EFFECTS OF ADMINISTRATION OF ESTROGEN OR DIPHENYLHYDANTOIN ON THE KINETICS OF DIPHENYLHYDANTOIN IN MAN

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The rate of disappearance of ^{131}I-diphenylhydantoin (^{131}I-DPH) from plasma and its hepatic uptake were studied during a control period and after estrogen administration in ten normal human subjects. Four subjects were given DPH and were studied in the same fashion as the estrogen-treated group. After intravenous injection of a tracer dose of ^{131}I-DPH (period of study: 30–150 min after ^{131}I-DPH administration), the estrogen-treated group showed an increase in the plasma half-time, a decrease in the fractional turnover rate, and a decrease in the distribution space of ^{131}I-DPH in comparison with control. The hepatic uptake was decreased in comparison with the control, which may be explained by an increase in the binding capacity of thyroxine-binding globulin (TBG) induced by estrogens. An increase in the distribution space of ^{131}I-DPH was observed in subjects treated with diphenylhydantoin. Consequently, the clearance rate was increased. No change in half-time or in turnover rate was observed. The hepatic uptake in the DPH-treated group was increased in comparison with control. This may be explained by a displacement of ^{131}I-DPH by DPH from the binding sites of TBG. This study showed, therefore, that changes in the binding capacity of TBG are associated with alterations in the peripheral metabolism of ^{131}I-DPH in man.

The specific interactions of thyroxine-binding globulin (TBG) with thyroxine (T_4) and triiodothyronine (T_3) have been demonstrated in vitro by electrophoresis and in vivo by turnover rate determinations (*1*).

Diphenylhydantoin (DPH) that is weakly bound to TBG in vitro and in vivo (*2–5*) can compete with T_3 and T_4 for binding sites on TBG and when administered in vivo it produces a marked decrease in the proportion of T_4 and T_3 bound to TBG (*6*). In addition, it has been shown that hyperestrogenia, whether exogenous or endogenous, is associated with an elevation of the thyroxine-binding capacity of TBG (*7–10*).

The purpose of this investigation was to study the effects of estrogen or DPH-induced alterations in the binding capacity of TBG on the disappearance of ^{131}I-diphenylhydantoin (^{131}I-DPH) from the circulation and on the uptake of ^{131}I-DPH by the liver.

MATERIALS AND METHODS

Estrogen-treated group. The group consisted of ten healthy volunteers who were not on medication and who were considered normal by clinical and laboratory findings. Two weeks after the control studies were done, each of these subjects was given the synthetic estrogen dienestrol (3–4-pp^1-diphenyl-dihydroxy 2:4 hexadieno, Glaxo Laboratories) in doses of 20–30 mg orally daily for 14–21 days in order to increase the binding capacity of TBG. Nausea occurred during the drug administration in all of these subjects but it was not severe enough to cause discontinuation of the medication.

DPH-treated group. Two weeks after the control studies, four normal subjects received 200 mg of diphenylhydantoin (Dilantin) by mouth 12 hr prior to the commencement of the study and again at the beginning of the study.

Iodine-131-DPH turnover studies were carried out before (control period) and again after either estrogen or DPH administration (treatment period) in individual subjects. All subjects were given Lugol's solution prior to the administration of ^{131}I-DPH. The volunteer subjects were informed about the nature of the procedure.

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TABLE 1. PLASMA KINETICS OF ^{131}I-DPH

