TOTAL AND FREE SERUM HALOPERIDOL LEVELS IN SCHIZOPHRENIC PATIENTS AND THE EFFECT OF AGE, THIORIDAZINE AND FATTY ACID ON HALOPERIDOL-SERUM PROTEIN BINDING *IN VITRO*

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- 1 Using radioimmunoassays, steady-state levels of total and free haloperidol (obtained by ultra-filtration and dialysis) have been determined in twenty-two patients on long term treatment receiving doses from 3 to 45 mg per day.
- 2 For the group, both total serum concentration and free drug concentration showed significant correlations ($P < 0.001$) with daily dose.
- 3 No significant correlation was observed between age of the patient and percentage free haloperidol in serum.
- 4 *In vitro* experiments using sera from twenty-three healthy volunteers showed significant negative correlations ($P < 0.01$) between age and percentage free haloperidol.
- 5 Thioridazine and oleic acid significantly enhanced the percentage of free haloperidol in normal human sera *in vitro*.

Introduction

Haloperidol is a widely used neuroleptic administered as an intramuscular depot injection or orally to suppress psychiatric disorders. Its use however, frequently induces extra pyramidal dysfunctions including akathisia and dystonic reactions which appear to be directly related to dosage. Persistent and sometimes irreversible dyskinesias of late onset may also occasionally occur especially with high doses or prolonged treatment (Kobayashi, 1977). Current evidence from animal studies suggests that supersensitivity to dopamine agonists at striatal dopamine receptors induced by prolonged exposure to neuroleptics may be the origin of this latter dysfunction (Snyder, 1979). Factors which increase the brain concentration of neuroleptics may therefore increase the probability of the development of tardive dyskinesia and other side-effects. Since the incidence of tardive dyskinesia correlates positively with high doses of neuroleptic and/or duration of drug treatment, particularly in patients older than 55 years (Crane, 1974), we have monitored the serum levels of total haloperidol and the 'free' drug in patients undergoing long-term drug treatment to ascertain whether the fractional binding of haloperidol to serum varies with age and concomitantly to establish whether the circulating free level is related to the dosage which is reported to be the case for total serum levels of haloperidol and dose (Forsman & Öhman, 1977).

In addition we have examined *in vitro* factors which have been reported to affect the binding of haloperidol and other neuroleptics in human serum. These include the age of the individual which has been shown to correlate positively with the free fraction of fluphenazine in serum *in vitro* (Rowell, Hui, Fairbairn & Eccleston, 1980), and the co-incubation of thioridazine which is extensively bound to human serum (>99%) (Nyberg, Axelson & Martenson, 1978) and has been demonstrated to cause elevated free fractions of fluphenazine in serum *in vitro* (Rowell *et al.*, 1980). We have also examined the effect of the fatty acid, oleic acid, on haloperidol-serum binding since intake of food has been shown to increase total haloperidol serum levels during pharmacokinetics experiments on volunteers (Forsman & Öhman, 1976).

Methods

1. Total and free serum haloperidol levels

The subjects were 22 patients with a diagnosis of schizophrenia: 13 male and 9 female aged between 22 and 79 years. Before participating in the study, all patients gave fully informed consent.

The twenty-two subjects were already established

on haloperidol by oral dose for a period of at least six months and were receiving no other medication (Table 1). The total daily dose varied between 3 to 45 mg and was given twice to four times daily as 1.5 to 20 mg tablets.

Blood samples were taken between 9.30 and 10.30 in the morning prior to medication. The serum samples were obtained by immediate centrifugation of the bloods, the serum above the clotted blood cells and proteins was then used in the ultrafiltration study. Ultrafiltration was carried out on half of each serum sample at 37°C using an Amicon MMCA multi-micro system and Diaflo membranes (PM 10, 25 mm diameter) to obtain the 'free' drug fraction of each sample, the remaining serum being analysed for total serum levels. All serum samples and separated 'free' fractions were refrigerated at 4°C before estimation. All estimations were performed in duplicate within 12 h of sampling to avoid the possible effect of freezing and thawing on drug-serum binding.

