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INFLUENCE OF MONTMORILLONITE ON THE DISSOLUTION AND BIOAVAILABILITY OF PHENYTOIN

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Key words: Dissolution of phenytoin-montmorillonite mixtures; improvement of dissolution rate of phenytoin; solvent deposition of phenytoin on montmorillonite. Bioavailability of phenytoin; Influence of montmorillonite on the absorption of phenytoin.

ABSTRACT

The dissolution of phenytoin were studied from various phenytoin-montmorillonite combinations. Firstly, phenytoin-montmorillonite combinations were obtained by precipitating phenytoin from two different solvents on to montmorillonite using drug to montmorillonite ratios. Secondly, physical mixtures of phenytoin and montmorillonite were prepared in four different ratios. The twelve phenytoin-montmorillonite combinations were compressed into tablets and the dissolution rates were determined. The dissolution rates of phenytoin from phenytoin sodium and phenytoin were determined and compared with the dissolution rates of the combinations. The montmorillonite increased the dissolution rate

of phenytoin from all the combinations to such an extent that the dissolution rates compared well with those obtained from phenytoin sodium.

The bioavailability of phenytoin from three different phenytoin-montmorillonite mixtures were compared with the bioavailability from a phenytoin sodium capsule in four volunteers. More phenytoin were absorbed from the phenytoin-montmorillonite mixtures than from the phenytoin sodium. The absorption rate of phenytoin from the three different montmorillonite mixtures were faster than from the phenytoin sodium capsule for the first hour, after administration. After one hour the phenytoin blood levels from the phenytoin-montmorillonite mixtures started to level of to reach lower peak concentrations in plasma than that obtained from phenytoin sodium. The total amount phenytoin absorbed from two of the phenytoin-montmorillonite mixtures (1:1 and 1:9 ratios) was more than that absorbed from phenytoin sodium. The advantages of combining phenytoin and montmorillonite for the improvement of the bioavailability of phenytoin have clearly been demonstrated.

INTRODUCTION

The dissolution rates of poorly water soluble drugs can be increased by adsorption to finely divided solids. Increased dissolution rates have been reported for various drugs adsorbed to montmorillonite, for example: griseofulvin[1], indomethacin[1], prednisone[1], chlorpheniramine maleate[2], propoxyphene hydrochloride[2], amphetamine sulphate[2], chlorpropamide[3], tolbutamide[3], acetohexamide[3] and metronidasole[4]. In general, the dissolution rates tended to increase with an increasing ratio of montmorillonite.

Phenytoin, an anti-epileptic agent, has some physicochemical and pharmacokinetic properties which make it liable to bioavailability problems[5]. It has been classified as a drug with a high risk potential

with respect to bioavailability problems. To improve the dissolution rate of phenytoin and thus the bioavailability, extensive research has been done over the past two decades. Various techniques were used to improve the dissolution rate of phenytoin by (a) increasing the surface area available for dissolution by solid dispersions using polyethylene glycol (PEG) 4000[6] and PEG 6000[7], (b) changing the crystalline form to the amorphous form[8,9,10,11,12,13] (c) changing the original drug to a more soluble salt[10], (d) complexation formation with cyclodextrins[13,14] and (e) improving the wettability by using surface active agents[15].

Although many studies on the adsorption of poorly water soluble drugs on montmorillonite have been done no studies on the influence of montmorillonite on the properties of phenytoin has been done.

The purpose of this paper was (a) to investigate the influence of montmorillonite on the dissolution rate of phenytoin from various phenytoin-montmorillonite combinations and (b) to compare the absorption of phenytoin from phenytoin-montmorillonite mixtures with the absorption from a phenytoin sodium capsule after oral administration to human volunteers. The aim with the phenytoin-montmorillonite mixtures was to obtain a fast absorption of phenytoin and producing longer blood levels over a longer time span than obtained with phenytoin sodium.

EXPERIMENTAL

Reagents and materials - The following materials were used: phenytoin[16], phenytoin sodium[17], montmorillonite[18], and microcrystalline cellulose[19]. The two solvents (acetone and methanol) were of analytical grades and were used as received. Commercial available phenytoin sodium capsules (100 mg)[17].

Precipitation of phenytoin on montmorillonite - One gram of pure phenytoin was dissolved in 200 ml solvent (methanol or acetone). An

accurately weighed amount of montmorillonite was suspended in the drug solution and stirred. The solvent was removed in a rotovap at 35°C, under a slight vacuum. The precipitate was dried at 45°C to a constant weight and passed through a #100-mesh sieve. By varying the quantity of montmorillonite, adsorbates were prepared to contain drug-montmorillonite ratios of 1:1; 1:4; 1:9 and 1:20 (weight by weight).

