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Effect of Phenytoin on the Salivary Composition in Handicapped Patients

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Abstract

It is well recognized that chronic anticonvulsant drug therapy produces significant disorders of mineral and vitamin D metabolism, as well as possible gingival enlargement. The purpose of this study was to evaluate the effect of Phenytoin (PHT) on the main components of saliva. Blood plasma and whole saliva were collected from 36 severely handicapped patients who had been administered PHT for a long period of time in an asylum and from 36 healthy controls. One portion of the blood plasma from the patients was ultrafiltered and the PHT concentration was determined by ELISA. The concentrations of calcium (Ca) and inorganic phosphate (Pi) were significantly higher ($p<0.001$) in whole saliva from the PHT group, while no significant changes in unstimulated flow rate, pH, and the other components were found. There were no group differences in the mean plasma concentrations of Ca and Pi, but the value of the product of these two was lower in the PHT group than in the control group ($p<0.02$).

Key words: Phenytoin, salivary calcium, salivary phosphate

Introduction

Phenytoin (PHT) was synthesized in 1908 by Biltz[1] and introduced as an antiepileptic agent in 1938 by Merritt and Putnam[2]. Its efficacy in controlling epileptic seizures has been

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confirmed and it is estimated that currently there are well over two million people taking this drug[3]. However, a number of untoward reactions have been observed in patients receiving chronic anticonvulsant drug therapy[4]. Kruse[5] reported that 15% of young epileptics showed x-ray evidence of osteomalacia. In other patients, different types of bone disease have been reported, accompanied by reduced serum calcium, inorganic phosphate and 25-hydroxyvitamin D concentrations, elevated serum alkaline phosphatase and immuno-reactive parathyroid hormone concentrations, reduced urinary calcium, and increased urinary hydroxyproline excretion[6-10].

Most severely-retarded individuals have been shown to exhibit a high incidence of oral disease[11,12]. Since the retardation is frequently associated with seizure disorders, many of them have a history of epilepsy and receive anticonvulsant medications[13]. The importance of saliva in providing oral self-protection mechanisms is well established[14] and the possibility of altered salivary composition by PHT has not been explored. Several studies have reported good linear correlations between PHT concentrations in plasma and those in saliva[15-18].

The aim of our present study was to measure plasma and salivary levels of PHT in severely mentally retarded individuals, and also evaluate whether PHT altered the electrolyte composition of plasma and whole saliva.

Materials and Methods

The 36 experimental subjects, 20 male and 16 female, were aged 12-32 years (mean age 22 years) and had been administered PHT for more than 9 years in an asylum for epileptics. All of them were receiving 200 mg PHT as the average daily dose ; 26 subjects were receiving PHT alone, while the others also received some of the major anticonvulsant drugs, phenobarbital and primidon. Control subjects were 18 male and 18 female university students of 21 year mean age.

The saliva and blood were collected at the same time of day (2 : 00 p. m.)[19], from patients and controls, at least 2 hours after the last intake of food or drink. About 1 ml of unstimulated parotid saliva (from 17 of the patients) could be collected with a Lashley cannula[20], and unstimulated whole saliva was collected from the oral cavity of all subjects with a plastic suction tube. The secretion volumes were determined immediately, and the saliva centrifuged at 1,500 g for 15 minutes. One portion of the blood plasma was ultrafiltered with "Ultra Free" (Worthington Diagnostic Division, Millipore Corp.)[21] for preparing protein-free plasma. The intact plasma, ultrafiltered plasma, and saliva specimens were frozen and stored at −20°C until analyzed. The levels of PHT were determined by an enzyme-linked immunosorbent assay technique[22] (Automatic Clinical Analyzer, DuPont).

Parotid saliva was analyzed only for PHT but plasma was analyzed for PHT, calcium, and inorganic phosphate. Whole saliva was analyzed for several other components: pH, by electrode (pH meter HM-5EST, Toa) ; sodium and potassium, by flame photometry (460 Flame

Photometer, Corning); calcium and magnesium, by EDTA titrator (Ca-Mg meter C-50, Joko); inorganic phosphate, by Fiske-Subbarow (Reagent, Yatoron); alkaline phosphatase, by Kind-King (Reagent, Sinotest); Pco_2, by electrode (IL meter 813, IL); chloride, by coulometry (Chloride meter C-50, Joko). The saliva collection and analysis were repeated five times on separate days from the patients and the mean values were compared with those of the saliva from the 36 healthy control subjects.

The data from the two groups were compared by the unpaired Student t-test. Correlations between some parameters were tested by linear regression analysis.