Six samples of fresh (unfrozen) human serum (1.0 ml) from schizophrenic patients receiving haloperidol were dialysed for 21 h at 37°C in phosphate buffer pre-soaked dialysis bags (Visking tubing, 8/32 in) against phosphate buffer (1.0 ml, 0.05M phosphate buffer, pH 7.4, containing 0.1% sodium azide), in stoppered test-tubes which were agitated using a rotating inverter. The levels of haloperidol obtained by analysis of the dialysates, retentates and total

serum levels were used to obtain an estimate of the binding of drug to the dialysis membrane.

2. The effect of age and thioridazine on haloperidol serum levels in vitro

The subjects were 23 healthy individuals, 12 male and 11 female aged between 20 and 72 years none of whom was receiving medication.

Blood samples were taken between 09.30 h and 10.30 h and separated by centrifugation immediately after bleeding. To samples of fresh (unfrozen) human serum (0.9 ml) were added haloperidol solution in distilled water (50 µl of 2×10^{-6} M) and distilled water (50 µl) to give an initial haloperidol concentration of 37.5 ng/ml.

Identical samples were treated with haloperidol as before plus thioridazine solution in distilled water (50 µl; 2×10^{-6} M).

All samples were then dialysed for 21 h at 37°C in phosphate buffer pre-soaked dialysis bags (Visking tubing 8/32 in) against phosphate buffer (1.0 ml of 0.05 M phosphate solution of pH 7.4 containing 0.1% sodium azide). All samples were incubated in stoppered test-tubes, being agitated using a rotating inverter. Six samples were also subjected to ultrafiltration (using Amicon Model 12 UF cell, Diaflo PM 10 membrane) at 37°C. As control, dialysis was performed as described but in the absence of both drugs

Table 1 Total, free and percentage free haloperidol levels in sera from 22 schizophrenic patients.

Subject	Age (years)	Sex	Total daily dose (mg)	Total drug (ng/ml)	Serum haloperidol	
					Free drug (ng/ml)	Free drug (%)
1	50	M	30	14.5 ± 1.2	1.5 ± 0.10	10.3
2	57	M	9	12.2 ± 1.0	1.8 ± 0.15	14.7
3	43	F	9	8.0 ± 0.50	0.56 ± 0.05	7.0
4	66	F	4.5	2.1 ± 0.10	<0.375	—
5	77	F	4.5	1.7 ± 0.15	<0.375	—
6	79	F	4.5	5.4 ± 0.5	<0.375	7.0
7	62	F	4.5	<0.375	<0.375	—
8	65	M	40	28.0 ± 2.1	4.31 ± 0.25	15.4
9	47	M	45	31.9 ± 2.5	2.44 ± 0.13	7.6
10	56	M	45	38.8 ± 3.0	3.94 ± 0.25	11.7
11	50	F	6	2.0 ± 0.15	<0.375	—
12	57	M	30	31.8 ± 2.0	3.0 ± 0.20	9.4
13	56	M	45	32.8 ± 3.15	6.38 ± 0.61	19.4
14	22	M	45	32.6 ± 3.0	6.38 ± 0.50	19.5
15	42	M	30	32.3 ± 3.0	3.2 ± 0.25	10.0
16	70	M	9	2.05 ± 0.2	<0.375	—
17	48	M	3	<0.375	<0.375	—
18	38	M	4.5	1.75 ± 0.10	<0.375	—
19	58	M	10	6.50 ± 0.35	1.06 ± 0.07	16.3
20	45	F	10	8.75 ± 0.55	1.50 ± 0.10	17.1
21	39	F	15	22.0 ± 2.1	2.01 ± 0.10	9.1
22	67	F	4.5	1.00 ± 0.07	<0.375	—

or using only thioridazine with the serum samples. All determinations were performed in duplicate. Only the samples subjected to dialysis were in contact with sodium azide.

