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Absorption rate limited metabolism of salicylate in man: a consideration in bioavailability assessment

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Summary

To study the effect of absorption rate on the urinary excretion of the unchanged salicycic acid and its consequence on bioavailability tests, 500 mg tablets of a regular (A) and two slow-dissolved (B and C) acetylsalicylic acid were administered to 8 volunteers in a cross-over fashion. Salicylic acid in plasma and urine and total metabolites in urine were determined. Comparison of area under plasma level–time curves (AUC) failed to show any significant differences between 3 products. However, the flow dissolution rate caused reduction in maximum plasma levels and delayed the time of their occurrence. The cumulative total metabolites (ΣMu) and salicylic acid (as % of ΣMu) were significantly less following administration of B and C. It is concluded that the extent of absorption of slow-dissolved tablets is significantly lower than regular product although it is not reflected on the observed AUCs. Therefore, when salicylate products being tested have different absorption rates, the cumulative excretion of total metabolites seems to be a more valid measure of the relative extent of bioavailability than AUC. The reduction in the cumulative salicylic acid (as % of ΣMu) excreted in the urine may indicate an absorption rate limited metabolism phenomenon.

Introduction

Plasma salicylate levels exhibit considerable inter-subject variations amongst patients ingesting the same dose of a given acetylsalicylic acid (ASA) formulation. Factors such as body weight (Cummings and Martin, 1964), plasma albumin concentration (Reynolds and Cluff, 1960), urine pH (Smith et al., 1946), metabolic

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capacity (Paulus et al., 1971; Gibson et al., 1975), genetic differences (Furst et al., 1977) and induction of metabolism (Furst et al., 1977; Rumble et al., 1980) have been suggested to cause the above-mentioned variations.

It is well documented that, due to the capacity-limited metabolism of salicylates, the relationship between the ingested dose and plasma levels of this drug is non-linear (Levy, 1965; Levy, 1979; Mandelli and Tognoni, 1980). As a result, although the recovery of the total metabolites in urine is almost complete, the proportions of various metabolities vary with dose (Levy, 1965; Gibson et al., 1975). After oral administration of single doses of 250–2000 mg ASA to 4 subjects, Levy (1965) noticed that the fraction excreted as salicyluric acid decreased while the fraction eliminated as unchanged salicylic acid and salicyl glucuronides increased with elevating the dose. In another study (Gibson et al., 1975), however, increasing the daily dose in 4 patients, under long-term treatment with ASA, decreased the proportion of salicyluric acid and salicyl phenolic glucuronide and increased the fraction of unchanged salicylic acid excreted in urine. These reports suggest that, in high doses, excretion of the unchanged salicylic acid may be an important excretory pathway for salicylates. When the adult human body content of salicylate exceeds 200–300 mg, its saturable metabolic pathways reach their maximum rates (Levy, 1965). This amount seems to be achieved after ingestion of a conventional fast-dissolving tablet of ASA. However, the body content of drugs also depends partly on the absorption rate. Therefore, it is conceivably that, similar to the dose size, the rate of absorption may also affect the fraction of a dose of salicylate excreted as unchanged salicylic acid. The present study was designed to examine this suggestion. It also examines if such a phenomenon has any consequences on the bioavailability tests using plasma salicylate data.

Experimental

Dosage forms and dissolution tests

Three different commercially available ASA tablets were purchased. Product A [1] was a 500 mg regular disintegrating tablet and B [2] and C [3] were 500 mg ethylcellulose microencapsulated tablets of ASA. The latter products are prepared by moulding ASA into individually coated microspherules, dispersing them in a ethylcellulose matrix and compressing into tablets (Green, 1966).

The dissolution profiles of the products were measured in 1000 ml distilled water at 30°C using the rotating basket method (American Pharmaceutical Association, 1975) at 50 rpm. Samples were taken at the time intervals shown in Fig. 1 in 1-ml aliquots and an equal volume of water was added to the beaker. Trinder's (1954) method was used to determine the dissolved drug. Each point on Fig. 1 is the mean of 4 separate tests.

[1] Bayer, Iran.
[2] Specia, Iran.
[3] Bayer, Iran.

