Research Article

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Advanced Techniques in Clinical Microbiology

2017
Vol.1 No.1:7

The Isomers of Clomiphene Citrate have Dissimilar Dispositions Once Ingested: Results of a Mouse ADME Study

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Received date: January 17, 2017; **Accepted date:** January 18, 2017; **Published date:** January 25, 2017

Citation: Fontenot GK, Wiehle RD, Hsu K, et al. The Isomers of Clomiphene Citrate have Dissimilar Dispositions Once Ingested: Results of a Mouse ADME Study. Adv Tech Clin Microbiol. 2017, 1:1.

Abstract

Background: Clomiphene Citrate is composed of two structural isomers: cis- or Zuclomiphene citrate (Zu) and trans- or Enclomiphene citrate (En). Clomiphene, as well as isolate isomers, has recently been shown to have effects on Ebola virus infections. It has been shown that the enclomiphene molecule is an estrogen antagonist and that zuclomiphene is inactive. We demonstrate here that the two isomers have different fates once ingested and the tissues that absorb each are distinct from each other to lead to different biologic effects.

Methods and findings: We studied these molecules by employing 14C-labelled versions of each in C-57 black mice. Mice were given the same oral dose, but sacrificed at different time periods. Each isomer could be followed separately. Enclomiphene was rapidly lost such that the majority was found in low amounts after 24 h. Zuclomiphene was distributed to more organs and remained associated with discrete tissues for longer periods of time. Remarkable exceptions were the pigmented organs of the eye, which retained both compounds. Notable was the specific absorption in individual tissues and the lack of clearance in certain cases. The ratio of zuclomiphene to enclomiphene (Zu/En) demonstrated the promiscuous nature of the zuclomiphene and the specific absorption. The tissue/plasma ratios demonstrated those tissues that were accrued or failed to clear compounds. Important differences were found in the lack of clearance of isomers in the eye, gall blabber/bile, brain, lung, fat, adrenals, kidneys and reproductive tissues.

Conclusion: The adverse effects of Clomiphene citrate in the eye and male reproductive organs may be rationalized by lack of clearance of higher levels of zuclomiphene as well as effects of Ebola virus infection. An additional element of this study was to determine levels that would not be expected to represent a significant radiation exposure risk to human male volunteers in future ADME studies.

Keywords: Clomiphene; Enclomiphene; Zuclomiphene; Blood-brain barrier; Metabolism; Uveal tract

Introduction

Clomiphene citrate has been approved for use in women to induce ovulation [1-3] and has been used off-label in men to raise serum testosterone [4-9]. The latter use was rationalized early [10] by the recognition that a bolus dose of Clomiphene citrate could be used as a diagnostic tool to determine the functionality of the hypothalamic-pituitary axis by the stimulation of release of Luteinizing Hormone (LH) in a subject with low gonadotropins. Clomiphenes and its isolated isomes, have also been shown to have an effect on progression of disease after Ebola virus infection in cell culture and mouse model systems [11, 12].

Clomiphene citrate is considered to be a SERM, i.e., a compound possessing estrogen agonist or antagonist properties depending on the tissue. Clomiphene citrate was described as a mixture of two isoforms, enclomiphene (trans-isomer) and zuclomiphene (cis-isomer), with estrogen agonist or antagonist properties [13-16]. The structure of the isomers described in the initial papers is shown in **Figure 1**. Clomiphene citrate is available commercially as a mixture of the two isomers. Clomiphene citrate is approximately 62% enclomiphene and 38% zuclomiphene.

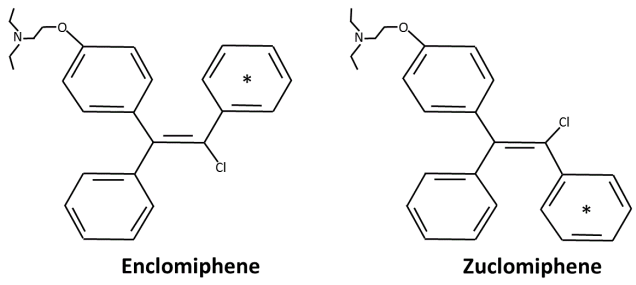

Figure 1 Structure of the isomers.

