Disposition of Norgestimate in the Presence and Absence of Ethinyl Estradiol after Oral Administration to Humans

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Abstract □ The disposition of radioactivity following oral administration of ^{14}C-norgestimate was compared to that following administration of the drug in combination with ^{3}H-ethinyl estradiol in humans. Seven normal, healthy female subjects were each administered one capsule orally containing ^{14}C-norgestimate either alone (74.4 μCi, 0.50 mg) or in combination (73.5 μCi, 0.49 mg) with ^{3}H-ethinyl estradiol (103 μCi, 0.14 mg) in polyethylene glycol 400. Peak levels of radioactivity due to carbon-14 and tritium in plasma occurred within 2 hr after drug administration, followed by distribution and elimination phases. The mean apparent elimination half-life and mean cumulative elimination of radioactivity in the urine and feces following ^{14}C-norgestimate administration were not significantly different than those following administration of the combination dose. Approximately 50% of the administered radioactivity due to carbon-14 was excreted in the urine following administration of ^{14}C-norgestimate both in the presence and absence of coadministered ^{3}H-ethinyl estradiol.

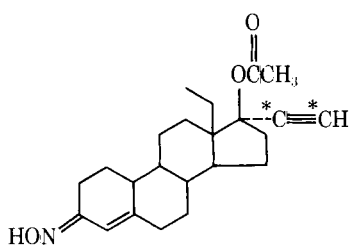
Keyphrases □ Norgestimate—disposition after oral administration alone and combined with ethinyl estradiol in humans □ Disposition—norgestimate after oral administration alone and combined with ethinyl estradiol in humans □ Progestins—norgestimate, disposition after oral administration alone and combined with ethinyl estradiol in humans

Norgestimate[1], *d*-17β-acetoxy-13β-ethyl-17α-ethynyl-4-gonene-3-one oxime (I), is a lipophilic steroid with progestational and antifertility activity in a number of laboratory animal species (1, 2). It is currently being studied in humans as a low dose oral contraceptive in combination with ethinyl estradiol (3–5). Drug disposition studies in rats, dogs, and monkeys were conducted previously (6), and the metabolism of ^{14}C-norgestimate in humans was briefly described (7).

The present study determined the absorption characteristics of ^{14}C-norgestimate in humans following oral administration of the compound alone or in combination with ^{3}H-ethinyl estradiol. Preliminary information on the pharmacokinetics and disposition of the progestin was also obtained.

EXPERIMENTAL

Materials—^{14}C-Norgestimate (specific activity ~150 μCi/mg) was synthesized[2], and its radiochemical purity was determined by TLC to be greater than 95%. ^{3}H-Ethinyl estradiol[3] was mixed with unlabeled ethinyl estradiol and repurified. The resulting compound (specific activity ~714 μCi/mg) had a radiochemical purity of >95%.



I: * = position of ^{14}C-label

[1] Ortho Pharmaceutical Corp., Raritan, N.J.
[2] Roussel UCLAF, Paris, France.
[3] New England Nuclear Corp., Boston, Mass.

Table I—Subjects Enrolled

Dosage Form Administered	Subject	Age, years	Height, cm	Weight, kg
^{14}C-Norgestimate	PP	35	163	67.3
	KC	33	160	75.9
	MS	40	163	59.5
	JG	40	159	67.7
^{14}C-Norgestimate and ^{3}H-ethinyl estradiol	KB	29	163	55.0
	LS	37	163	56.8
	KK	35	164	57.0

Samples of whole blood, plasma, and fecal homogenate were combusted in a tissue oxidizer[4], and the radioactive content[5] was determined by liquid scintillation spectrometry[6]. Internal standardization was used to correct for quenching. The efficiency of the tissue oxidizer was determined by combusting known amounts of radioactivity.

A radiochromatogram scanner[7] was used to determine the radiochemical purity of the isotope. Blenders[8] were employed to homogenize feces.

Dosage Forms—The dosage forms were prepared in the following manner. Polyethylene glycol 400 (1.5 ml) was added to a solution containing 3.0 mg of ^{14}C-norgestimate in 2.0 ml of alcohol USP. For coadministration of ^{3}H-ethinyl estradiol, polyethylene glycol 400 (1.5 ml) was added to a solution containing 3.0 mg of ^{14}C-norgestimate and 0.841 mg of ^{3}H-ethinyl estradiol in 2.0 ml of alcohol USP. The alcohol was then evaporated by heating the mixtures at 60° with constant stirring for 3 hr.