Preparation of the physical mixtures of phenytoin and montmorillonite - Phenytoin and montmorillonite were mixed in the ratios of 1:1; 1:4; 1:9; and 1:20 in a Turbula-mixer for five minutes.

Compression of tablets - Tablets which contained 15 mg of pure drug were compressed from the phenytoin precipitates and the physical mixtures, after mixing with a constant ratio microcrystalline cellulose.

Disintegration - The disintegration test of the British Pharmacopoeia (1980) was used for all the tablets as well as for the commercially available capsule.

Dissolution - The dissolution studies were conducted with the USP XII Apparatus 2. The tablet, or tablets in the case of the 1:20 drug-montmorillonite combinations, was added to the dissolution beaker containing 900 ml of purified water at 37°C and stirred at 50 rpm. Five milliliter samples were withdrawn at various time intervals and assayed for drug content using high pressure liquid chromatography (United States Pharmacopoeia, XXI, 1985, 4th Supplement). To maintain sink conditions, 5 ml of fresh medium were added after the removal of each sample. The dissolution determination for each phenytoin product was done in duplicate.

Scanning electron micrograph (SEM)-photos[20] were taken and **X-ray diffraction grams** and **differential scanning calorometry thermograms** were recorded of the pure phenytoin crystals and the phenytoin crystals after recristallisation from acetone or methanol.

Volunteers and dosage - the four females who participated in this study were normal healthy volunteers as determined by medical examination and

routine laboratory tests. The volunteers received the following treatments during a complete cross-over trial as single doses containing 100 mg phenytoin:

Treatment a = one capsule containing phenytoin sodium equivavalent to 100 mg phenytoin as a commercially available product (Lennon (Pty) Ltd). This capsule had a comparable bioequivalence to that of Epanutin capsules (Parke Davis)[21].

Treatment b = two phenytoin-montmorillonite tablets of 375 mg each (1:1 ratio of phenytoin to montmorillonite) equivalent to 100 mg phenytoin.

Treatment c = four phenytoin-montmorillonite tablets of 500 mg each (1:4 ratio of phenytoin-montmorillonite) equivalent to 100 mg phenytoin.

Treatment d = six phenytoin-montmorillonite tablets of 500 mg each (1:9 ratio of phenytoin to montmorillonite) equivalent to 100 mg phenytoin.

Trial volunteers were fasted for twelve hours before the trial.

The treatment was administered with 250 ml of water.

Sample collection - 5 ml venous blood samples were obtained on the following times after administration: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 4,0; 5,0; 6,0; 8,0; 10,0; 12,0; 24,0; 36,0; 48,0 and 72,0 hours. The plasma samples obtained were frozen until analysis.

Analysis of plasma samples - The plasma samples were analysed with assayed using a Abbott TDx apparatus[21].

Calculation of bioavailability parameters - the accepted bioavailability parameters were calculated from the phenytoin plasma concentrations. The area under the drug concentration versus time curve (AUC) was

calculated with the trapezoidal rule. The peak drug concentration in plasma (Cmax) and the time to reach this peak (tmax) was taken from the raw data.

RESULTS AND DISCUSSION

The scanning electron micrograph (SEM) photos showed a size difference between the precipitated crystals from acetone and methanol. The phenytoin precipitated from both solvents was not arranged in an orderly fixed pattern on the surface of the montmorillonite. X-ray diffractograms indicated that there were no differences in the crystal structure of the pure phenytoin crystals and those recrystallised from the solvents could be detected by the differential scanning calorometry thermograms. This indicated that no chemical interaction between phenytoin and montmorillonite occured that could be detected with this technique[23].

The mean disintegration times of the 1:1 ratio phenytoin-montmorillonite tablets and the capsule complied with the B.P. (1980) specifications. The other disintigration times varied between 16 and 25 minutes. The more montmorillonite present in a tablet the longer the disintegration time.

The results of the dissolution tests of the tablets of the precipitates and physical mixtures were compared with the dissolution of a phenytoin sodium capsule and a reference tablet containing only phenytoin and microcrystalline cellulose. These results can be seen in figures 1 to 4.

The dissolution rate of the phenytoin-montmorillonite precipitates prepared from acetone and methanol as well as the physical mixture of phenytoin and montmorillonite differed significant from the dissolution rate of the reference tablet containing pure phenytoin (15 mg) and microcrystalline cellulose. It is thus clear that the presence of montmorillonite improves the dissolution properties of phenytoin (see figures 2 to 4).