Subjects

Thirteen normal healthy volunteers whose general characteristics are shown in Table 1 with no history or signs of gastrointestinal disturbances or liver and kidney dysfunctions were chosen. They were told about the objective of the experiment and participated in the project voluntarily.

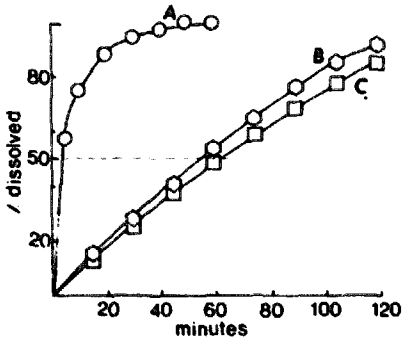

Fig. 1. Dissolution profile of acetylsalicylic acid tablets. A, regular, B and C, two different microencapsulated formulations.

Plasma levels and urinary excretion studies

To 8 fasting subjects 500 mg tablets of A, B and C with were administered with 250 ml water on different occasions. At least a one-week interval was maintained between each test. Food was allowed not sooner that 1 h after dosing. No other drugs were allowed for at least one week prior to and during the experiments. Blood

TABLE 1

General characteristics of subjects. F. female and M. male

Subject	Sex	Weight (kg)	Age (years)
F.K.	F	51	26
K.A.	F	59	18
H.M.	F	59	19
E.Y.	F	76	43
A.L.	F	80	23
A.B.	F	54	32
M.R.	M	63	36
K.D.	F	52	27
R.S.	M	60	39
T.K.	F	62	23
M.K.	F	52	23
H.K.	M	80	34
Z.A.	F	57	29
Mean		61.9	28.6
Standard deviation		10.3	7.7

TABLE 2

Plasma salicylate maximum concentrations (C_{max}, μg/ml), time of their attainment (T_{max}, h) and concentrations at 12 h post-dosing (C_{12}, μg/ml) following oral administration of 500 mg tablets of a regular (A) and two microencapsulated (B and C) acetylsalicylic acid in man

Subject	B			C			A		
	T_{max}	C_{max}	C_{12}	T_{max}	C_{max}	C_{12}	T_{max}	C_{max}	C_{12}
F.K.	4	34.12	6.92	4	41.50	6.80	2	54.32	4.10
K.A.	4	50.66	5.73	3	39.95	6.56	2	75.55	6.25
H.M.	5	33.66	10.23	4	46.00	12.03	3	50.50	6.40
E.Y.	4	48.85	2.68	4	58.40	9.92	2	68.10	3.85
A.L.	3	24.50	2.12	2	26.01	4.18	1	36.12	3.82
A.B.	4	33.24	4.81	4	42.10	4.75	2	36.89	1.60
M.R.	4	37.18	5.52	3	38.09	4.00	1	64.75	3.79
K.D.	2	38.04	4.20	4	32.81	6.12	2	49.46	5.85
Mean	3.75	37.53	6.53	3.50	40.61	6.80	1.88	54.46	4.46
Standard deviation	0.89	8.58	3.41	0.75	9.48	2.84	0.64	14.26	1.62
Coefficient of variation (%)	24	23	52	22	23	42	34	26	36
Statistical differences *:									
			T_{max}:	B	C	A			
			C_{max}:	B	C	A			
			C_{12}:	B	C	A			

* Determined by the Duncan's New Multiple test at $\alpha = 0.05$. Means not significantly different are connected with a line.

samples were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 12 h post-administration with heparinized syringes. The samples were then centrifuged and plasma was separated and extracted.

Simultaneous urine samples were collected from 3 of the above subjects until 48 h post-administration. Five more subjects were also included in this part of the experiment from which no blood samples were collected.

Assay

Salicylate contents were extracted from 1 ml of plasma or 5 ml of urine into 15 ml ether and analyzed using a high-pressure liquid chromatography method described elsewhere (Jamali and Keshavarz, 1981).

The colorimetric method of Trinder (1954) was used to determine total metabolites excreted in urine.

