SERUM α_1 -ACID GLYCOPROTEIN AND THE BINDING OF DRUGS IN OBESITY

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Although clinically relevant, drug-protein interactions in the morbidly obese population have not been studied thoroughly. The objective of this study was to evaluate serum chemistry profiles and the degree of serum protein binding of propranolol, diazepam and phenytoin in the serum of four female, morbidly obese (> 190% of ideal body weight) and eight control female subjects. Serum triglyceride concentrations were higher and high-density lipoproteins were lower in the obese subjects than in the control group. Serum albumin and total protein concentrations in the obese were not different from controls. Unexpectedly, α_1 -acid glycoprotein concentrations were doubled in the obese subjects (mean obese value 121 mg/100 ml vs 62.9 mg/100 ml for the control subjects). Obese subjects had a mean fraction unbound (f_u) for propranolol of 0.086, which was significantly different from the controls ($f_u = 0.123$). The binding of diazepam was decreased slightly in the obese subjects. The binding of phenytoin was similar in both groups.

The altered serum chemistry of obesity may play a significant role in the drug management of the obese patient by altering drug-protein interactions.

Keywords α_1 -acid glycoprotein obesity drug binding

Introduction

The severely obese patient represents, from the drug management and theoretical pharmacokinetic perspectives, a complex and intriguing physiological state (Abernethy & Greenblatt, 1982). The assumption that the obese individual is simply a normal person plus a compartment of adipose tissue is an oversimplification of their true physiological state. The dosing of drugs in the obese population is truly a therapeutic dilemma. While the role of serum protein binding in determining drug effect and disposition has been clearly established (Evans *et al.*, 1973; Wilkinson & Shand, 1975; Yacobi & Levy, 1975), drug-protein interactions in the morbidly obese have been examined only recently. The serum protein binding of several benzodiazepines (Abernethy *et al.*, 1981, 1983) and thiopentone (Mayersohn *et al.*, 1981) was reported to be unaffected by the obese state. To our knowledge, the binding of basic compounds and serum α_1 -acid glycoprotein (AAG) concentrations have not been reported in the morbidly obese population.

The specific aim of this study was to evaluate the serum chemistry profiles and the degree of serum protein binding of three model compounds, propranolol (PP), phenytoin (DPH) and diazepam (DZ) in the serum of morbidly obese subjects and control subjects.

Methods

Four morbidly obese (> 190% of ideal body weight) female patients between 27 and 33 years of age were studied prior to gastric by-pass operation. These patients were drug-free, otherwise healthy adults. Eight healthy female volunteers between 23 and 27 years of age served as the control population (within 15% of ideal body weight). All samples were obtained at approximately the same time of year and the medical history of the subjects did not reveal the presence of disease states or other factors which might influence the serum chemistry (i.e. AAG) or drug-protein binding profile. Blood samples were obtained by venepuncture using plastic syringes containing no anticoagulant. The blood was allowed to clot, serum samples were harvested and frozen (-20°C) until used.

The protein binding of PP, DPH and DZ was determined by equilibrium dialysis using a standard dialysis membrane (Spectrapor No. 2, Spectrum Medical Industries, Los Angeles, CA) in 1 ml plexiglass cells (Bolab Inc., Lake Havasu, AZ). PP, DPH and DZ were added to separate buffer solutions (0.134 M phosphate, pH 7.4) to achieve serum concentrations at the end of dialysis which were in the therapeutic concentration range for each compound (PP: 100 ng/ml; DPH: 15 $\mu\text{g/ml}$; DZ: 1 $\mu\text{g/ml}$). A trace amount of tritiated drug; $\pm 4\text{-}[^3\text{H}]$ -propranolol

HCl; 5,5-Phenyl-4-[^3H]-(*N*)diphenylhydantoin; methyl-[^3H] diazepam (New England Nuclear, Boston, MA) was also added to the buffer solutions. Serum samples (1 ml) from each subject were dialyzed in triplicate against 1 ml of one of the buffer solutions for 12 h at 37°C. Aliquots of 100 μl of serum and buffer were measured at the end of dialysis. These concentration values were corrected for quenching by the external standard ratio method. The ratio of the buffer to serum drug concentration was designated the fraction unbound (f_u).

Total serum proteins were assayed using the Biuret method (Gornall *et al.*, 1949), albumin concentrations were quantitated by gel electrophoresis (Sepratek, Gelman Sciences, Ann Arbor, MI), free fatty acids (FFA) were measured colorimetrically (Laurell & Tibbling, 1967) and AAG levels were quantitated by radial immunodiffusion (Mancini *et al.*, 1965), using commercially available plates (M-Partigen, Calbiochem-Behring, LaJolla, CA). In addition, standard clinical laboratory methods were employed to measure serum concentrations of triglycerides, cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL). Wilcoxon two sample test was used for statistical analysis.

Results

Table 1 presents the mean serum chemistry values for the obese and control populations. Mean serum triglyceride concentration for the obese subjects was 262 mg 100 ml which was statistically different ($P < 0.05$) from the control mean (80.4 mg 100 ml). Mean serum cholesterol and FFA concentrations appeared to be slightly elevated in the obese population, but

did not reach statistical significance. Serum albumin and total protein concentrations were not different from the control population. Serum HDL concentrations were substantially decreased in the obese, while LDL concentrations were unaffected (Table 1).

Serum concentration of AAG was doubled in the obese population (Table 1). The mean AAG concentration in the obese subjects was 121 mg 100 ml compared to the control mean of 62.9 mg 100 ml. Furthermore, there was no overlap of the two sets of values (obese range: 96.1–133 mg 100 ml vs control range: 35.8–92.0 mg 100 ml) which resulted in the difference between these two populations being statistically significant ($P < 0.05$). To further substantiate the AAG findings, serum AAG concentrations were measured from four additional obese females. Their values were in excellent agreement with the present data (AAG range 98.6–121 mg 100 ml). Protein binding studies were not performed on these additional serum samples.