3. The effect of fatty acid on haloperidol-serum protein binding in vitro

Fresh (unfrozen) human serum (20 ml) was defatted as described by Chen (1967), using activated charcoal (0.4 g). To each aliquot of defatted human serum (1.8 ml, pH 7.4) was added haloperidol (0.1 ml, 2×10^{-6} M) in distilled water and sodium oleate (0.1 ml; 86.4 – 10.8 mmol in phosphate buffer, pH 7.4, 0.05 M) to give solutions of composition sodium oleate to human serum albumin of 8:1 to 1:1 moles respectively.

The serum samples were incubated for 1.5 h at 37°C and then subjected to ultrafiltration (using an Amicon Model 12 UG cell and Diaflo PM 10 membranes) at 37°C. All determinations were performed in duplicate. No samples were in contact with sodium azide.

Method of estimation

Haloperidol was estimated by radioimmunoassay using [^3H]-spiroperidol and [^{125}I]-haloperidol labels (Rowell, Hui & Kamel, 1981). Each sample (200 μl) was assayed in duplicate. The radioimmunoassay was specific for butyrophenones, showing less than 3% cross-reaction with the major haloperidol metabolites and less than 0.01% cross-reaction to thioridazine. Over the range from 1×10^{-7} – 1×10^{-9} M the recovery was $101.0 \pm 1.01\%$ and the assay precision was 3.7 – 8.7% and 5.3 – 10.6% (% coefficient of variation, c.v.) for *intra* and *inter* assays respectively. The results presented are those obtained using the [^3H]-spiroperidol label (x) in the assay; the results using [^{125}I]-haloperidol (y) showed excellent agreement with those quoted ($r = 0.989$, $n = 21$, $y = 0.931x + 0.168$).

Results

1. Free serum haloperidol levels after dialysis and ultrafiltration

Excellent correlations were observed between the results obtained using dialysis (x) or ultrafiltration (y) to separate bound and free drug in sera from patients or haloperidol spiked serum samples (typically, $r = 0.99$, $n = 6$, $y = 0.967x + 0.586$). Analysis of total drug levels and those in dialysates and retentates demonstrated that binding to the dialysis membrane was less than the % c.v. of the *intra* assay precision (i.e. < 9%). The correlation observed for the results obtained using dialysis and ultrafiltration indicates that binding of the drug to the PM 10 membrane is

also insignificant and that corrections for the Donnan Effect during the dialysis need not be applied.

2. Total and free serum haloperidol levels

A significant correlation between total daily dose of haloperidol and total drug serum levels was observed for twenty of the patients ($r = 0.93$, $P < 0.001$). Two serum samples had undetectable haloperidol levels, possibly due to poor compliance, and are not included in the regression data which is shown in Figure 1. A

Figure 1 Correlation between dose of haloperidol and serum levels ($r = 0.93$, $P < 0.001$, $n = 20$).

significant correlation between total daily dose of haloperidol and free drug level was also observed for the fourteen samples having free haloperidol levels above the limit of detection of the assay (0.375 ng/ml) ($r = 0.83$, $P < 0.001$) (Figure 2). The total and free serum levels and the percentage free drug together with details of the patients and their daily doses are shown in Table 1.

No significant correlation was observed between the age of the patients and the free fraction of haloperidol in serum ($r = 0.26$). The mean percentage of free drug was 12.5% ($\pm 4.3\%$ s.d.) for fourteen patients.

Figure 2 Correlation between dose of haloperidol and unbound serum levels ($r = 0.83$, $P < 0.001$, $n = 14$).

3. The effect of age and thioridazine on free haloperidol serum levels in vitro

The mean value for the percentage free haloperidol was 10.9% ($\pm 3.2\%$ s.d.) for the twenty-three serum samples examined. This value was not significantly different from the *in vivo* binding value ($t = 1.43$, $P > 0.1$, $n = 41$). Co-incubation of thioridazine significantly increased the mean free value to 15.4% ($\pm 3.5\%$ s.d.) for the same serum samples ($t = 4.7$, $P < 0.001$). No significant correlation was observed between age and free fraction in the presence of thioridazine.