Results

Table 1 shows the mean values and ranges for PHT in all specimens in this study. The mean concentrations of non-protein-bound (free) PHT were almost the same in ultrafiltered plasma, whole saliva and parotid saliva. The free PHT concentration as a percentage of total plasma phenytoin concentration (in non-ultrafiltered plasma) was 15.3%±2.8 (mean ±S.D.) for

Table 1 Comparison of PHT Concentrations in Plasma and Saliva (μg/ml)

	Unfiltered Plasma	Ultrafiltered Plasma	Whole Saliva	Parotid Saliva
Mean	8.5	1.2	1.5	1.3
S.D.				
Range	1.4-20.8	0.8-2.0	0.9-3.8	0.9-2.3
No. of Subjects	31	31	31	17

Table 2 Correlation Between Total PHT and Free PHT Concentrations (μg/ml) in Plasma and Saliva

	r_{xy}	Y = bx + C	N	Sig.
PHT Conc. in Unfiltered Plasma vs.				
free PHT of plasma	0.836	Y=0.03x + 0.94	31	
whole saliva PHT conc.	0.869	Y=0.09x + 0.79	33	p<0.001
parotid saliva PHT conc.	0.952	Y=0.04x + 0.93	17	
PHT Conc. in Ultrafiltered Plasma vs.				
whole saliva PHT conc.	0.893	Y=2.56x - 1.53	32	p<0.001
parotid saliva PHT conc.	0.972	Y=0.83x + 0.19	15	

N: Number of subjects
Sig: Significance
Conc: Concentration

ultrafiltered plasma, 19.0%±3.4 for parotid saliva and 14.3%±2.4 for whole saliva. Table 2 shows statistically significant correlations between total and free PHT concentrations in plasma and saliva. Correlations were higher between PHT concentrations in plasma and in parotid rather than whole saliva.

Data for salivary flow rate and composition are presented in Table 3. The calcium (Ca) and inorganic phosphate (Pi) concentrations for the PHT-administered group were significantly higher than those of the control group ($p<0.001$), while no significant changes in unstimulated flow rate, pH and the concentrations of the other components were found.

Tabel 4 shows a comparison of the Ca and Pi concentrations in the plasma of the control and PHT administered subjects. The mean Ca concentration for the PHT-administered group was slightly, but statistically not significantly lower than that of the controls, which fell in the

Table 3 Comparison of Compositions of Unstimulated Whole Saliva (Mean ± S. D.) in Control Subjects and Those Receiving Chronic Administration of PHT

	Control	N	PHT Adminst.	N	Sig.
Unstim. flow rate(ml/min)	0.68 ± 0.24	36	0.53 ± 0.36	36	
pH	7.41 ± 0.36	36	6.97 ± 0.43	36	
Pco_2 (mm Hg)	14.20 ± 6.50	32	15.10 ± 5.40	30	
Na (mEq/L)	14.00 ± 10.10	35	15.00 ± 8.90	31	N.S.
K (mEq/L)	19.70 ± 4.28	35	23.00 ± 4.20	31	
Mg (mEq/l)	0.64 ± 0.31	31	0.55 ± 0.26	30	
Cl (mEq/l)	20.20 ± 11.96	35	16.70 ± 9.56	34	
ALP (IU/L)	3.60 ± 2.10	29	2.60 ± 1.30	30	
Ca (mmol/l)	1.10 ± 0.80	35	2.40 ± 1.00	35	p<0.001
Pi (mmol/l)	3.40 ± 1.30	35	6.20 ± 2.20	34	

N.S. = not significant

Table 4 Comparison of Calcium and Inorganic Phosphate Concentrations (Mean ± S. D.) in the Plasma of Control Subjects and Those Receiving Chronic Administration of PHT

	Control	N	PHT Adminst.	N	Sig.
Ca (mmol/l)	2.3 ± 0.2	35	2.1 ± 0.2	36	N.S.
Pi (mmol/l)	1.1 ± 0.1	35	1.0 ± 0.1	36	N.S.
Ca x Pi $(mmol/l)^2$	2.5 ± 0.7	35	2.1 ± 0.5	36	p<0.02

N.S. = not significant

normal range (Ca ; 2.3-2.5 mM)[23]. The Pi concentrations for both groups were within the normal range (Pi ; 0.7-1.4 mM)[23]. There were no significant differences in the mean concentrations of plasma Ca and Pi between the two groups, but the product of the two values was significantly lower in the PHT-administered group than in the control group ($p<0.02$).