Enclomiphene citrate has anti-estrogenic properties [17] and appears to block the negative feedback inhibitory effects of estradiol on the hypothalamic-pituitary axis resulting in increased levels in both LH and follicle stimulating hormone

(FSH) which stimulate endogenous intragonadal testosterone production and spermatogenesis in men [8, 18].

Clomiphene citrate presented no adverse sequelae due to zuclomiphene which is, at worst, an inactive congener. That view was not blunted despite the known much longer half-life of zuclomiphene [19, 20] and the absence of estrogen antagonism [17, 21], the key feature of the activity of the mixture. Early ADME studies of Clomiphene citrate describe loss through the feces and urine after a single dose [13, 22]. Previous descriptions of The ADME profiles of Clomiphene citrate are thus inadequate since the longer-lived zuclomiphene would be in excess over the more active enclomiphene.

We have determined that one of the two isomers of Clomiphene citrate, Zuclomiphene citrate, a component of a drug believed to be safe, has deleterious effects on the male reproductive organs of mice [23]. There are known effects of clomiphene on the eyes [24-26] as has been recognized in the product label [27]. These effects might be a result of an accumulation of the zuclomiphene isomer over time.

Materials and Methods

The ^{14}C-labelling of Enclomiphene and Zuclomiphene was done at Ricerca Concord, OH. The labeled compounds as citrate salts were done after synthesis. The positon of the 14C-label was distributed among the carbons of one of the rings **(Figure 1)**. The in-life dosing to C-57 black mice was performed at Covance Laboratories Inc. Madison, WI under the direction of Randall Press. All procedures performed in animals were under the guidance of the "Guide for the Care and Use of Laboratory Animals". Tissue distribution of 14CEnclomiphene related radioactivity was assessed following a single oral administration of 14CEnclomiphene citrate to male and female pigmented (C57 black) mice (Group 1). Tissue distribution of 14CZuclomiphene related radioactivity was also investigated following a single oral administration of 14CZuclomiphene citrate to male pigmented (C57 Black) mice (Group 2). Dose formulations were prepared as solutions on the day of dose administration by combining appropriate amounts of radiolabeled and nonradiolabeled Enclomiphene citrate or radiolabeled and non-radiolabeled Zuclomiphene citrate in 0.5% methylcellulose and 0.2% Tween 80 in reverse osmosis water at a nominal concentration of 2.0 mg/mL and a target radioactivity dose of 100 µCi/20 mg/kg. In Group 1, two animals/sex/time points were sacrificed at 0.25, 1, 4, 24, 72, 168, 240, 336, 504 and 840 h. For Group 2, two animals/time points were sacrificed at 1, 4, 24, 72, 168, 240, 336, 504, 840 and 1008 h post dose. Blood was collected and plasma was harvested at specified time points and carcasses were prepared for QWBA. Sections were collected from one carcass per time point and exposed to phosphor imaging screens. The images were processed for determination of the radioactivity concentrations in selected tissues. Blood and plasma were analyzed for concentrations of radioactivity using liquid scintillation counting (LSC). The radioactivity concentrations were expressed as ng equivalents 14CEnclomiphene/g or ng equivalents 14CZuclomiphene/g of sample, as applicable. The pharmacokinetic analysis of 14CEnclomiphene citratederived and 14CZuclomiphene citrate-derived radioactivity were conducted for blood, plasma and tissues and radiation dosimetry parameters were calculated. There was no attempt to separate and thus determine the identity of the 14C-derived products, i.e., to determine the metabolites of each drug. A description of the kinds of metabolites derived from each of the drugs in women has already been shown [28, 29]. We have also determined metabolites of each in liver hepatocytes of four species and characterized the metabolites detected in dogs after both acute and chronic dosing using non-labelled materials (internally generated data not shown).

Results

Test article and dose formulation analysis

Radiopurity and stability

The HPLC analyses performed by Covance showed the radiopurities of 14C-Enclomiphene and 14C-Zuclomiphene to be 98.2 and 96.6%, respectively, prior to dose preparation. The mean radiopurity values from HPLC analysis for 14C-Enclomiphene of predose and postdose aliquots were 98.0 and 97.9%, respectively. The mean radiopurity values from HPLC analysis for 14C-Zuclomiphene of pre-dose and post-dose aliquots were 97.2 and 97.3%, respectively. These values confirmed stability of the test articles under conditions of the study.

