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A NON-CHROMATOGRAPHIC RADIOIMMUNOASSAY FOR 3-OXO DESOGESTREL

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Summary—A non-chromatographic radioimmunoassay for 3-oxo desogestrel (13β-ethyl-17-hydroxy-11-methylene-18,19-dinor-pregn-4-en-20-yn-3-one), the biologically-active metabolite of desogestrel (13β-ethyl-11-methylene-18,19-dinor-pregn-4-en-20-yn-17-ol), has been developed to facilitate studies of the pharmacokinetics of this steroid. The method uses an antiserum raised against levonorgestrel (13β-ethyl-17-hydroxy-18,19-dinor-pregn-4-en-20-yn-3-one). None of the steroids tested which showed significant cross-reactions are believed to be present in plasma after ingestion of desogestrel; furthermore, dilutions of standards and unknowns gave parallel responses in the assay. Intra- and inter-assay coefficients of variation were 12.9 and 11.8% respectively. The sensitivity of the assay was approx 0.02 ng/ml. The peak concentrations of 3-oxo desogestrel after a 150 μg dose of desogestrel in three subjects were between 0.48–0.71 ng/ml, and in two subjects 3-oxo desogestrel was still detectable 24 h after dosing.

INTRODUCTION

Desogestrel (13β-ethyl-11-methylene-18,19-dinor-pregn-4-en-20-yn-17-ol) [Fig. 1] is a synthetic progestogen which is used in the oral contraceptive Marvelon® (Organon International B.V., Oss, The Netherlands). The affinity of desogestrel for the progesterone receptor in human myometrium is relatively low, whereas that of its metabolite 3-oxo desogestrel (13β-ethyl-17-hydroxy-11-methylene-18,19-dinor-pregn-4-en-20-yn-3-one; Fig. 1) is considerably greater. It has therefore been proposed that the progestational activity of desogestrel is due to conversion to 3-oxo desogestrel, in a manner analogous to the metabolism of lynestrenol (19-nor-pregn-4-en-20-yn-17-ol) to norethisterone (17-hydroxy-19-pregn-4-en-20-yn-3-one) [1].

Little is known about the pharmacokinetics of 3-oxo desogestrel after administration of desogestrel. Viinikka[2] measured plasma concentrations of 3-oxo desogestrel in a single subject after a 2.5 mg dose of desogestrel, and in four subjects after a 50 μg dose [3]. 3-Oxo desogestrel was measured by radioimmunoassay after chromatographic separation from desogestrel [2]. The use of chromatography, however, reduces the number of samples which can be assayed and thus renders detailed pharmacokinetic studies, in which large numbers of blood samples are required, difficult. In order to carry out a study of the pharmacokinetics of 3-oxo desogestrel, we have therefore developed a non-chromatographic radioimmunoassay for this steroid.

EXPERIMENTAL

Materials

All reagents were purchased from B.D.H. Ltd, Liverpool, U.K., unless otherwise stated.

[16-^{3}H] 3-Oxo desogestrel (25 Ci/mmol) was donated by Organon International B.V. (Oss, The Netherlands), as were unlabelled desogestrel, 3-oxo desogestrel, 3β-hydroxy desogestrel, 3β-hydroxy,5α-dihydro desogestrel, 3α-hydroxy,5α dihydro desogestrel, 3-oxo,5α dihydro desogestrel, levonorgestrel and norethisterone.

The assay buffer was a 0.1 M phosphate buffer (pH 7.0), containing 0.9% (w/v) sodium chloride, 0.1% gelatine and 0.1% merthiolate. Dextran T-40 was obtained from Pharmacia (Uppsala, Sweden), and activated charcoal from Sigma Chemical Co. (Poole, U.K.). A Dextran-coated charcoal suspension was prepared by mixing 0.075 g of Dextran and 0.75 g of charcoal in 200 ml of buffer.

The antiserum was raised in rabbits immunised against levonorgestrel-3-(*O*-carboxymethyl)oxime conjugated to bovine serum albumin [4]. Previous

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The following steroid names are used in this paper: desogestrel, 13β-ethyl-11-methylene-18,19-dinor-pregn-4-en-20-yn-17-ol; 3-oxo desogestrel, 13β-ethyl-17-hydroxy-11-methylene-18,19-dinor-pregn-4-en-20-yn-3-one; 3β-hydroxy desogestrel, 13β-ethyl-11-methylene-18,19-dinor-pregn-4-en-20-yne-3β,17-diol; 3-oxo, 5α dihydro desogestrel, 13β-ethyl-11-methylene-18,19-dinor-5α,-pregn-20-yn-3-one; 3β-hydroxy-5α dihydro desogestrel, 13β-ethyl-11-methylene-18,19-dinor-5α, pregn-20-yne-3β,17-diol; 3α-hydroxy 5α dihydro desogestrel, 13β-ethyl-11-methylene-18,19-dinor-5α,pregn-20-yne-3α,17-diol; lynestrenol, 19-nor-pregn-4-en-20-yn-17-ol; norethisterone, 17-hydroxy-19-nor-pregn-4-en-20-yn-3-one; levonorgestrel, 13β-ethyl-17-hydroxy-18,19-dinor-pregn-4-en-20-yn-3-one; ethynyloestradiol, 17α-ethynyl-1,3,5 (10)-estratriene-3,17β-diol.

