PALM KERNEL OIL BLENDS AS SUPPOSITORY BASES IN THE DELIVERY OF ASPIRIN

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ABSTRACT: Rectal delivery of drugs has been proven to be effective in terms of drug absorption and distribution comparable with other routes such as oral, buccal, sublingual or even nasal. In this study, two new suppository bases were developed using combinations of locally sourced hydrogenated palm kernel oil, hydrogenated palm kernel stearin and hydrogenated palm kernel olein with mixtures of stearic acid and glyceryl monostearate. When formulated with aspirin, these bases produced suppositories with acceptable characteristics. These aspirin suppositories were tested on twelve healthy subjects after an approval from the Medical Ethics Committee, University of Malaya had been procured. We quantified aspirin from the urine samples of the subjects to determine the relative availability of the different suppository preparations relative to an oral dose. The excretion of salicylic acid, one of the metabolite of aspirin in human urine taking aspirin was quantified. The F value was found to range from 1.16 to 1.38. Hence, the excretion results showed that these palm kernel oil blends are suitable suppository bases. *(JUMMEC 2007; 10(2):43-50)*

KEYWORDS: Rectal delivery, palm kernel oil, suppository, aspirin, urine.

Introduction

Suppositories use oil only in the form of hard butter. Suppository bases have evolved from the traditional cocoa butter (theobroma) to currently available commercial bases such as the Witepsol(r) and Wecobee(r) which are made from the lauric component of coconut oil. These bases are replacing theobroma as it exhibits problems in the preparation and storage stages of the finished suppository product (1). As Malaysia is blessed with an abundant production of palm oil or palm kernel oil, new suppository bases can be formulated which can have characteristics similar or superior to the currently available commercial suppository bases mentioned earlier. For instance, the suppository bases made from palm oil and palm kernel oil can be made to be more robust and can be exposed to extreme temperatures without affecting the integrity of the finished product in terms of quality and effectiveness. This study was designed to determine the suitability of palm kernel oil blends as a base in the production of suppositories. In terms of drug release from the proposed suppository bases in human subjects, we only sampled urine from subjects taking aspirin in the form of suppositories made from the two selected blends of palm kernel oils and an oral capsule preparation. The interpretation of urine data is very straight forward as shown by Richardson (2) in his study on urine. Approval from the Medical Ethics Committee, University of Malaya on this study had been procured (Reference number: MEC 308.4).

Material and Methods

Material used in preparation of suppository bases
Hydrogenated palm kernel oil (batch no: 0040933801) and hydrogenated palm kernel stearin (batch no: 0091420002) were procured from Cargill (M) Sdn. Bhd., stearic acid (batch no: Tristar149) was donated by Hesego Industry (M) Sdn. Bhd. and glyceryl monostearate (batch no: E01/096) was donated by Esterchem (M) Sdn. Bhd.

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Preparation of palm kernel oil blend (PKOB) suppository bases

The PKOB suppository base was prepared by blending hydrogenated palm kernel oil and hydrogenated palm kernel stearin in the ratio of 9:1 for suppository base A and in the ration of 8:2 for suppository base B, with the addition of 5% stearic acid and 5% glyceryl monostearate using an Erweka mixer (Model No. AR 402) set at a temperature of 45ºC and with a stirrer speed of 100 rpm for each preparation. After being thoroughly mixed, the blends were set to solidify at 25ºC for one week before being refrigerated at 4ºC. The amount of each component in the bases was determined earlier by trial and error where different blends were prepared and tested for good characteristics as suppository bases such as good moulding characteristics with easy release of the resulting suppositories from the mould and producing suppositories with a melting point close to body temperature and with good liquefaction time and hardness.

Preparation of Samples

The oral aspirin capsules were prepared by placing 600mg of acetyl salicylic acid (obtained from Sigma Chemical Company (USA)) into a medium-sized empty capsule. For the rectal dosage, suppositories of 600 mg aspirin were prepared using the two different types of suppository bases, A and B. The double casting method was employed for the two 600 mg aspirin suppository preparations.

Study Protocol

A number of twelve volunteers weighing between 50 kg to 75 kg were identified where their inclusion criteria were as follows:

1. Aged above 18.
2. Were not allergic to aspirin or any other salicylic preparations
3. No history of allergy to any drug or preparation.
4. Did not suffer from any gastritis condition
5. No history of any stomach or intestinal bleeding
6. No history of stomach or intestinal ulcer
7. No history of asthmatic disease
8. No history of renal diseases
9. No history of liver diseases
10. No history of bowel disease such as hemorrhoids, bleeding or carcinoma
11. Normal bowel movement

A three-way cross-over study was carried out starting with blank urine samples collection from every subject before taking any aspirin preparation. The comparator oral dosage of 600 mg aspirin capsule was taken with approximately 300 ml of water. All subjects continued to drink approximately 200 ml of fluid per hour. Urine was collected after 30 minutes and then after one hour, then continuously collected at hourly intervals for seven hours. These samples were then analysed using HPLC.