A 0.25-ml aliquot of polyethylene glycol 400 containing either 0.5 mg of ^{14}C-norgestimate or 0.5 mg of ^{14}C-norgestimate and 0.14 mg of ^{3}H-

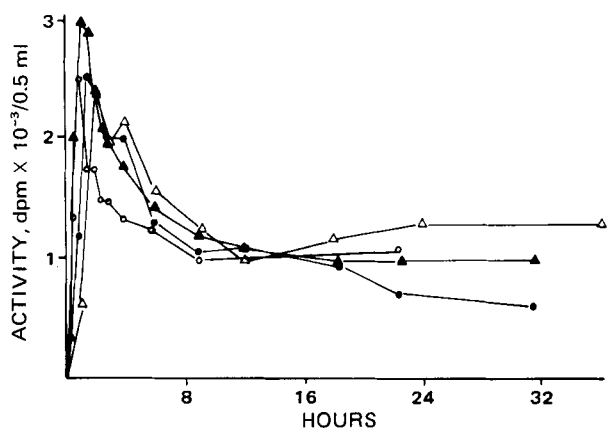


Figure 1—*Radioactivity in plasma following oral administration of ^{14}C-norgestimate (74.4 μCi, 0.50 mg) in polyethylene glycol 400 (0.25 ml) in soft gelatin capsules to Subjects PP (△), MS (▲), JG (●), and KC (○).*

[4] Model 306, Packard Instruments, Downers Grove, Ill.
[5] Carbon-14 content dissolved in Permafluor V and Carbo-Sorb; tritium content dissolved in Monophase-40. These scintillation cocktails were obtained from Packard Instruments, Downers Grove, Ill.
[6] Model SL-30, Intertechnique Inc., Westwood, N.J.
[7] Model 7200, Packard Instruments, Downers Grove, Ill.
[8] Waring Products Corp., Winsted, Conn.

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Table II—Apparent Half-Life of Radioactivity Due to Carbon-14 in Plasma and Elimination Rate Constant following Oral Administration of ^{14}C-Norgestimate

^{14}C-Norgestimate Alone[a]			^{14}C-Norgestimate and ^{3}H-Ethinyl Estradiol[b]		
Subject	Half-Life of Radioactivity Due to Carbon-14, hr	Elimination Rate Constant, hr^{-1}	Subject	Half-Life of Radioactivity Due to Carbon-14, hr	Elimination Rate Constant, hr^{-1}
PP	45	0.015	KB	49	0.014
MS	58	0.012	LS	56	0.012
JG	71	0.010	KK	59	0.012
KC	64	0.011			
Mean ± *SE*	59.5 ± 5.5	0.012 ± 0.001	Mean ± *SE*	54.7 ± 3.0	0.013 ± 0.001
Range	45 – 71	0.010–0.015	Range	44 – 59	0.012–0.014

[a] ^{14}C-Norgestimate (74.4 μCi, 0.50 mg) in polyethylene glycol 400 (0.25 ml) in soft gelatin capsule. [b] ^{14}C-Norgestimate (73.5 μCi, 0.49 mg) in combination with ^{3}H-ethinyl estradiol (103 μCi, 0.14 mg) in polyethylene glycol 400 (0.25 ml) in soft gelatin capsule.

ethinyl estradiol was injected into each preweighed 6-minum soft gelatin capsule. The capsules were then reweighed to confirm the quantity of added solution and were sealed by means of an aqueous gelatin solution.

One representative capsule of each dosage form was assayed for radiochemical purity, radioactive content due to carbon-14 (and tritium), and drug content immediately prior to initiating the clinical trials. The radiochemical purity was greater than 95%, and the capsules contained 73.5 μCi (0.49 mg) of ^{14}C-norgestimate and 103 μCi (0.14 mg) of ^{3}H-ethinyl estradiol in the combination and 74.4 μCi (0.50 mg) of ^{14}C-norgestimate in the norgestimate alone capsule.

Subjects—Seven healthy female subjects (Table I) reported that they had not taken any steroid for at least 3 months prior to the study and had taken no other medication for at least 2 weeks prior to the study. Assay of radioactivity in whole blood, plasma, urine, and fecal samples from the subjects, obtained 1–3 days prior to initiation of the study, indicated the presence of only background levels of radioactivity.

Methods—After a fast of at least 8 hr, the subjects each ingested one norgestimate capsule or a capsule containing norgestimate and ethinyl estradiol followed by 240 ml of water. Water was permitted *ad libitum*, and a normal diet was permitted commencing 4 hr after drug administration.

Venous blood was collected into heparinized tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 16, 18, 24, 36, 48, 72, 96, and 120 hr and at 7 and 14 days. Single urine and fecal samples were collected on the day prior to drug administration, and all urine and feces were collected for 2 weeks thereafter. Urine and feces were frozen immediately after collection.

Two 0.5-ml aliquots of whole blood from each blood sample were placed into tissue sample cups[9] immediately after each collection. The aliquots were subsequently combusted and assayed for radioactive content. Plasma was separated from the remainder of each whole blood sample, and both the plasma and red cell pellets were frozen. Two 0.5-ml aliquots of plasma were subsequently combusted and assayed for radioactive content.