The elevations in serum concentrations of AAG were reflected in the binding of PP (Table 1). The obese subjects ($n = 4$) had a mean f_u for PP of 0.086 compared to the control population ($n = 8$) mean of 0.123. Again there was no overlap in f_u values (obese range: 0.069–0.104 vs control range 0.109–0.149) which resulted in the difference between the means being statistically significant ($P < 0.05$). Regression analysis of the ratio of bound free PP vs AAG concentration for all of the data (Figure 1), revealed that ~78% of the variability of PP binding could be accounted for by considering AAG concentrations.

The serum protein binding of DZ was slightly decreased in the obese subjects (Table 1). However, this difference failed to achieve statistical significance ($P < 0.05$) using nonparametric analysis due to the over-

Table 1 Serum chemistry and binding parameters (mean value $\pm$ s.d.)

Serum parameter	Control $n = 8$	Obese $n = 4$
<i>Lipid concentration</i>		
Triglycerides (mg 100 ml)	80.4 $\pm$ 28.6	262.0 $\pm$ 63.0*
Cholesterol (mg 100 ml)	192.0 $\pm$ 33.0	230.0 $\pm$ 30.0
Free fatty acids (mg 100 ml)	14.5 $\pm$ 5.9	21.3 $\pm$ 12.7
<i>Protein concentration</i>		
Total protein (g 100 ml)	7.53 $\pm$ 0.40	7.73 $\pm$ 0.32
Albumin (g 100 ml)	4.2 $\pm$ 0.2	4.0 $\pm$ 0.2
AAG (mg 100 ml)	62.9 $\pm$ 18.8	121.0 $\pm$ 17.0*
HDL (mg 100 ml)	35.1 $\pm$ 4.5	21.5 $\pm$ 3.7*
LDL (mg 100 ml)	141.0 $\pm$ 31.0	156.0 $\pm$ 28.0
<i>Fraction unbound</i>		
Propranolol	0.123 $\pm$ 0.013	0.086 $\pm$ 0.014*
Diazepam	0.021 $\pm$ 0.002	0.032 $\pm$ 0.009
Phenytoin	0.130 $\pm$ 0.008	0.140 $\pm$ 0.017

* Statistically significant ($P < 0.05$)

Figure 1 Influence of serum concentrations of AAG on the ratio of bound/free propranolol in lean (○) and morbidly obese (●) female subjects ($r^2 = 0.785$, $P < 0.01$).

lap of one of the obese values (0.021, 0.028, 0.035 and 0.042) into the control range (0.018–0.024).

In contrast to PP and DZ, the binding of DPH appeared to be relatively unaffected by the obese state (Table 1). The mean f_u value for DPH of the obese subjects was 0.140 as compared to a similar value (0.130) for the control population.

Discussion

The results of the present study indicate that the serum chemistry profile of the obese patient is remarkably different than that of a comparable control population. Moreover, these alterations in serum chemistries result in changes in the binding of drugs to serum components, which in turn may influence drug disposition and pharmacological effect.

Lipid abnormalities, particularly elevated triglycerides, are often associated with obesity (Kannel *et al.*, 1979). In addition, serum FFA concentrations have been shown to increase with increasing degree of obesity (Dole, 1956), while high density lipoprotein (HDL) concentrations are reported to be negatively

correlated with obesity (Gordon *et al.*, 1977; Kannel *et al.*, 1979). The serum chemistry data (Table 1) confirms these trends in the present subjects.

In addition to the altered lipid concentrations, the present study has demonstrated a dramatic increase in the serum concentration of AAG in the morbidly obese. This particular serum protein has been widely studied due to its important role in the binding of basic drugs (Piafsky *et al.*, 1978; Pike *et al.*, 1981), and the fluctuations in the concentration of AAG due to disease states (Piafsky, 1980; Piafsky *et al.*, 1978; Jackson *et al.*, 1982), stress (Fremstad *et al.*, 1976; Routledge *et al.*, 1981a; Edwards *et al.*, 1982) and concomitant medication (Routledge *et al.*, 1981b; Tiula & Nuevonen, 1982).

Consistent with this elevation in AAG was the increase in the serum binding of PP. The doubling of AAG resulted in a 30% decrease in the fraction unbound of PP at comparable total PP concentrations. Since the extraction of PP is blood flow limited, this decrease in the fraction unbound should not alter the clearance of PP. However, this binding change could mean a parallel decrease in the therapeutic effect of PP. This observation remains to be confirmed.

In contrast to AAG, serum albumin and total serum protein concentrations were unaffected by the obese state. As a result, the serum binding of DPH appeared to be unaltered in these morbidly obese patients (Table 1).

The binding of DZ to obese serum was decreased slightly in the present study. However, this difference was not statistically significant using a nonparametric test, apparently due to the large intersubject variability and the small sample size of the obese group. A trend for decreased binding of DZ is inconsistent with previous studies (Abernethy *et al.*, 1981, 1983) which found no influence of obesity on the binding of several benzodiazepines, including DZ. This aspect of the present data requires further study.

The clinical significance of the present results should be assessed cautiously. The present population was morbidly obese. This altered physiological state is not necessarily representative of the moderately obese, which is a far more common condition. However, the pharmacokinetic and the pharmacodynamic implications of altered protein binding are well documented and as a result, drug therapy in this severe physiological state may be much more complicated than first believed.

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