Discussion

The results of this study demonstrate that measurements of salivary phenytoin concentrations provide an index of the free plasma level of PHT. These findings support the concept that the non-protein-bound (free) fraction of this drug is secreted by the salivary glands and remains in equilibrium with the free drug in plasma [15-18,24]. Killmann and Thaysen[25] and Borzelleca and Putney[26] have reported that the rate of diffusion across lipid membranes depends on lipid solubility characteristics and on the degree of ionization of the drug. Since PHT has an ionization constant(pKa) of 9.2[27] and the pH of plasma is about 7.4 and that of saliva between 6-8[28] depending on flow rate, very little drug should be ionized in either fluid. Because PHT can be considered to possess good lipid solubility characteristics[27], the movement of PHT across lipid membranes would not be hindered.

Paxton et al.[17] have studied the relationship between the salivary concentration of PHT and flow rate and concluded that the concentration of PHT in saliva was independent of flow rate. The results of our study support their findings.

The apparent association between long-term PHT therapy and hypocalcaemia and osteomalacia was first reported by Kruse[5]. Current evidence indicates that reduced 25-hydroxycholecalciferol concentrations observed in this disorder are due to increased hepatic microsomal catabolism of vitamin D and 25-hydroxycholecalciferol or to a direct inhibition of the hepatic conversion of vitamin D to 25-hydroxycholecalciferol[8]. Normally the concentrations of serum Ca and Pi are inversely correlated, which keeps the product relatively constant[29]. Once osteomalacia has occured as a result of a deficiency of vitamin D, the concentrations of serum Ca and Pi are reduced, accompanied by elevated serum alkaline phosphatase[30]. In our subjects, although the mean concentration of plasma Pi was within the normal range, the mean concentration of plasma Ca was slightly, but not significantly, lower but the products of the Ca and Pi concentrations fell significantly below the normal range and those of the control group ($p<0.02$) (Table 4).

Although there have been many studies of the effect of PHT on gingival overgrowth[31-33] there have been few studies of its effects on salivary constituents. Davis[34] reported that parotid salivary flow rates for fifteen cerebral palsy patients were significantly lower than those of a control group ($p<0.001$). However, the salivary composition was within the normal range when flow rate was considered. Our investigation on whole saliva flow rates showed no significant difference between the PHT-administered group and the control group. The most extensive survey of the Ca and Pi concentrations and flow rate of whole saliva were carried out

by Becks & Wainwright [35,36]. They studied over 600 subjects and found that the mean Ca and Pi concentrations were 5.8 mg/dl (1.5 mM) (range 2.2-11.3 mg/dl) and 16.8 mg/dl (5.4 mM) (range 6.1-71.0 mg/dl), respectively, and that mean flow rate was 19 ml/hour (0.32 ml/min.) (range 0.5-111 ml/hour). There is great variation in these values, but more recent figures, while giving similar average values, do not show such wide variation[37]. As the composition of saliva varies especially with flow rate[38-40], it is very important to consider the effects of flow rate on saliva composition. Becks & Wainwright[41] also found that the unstimulated whole saliva of slow secretors contained slightly higher Ca and considerably higher Pi concentrations than those of rapid secretors. As compared with their report[41], the flow rates in both the PHT-administered group and the control group were higher (0.53 ml/min ± 0.36, 0.68 ml/min ± 0.24, respectively). This could explain the lower Ca and Pi concentrations in whole saliva of the control group (1.1 mM ± 0.8, 3.4 mM ± 1.3, respectively). However, despite the higher flow rates, the Ca and Pi concentrations in whole saliva of the PHT-administered group showed higher values (2.4 mM ± 1.0, 6.2 mM ±2.2, respectively) than in the controls or in those reported by Becks & Wainwright[35,36].

The mechanisms for the effect of PHT on whole salivary Ca and Pi in our study are not clear. Hassell et al.[33] have detected a positive correlation between gingival overgrowth severity and calculus accumulation in 77 institutionalized persons taking PHT. Our results suggest that effects of PHT in increasing salivary calcium and phosphate levels may be one factor responsible for the accumulation of calculus in the mouths of those patients receiving PHT who also have poor oral hygiene.

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Phenytoinの唾液成分に及ぼす影響について

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抄　録

抗てんかん剤の長期服用は，歯肉増殖を誘発したり，体内の無機質，ビタミンD代謝に障害を及ぼすことが一般によく知られている。今回我々は，phenytoin（PHT）の唾液中成分に及ぼす影響について検討を行った。

施設にて長期間PHTを服用している36人の重症心身障害児より，血漿と混合唾液を採取した。血漿の一部は超濾過し，ELISAにてPHT濃度を測定した。血中および混合唾液中の成分を36人の健康人と比較した結果，唾液中のカルシウムと無機リンは，コントロール群に比較し，著しく高い値を示した。一方，安静時唾液，PH，その他の成分については差は認められなかった。血漿中カルシウムと無機リンの比較では，両グループに差はみられなかったが，カルシウムとリンの積の比較では，PHT投与群の方が低い値を示した。

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