Table 1. Cross-reactions of the levonorgestrel antiserum with A-ring metabolites of desogestrel, and with norethisterone and levonorgestrel

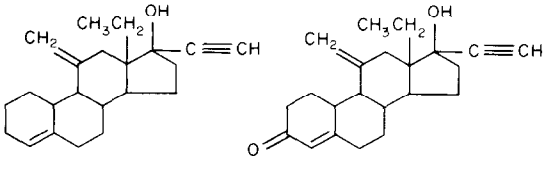
Steroid	Cross-reaction (%)
Desogestrel	0
3-Oxo desogestrel	100
3β-Hydroxy desogestrel	65.9
3-Oxo, 5α dihydro desogestrel	14.1
3α-Hydroxy-5α dihydro desogestrel	17.8
3β-Hydroxy-5α dihydro desogestrel	30.7
Norethisterone	68.8
Levonorgestrel	371.3

experience in our laboratory has shown that this antiserum can be used to measure 3-oxo desogestrel (W. L. Allen and D. J. Back, unpublished observations).

Methods

Radioimmunoassay. Plasma samples (0.2–0.4 ml) were centrifuged for 5 min at 1500 *g* before assay. They were then extracted with Aristar grade diethyl ether (3 ml) by shaking for 1 min on a multi-tube Vortex mixer (S.M.I. Ltd, Emeryville, U.S.A.). After centrifugation for 2 min at 1500 *g* to facilitate separation of the phases, the samples were frozen in a methanol–dry ice mixture, and the ether decanted into clean polypropylene test tubes (L.I.P. Ltd, Shipley, U.K.). The ether was then evaporated under vacuum in a Vortex evaporator (Searle Analytic, supplied by Baird and Tatlock Ltd, Romford, U.K.), and the residues were preincubated for 1 h at 37°C with 0.4 ml of antiserum diluted 1:20000 with assay buffer. Tritiated 3-oxo desogestrel (ca 15,000 dpm/0.1 ml buffer) was then added, and the samples incubated for 2 h at room temperature. The tubes were cooled to 4°C in an ice bath, and 0.5 ml of Dextran-coated charcoal suspension added. After 30 min, they were centrifuged at 4°C for 10 min (1500 *g*), and the supernatants decanted into 4 ml of NE 299 liquid scintillation cocktail (Packard Instruments Ltd, Reading, U.K.). A series of standards, covering the range 0–500 pg, was extracted from 0.4 ml of plasma obtained from adult men (blank plasma), and assayed in parallel with the unknowns.

Human volunteer studies. Plasma concentrations of 3-oxo desogestrel were measured in three adult men, weighing between 57–70 kg, after ingestion of a single Marvelon® tablet (150 μg desogestrel + 30 μg ethynyloestradiol). Blood samples (10 ml) were taken into heparinised tubes from a forearm vein via an indwelling catheter prior to dosing, and at 30 min, 1, 2, 3, 4, 6 and 8 h after dosing. The catheters were removed after 8 h, and further blood samples taken by venepuncture at 10–12 and 24 h. Samples were centrifuged as soon as possible, and the plasma stored at −20°C.



Desogestrel 3-oxo desogestrel

Fig. 1. Structures of desogestrel and 3-oxo desogestrel.

RESULTS

The specificity of the assay was tested by measuring the cross-reactions of the antiserum with various metabolites of desogestrel. The results, measured at 50% inhibition of binding, are listed in Table 1. There was no cross-reaction with desogestrel, and there appears to be no evidence that any of the cross-reacting metabolites tested are present in significant concentrations after administration of desogestrel (H. Hasenack, personal communication). Dilutions of standards and unknowns gave parallel responses in the assay (Fig. 2), which suggests a lack of interference from cross-reacting steroids.

Assessment of the assay's accuracy required indirect evidence. The commonly-used test of measuring the recovery of steroid added to plasma was inappropriate in this case because the standards were themselves extracted from plasma; under these circumstances one would expect quantitative recovery of added steroid. Evidence for the accuracy of the assay comes from the demonstration of parallelism between increasing volumes of standard and unknown (Fig. 2). Such parallelism experiments are frequently used to test for bias [5].

