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In vivo–in vitro correlations of salicylate saliva levels and continuous flow cell dissolution rates

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Summary

The dissolution rate of a standard tablet containing acetylsalicylic acid was measured with a continuous flow cell method. The same tablet was given to 14 subjects and salicylate saliva levels were monitored. Using the Wagner-Nelson method, the absorption kinetics were determined to follow a first-order process. The absorption rate constant was calculated and compared with the dissolution rate constant of the in vitro experiment. Under the same conditions 3 other tablets containing acetylsalicylic acid were investigated. From the obtained dissolution data the salicylate saliva levels were predicted and compared with the measured concentrations. Their good agreement indicated the ability of continuous flow systems to give valid information for quality control and tablet formulation screening.

Introduction

The primary purposes of in vitro dissolution studies are to aid in screening tablet formulations and to ensure lot-to-lot uniformity in manufacturing. Furthermore, if the in vitro data can be correlated with results from in vivo absorption experiments and if this correlation can be described mathematically, dissolution data might be used to predict the time course of drug concentrations in the body.

Many different dissolution models have been described in the literature. Continuous flow cell methods have often been shown to be more differentiating than other methods (Tingstad and Riegelman, 1970; Dibbern and Wirbitzky, 1971) and give good in vitro–in vivo correlations for different drugs (Gröning, 1980; Steinbach et al., 1978; Steinbach et al., 1980). In the present study a continuous flow cell

(Dibbern and Wirbitzky, 1971) with a modified set up (Derendorf and Rohdewald, 1981) was used to predict salicylate saliva levels for acetylsalicylic acid tablets with different dissolution profiles. Saliva was chosen as the in vivo reference fluid as it reflects the free, non-protein-bound salicylate level. Monitoring salicylate levels in saliva may provide a simple non-invasive technique that was proposed to be routinely used in patients with rheumatoid arthritis (Dromgoole and Furst, 1980).

Materials and Methods

Subjects

Every drug was given to a group of 14 volunteers aged 21–36 years. In addition to that two preparations (tablets C and D) were also given to another group of 14 volunteers in the same age range. All subjects were informed about the experimental protocol and signed an informed consent form.

Investigated tablets

The 4 different tablets studied, their composition and the manufacturers are listed in Table 1.

Drug application

Each tablet was evaluated in a double-blind, cross-over design. The volunteers swallowed the tablets with a total of 200 ml water and rinsed their mouths to make sure that no drug remained there that could interfere with the saliva analysis. The subjects were not allowed to take part in another experiment for at least 48 h.

Saliva level monitoring

Saliva samples were taken just before drug application and at 20, 40, 60, 90, 120

TABLE 1

COMPOSITION AND MANUFACTURERS OF THE INVESTIGATED TABLETS

Code	Compounds in one tablet	No. of tablets given	Manufacturer
A	500 mg acetylsalicylic acid	1 and 2	Bayer AG, 5090 Leverkussen
B	250 mg acetylsalicylic acid	4	Togal AG, 8000 München
	42 mg lithium citrate		
	1.5 mg quinine dihydrochloride		
C	250 mg acetylsalicylic acid	2	Thomae GmbH, 7950 Biberach
	200 mg paracetamol		
	50 mg caffeine		
D	250 mg aluminium salt of acetylsalicylic acid	2	Mack, 7918 Illertissen
	200 mg paracetamol		
	50 mg caffeine		

and 150 min after drug intake. During the sample time electrical stimuli were applied to the teeth of the subjects to elicit pain and measure the analgesic activity of these drugs (Rohdewald et al., 1982). A side-effect of this procedure was a stimulation of saliva flow that supported sampling. The assumption was made that the artifical production of pain and saliva flow did not affect the absorption kinetics. The subjects emptied their mouths into glass vials. The samples were frozen at −20°C until analyzed by high-performance thin-layer chromatography (HPTLC).