Concentrations of radioactivity in blood and plasma

Group 1, 14C-Enclomiphene

After a single 20 mg/kg oral dose of 14C-Enclomiphene to male and female mice, the maximum average plasma concentrations of radioactivity were 1370 and 2140 ng equivalents 14C-Enclomiphene/g, respectively, observed at 1 and 4 h post-dose, respectively, concentrations declined to BLQ, by 240 h post-dose. The mean concentration versus time profile is presented graphically in **Figure 2A**. All reported concentrations are in ng equivalents of free base/g of matrix.

Group 2, 14C-Zuclomiphene

After a single 20 mg/kg oral dose of 14C-Zuclomiphene to male mice, the maximum average plasma concentration of radioactivity was 697 ng equivalents 14C-Zuclomiphene/g, observed at 4 h post-dose, concentrations declined to BLQ by 336 h post-dose. The mean concentration versus time profile is presented graphically in **Figure 2B**.

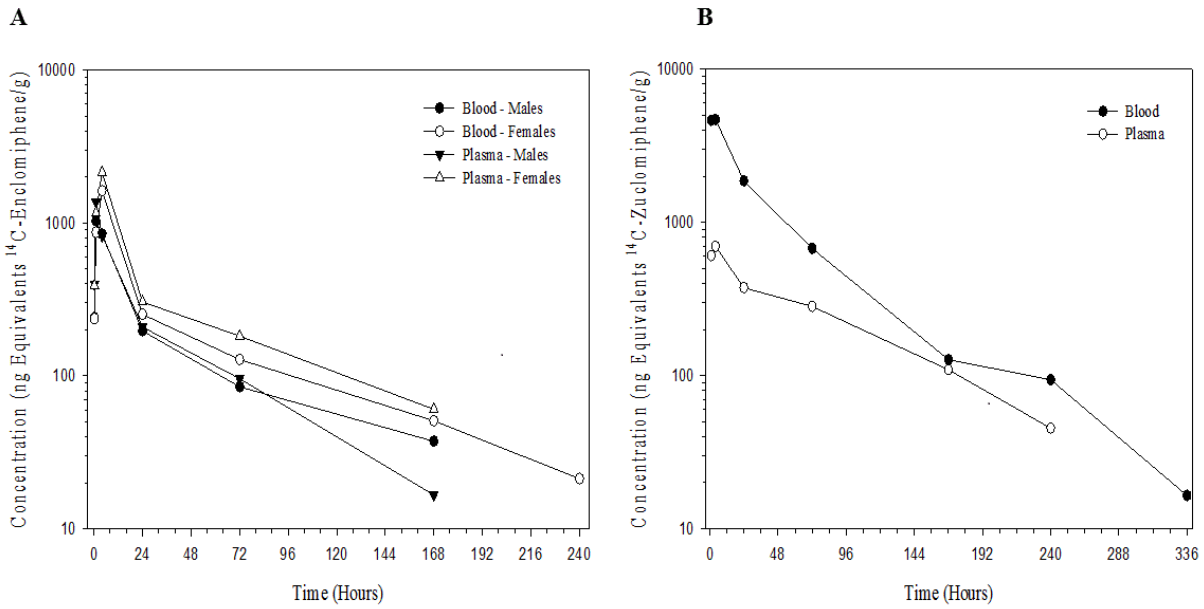

Figure 2 Mean concentration versus time profile.

Tissue distribution of radioactivity by QWBA

Group 1, 14C-Enclomiphene

The concentrations of radioactivity in tissues are presented in **Table 1** (for males) and **Table 2** (for females). All reported concentrations are in ng equivalents of free base/g of matrix.

Tissue	4 h	Tissue	24 h
Bile	728000	Eye uveal tract	6680
Gall bladder	583000	Liver	4180
Liver	29800	Gall bladder	3960
Pancreas	14200	Bile	2420
Kidney cortex	12700	Harderian gland	2210
Kidney medulla	12700	Eye(s)	1560
Kidneyls)	12700	Pancreas	1380
Harderian gland	11600	Intra-orbital lacrimal gland	1280
Eye uveal tract	10300	Kidney cortex	1220
Lung(s)	9840	Preputial gl	1170
Urine	9710	Kidneyls)	1130
Small intestine	9340	Kidney medulla	1040
Pituitary gland	6160	Cecum	977
Urinary bladder	6010	Pituitary gland	970
Esophagus	5690	Prostate gland	961
Exorbital lacrimal gland	5550	Small intestine	689
Intra-orbital lacrimal gland	5020	Exorbital lacrimal gland	676