Intra- and inter-assay variations were determined by assaying aliquots of a plasma sample in one batch ($n = 8$), and in a series of batches ($n = 6$). Intra- and inter-assay coefficients of variation were 12.9 and 11.8% respectively, at a sample concentration of approx 0.2 ng/ml.

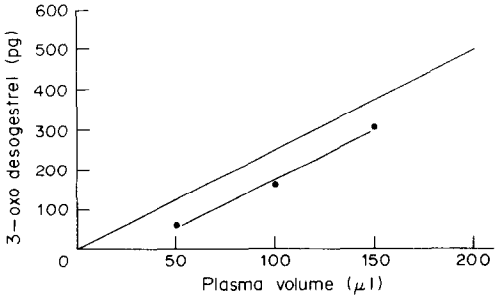


Fig. 2. Parallelism between standard and unknown in the radioimmunoassay for 3-oxo desogestrel. The line passing through the origin represents the 3-oxo desogestrel content of various volumes of standard solution (2.5 ng/ml plasma). The line defined by the closed circles represents the 3-oxo desogestrel content of different volumes of an unknown sample. In each case, the volume was made up to 200 μl with plasma which did not contain 3-oxo desogestrel.

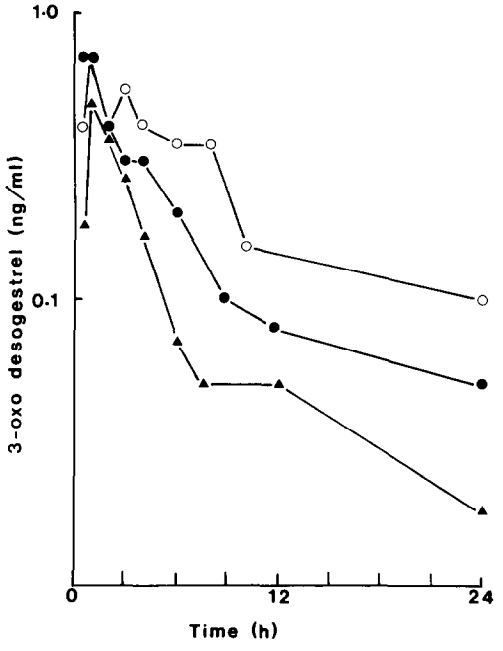


Fig. 3. Plasma concentrations of 3-oxo desogestrel in three subjects at various times after ingestion of a single Marvelon® tablet.

The sensitivity of the assay was expressed as the lower 95% confidence limit for determination of a blank sample [6]. This was approx 0.02 ng/ml.

Plasma concentrations of 3-oxo desogestrel after oral administration of desogestrel are shown in Fig. 3. Peak concentrations of 0.48–0.71 ng/ml were observed 1 h after dosing, and concentrations then decreased over 12–24 h. The half-lives of elimination for the first 12 h in the three subjects were between 3.1–5.3 h. In two of the three subjects, the concentration remaining after 24 h was greater than the sensitivity of the assay.

DISCUSSION

An unusual feature of this assay is the use of an antiserum raised against levonorgestrel. We have previously found (W. L. Allen and D. J. Back, unpublished observations) that plasma from women who were taking oral contraceptives which contained desogestrel showed measurable binding in an assay for levonorgestrel. In the assay reported here, the antiserum showed significant cross-reactions with a number of A-ring metabolites of desogestrel. None of the metabolites tested, however, appear to be present in significant concentrations. There was no cross-reaction with desogestrel itself. These findings suggest that a chromatographic step is unnecessary. Further evidence for the specificity of the assay comes from the demonstration of parallelism between standard and unknown, although this does not preclude interference from some factor whose affinity for the antiserum is similar to that of 3-oxo desogestrel [5].

The precision of the assay is within acceptable limits, and the sensitivity (0.02 ng/ml) is such that it is possible to measure 3-oxo desogestrel for up to 24 h after a 150 μg dose of desogestrel.

The peak concentrations of 3-oxo desogestrel after ingestion of a 150 μg dose of desogestrel are comparable to data reported by others. Viinikka[2] reported a peak concentration of 12.7 ng/ml after a 2.5 mg dose of desogestrel, and Viinikka *et al.*[3] reported peak concentrations of approx 0.2–0.4 ng/ml after a 50 μg dose. In both of these studies, 3-oxo desogestrel was measured by radioimmunoassay after Celite column chromatography. This apparent agreement between a chromatographic and a non-chromatographic assay provides further evidence for the reliability of the assay.

The concentrations of 3-oxo desogestrel reported here are an order of magnitude lower than the levonorgestrel concentrations measured after an equivalent dose [4]. They are also considerably lower than the norethisterone concentrations measured after a 1 mg dose [7]. Further studies are needed of the pharmacokinetics of 3-oxo desogestrel; the assay described in this paper appears to be suitable for such studies.

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