Dissolution method

The dissolution method used has been described in detail before (Derendorf and Rohdewald, 1981). In a water bath of 37°C continuous flow cells (Dibbern and Wirbitzky, 1971) rotate for 360° forth and back with 1.2 cpm. The solvent used was gastric fluid USP XX, the flow rate 15 ml/min. Fractions were sampled at 3, 6, 9, 12, 18, 24, 30, 45 and 60 min and analyzed by HPTLC. Six tablets from 3 different lots of each of the four preparations were individually investigated. Before the dissolution experiment 15 tablets from the same lots have been tested for aspirin content. All tablets showed aspirin content with less than ±5% deviation of the label (Derendorf and Rohdewald, 1981).

Chromatography

The analytical method used for the dissolution experiments as well as for the saliva monitoring was quantitative high-performance thin-layer chromatography (Drehsen and Rohdewald, 1981; Derendorf and Rohdewald, 1981). Salicylic acid was measured either by in situ remission at 230 nm or by fluorescence with an excitation wavelength of 314 nm and an emission wavelength of 390 nm. The quantitative analysis of the chromatograms was done by integration of the peaks and calibration curves of standard solution chromatographed on the same plate.

Results

Evaluation of the absorption profile of a standard

Tablet A is the most common preparation containing acetylsalicylic acid and therefore was considered as the standard tablet of this study. In the first step it was investigated whether there is a correlation of the dissolution profile of tablet A and the rate of absorption. To get more information about the kinetics of the absorption the saliva data were analyzed by the method of Wagner and Nelson (1963). This method enables to isolate the absorption process for a one-compartment-body model under the assumption of first-order elimination kinetics. In the investigated dose range this assumption was accepted, the elimination constant k_e for salicylic acid is 0.0015 min^{-1} (Dromgoole and Furst, 1980). With the Wagner-Nelson method the fraction of the dose, that was absorbed at the times of saliva sampling, was calculated:

$$f_t^{abs} = \frac{A_t^{abs}}{D_0} = \frac{A_t^b + A_t^{el}}{D_0} = \frac{c_t \cdot V_b + k_e \cdot V_b \cdot AUC_t}{D_0} = \frac{V_b}{D_0}(c_t + k_e AUC_t) \quad (1)$$

where f_t^{abs} is the fraction of the dose absorbed at time t, A_t^{abs} is the amount of the drug absorbed at time t, D_0 is the dose, A_t^b is the amount of the drug in the body at time t, A_t^{el} is the amount of the drug already eliminated from the body at time t, c_t is the saliva concentration of the drug at time t, V_b is the volume of distribution referenced to the saliva analysis, k_e is the first-order elimination rate constant for salicylic acid (0.0015 min^{-1}) and AUC_t is the area under the saliva level–time curve up to time t. The volume of distribution V_b referenced to saliva analysis can be calculated under the assumption that the relative bioavailability of the standard is 100%. As $f_{t=\infty}^{abs}$ equals 1 and $C_\infty = 0$, Eqn. 1 can be rearranged for $t = \infty$ to calculate V_b:

$$V_b = \frac{D_0}{k_e \cdot AUC_\infty} \tag{2}$$

The results are given in Table 2 for the saliva data measured after application of two different doses of preparation A. They indicate that absorption profile of A is apparent first-order and can reasonably be fitted with an absorption rate constant k_a of 0.024 min^{-1} (Fig. 1A). The absorption curves for both doses are superimposable which is a strong indication that in the investigated dose range the pharmacokinetics follow first-order processes. The area under the curve at infinite time (AUC_∞) times k_e (see Table 2) approaches 0.75 $\mu g \cdot ml^{-1}$ and 1.5 $\mu g \cdot ml^{-1}$, respectively, for the 500 mg and 1000 mg doses. The volume of distribution V_b, referenced to saliva is 666 l (Eqn. 2).