Estimation of the half-life of total radioactivity and its corresponding elimination rate constant was made from a linear regression analysis of the terminal exponential phase of a plot of the logarithm of radioactivity in plasma *versus* time.

Each fecal specimen was diluted to a constant volume (500 ml) with methanol–water (1:1) and homogenized, and aliquots (0.5 ml) were removed and assayed for radioactive content.

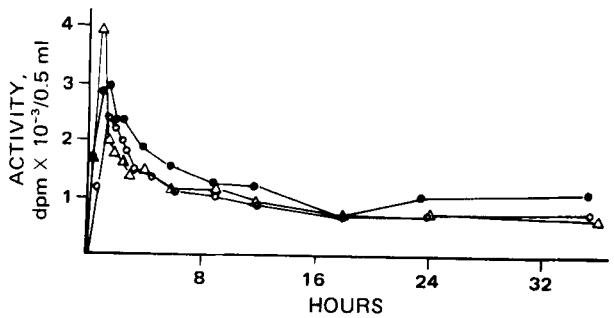

Figure 2—*Radioactivity in plasma following oral administration of ^{14}C-norgestimate (73.5 μCi, 0.49 mg) and ^{3}H-ethinyl estradiol (103 μCi, 0.14 mg) in polyethylene glycol 400 (0.25 ml) in soft gelatin capsules to Subjects KB (●), LS (△), and KK (○).*

[9] Combustocones, Packard Instruments, Downers Grove, Ill.

RESULTS

Absorption of radioactivity after oral administration of ^{14}C-norgestimate alone or in combination with ethinyl estradiol in all seven subjects was rapid. Peak levels of total radioactivity in plasma occurred within 2 hr, followed by distinct distribution and elimination phases (Figs. 1 and 2). The half-life of radioactivity in plasma due to carbon-14 was 45–71 hr (mean ± *SE* = 59.5 ± 5.5 hr) following administration of ^{14}C-norgestimate alone and 49–59 hr (mean ± *SE* = 54.7 ± 3.0 hr) after administration of the drug in combination with ^{3}H-ethinyl estradiol (Table II). The mean elimination rate constants were 0.012 and 0.013 hr^{-1}, respectively (Table II). The apparent half-life of radioactivity in plasma due to tritium was 45–84 hr (mean ± *SE* = 60.4 ± 12.1 hr), and the mean elimination rate constant for tritium was 0.012 hr^{-1}.

Cumulative elimination of radioactivity due to carbon-14 in the urine and feces is summarized in Tables III and IV. Following oral administration of ^{14}C-norgestimate, approximately 64–94% (mean ± *SE* = 83.5 ± 7.0%) of the administered radioactivity due to carbon-14 was eliminated within the 2 week-collection period. About 45–49% (mean ± *SE* = 46.8 ± 1.0%) of the radioactivity was excreted in the urine, while 16–49% (mean ± *SE* = 36.8 ± 7.3%) was recovered in the feces.

Following oral administration of ^{14}C-norgestimate in combination with ^{3}H-ethinyl estradiol, approximately 82–89% (mean ± *SE* = 85.6 ± 2.1%) of the administered radioactivity due to carbon-14 was eliminated during 2 weeks. About 35–49% (mean ± *SE* = 43.6 ± 4.4%) was excreted in the urine while 39–47% (mean ± *SE* = 41.9 ± 2.5%) was recovered in the feces. Approximately 64–90% (mean ± *SE* = 77.7 ± 7.4%) of the administered radioactivity due to tritium was eliminated within the collection period. Approximately 30–50% (mean ± *SE* = 43.5 ± 6.5%) of the radioactivity due to tritium was excreted in the urine, while 14–49% (mean ± *SE* = 34.2 ± 10.3%) was found in the feces.

DISCUSSION

The disposition of radioactivity following oral administration of ^{14}C-norgestimate was compared to that following administration of the drug in combination with ^{3}H-ethinyl estradiol in normal healthy adult female subjects. The mean apparent elimination half-life and mean cumulative elimination of radioactivity in the urine and feces following ^{14}C-norgestimate administration were not significantly different than those following the combination dose. These data are in general agreement with findings following oral administration of other radiolabeled progestins to human subjects (8, 9).

Table III—Cumulative Elimination of Radioactivity Due to Carbon-14 in Urine and Feces following Oral Administration of ^{14}C-Norgestimate[a]

Cumulative Percent Dose Eliminated	Subject PP	MS	JG	KC	Mean ± *SE*
During first 3 days	44	83	70	40	59.5 ± 10.5
During first 7 days	78	94	89	60	80.3 ± 7.5
During first 14 days	83	94	93	64	83.5 ± 7.0
In urine	45	45	49	48	46.8 ± 1.0
In feces	38	49	44	16	36.8 ± 7.3

[a] ^{14}C-Norgestimate (74.4 μCi, 0.50 mg) in polyethylene glycol (0.25 ml) in soft gelatin capsule.