After an interval of seven days of the wash out period the same subject came back for the rectal aspirin preparation, suppository A and the same procedure as above was applied. Again after an interval of another 7 days of the wash out period the same subject came back for the rectal aspirin preparation, suppository B and the same procedure as above was again applied.

Determination of Urine Salicylic Acid Using HPLC

The HPLC used was the Waters(r) Alliance System 2690 with auto capability and Millenium 32 Chromatography Manager Software. The detector attached was the Waters(r) 996 Photodiode Array Detector. The elution was performed from a reverse phase, Supelco(r) (Bellafonte, USA), 3 micron ODS 2, 5cm $\times$ 4.6mm column, with a mobile phase of a mixture of methanol (60%) and water (40%). The flow rate used was 1ml/min and the detection was done at 254nm. The injection volume used was 20 µl.

Standards curves for salicylic acid were created by injecting the HPLC with a range of different concentrations of its standard solution and 100 mcg/ml of methyl paraben as the internal standard in fresh urine. The salicylic acid concentrations used were 15, 25, 50, 75, 100, 150 and 200 mcg/ml. The peak ratios of aspirin against methyl paraben (as internal standard) were used to plot the standard curve. Methyl paraben was used as the internal standard as the structure of both salicylic acid and methyl paraben were comparable (Figure 1). To determine reproducibility of the results, tests were carried out at three different times in a day (morning, afternoon and evening) for three different days. Fresh standards and samples were prepared for each time. Fresh urine was spiked with salicylic acid in three concentrations: 60, 90 and 180 µg/ml. This mixture was than processed and quantified in the same way as the samples of urine collected from the subjects for quantification of salicylic acid. This was to determine the accuracy of the method used in this study.

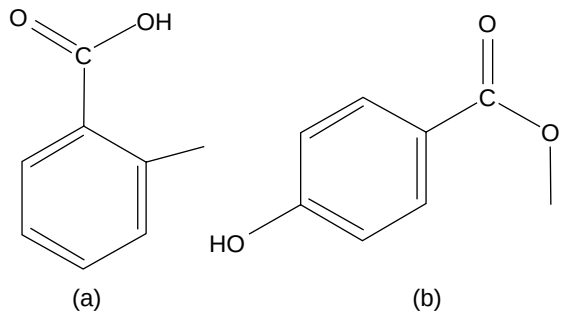

Figure 1. Structure of salicylic acid (a) and structure of methyl paraben (b)

Urine from subjects who had taken the aspirin preparation was analysed employing the following procedures:
A 2 ml aliquot of each of the urine samples was taken and mixed with concentrated ammonia solution and heated at 100°C for ten minutes. This was to break down the metabolic conjugates to release salicylic acid. The solutions were cooled and neutralized with 1M HCl solution, then transferred into 10 ml volumetric flasks where 1mg of methyl paraben was made up to 10 ml with distilled water. A 20 µl volume of the resulting solution was analyzed using the HPLC. The results were then compared with the standard curves obtained previously to establish the concentrations of the salicylic acid metabolites in the urine samples. Three injections were done for each sample, and the mean peak height ratio was taken for calculation of the sample concentrations.

Results and Discussion

Rectal delivery of drugs has been proven to be effective in terms of drug absorption and distribution in comparison with other routes such as oral, buccal, sublingual or even nasal (3). Aspirin was taken as the model drug in this study. It is a well-known drug in terms of its pharmacokinetics and its toxicity as it has been in the market for quite some time (4,5,6). Upon approval of the University of Malaya Ethics Committee, aspirin suppositories were prepared from the two blends and were tested on human subjects. Urine was sampled from the subjects and was assayed using HPLC.

The HPLC method exhibited good precision, where the RSD was found to be less than 2.8%. Figures 2 and 3 show representative chromatograms produced from this method. The HPLC method also showed a good degree of reproducibility where the statistical one-way anova analysis of the results from intra-day (morning, afternoon and evening) and for three different days showed equality of variances ($p>0.05$). Linearity was proven (Figure 4) when the standard concentration was plotted against the response (peak height ratio). The accuracy was determined by spiking three known amounts of salicylic acid to fresh urine (7), processing the urine, and quantifying the salicylic acid content again using the same method (Table 1). The paired t-test statistical analysis showed that there was no significant

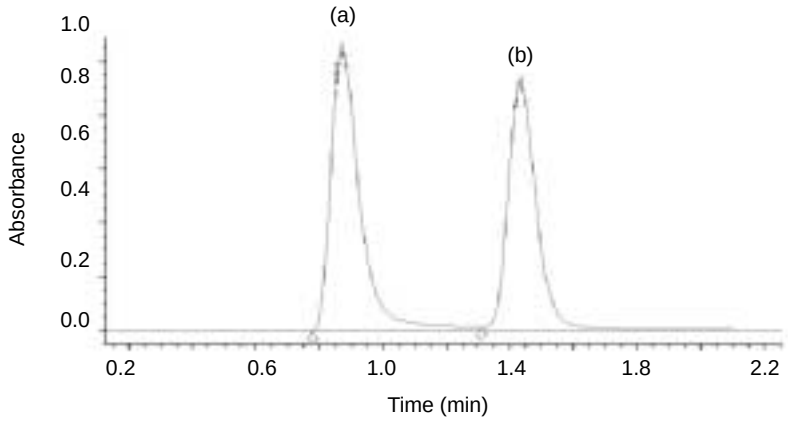

Figure 2. HPLC peaks for salicylic acid standard solution with concentration 50 µg/ml (a) and methyl paraben (internal standard) (b)