TABLE 2

CALCULATION OF THE FRACTION OF THE DOSE THAT WAS ABSORBED AT THE TIMES OF SALIVA SAMPLING (f_t^{abs}) FOR 2 DIFFERENT DOSES OF TABLET A USING THE WAGNER-NELSON METHOD (SEE EQN. 1 IN THE TEXT)

Time t (min)	C_t ($\mu g \cdot ml^{-1}$)	AUC_t ($\mu g \cdot ml^{-1} \cdot min$)	$k_e \cdot AUC_t$ ($\mu g \cdot ml^{-1}$)	$C_t + k_e AUC_t$ ($\mu g \cdot ml^{-1}$)	f_t^{abs}
(a) Dose D_0 = 500 mg					
20	0.28	2.8	0.0042	0.2842	0.329
40	0.43	9.9	0.0149	0.4449	0.593
60	0.51	19.3	0.0290	0.5390	0.719
90	0.45	33.7	0.0506	0.5006	0.667
120	0.63	49.9	0.0749	0.7049	0.940
150	0.50	66.9	0.1003	0.6003	0.800
(b) Dose D_0 = 1000 mg					
20	0.50	5.0	0.0075	0.5075	0.338
40	0.82	18.2	0.0273	0.8473	0.565
60	1.08	37.2	0.0558	1.1358	0.757
90	1.28	72.6	0.1089	1.3889	0.926
120	1.40	112.8	0.1692	1.5692	1.046
150	1.14	150.9	0.2264	1.3664	0.911

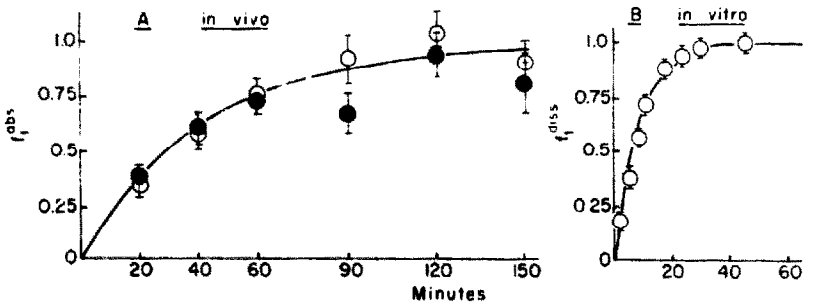

Fig. 1. A: in vivo absorption profile of 1 tablet A (●) and 2 tablets A (○) calculated from salicylate saliva levels using the Wagner-Nelson method (means of 14 subjects ± S.E.M., see Eqn. 1 in the text). B: in vitro dissolution profile of tablet A calculated from the amount drug dissolved at certain times (means of 6 tablets ± S.E.M., see Eqn. 3 in the text).

Calibration of the in vitro data with the standard

Fig 1B shows the dissolution profile of tablet A in the continuous flow cell system. The dissolution process can be interpreted to be first-order and was fitted by non-linear regression with a dissolution rate constant $k_{in\,vitro}$ of 0.115 min^{-1}. The fraction of the drug dissolved at time t, f_t^{diss}, can be calculated

$$f_t^{diss} = f_\infty^{diss}(1 - e^{-k_{in\,vitro} \cdot t}) \qquad (3)$$

For the standard preparations the in vitro dissolution process appears to be 4.8 times faster than the in vivo absorption process. This correlation was taken to

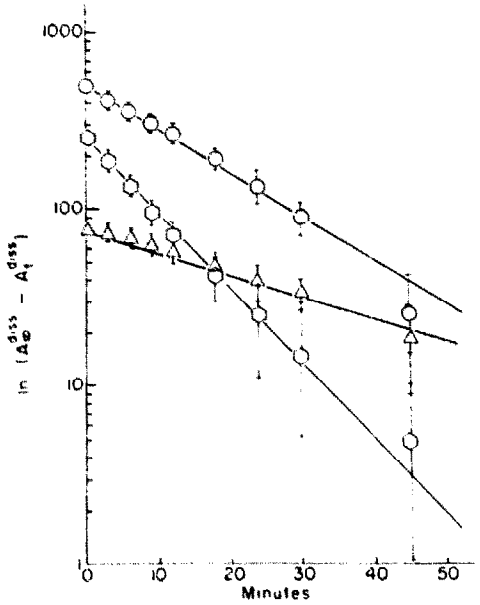

Fig. 2. Plots of the amounts to be dissolved against time for tablets B (○), C (o) and D (△) to prove first-order kinetics and determine the dissolution rate constant $k_{in\,vitro}$ (means of 6 tablets ± S.E.M., see Eqn. 4 in the text)

evaluate the results of 3 other tablets that were investigated under exactly the same conditions.