Salivary(s)	4260	Large intestine	635
Spleen	4180	Salivary(s)	469
Thyroid	4120	Epididymis	456
Bone marrow	3850	Thyroid	444
Lymph node(s)	3760	Testisles)	442
Diaphragm	3010	Diaphragm	440
Adrenals	2880	Thymus	436
Cecum	2830	Lymph node(s)	431
Fat (brown)	2810	Lung(s)	384
Stomach	2770	Esophagus	383
Thymus	2760	Spleen	349
Eye Is)	2450	Urinary bladder	302
Large intestine	2360	Fat (brown)	258
Myocardium	1840	Skin (pigmented)	233
Preputial gland	1660	Bone marrow	222
Prostate gland	1390	Adrenals	221
Skin (pigmented)	1260	Stomach	197
Fat (abdominal)	1210	Fat (abdominal)	BLQ
Muscle	919	Muscle	BLQ
Brain cerebrum	855	Brain cerebrum	BLQ
Seminal vesicle(s)	823	Seminal vesicle(s)	BLQ
Blood	815	Blood	BLQ
Brain cerebellum	786	Brain cerebellum	BLQ
Spinal cord	716	Spinal cord	BLQ
Brain medulla	678	Brain medulla	BLQ
Bone	672	Bone	BLQ
Testisles)	669	Myocardium	BLQ
Brain olfactory lobe	595	Brain olfactory lobe	BLQ
Nasal turbinates	417	Nasal turbinates	BLQ
Epididymis	382	Urine	BLQ
Eye lens	BLQ	Eye lens	BLQ

Tissue	4 h	Tissue	24 h
Bile	383000	Gall bladder	19400
Gall bladder	256000	Bile	16400
Liver	28500	Liver	7650
Kidney medulla	12100	Cecum	4310
Pancreas	11700	Eye uveal tract	3720

Harderian gland	11400	Small intestine	2890
Small intestine	10500	Kidney cortex	1970
Lung(si_	10400	Kidney(s)	1940
Cecum	9340	Kidney medulla	1870
Kidney(s)	8830	Pancreas	1510
Eye uveal tract	7350	Harderian gland	1470
Kidney cortex	5800	Large intestine	1170
Large intestine	5200	Intra-orbital lacrimal gland	1140
Adrenals	4310	Stomach	1090
Exorbital lacrimal gland	4130	Adrenals	997
Pituitary gland	3810	Thyroid	916
Esophagus	3340	Diaphragm	901
Spleen	3310	Esophagus	778
Salivary(s)	3240	Eye(s)	773
Lymph node(s)	3160	Exorbital lacrimal gland	730
0vary(ies)	3000	lung(s)	713
Thyroid	2920	Ovary(ies)	628
Bone marrow	2880	Spleen	577
Fat (brown)	2860	Pituitary gland	542
Intra-orbital lacrimal gland	2720	Fat (brown)	458
Diaphragm	2710	Uterus	458
Urine	2700	Salivary(s)	448
Stomach	2610	Urinary bladder	423
Thymus	1990	Urine	423
Fat (abdominal)	1780	Lymph node(s)	377
Eye(s)	1520	Thymus	304
Uterus	1480	Blood	236
Myocardium	1440	Myocardium	198
Urinary bladder	1290	Fat (abdominal)	197
Blood	1060	Bone marrow	BLQ
Skin (pigmented)	999	Skin (pigmented)	BLQ
Muscle	687	Muscle	BLQ
Brain medulla	594	Brain medulla	BLQ
Spinal cord	558	Spinal cord	BLQ
Brain cerebrum	549	Brain cerebrum	BLQ
Brain cerebellum	454	Brain cerebellum	BLQ
Brain olfactory lobe	427	Brain olfactory lobe	BLQ
Nasal turbinates	397	Nasal turbinates	BLQ
Bone	292	Bone	BLQ

Eye lens	BLQ	Eye lens	BLQ

The single 20 mg/kg oral dose of 14C-Enclomiphene to male and female pigmented mice was well absorbed and widely distributed to tissues by 1 h post-dose. Peak tissue concentrations were reached at 1 or 4 h post-dose and the concentrations of radioactivity in tissues declined over time in both genders. For both genders, the highest concentrations of drug-derived radioactivity were in the contents of the gastrointestinal (GI) tract and bile. The radioactivity concentrations in the contents of the GI tract were based on qualitative visual assessment of the autoradiographic data. In male and female mice, bile had a maximum concentration of 728000 and 383000 ng equivalents 14C-Enclomiphene/g, respectively, observed at 4 h post-dose. Hepato-biliary excretion was an important route of elimination.