4. The effect of oleic acid on haloperidol serum protein binding in vitro

Defatted human serum bound ninety per cent of the haloperidol and this level remained constant with mixtures of oleic acid and human serum albumin of mole ratios 1:1 to 4:1. Increasing the molar ratio of oleic acid above 4:1 decreased the serum albumin binding progressively so that at an 8:1 ratio the free percentage had increased to seventeen per cent. These results are illustrated in Figure 3.

Addition of 100 μ l aliquots from the ultrafiltrate

Figure 3 Variations of free haloperidol fraction with oleic acid concentrations in defatted human serum (ratio oleate/human serum albumin calculated assuming an albumin concentration of 0.6 mmol in serum).

obtained from the incubation of defatted human serum albumin and oleic acid (1:8 ratio) in the absence of haloperidol to the immunoassay in place of phosphate buffer gave a displacement curve with haloperidol standards which was identical to that obtained with only phosphate buffer as assay diluent. Thus it may be assumed that the presence of oleic acid did not affect the radioimmunoassay.

Discussion

The total serum haloperidol levels in the patients' samples showed a highly significant correlation with daily haloperidol dose. This correlation has been reported before together with the lack of significant correlation between age and serum concentration (Forsman & Öhman, 1977) which was also observed in this study.

The significant correlation between free haloperidol serum concentration and daily haloperidol dose is of interest and implies that the free level increases proportionally with increasing total serum levels thus maintaining a constant free fraction of drug under the steady state conditions examined. Thus no significant correlation of free haloperidol fraction with daily dose was observed the percentage of free haloperidol remaining reasonably constant at

12.5%. However wide inter-individual variations in total, free and fractional free levels for the same daily doses of haloperidol were observed.

The *in vitro* binding experiments showed a significant negative correlation ($P < 0.01$) between age and percentage free haloperidol. No such correlation was observed in the sera of schizophrenic patients. This suggests that the age-binding relationship is altered by schizophrenia or that metabolites of haloperidol, which were not co-incubated in the *in vitro* experiment, obscure the relationship *in vivo*. Alternatively, the weak *in vitro* correlation could be a spurious observation and more subjects are required to confirm or deny it.

The displacement of haloperidol from human serum by oleic acid appears to occur after the three primary fatty acid binding sites are saturated (Ashbrook, Spector, Santos & Fletcher, 1975). This phenomenon has also been observed *in vitro* for the acidic drug warfarin (Wilding, Feldhoff & Vessel, 1976) and the basic drug propranolol (Glasson, Zini, D'Athis, Tillement & Boissier, 1980). *In vivo*, the binding of fatty acids to serum albumin falls after meals containing high carbohydrate levels (Fernstrom & Wurtman, 1974). Such fatty acid depleted serum albumin would exhibit a higher affinity for haloperidol, decreasing its free serum level and increasing the total serum drug level if drug enters the plasma pool from surrounding tissue. Such changes in serum levels after meals have

been observed for L-tryptophan in rats (Fernstrom & Wurtman, 1974) and for diazepam in humans (Naranjo, Sellers & Khouw, 1980; Naranjo, Sellers, Giles & Abel, 1980). In the former study the enhanced bioavailability of the L-tryptophan in serum resulted in enhanced metabolism of serotonin in the brain.

free levels of neuroleptic drugs in serum also fluctuate with diet and to correlate individual responses with response to treatment and/or incidence of side effects.

The significant increase in the free fraction of haloperidol in the presence of thioridazine which was observed *in vitro* with equimolar mixtures of the drugs may also have *in vivo* relevance since the serum level of thioridazine may reach a thousand fold excess of haloperidol during co-administration. Also the *in vivo* clearance of phenytoin was altered by valproic acid co-administration (Frigo, Lecchini, Gatti, Perucca & Crema, 1979) in line with the increase in free fraction of phenytoin observed *in vivo* during valproate co-administration (Dahlqvist, Borga, Rane, Walsh & Sjöqvist, 1979) and the corresponding increase in free phenytoin fraction observed *in vitro* (Monks, Boobis, Wadsworth & Richens, 1978).

Further studies are planned to determine whether the *in vitro* phenomena described are manifested *in vivo*.

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