Evaluation of the in vitro results for the test tablets

To compare the results of standard and test tablets it is important to make sure that also the dissolution profile for the test tablets follows first-order kinetics. In a logarithmic plot of the difference between the amount dissolved at infinity, A_∞^{diss}, and the amount dissolved at time t, A_t^{diss}, against time a straight line indicates that this assumption is true.

$$\ln\left(A_\infty^{diss} - A_t^{diss}\right) = A_\infty^{diss} - k_{invitro} \cdot t \tag{4}$$

The dissolution data for the tablets B, C and D can be fitted by straight lines (Fig. 2). From the slope of these graphs it is possible to determine $k_{invitro}$: 0.0614 min^{-1} for B; 0.0920 min^{-1} for C and 0.0315 min^{-1} for D. The intercept gives the amount dissolved at infinity and equals the dose for B and C. For tablet D the intercept is only 75 mg, which means that either the drug is not dissolvable under the conditions of the experiment or the dissolution profile cannot be described sufficiently by a simple first-order term.

Prediction of the in vivo saliva level from the in vitro dissolution data

Knowing the in vitro dissolution rate constant, $k_{invitro}$, for the test tablets and regarding the correlation between $k_{invitro}$ and the apparent absorption constant k_a for the standard it is now possible to predict an apparent absorption constant also for B, C and D. With this information it is possible to predict the saliva level curve as the saliva concentration can be calculated according to Eqn. 5

$$C_t = \frac{f_\infty^{abs} \cdot D_0 \cdot k_a}{V_b \cdot (k_a - k_e)} \cdot \left(e^{-k_e t} - e^{-k_a t}\right) \tag{5}$$

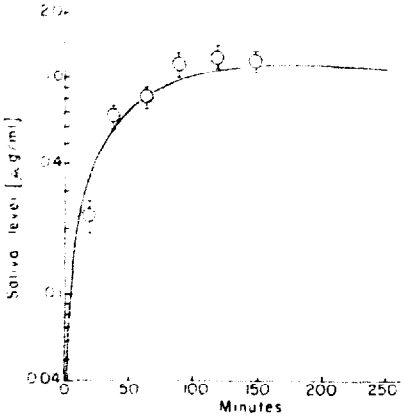

Fig. 3. Salicylate saliva levels after application of 4 tablets B (○) and predicted saliva level–time curve calculated from the in vitro dissolution data (means of 14 subjects ± S.E.M., see Eqn. 5 in the text).

Fig. 3 compares the calculated saliva level curve with the experimental data for tablet B (1000 mg). Reasonable agreement for predicted and measured levels is obtained ($r = 0.983$). Also in the case of the 500 mg dose of tablet C a good prediction can be made by calculating the absorption constant from the in vitro dissolution rate constant (Fig. 4).

For tablets B and C the total amount of acetylsalicylic acid goes into solution, whereas for D the extrapolated value from the intercept of Fig. 2 is only 75 mg per tablet. This is equivalent to a fraction of the dose of 0.33 as 250 mg aluminum acetylsalicylate contain 224 mg acetylsalicylic acid. When this fraction was used to calculate the saliva level of D, using $f_{\infty}^{abs} = 0.33$ and $k_a = 0.007\ min^{-1}$, a good prediction was possible. Fig. 4 shows clearly the difference in the saliva levels of C and D, which are of identical composition with D just containing the aluminum salt of acetylsalicylic acid. This difference could be very well predicted from the in vitro experiments.