The highest peak tissue radioactivity concentrations in males were in gall bladder, liver, kidney cortex, kidney and pancreas with values of 583000, 46800, 16800, 15600 and 14200 ng equivalents 14C-Enclomiphene/g, respectively. All other analyzed tissues had peak concentration values less than 13000 ng equivalents 14C-Enclomiphene/g. For females, the highest peak tissue radioactivity concentrations were gall bladder, liver, kidney medulla, pancreas and harderian gland with values of 256000, 51700, 12100, 11700 and 11400 ng equivalents 14C-Enclomiphene/g, respectively. All other analyzed tissues had peak concentration values less than 11000 ng equivalents 14C-Enclomiphene/g. The lowest peak concentrations were detected in nasal turbinates (448) of males and bone (292) of females.

Group 2, 14C-Zuclomiphene

The concentrations of radioactivity in tissues are presented in **Table 3** (males only). All reported concentrations are in ng equivalents of free base/g of matrix.

Tissue	4 h	Tissue	24 h
Bile	105000	Harderian gland	43900
Gall bladder	90200	Bile	39800
lung(s)	72400	Gall bladder	33600
Kidneycortex	53500	lung(s)	33600
Kidneyls)	43700	Eye uveal tract	30000
Pituitary gland	34200	Kidneycortex	29400
Kidney medulla	32400	Kidney(s)	24600
Spleen	31600	Liver	18200
Harderian gland	29300	Kidney medulla	17800
Liver	29100	Exorbital lacrimal gland	15000
Adrenals	26100	Adrenals	14800
Pancreas	24900	Intra-orbital lacrimal gland	14000
Small intestine	21900	Spleen	13500
Cecum	21000	Pancreas	12700
Salivary(s)	20300	Salivary(s)	12700
Exorbital lacrimal gland	20000	Pituitary gland	12600
Fat (brown)	19800	Thymus	11700
Bone marrow	19500	Fat (brown)	11400
Intra-orbital lacrimal gland	18700	Lymph node(s)	10600
Eye uveal tract	18400	Epididymis	10500
Thyroid	17400	Small intestine	10000
Diaphragm	15200	Bone marrow	9930

Stomach	14900	Urinary bladder	9630
Myocardium	14700	Cecum	9260
Fat (abdominal)	14000	Diaphragm	8190
Thymus	12500	Thyroid	7410
Lymph node(s)	11900	Brain cerebrum	7110
Esophagus	10700	Testisles)	6810
Urinary bladder	9140	Myocardium	6790
Large intestine	7820	Large intestine	6070
Preputial gland	7630	Eye(s>	5760
Brain cerebrum	7070	Fat (abdominal)	5530
Muscle	5860	Prostate gland	4760
Brain cerebellum	5310	Spinal cord	4460
Spinal cord	5000	Stomach	4170
Seminal vesicle(s)	4630	Nasal turbinates	4020
Brain medulla	4620	Brain cerebellum	3920
Prostate gland	4460	Brain medulla	3800
Brain olfactory lobe	4390	Esophagus	3770
Epididymis	4390	Seminal vesicle(s)	3490
Nasal turbinates	4290	Brain olfactory lobe	3300
Blood	4250	Skin (pigmented)	3220
Bone	3060	Preputial gland	3140
Eye Is)	2840	Muscle	2910
Testis(es)	2830	Urine	2530
Skin (pigmented)	2680	Blood	1670
Urine	2350	Bone	1440
Eye lens	BLQ	Eye lens	302

14C-Zuclomiphene was well absorbed and widely distributed to tissues by 1 h post-dose. Peak tissue concentrations were reached at 1 or 4 h post-dose for most tissues, with some tissues having peak concentrations at 24 and 72 h post-dose. The highest concentrations of drug-derived radioactivity were in the contents of the GI tract and bile. The radioactivity concentrations in the contents of the GI tract were based on qualitative visual assessment of the autoradiographic data. Bile had a maximum concentration of 105000 ng equivalents 14C-Zuclomiphene/g observed at 4 hours post-dose. Hepato-biliary excretion was an important route of elimination.