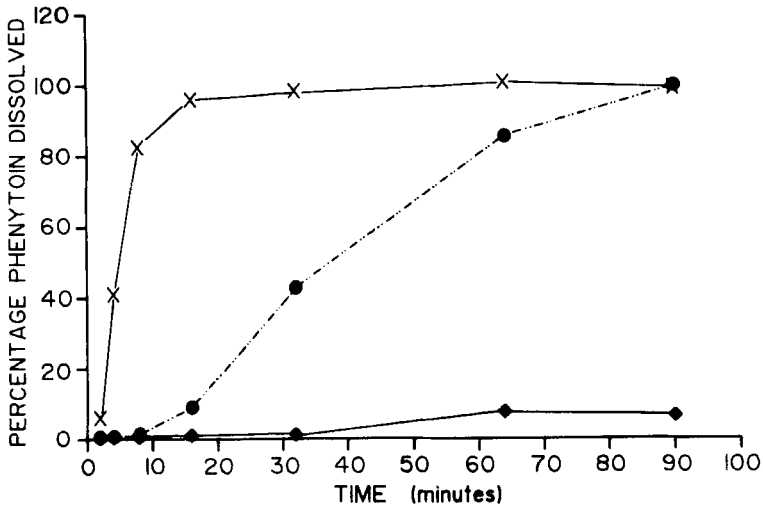

FIGURE 1 PERCENTAGE PHENYTOIN DISSOLVED FROM A PHENYTOIN TABLET (—◆—), PHENYTOIN SODIUM CAPSULE (- ● -) AND A PHENYTOIN SODIUM TABLET (—x—).

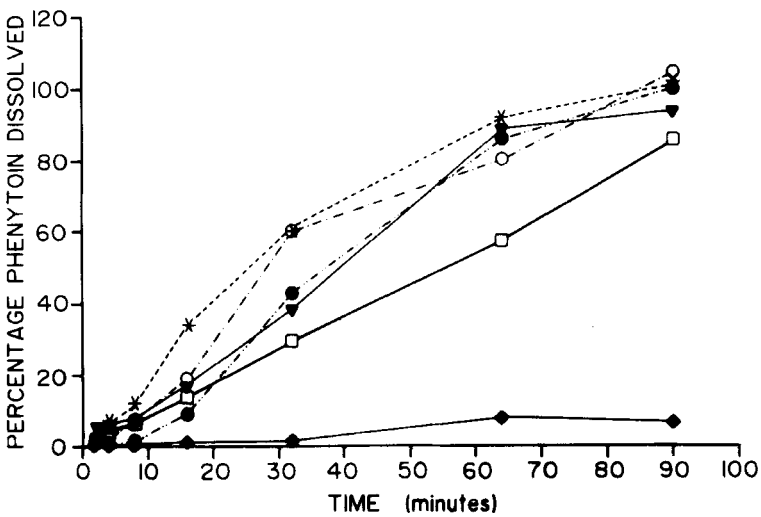

FIGURE 2 PERCENTAGE PHENYTOIN DISSOLVED FROM THE 1:1 (—▼—), 1:4 (--*--), 1:9 (—□—) AND 1:20 (-○-) ACETONE ADSORBATES AS WELL AS THE PHENYTOIN TABLET (—◆—) AND THE PHENYTOIN SODIUM CAPSULE (- ● -).

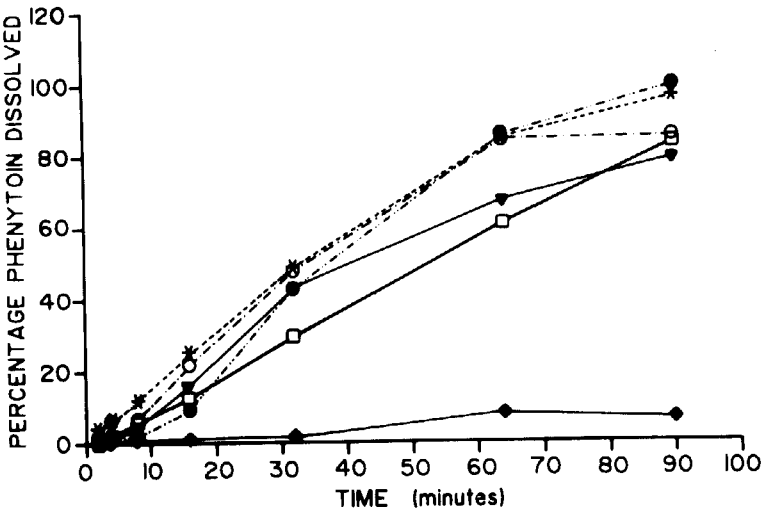

FIGURE 3 PERCENTAGE PHENYTOIN DISSOLVED FROM THE 1:1 (—▼—), 1:4 (--*--), 1:9 (—□—) AND 1:20 (-·○·-) METHANOL ADSORBATES AS WELL AS THE PHENYTOIN TABLET (—◆—) AND THE PHENYTOIN SODIUM CAPSULE (-·●·-).