Evaluation of data

Plasma concentrations were plotted versus time and the areas under salicylic acid plasma level–time curves from the time of administration until 12 h post-dosing (AUC_{0-12}) were measured using a planimeter. The total area ($AUC_{0-\infty}$) was calculated using the equation:

$$AUC_{0-\infty} = AUC_{0-12} + C_{12}/\beta \quad (1)$$

where C_{12} is the last plasma sample measured and β is the elimination rate constant. Cumulative total metabolites (ΣMu) and salicylic acid (ΣXu) excreted in urine were determined. Calculated ΣXu was converted on a proportionality value ($\Sigma Xu/\Sigma Mu \times 100$) to correct ΣXu for differences in ΣMus. The statistical differences between the maximum plasma concentrations (C_{max}) and the time of their attainment (T_{max}), plasma concentrations after 12 h (C_{12}), AUCs, ΣMus and corrected ΣXus as the percentage of ΣMus were determined using the Duncan's New Multiple test at $\alpha = 0.05$ (Tables 2–5).

Results and discussion

Dissolution profiles, depicted in Fig. 1, indicated that microencapsulated tablets have substantially slower rates of dissolution than the regular ASA product. The time required for dissolution of 50% of ASA was 3–5 min for regular and 56–65 min for slow-dissolving tablets. The delayed dissolution seems to affect T_{max} and C_{max} values. While no significant differences were noted between products B and C, the average C_{max} was 31 and 25% lower than regular ASA for products B and C, respectively (Table 2). Meanwhile, compared to the regular ASA, the mean T_{max} was delayed by 2.0 and 1.6 h for B and C, respectively, indicating a slower absorption rate.

The delayed absorption of ASA from microencapsulated tablets have also been noted by Green (1966) and Delacoux et al. (1970). Green (1966) administered 650 mg ASA as regular tablets every 4 or 6 h and 1300 mg ASA as microencapsulated tablets every 8 or 12 h for 5 or more drugs. Considering the data from the first dose,

it is apparent that C_{max} is achieved within 1–2 h for regular and about 6 h for the slowly absorbed product. Although the first dose of microencapsulated ASA was twice as much as that of the regular tablet, within the first few hours the plasma salicylate levels were higher following ingestion of the latter product. Delacoux et al. (1970) administered single oral doses of 1 g ASA as regular or microencapsulated tablets and collected blood samples for 3 and 4 h, respectively. While the maximum mean concentration (73.2 μg/ml) of regular ASA was attained in 2 h, following administration of the slowly absorbed tablet, the salicylate plasma levels were still elevating when the blood sampling was terminated (i.e. 4 h post-medication).

In this work, however, the mean AUCs observed following administration of all 3 products were not significantly different (Table 3). The salicylate plasma levels obtained from subject M.R. were closest to the mean values. Therefore, they are shown in Fig. 2 as the representative data following administration of single oral doses of 500 mg tablets of A, B and C. Green (1966) also assumed a comparable bioavailability for his examined products. His suggestion was based on the comparable mean steady-state plasma salicylate levels obtained following administration of equal daily doses, but on different schedules, of two different products. The steady-state plasma salicylate levels observed by this author showed very large inter-subject variations.

The relative bioavailability of the products, calculated from AUC_{0-12}, gave

TABLE 3

Area under plasma salicylate concentration–time curves (AUC, μg/ml/h), from zero to 12 h (0–12) and total (0–∞), following oral administration of 500 mg tablets of a regular (A) and two microencapsulated (B and C) acetylsalicylic acid in man

Subject	B		C		A	
	0–12	0–∞	0–12	0–∞	0–12	0–∞
F.K.	245.23	281.05	304.18	330.54	242.87	258.76
K.A.	280.60	303.61	365.45	391.79	459.81	484.91
H.M.	303.65	348.32	358.45	410.98	248.40	276.28
E.Y.	413.60	422.90	332.20	366.57	352.00	365.37
A.L.	132.05	142.44	150.91	171.40	146.20	164.92
A.B.	238.16	253.48	235.80	250.93	193.30	198.39
M.R.	268.81	290.20	224.01	239.51	299.47	314.16
K.D.	246.10	265.77	240.52	269.19	304.18	331.58
Mean	266.03	288.47	276.43	303.86	280.78	299.30
Standard deviation	78.35	80.18	75.43	84.13	97.29	100.35
Coefficient of variation (%)	29	28	27	28	35	33
Statistical differences *		B		C		A