Dosimetry

Group 1, 14C-Enclomiphene

Dosimetry calculations from pigmented male mice data predict that uveal tract, eye, liver, pancreas and renal cortex will be exposed to the highest doses of radiation in humans following a single oral dose of 14C-Enclomiphene. In man, these matrices are estimated to be exposed to 215, 53.1, 11.4, 8.45 and 6.02 mRad or mrem, respectively (2.15, 0.531, 0.114, 0.0845 and 0.0602 mGy, respectively).

Dosimetry calculations from pigmented female mice data predict that uveal tract, eye, liver, small intestine and pancreas will be exposed to the highest doses of radiation in humans following a single oral dose of 14C-Enclomiphene. In women, these matrices are estimated to be exposed to 186, 49.7, 16.7, 9.93 and 8.01 mRad or mrem, respectively (1.86, 0.497, 0.167, 0.0993 and 0.0801 mGy, respectively).

Group 2, 14C-Zuclomiphene

Dosimetry calculations from pigmented male mice data predict that uveal tract, epididymis, eye, testes and renal cortex will be exposed to the highest doses of radiation in humans following a single oral dose of 14C-Zuclomiphene. In man, these matrices are estimated to be exposed to 365, 185, 91.8, 88.9

and 62.0 mRad or mrem, respectively (3.65, 1.85, 0.918, 0.889 and 0.620 mGy, respectively).

Discussion

There were no apparent gender related differences in the tissue concentration data for male and female mice. Tissue to plasma concentration ratios were greater than one for most tissues through 24 h post-dose and were all above one where measurable through 168 h post-dose. Radioactivity concentrations in central nervous system tissues protected by the blood:brain barrier (cerebellum, cerebrum, medulla, olfactory lobe and spinal cord) were low and dropped to non-measurable levels by 24 h post-dose. Low levels of 14C-Enclomiphene-derived radioactivity distributed to the reproductive tissues of the male and female mice, but cleared by 72 h post-dose. Elimination was nearly complete for most tissues by 72 h post-dose. By the final sampling time of 840 h post-dose, no tissues, other than the eye and uveal tract of the eye, contained measurable concentrations of radioactivity.

The accrual in 47 male tissues individually assessed are summed and given for both zuclomiphene and enclomiphene in **Figure 3**. We chose to use "accrual" to indicate amounts found in tissue in distinction to "accumulation" which might indicate an active process of tissue uptake or lack of clearance over that found in biological tissues. Peak tissue concentrations were reached at 1 or 4 h post-dose for most tissues, with some tissues having peak concentrations at 24 and 72 h post dose. There were major differences in the uptake and disposition of zuclomiphene and enclomiphene. Enclomiphene has been shown to have an approximate half-life of 7 h compared to the 14 day half-life of zuclomiphene. Every tissue examined demonstrated high lack of clearance of 14C-zuclomiphene over plasma at 1, 4, 24 and 72 h post dose **(Figure 4)**. These differences were an effect of the differing half-lives of the two isomers. We propose there is not a differential accumulation of the two isomers, rather a difference of half-life and of each of the isomers resulting in a differing elimination of each isomer in a specific tissue.

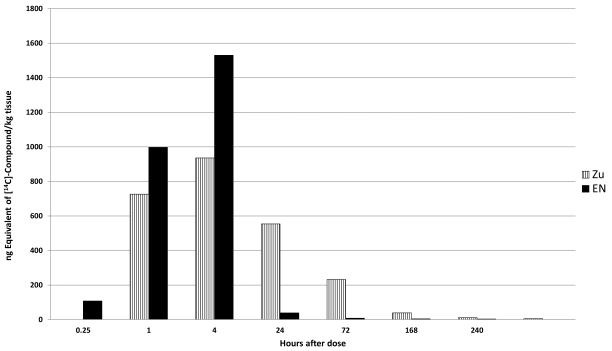

Figure 3 Accrual in 47 male tissues for both zuclomiphene and enclomiphene.