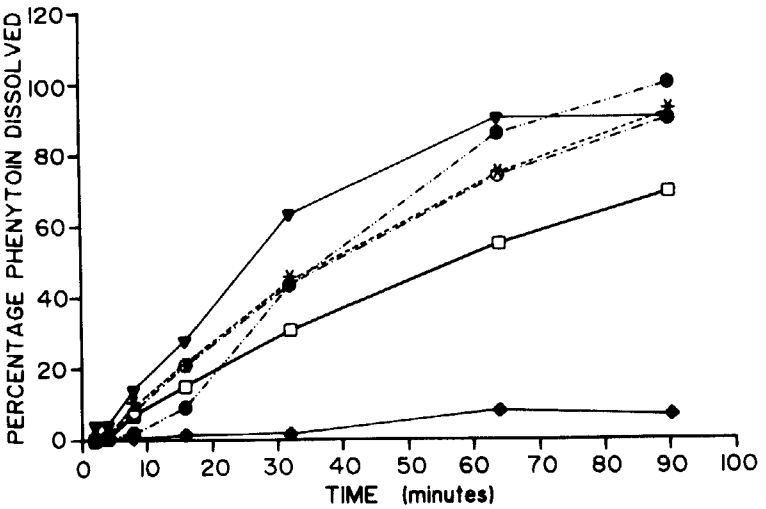

FIGURE 4 PERCENTAGE PHENYTOIN DISSOLVED FROM THE 1:1 (—▼—), 1:4 (--*--), 1:9 (—□—) AND 1:20 (-·○·-) PHYSICAL MIXTURES AS WELL AS THE PHENYTOIN TABLET (—◆—) AND THE PHENYTOIN SODIUM CAPSULE (-·●·-).

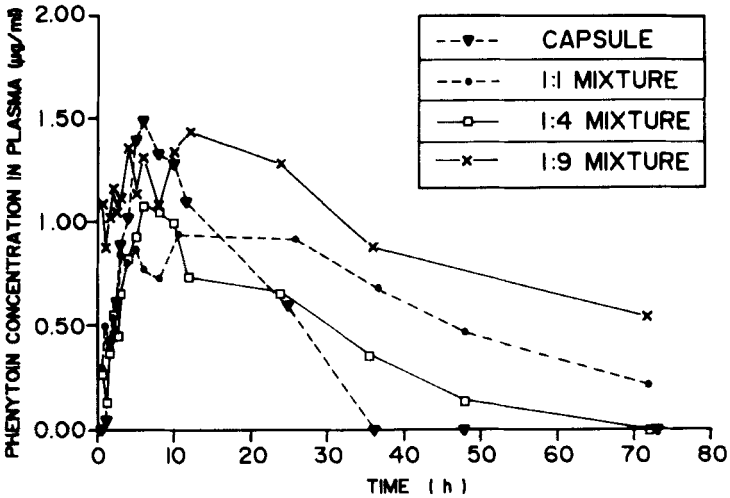

FIGURE 5: Mean phenytoin concentration in plasma for four volunteers after administation of 100 mg phenytoin as phenytoin-montmorillonite mixtures (1:1; 1:4; 1:9) and as a commercial capsule

To evaluate the differences between the dissolution properties of the various ratios montmorillonite present the Student-Newman-Keuls multiple comparison test was used[24].

The 1:9 ratio of the precipitates and the physical mixture had the slowest dissolution in comparison with the other ratios. Considering the dissolution profiles of the 1:1, 1:4 and 1:20 ratios, there was no clear correlation between the amount of montmorillonite present in the mixtures and the improvement in the dissolution rate. However, all the ratios of the acetone and methanol precipitates and the physical mixtures, showed a significant increase ($p = 0,05$) in the amount dissolved after 32 minutes compared to the reference tablet (phenytoin and microcrystalline cellulose) after 32 minutes.

In figure 5 the mean phenytoin concentrations in plasma for the four volunteers after administration of the different treatments are plotted versus time.