Subject	Sex and age		^{131}I-DPH half-time (min)	k (%/hr)	DS (liters)	Cl (liters/hr)
Estrogen-treated						
1	F,38	Control	88.3	47.0	12.5	5.8
		Estrogen	146.6	28.3	7.1	2.0
2	M,27	Control	110.8	37.5	8.3	3.1
		Estrogen	252.6	16.4	8.2	1.3
3	F,37	Control	91.6	45.3	7.2	3.2
		Estrogen	146.3	28.4	5.8	1.6
4	M,42	Control	105.0	39.6	7.1	2.8
		Estrogen	148.0	28.0	3.3	0.9
5	F,32	Control	69.0	60.2	10.0	6.0
		Estrogen	183.0	22.7	5.1	1.1
6	F,28	Control	77.6	53.5	6.6	3.5
		Estrogen	129.9	32.0	3.5	1.1
7	F,29	Control	74.9	55.5	9.0	4.9
		Estrogen	133.3	31.1	5.5	1.7
8	M,36	Control	120.8	34.4	8.8	3.5
		Estrogen	160.5	25.8	5.2	1.3
9	M,44	Control	98.3	42.2	11.0	4.6
		Estrogen	246.0	16.9	6.0	1.0
10	M,44	Control	123.3	33.7	15.3	5.1
		Estrogen	151.5	27.4	7.8	2.1
Control	Mean	—	95.9	44.8	9.5	4.2
	s.d.	—	±19.0	±9.1	±2.7	±1.1
Estrogen	Mean	—	169.7	25.7	5.7	1.4
	s.d.	—	±44.4	±5.4	±1.6	±0.4
	p	—	<0.001	<0.001	<0.001	<0.001
DPH-treated						
11	F,38	Control	67.4	61.6	10.1	6.2
		DPH	78.3	53.1	14.2	7.5
12	M,33	Control	66.2	62.8	9.5	5.9
		DPH	64.9	64.0	16.6	10.6
13	M,23	Control	78.8	52.7	6.8	3.5
		DPH	76.3	54.4	14.9	8.1
14	M,32	Control	79.6	52.2	10.2	5.3
		DPH	81.6	50.9	13.3	6.7
Control	Mean	—	73.0	57.3	9.1	5.2
	s.d.	—	±7.1	±5.6	±1.5	±1.2
DPH	Mean	—	75.2	55.6	14.7	8.2
	s.d.	—	±7.2	±5.7	±1.3	±1.6
	p	—	NS	NS	<0.001	<0.001

^{131}I-DPH, diphenylhydantoin labeled with ^{131}I; DS, ^{131}I-DPH distribution space = 100% dose/% dose/liter plasma at zero time; k, fractional turnover rate = 0.693/^{131}I-DPH half-time; Cl, clearance rate (DS.k); s.d., standard deviation; p, probability value; and NS, not significant ($p > 0.05$).

The ^{131}I-labeled DPH used in this investigation was obtained from the laboratories of the Atomic Energy Commission of Argentina (*11*). It was prepared in an aqueous propylene-glycol solution with a specific activity of approximately 1.1 mCi/mg. The preparation was found to be 95% pure ^{131}I-DPH (*11*). The radiopharmaceutical was utilized within 3 days of its preparation in order to avoid spontaneous deiodination. Each subject was given a single intravenous injection of 0.7 μCi/kg body weight of ^{131}I-DPH, a dose averaging 42 μCi.

Heparinized plasma samples were obtained at 15-min intervals for at least 150 min following injection of the ^{131}I-DPH. Aliquots of plasma (2 ml) were filtered through an anion-exchange resin (Amberlite-IRA-400) that removed the contaminating iodides but did not retard the ^{131}I-DPH. In addition, ^{131}I-DPH standards were run through the columns. Plasma ^{131}I-DPH radioactivity was then determined in the effluent volume using a well scintillation gamma counter (Searle Radiographics) and the net counts per minute plotted on semilog paper. Using the data obtained between 30 and 150 min after the injection, regression lines were plotted by the method of least squares (*12*). Using these lines, the plasma ^{131}I-DPH half-life, the fractional turnover rate, the distribution space, and the clearance rates were then calculated.

The liver radioactivity was estimated by placing the detector (Renaltron IV, Searle Radiographics) 15 cm from the right costal wall in the seventh intercostal space at the anterior axillary line. The hepatic

radioactivity was measured continuously after ^{131}I-DPH administration. A standard, prepared by mixing ^{131}I-DPH with plasma and diluting to 1,000 ml with water, was counted in the same manner as the patients' livers. The results of the liver counts are expressed as percent of the standard. Statistical analyses were performed using the Student-Fisher test (*12*).

The thyroxine-binding capacity of TBG was determined in the subjects by the method of Ingbar (*13*). The areas of radioactivity of the electrophoretic strips were calculated by a digital integrator (Actigraph III, Searle Radiographics).

RESULTS

In vivo: (Tables 1, 2; Figs. 1, 2). *Estrogen group.* During the control period, the ^{131}I-DPH half-time was 95.9 ± 19 min, the fractional turnover rate was 44.8 ± 9.1%/hr, the distribution space was 9.5 ± 2.7 liters, and the clearance rate was 4.2 ± 1.1 liters/hr. Thirty minutes after the injection of ^{131}I-DPH, a mean value of 12.5 ± 0.3% of the standard had been taken up by the liver. This decreased to 7.8 ± 0.4% at 150 min.

After the administration of estrogens and associated with an increase in the binding capacity of TBG on electrophoresis, ^{131}I-DPH half-time increased to 169.7 ± 44.4 min and fractional turnover rate decreased to 25.7 ± 5.4%/hr. There was a contraction of the distribution space to 5.7 ± 1.6 liters and the clearance rate decreased to 1.4 ± 0.4 liters/hr. The estrogen-treated subjects had a mean 30-min hepatic radioactivity of 6.7 ± 0.4% of the standard; thus, the radioactivity in the liver was less than during the control period.