* Determined by the Duncan's New Multiple test at $\alpha = 0.05$. Means not significantly different are connected with a line.

essentially the same answers as those from $AUC_{0-\infty}$. This is in line with the suggestion of Wagner (1975) that in measuring bioavailability, the area from zero to any time, t, can be used providing some information relating to the equality of the

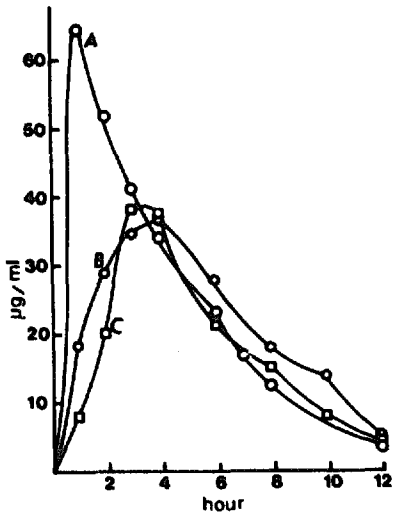

Fig. 2. Plasma salicylate concentration–time curves following oral administration of 500 mg tablets of a regular (A) and two different microencapsulated (B and C) acetylsalicylic acid to subject M.R. These curves represent the closest date to the mean values for the group.

results from AUC_{0-t} and $AUC_{0-\infty}$ are made available. We observed no significant differences between salicylate levels 12 h after the administration (Table 2). At low salicylate concentrations, the elimination kinetics of the drug were first-order. The study was of a cross-over type, thus, equal biological half-lives were expected following administration of all products to the same subject. Therefore, since in the post-distribution–absorption phase, AUC is a function of concentration and the biological half-life (Eqn. 1), in this study, $AUC_{12-\infty}$ does not play a significant role in assessing the relative bioavailability. Furthermore, as compared to the area of the first 12 h, the remaining area constituted a minor, if not negligible, part of the total AUC (Table 3).

Using the Trinder method (1954) the per cent dose detected as total metabolites in urine was in close agreement with that reported by Needham et al. (1978).

Cumulative excretion of total metabolites, however, depicts a completely different picture from that observed when AUCs were being analyzed. Unfortunately, due to some technical problems, simultaneous blood and urine samples were only collected from 3 of the subjects. Nevertheless, the data clearly indicate averages of 21 and 31% reduction in ΣMu following administration of B and C as compared to A, respectively (Table 4). This may suggest that the absorption of ASA from microencapsulated tablets is more erratic and incomplete than regular tablets although it is not reflected on AUCs. The diminished bioavailability is expected since microencapsulation may cause a delayed dissolution rate giving rise to a slower and more erratic rate of absorption. Therefore, such a dosage form may bypass the absorptive sites

TABLE 4

Cumulative urinary excretion of the total metabolites (mg), measured using the Trinder method, following administration of 500 mg tablets of a regular (A) and two microencapsulated (B and C) acetylsalicylic acid in man

Subject	C	B	A
R.S.	206.02	244.12	271.37
T.K.	200.81	180.28	276.24
M.K.	170.43	275.44	281.05
H.K.	150.95	161.28	224.24
Z.A.	138.75	214.17	228.80
A.B.	216.18	233.46	266.02
M.R.	161.90	175.62	257.10
K.D.	251.50	222.12	364.25
Mean	187.07	213.31	271.15
Standard deviation	37.98	38.75	43.11
Coefficient of variation (%)	20	18	16
Statistical differences *	C	B	A

* Determined by the Duncan's New Multiple test at $\alpha = 0.05$. Means not significantly different are connected with a line.

before complete absorption occurs (Jamali and Axelson, 1977).

The calculated coefficients of variation for AUC_{0-12} were 35, 39 and 27% (Table 3) and for ΣMu were 16, 18 and 20% (Table 4) with respect to A, B and C. The larger inter-subject variation with regard to the observed AUCs as compared to ΣMus may be responsible for the failure of the method to detect the possible difference between the products. Even greater variations in salicylate plasma levels have been observed by other investigators following single (Ekenved et al., 1975) and multiple (Green, 1966; Paulus et al., 1971; Ekstrand et al., 1979) dosing. After administration of 3, 4.5 and 6 g/day of ASA to rheumatoid patients, the mean steady salicylate levels attained are reported to be 694, 1055 and 1275 μmol/l with respective coefficients of variation of 36, 64 and 23% (Ekstrand et al., 1979).