TABLE 1: MEAN BIOAVAILABILITY PARAMETERS FOR THE DIFFERENT PHENYTOIN TREATMENTS

PARAMETER	TREATMENT			
	Capsule	Phenytoin-montmorillonite mixture		
		1:1	1:4	1:9
tmax	4,36 (1,96)*	9,13 (1,47)	6,85 (3,45)	7,71 (4,33)
Cmax	2,30 (0,71)	1,54 (0,52)	1,27 (0,29)	1,93 (0,50)
AUC	46,58 (17,10)	64,64 (16,43)	43,37 (9,22)	70,72 (13,98)

* Standard deviation in brackets.

The appropriate bioavailability parameters were obtained by non-compartmental methods and in table 1 the mean bioavailability parameters calculated for the different treatments are listed with the standard deviation in brackets.

The bioavailability ratios of the different parameters of the 1:1, 1:4 and 1:9 mixtures were determined with the phenytoin sodium capsule as reference. A statistical comparison between the different treatments were done according to the interval method[25] with a Bonferroni-correction[26]. The 90% confidence intervals for the different bioavailability ratios are given in table 2.

The tmax for the phenytoin sodium was much faster than for any of the tmax from the phenytoin-montmorillonite mixtures. The absorption rates of phenytoin form the phenytoin-montmorillonite mixtures were faster for the first hour after administration than the absorption rate from

TABLE 2: NON-SYMMETRICAL 90% CONFIDENCE INTERVALS FOR THE MEAN BIOAVAILABILITY RATIOS OF THE PHENYTOIN-MONTMORILLONITE MIXTURES TO THE PHENYTOIN SODIUM

PARAMETER	LOWER LIMIT	UPPER LIMIT
tmax 1:1	0,89	6,16
tmax 1:4	0,62	4,31
tmax 1:9	0,66	4,60
Cmax 1:1	0,44	1,00
Cmax 1:4	0,33	1,85
Cmax 1:9	0,56	1,28
AUC 1:1	0,84	2,42
AUC 1:4	0,57	1,63
AUC 1:9	0,93	2,67

TABLE 3: MEAN BIOAVAILABILITY RATIOS OF THE PHENYTOIN-MONTMORILLONITE MIXTURES TO THE PHENYTOIN SODIUM

PARAMETER	MEAN RATIO
tmax 1:1	2,66 (1,72)*
tmax 1:4	1,85 (1,00)
tmax 1:9	1,92 (0,80)
Cmax 1:1	0,67 (0,10)
Cmax 1:4	0,57 (0,10)
Cmax 1:9	0,87 (0,20)
AUC 1:1	1,48 (0,50)
AUC 1:4	0,99 (0,32)
AUC 1:9	1,62 (0,39)

* Standard deviation in brackets.

phenytoin sodium (see figure 5). The phenytoin concentration in plasma for the phenytoin-montmorillonite mixtures reached a plato after 1 to 2 hours and peak later and at a lower concentration than the phenytoin sodium.

The mean bioavailability ratios of the 3 parameters of the phenytoin-montmorillonite mixtures with the capsule as reference are given in table 3.

CONCLUSION

1. The in vivo tests of the different phenytoin-montmorillonite mixtures showed that:

   a. the rate of absorption of phenytoin was comparable to the rate of absorption obtained from the phenytoin sodium capsule,

   b. the amount of phenytoin absorbed from all the mixtures was more than that absorped from the capsule, except for the 1:4-mixture (which was slightly less), and

   c. lower maximum phenytoin concentrations were found after administration of the phenytoin-montmorillonite mixtures.

2. Over all the bioavailability of phenytoin was improved by using phenytoin-montmorillonite mixtures thus decreasing irratic absorption.

3. The in vitro results confirmed what happened in vitro. Dissolution studies showed that phenytoin dissolved much faster from the phenytoin-montmorillonite mixtures than from pure phenytoin, but it showed a comparable dissolution with the phenytoin sodium capsule.

4. From the results of this study, the following possibilities came forward:

   a. Phenytoin-montmorillonite mixtures can be used instead of phenytoin sodium.

   b. Because phenytoin-montmorillonite mixtures resulted in phenytoin concentration that lasted over a longer period than with phenytoin sodium, the possibility exists that lower doses and longer dosage intervals can be used.

RECOMMENDATIONS

1. It was clear that montmorillonite improves the dissolution and absorption of phenytoin and therefore further study regarding the influence of montmorillonite on the dissolution and absorption of other poorly water soluble drugs can be justified.

2. Research can be done on the in vivo behavior of phenytoin after multiple dosing of the phenytoin-montmorillonite mixtures to determine the blood levels of phenytoin at steady state from the montmorillonite mixtures.

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