Table IV—Cumulative Elimination of Radioactivity Due to Carbon-14 in Urine and Feces following Oral Administration of ^{14}C-Norgestimate in Combination with ^{3}H-Ethinyl Estradiol [a]

Cumulative Percent Dose Eliminated	Subject KB	LS	KK	Mean ± *SE*
During first 3 days	40.4	50.6	50.4	47.1 ± 3.4
During first 7 days	82.8	78.7	79.3	80.3 ± 1.3
During first 14 days	85.3	81.8	89.4	85.6 ± 2.1
In urine	46.4	35.0	49.4	43.6 ± 4.4
In feces	38.9	46.8	40.0	41.9 ± 2.5

[a] ^{14}C-Norgestimate (73.5 μCi, 0.49 mg) in combination with ^{3}H-ethinyl estradiol (103 μCi, 0.14 mg) in polyethylene glycol 400 (0.25 ml) in soft gelatin capsule.

Cumulative elimination of radioactivity in the seven subjects indicates that approximately half of the dose was excreted in the urine during the 14-day collection period, suggesting that a substantial fraction of the administrered radioactivity was absorbed. It cannot be assumed that the radioactivity recovered in the feces necessarily represents unabsorbed drug since studies with intravenously administered ^{14}C-norgestimate in dogs and rats demonstrated substantial biliary secretion of norgestimate and/or its metabolites (6). Estimates of the apparent half-life of radioactivity due to carbon-14 in the present study were consistent with those reported previously for dogs, rats, and monkeys (6).

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Molecular Connectivity Study of Muscarinic Receptor Affinity of Acetylcholine Antagonists

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Abstract □ A correlation between three molecular connectivity indexes and the muscarinic receptor affinity of 104 acetylcholine antagonists was found. Analysis of structure from these indexes reveals not only the importance of the onium and the bulky portions of the molecule but also their virtual independence of each other on the affinity. Analysis of the onium group portion of the molecules indicates that its contribution to the experimental affinity is virtually constant through a variety of structural variations. The influence of the bulky side chains, in contrast, is quite structure dependent. The equation relating connectivity indexes to muscarinic affinity of antagonists is capable of predicting the affinity of other antagonists as well as a number of agonist molecules.

Keyphrases □ Molecular connectivity indexes—various acetylcholine antagonists, correlated to muscarinic receptor affinity □ Muscarinic receptor affinity—various acetylcholine antagonists, correlated to molecular connectivity indexes □ Acetylcholine antagonists, various—molecular connectivity indexes correlated to muscarinic receptor affinity □ Topological indexes—molecular connectivity correlated to muscarinic receptor affinity of various acetylcholine antagonists □ Structure–activity relationships—molecular connectivity indexes of various acetylcholine antagonists correlated to muscarinic receptor affinity

Since the early observation that atropine is a competitive antagonist of acetylcholine muscarinic action, many molecules have been synthesized in efforts to develop clinically useful drugs. The most potent agents possess an onium group (usually quaternary) and a relatively bulky moiety bridged to the nitrogen by a three- to five-carbon chain. This bulky moiety is presumed to increase the affinity of the molecule by interacting through van der Waals forces beyond the region of the muscarinic receptor (1). Some similarities between antagonists and acetylcholine structure were summarized (2).

BACKGROUND

Several studies were directed toward a definition of the structural similarities and differences between muscarinic agonists and antagonists. Burgen (3) suggested that the onium group plays a major role in drug–receptor interaction among agonists while the bulky side-chain moiety is the salient feature for activity among antagonists. Increased drug–receptor interaction at the bulky side chain presumably weakens the interaction at the onium group, giving rise to a diminished agonist but predominant antagonist effect.

Barlow *et al.* (4) tested agonist and antagonist molecules and concluded that changes in the structure of the onium group produce similar effects on affinity for both series. In contrast to Burgen's (3) proposal, this view advocates a comparable role for the onium group among agonists and antagonists.

A subsequent study (5) on more than 100 acetylcholine analogs revealed that changes in the molecule affinity as the onium group was modified were not entirely independent of the bulky side chain. However, the interdependence of onium and the bulky side chain was not marked. Other factors could be operating besides binding influences, including realignment at the receptor due to onium group changes, leading to altered affinity.

Goldstein *et al.* (6) stated that competitive antagonism could result from an interaction at a different receptor near the acetylcholine receptor (7). The antagonist–receptor interaction would modify the agonist re-

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