Statistical evaluation of the in vitro and in vivo data

The 4 investigated tablets show statistical significant differences (t-test, $P < 0.05$) in their in vitro dissolution rates as well as in their salicylate saliva levels. At any point investigated the dissolution of D was slower than that of the other 3. Between A and C no statistical difference could be found whereas B showed a significant slower dissolution between 6 and 30 min. These in vitro differences are also reflected in the in vivo results. The salicylate levels of D, related to equal doses, are lower at

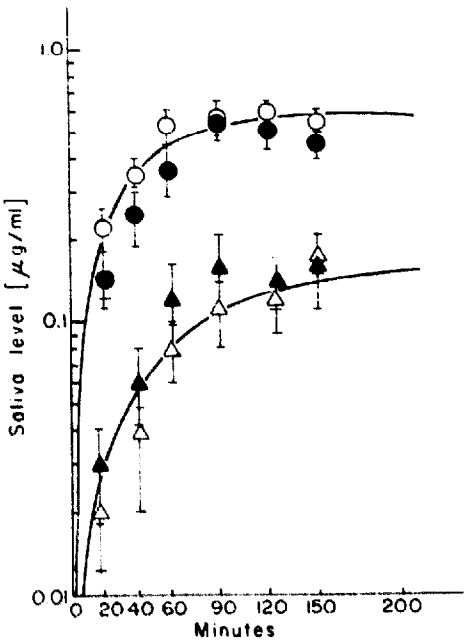

Fig. 4. Salicylate saliva levels after application of 2 tablets C (●) and D (▲) and the predicted saliva level–time curve calculated from the in vitro dissolution data (means of 14 subjects ±S.E.M., see Eqn. 5 in the text). The open signs refer to a repetitive study in another group of 14 volunteers under the same conditions to show reproducibility.

TABLE 3

CORRELATION COEFFICIENTS FOR THE COMPARISON OF EXPERIMENTAL AND THE FITTED DATA

Preparation	$k_{invitro}$	r
(A) In vitro dissolution data		
A	0.1150	0.997
B	0.0614	0.997
C	0.0920	0.998
D	0.0315	0.997
Preparation	**k_a**	**r**
(B) In vivo saliva levels		
A (1000 mg)	0.0240	0.970
A (500 mg)	0.0240	0.856
B	0.0128	0.983
C	0.0192	0.977
D	0.0070	0.926
C (repetition)	0.0192	0.951
D (repetition)	0.0070	0.964

any time investigated. Between A and C there is no statistical significant difference in salicylate saliva levels, whereas B shows a lower concentration in the 20 min sample.

This comparison also shows the correlation between in vitro and in vivo results and statistically underlines the ability of the in vitro experiment to differentiate among the different products studied.

The correlation coefficients for the comparison of all experimental and predicted data are summarized in Table 3.

Discussion

The results of the present study indicate that it may be possible to use continuous flow cell models to predict the time course of drug levels in the body. However, some precautions have to be taken. The translation of in vitro data to in vivo situations is only useful when a reproducible correlation for different dissolution and absorption rates in a certain dose range can be shown. For acetylsalicylic acid this is the case under the assumption that both processes follow first-order kinetics. This is a simplification of the true situation, as the 'absorption' step can be divided into the dissolution in the GI-tract, the absorption into the blood, the metabolism into salicylic acid and the diffusion into saliva. The dissolution step is the slowest process of these and determines the overall rate. The whole system appears to be a first-order system with apparent rate constant k_a. This simplification could be

shown to be appropriate and allowed good prediction of saliva levels from in vitro dissolution rates.

In the case of tablet D a good prediction for the salicylate saliva level could be observed assuming a relative bioavailability of only 33%. Slow and incomplete gastrointestinal absorption of the aluminum acetylsalicylate had been reported before (Levy and Sahli, 1962). However, the present data are not sufficient to give a final conclusion on the bioavailability of the aluminum salt even with the good agreement of measured and predicted data during the absorption phase. It is possible that the initial first-order process is followed by a zero-order absorption over a longer time that would lead to higher bioavailability. It is obvious (Fig. 4) that tablet D provides much lower salicylate level during the first hours after drug intake than the equivalent composed tablet C containing acetylsalicylic acid and that this difference could clearly be predicted from the in vitro experiment.

Continuous flow cell models are potent in vitro systems that also in the case of other drugs may allow predictive information for quality control purposes and tablet formulation screening.

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