DPH group. The half-time of ^{131}I-DPH and the fractional turnover rate were not changed significantly by administration of DPH. The distribution space and the clearance rate were increased after DPH administration in comparison with control (Table 1).

This DPH-treated group showed a marked increase in hepatic radioactivity at both 30 and 150 min after ^{131}I-DPH administration (Table 2).

TABLE 2. HEPATIC UPTAKE OF ^{131}I-DPH

Subjects (No.)	Percent of the standard 30 min	150 min	Probability value
Control (10)	12.5 ± 0.3*	7.8 ± 0.4	
Estrogen (10)	6.7 ± 0.4	2.9 ± 0.2	($p < 0.01$)
DPH-treated (4)	21.5 ± 1.1	16.5 ± 2.9	($p < 0.01$)

* Mean value ± standard error of the mean.

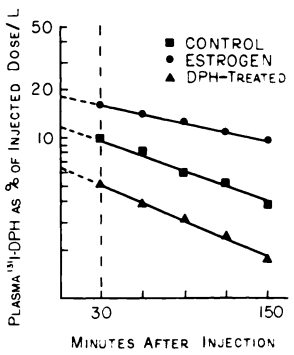

FIG. 1. Plasma disappearance curves of ^{131}I-DPH. Straight line 30 min after injection was utilized for determination of ^{131}I-DPH half-time in each group.

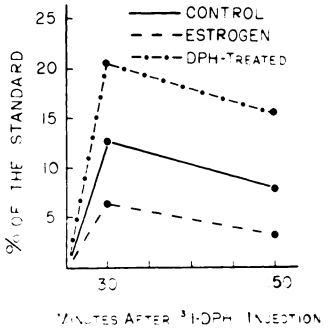

FIG. 2. Hepatic uptake of ^{131}I-DPH as percent of the standard.

In vitro. The following values were obtained for TBG binding capacity: (control: 25 ± 1.9 μg/100 ml plasma; estrogen-treated: 42 ± 1.7 μg/100 ml plasma ($p < 0.01$); DPH-treated: 16 ± 2 μg/100 ml plasma ($p < 0.05$).

DISCUSSION

Although the pharmacology of DPH has been extensively studied, there have been few studies dealing with the kinetics of labeled DPH in humans as affected by changes in the binding capacity of TBG. The administration of estrogens in the present study induced an increase in the binding capacity of TBG as demonstrated by electrophoresis. This is in agreement with a number of in vitro observations (*7,8*). Associated with this increased binding capacity, the estrogen-treated group had an increase in the ^{131}I-DPH half-time which was significantly longer than the control period (Fig. 1, Table 1). Thus, the exit of ^{131}I-DPH from the circulation to the tissues was delayed and the distribution space and turnover rate were consequently decreased. The present data show that ^{131}I-DPH plasma kinetics are affected by an increase in the binding capacity of TBG. Estrogens, however, may have also altered ^{131}I-DPH kinetics by changing tissue factors (e.g., tissue-binding proteins,

enzymes, etc.) (*1,10*). This possibility remains to be investigated.

After estrogen administration, less radioactivity was present in the liver in comparison with the control period (Fig. 2, Table 2). This finding might be explained either by an increased ability of plasma proteins to retain ^{131}I-DPH or by a direct effect of estrogens on the liver causing a decreased hepatic uptake or deiodination of ^{131}I-DPH.

The purpose of evaluating the DPH-treated group was to investigate whether DPH would acutely produce any alteration in ^{131}I-DPH kinetics. Neither the ^{131}I-DPH half-time nor the fractional turnover rate were altered by DPH treatment. However, the distribution space was increased after DPH administration (Table 1). Consequently, the percentage of ^{131}I-DPH retained in plasma during the period of study was lower than control, reflecting an increase in the clearance rate. These findings might be attributed to the administered DPH occupying binding sites on TBG (as demonstrated in vitro by the decrease in TBG-binding capacity) and therefore, decreasing the availability of sites to bind ^{131}I-DPH.

An increase in the hepatic uptake was demonstrated in the DPH-treated group (Fig. 2, Table 2). This observation might be explained by the more rapid exit of ^{131}I-DPH from plasma to liver produced by the administration of DPH. It is unlikely that the dose of DPH used in our study had produced any tissue alteration that could explain this finding.

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