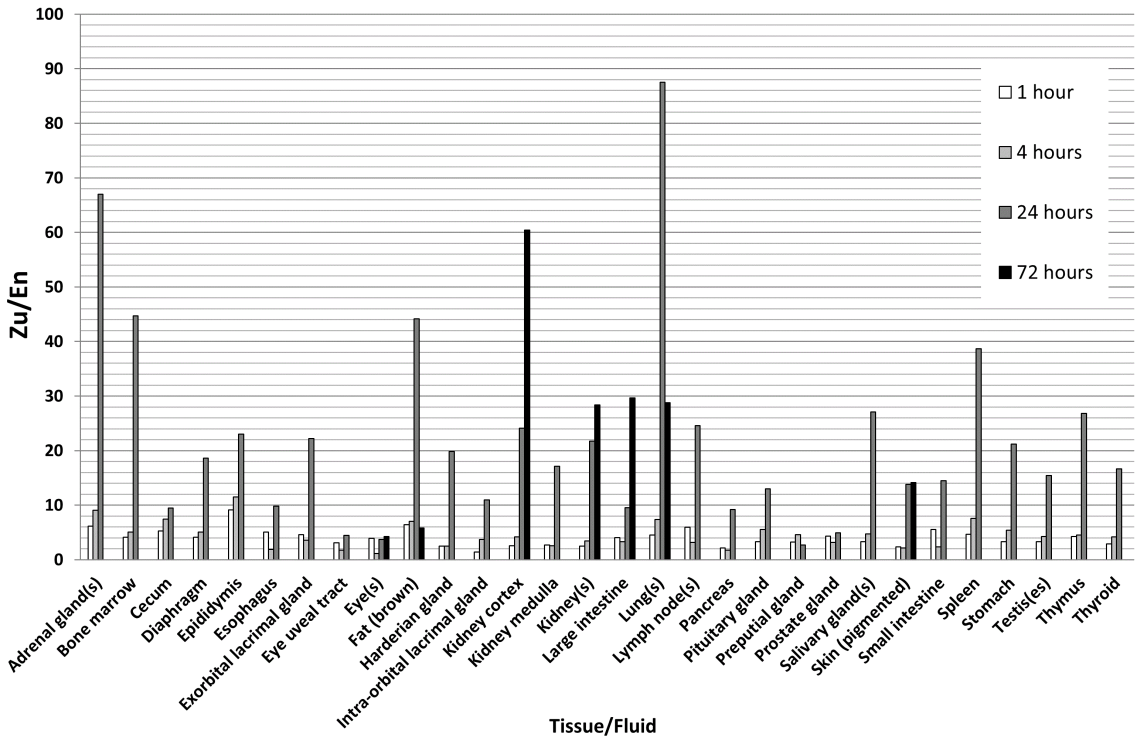

Figure 4 14C-zuclomiphene over plasma at 1, 4, 24 and 72 h post dose.

The highest concentrations of radioactivity in eye uveal tract (10300 and 7350 ng equivalents 14C-Enclomiphene/g in male and female pigmented mice, respectively) were observed at 4 h post-dose. Radioactivity concentrations in eye uveal tract

declined, but remained measurable through 840 h post-dose (1410 and 1150 ng equivalents 14C-Enclomiphene/g in male and female pigmented mice, respectively). The radioactivity concentrations in eye uveal tract at 840 h post-dose in male and female mice represented approximately 7- and 6-fold decreases in radioactivity concentrations, respectively, from the observed peak concentrations. The highest concentrations in pigmented skin were 1260 ng equivalents 14C-Enclomiphene/g at 4 h post-dose in males and 999 ng equivalents 14C-Enclomiphene/g at 4 h post-dose in females. The radioactivity concentrations in pigmented skin declined over time and dropped to BLQ by 72 and 24 h post-dose in male and female mice, respectively. These data suggested that 14C-Enclomiphene-related radioactivity was selectively associated with melanin-containing tissues of the eye.

For pigmented male mice, the 14C-Enclomiphene-derived radioactivity declined with half-life (T1/2) values ranging from 2.47 h (myocardium) to 1012 h (eye), with most matrices showing T1/2 values less than 20 h. The area under the concentration-time curve from 0 to infinity (AUC0-∞) ranged from 11064 ng equivalents 14C-Enclomipheneh/g in spinal cord to 7567172 ng equivalents 14C-Enclomipheneh/g in gall bladder. In pigmented female mice, the 14C-Enclomiphene-derived radioactivity declined with half-life values ranging from 5.17 hours (lung) to 707 h (eye), with most matrices showing T1/2 values less than 20 h. The AUC0-∞ ranged from 5115 ng equivalents 14C-Enclomipheneh/g in the olfactory lobe to 3973695 ng equivalents 14C-Enclomipheneh/g in the gall bladder.

