antiserum dilution 1:270; non-specific binding 4%; zero-concentration binding (B_0) 40%

now available for investigation, and work is in progress to characterise these antibodies further with regard to the metabolites of 4-OHA and to validate an RIA suitable for the analysis of clinical samples.

The availability of an assay using antisera described here should facilitate more extensive clinical pharmacokinetic studies designed to optimise the dose and route of administration of 4-OHA and to devise the most effective therapeutic regimen for this drug.

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