The observed corrected ΣXu also showed a significant reduction when microencapsulated tablets were given (Table 5) suggesting, perhaps, an absorption rate-controlled metabolism phenomenon. Levy (1965) reported that the percentage of salicylates excreted as unchanged salicylic acid increased upon increasing the dose. Percentage salicylic acid excreted was 2.0–5.1 and 3.6–7.3 following administration of 250 and 1000 mg ASA, respectively. In one subject these values were 2.5, 7.3 and 17.1% after ingestion of 250, 1000 and 2000 mg ASA, respectively. Following long-term treatment with ASA, Gibson et al. (1975) observed that when the daily 65 mg/kg dose was increased to 100 mg/kg, the unchanged salicylic acid elevated on

TABLE 5

Proportionality values for the cumulative unchanged salicylic acid excreted in urine after administration of 500 mg tablets of regular (A) and two microencapsulated (B and C) acetylsalicylic acid in man

Subject	C	B	A
R.S.	1.20	0.91	4.12
T.K.	1.93	2.38	9.12
M.K.	2.16	3.31	5.13
H.K.	0.95	2.01	6.00
Z.A.	1.35	0.82	3.90
A.B.	1.92	0.82	5.02
M.R.	2.39	1.78	5.94
K.D.	3.12	3.42	7.42
Mean	1.88	1.93	5.83
Standard deviation	0.71	1.06	1.74
Coefficient of variation (%)	38	55	30
Statistical differences *	C	B	A

* Determined by the Duncan's New Multiple test at $\alpha = 0.05$. Means not significantly different are connected with a line.

average by about 10% due, perhaps, to a limitation on the excretion of other metabolites. The present study indicates that even if the same dose is given but the rates of absorption of various products are significantly different, the metabolic pathway of salicylate may vary accordingly. Following administration of a fast disintegrating tablet of ASA with a T_{max} of approximately 2 h, the mean corrected value for ΣXu was 5.83% with a coefficient of variation of 30%. On the other hand, after ingestion of product B and C with a T_{max} of approximately 4 h, these values were 1.98 and 1.88% with coefficients of variation of 55 and 38%, respectively (Table 5). Therefore, the significant difference in proportionality values observed for ΣXus resulted after administration of the products examined is likely to be caused by the substantial differences in their absorption rates. This coupled with the large inter-subject variation may be an indication of a more efficient but inconsistent extent of metabolism of the slower-absorbed products examined. It is known that when the amount of salicylates in the body exceeds a certain level, their metabolic pathway becomes saturated (Levy, 1965). A similar phenomenon may very well be responsible for our observation. When a given salicylate formulation is rapidly absorbed, the metabolic system is likely to become saturated and a high quantity of the intact salicylic acid excreted in the urine. Conversely, a salicylate product with a slower absorption rate, such as a microencapsulated tablet, may reach the metabolic site at a slower rate, permitting a more complete metabolism. A change in the absorption rate may alter the amount of circulating salicylate in the body and hence in the plasma.

In assessing the total salicylate absorbed, Mayersohn (1978) recommended the determination of AUC for salicylic acid only over a relatively small dose range. However, based on the data presented here, it seems reasonable that, in addition to the dose size, the absorption rate be also accounted as an important factor in this respect. In other words, when the extent of absorption is being measured, comparison of AUC of various ASA products containing more than 500 mg of the drug, with large differences in their absorption rate, may not hold true. Alternatively, for such products, measuring the cumulative total metabolite excreted in urine seems to provide a more valid measure of the extent of absorption. This point, although without supporting data, has also been mentioned by Saltzman and Walkenstein (1981).

It is also worthy to emphasize that no significant differences were found between the plasma salicylate concentrations of the examined products 12 h after dosing (C_{12}). The observed mean values are shown in Table 2 and are 4.46, 6.53 and 6.80 with coefficients of variation of 36, 52 and 42% after administering products A, B and C, respectively. Therefore, the tested microencapsulated tablets may not be necessarily considered as longer acting products than regular ASA, although they have slower rates of absorption.

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