The highest peak tissue radioactivity concentrations in male mice were in gall bladder, lung, kidney cortex, liver and harderian gland with values of 90200, 72400, 53500, 45900 and 43900 ng equivalents 14C-Zuclomiphene/g, respectively. All other analyzed tissues had peak concentration values less than 43800 ng equivalents 14C-Zuclomiphene/g. The lowest peak concentrations were detected in plasma (644) and lens of eye (302). Elimination was nearly complete for most tissues by 504 h post-dose. By the final sampling time of 1008 hours post-dose, no tissues, other than the eye and uveal tract of the eye, contained measurable concentrations of radioactivity. Unlike 14C-Enclomiphene, tissue to plasma concentration ratios following single oral dosing with 14C-Zuclomiphene were greater than one for all tissues with measurable concentrations of radioactivity over the time course examined, except the lens of the eye. Radioactivity concentrations in central nervous system tissues protected by the blood:brain barrier (cerebellum, cerebrum, medulla, olfactory lobe and spinal cord) were low and dropped to non-measurable levels by 168 h post-dose. Unlike 14C-Enclomiphene, 14C-Zuclomiphene-derived radioactivity concentrations remained measurable in the non-circumventricular CNS tissues through 72 h post-dose. Low levels of 14C-Zuclomiphene-derived radioactivity distributed to the testis, but cleared by 240 h post-dose.

The highest concentration of radioactivity in eye uveal tract (30000 ng equivalents 14C-Zuclomiphene/g) was observed at 24 h post-dose. Radioactivity concentrations in eye uveal tract declined, but remained measurable through 1008 hours post-dose (1020 ng equivalents 14C-Zuclomiphene/g). The radioactivity concentrations in eye uveal tract at 1008 h post-dose represented approximately a 29-fold decrease in radioactivity concentration from the observed peak concentration. The highest concentration in pigmented skin was 3220 ng equivalents 14C-Zuclomiphene/g at 24 h post-dose. The radioactivity concentration in pigmented skin declined over time and dropped to BLQ by 168 h post-dose.

The 14C-Zuclomiphene-derived radioactivity declined with half-life (T1/2) values ranging from 18.7 h (urinary bladder) to 411 h (uveal tract of the eye), with most matrices showing T1/2 values less than 40 h. The AUC0-∞ ranged from 60644 ng equivalents 14C-Zuclomiphene h/g in plasma to 6204197 ng equivalents 14C-Zuclomipheneh/g in uveal tract of the eye.

Conclusion

In conclusion, 14C-Enclomiphene and 14C-Zuclomiphene-related radioactivity were widely distributed in tissues and organs of mice and were selectively associated with melanin-containing tissues. For 14C-Enclomiphene, there were no apparent gender related differences in the tissue concentration data for male and female mice. In general, 14C-Zuclomiphene-related radioactivity had a longer biological half-life in most tissues as compared to 14C-Enclomiphene. Unlike 14C-Enclomiphene, 14C-Zuclomiphene-related radioactivity was preferentially distributed to the cellular components of blood and tissue to plasma concentration ratios were greater than one for all tissues with measurable concentrations of radioactivity over the time course examined, except the lens of the eye. Based on the pharmacokinetic and dosimetry data, administration of a single 100 µCi (3.7 MBq) oral dose of 14C-Enclomiphene would not be expected to represent a significant radiation exposure risk to human male or female volunteers. Administration of an oral dose of 14C-Zuclomiphene at the same level would not be expected to represent a significant radiation exposure risk to human male volunteers.

Acknowledgement

Repros would like to acknowledge and thank Randy Press and Erin Ballard at Covance for their work in performing this study.

Authorship Contributions

Participated in research design: Fontenot, Wiehle, Podolski.

Conducted experiments: Covance Laboratories Inc. Madison, WI (Under direction of Randall Press).

Contributed new reagents: Hsu

Performed data analysis: Fontenot, Wiehle

Wrote or Contributed to writing of the manuscript: Fontenot, Wiehle, Podolski.

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