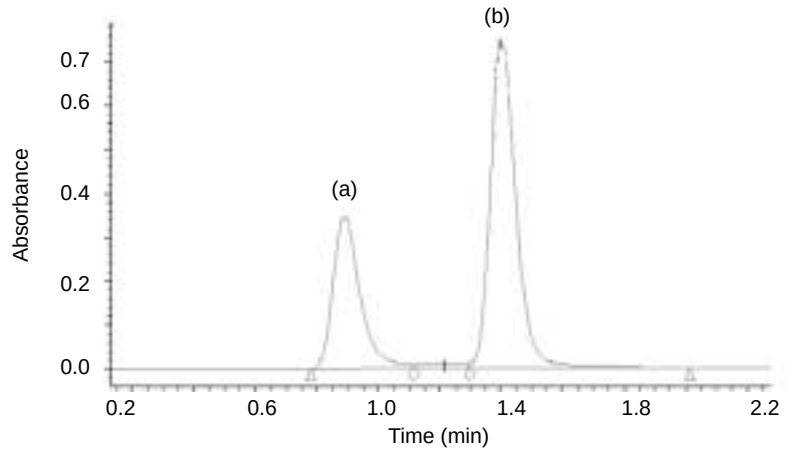

Figure 3. HPLC peaks for salicylic acid (a) and methyl paraben (b) captured from the urine of a subject after 60 minutes of introduction of 600mg salicylic acid suppository from base A

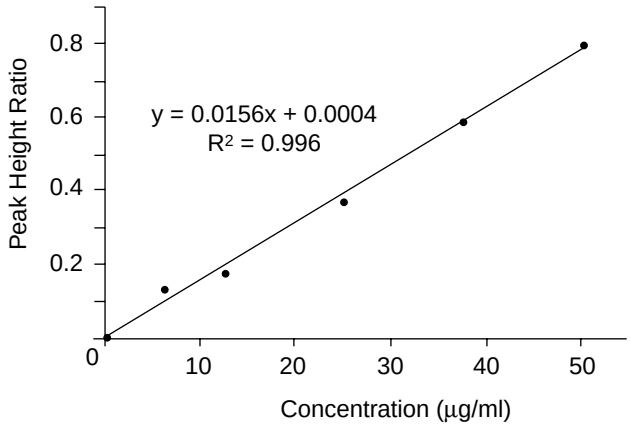

Figure 4. Standard curve for salicylic acid
The results in the graph are the mean values for injections done in the morning, afternoon and evening of three different days where the RSD was less than 2.8%, and the one-way anova proves the equality of the variances ($p>0.05$)

Table 1. Results of HPLC analysis of urine samples spiked with a known amount of salicylic acid

Actual Amount of salicylic spiked into urine (μg/ml)	Peak Height Ratio with internal standard	Amount of salicylic acid detemined by the method employed (μg/ml)	Percentage amount extracted from urine sample	*p*-value determined using the paired *t*-test statistical analysis
60	1.04 ±0.02	60.58 ± 1.17	100.97± 1.94	0.48
90	1.58 ± 0.07	91.47 ± 3.96	101.64 ± 1.44	0.59
180	3.23 ± 0.04	185.69 ± 2.40	103.16 ± 1.33	0.06

Each data is obtained from three repeated tests and expressed as mean ±SD (n=5)

difference between the actual amount spiked and the amount determined through analysis using the same method.

Table 2 shows the results of the amounts of aspirin being quantified from the urine, at certain intervals, of all the subjects involved. Figure 5 shows the salicylic acid concentration in the urine, plotted against time. The two suppository dosages with different bases show similarity in curve patterns with regard to the oral dosage. This indirectly suggests that the absorption and the elimination patterns of aspirin in the two rectal dosage forms and the oral dosage form are similar.

If plasma aspirin level was used in this study with the assumption of similarity in the distribution volume and elimination rate, the area under the curve (AUC) can be directly employed to compare bioavailability since the dosage between the compared preparations is the same (8,9).

$$F = \frac{[F]^A}{[F]^B} = \frac{[AUC]^A}{[AUC]^B} \qquad \text{---------- Eq. 1}$$

F is the bioavailability of product A compared relative to product B. In our case, we quantified aspirin from the urine samples to determine the relative availability

Table 2. Salicylic acid released in the urine of subjects who had taken 600mg salicylic acid orally or rectally

Dosage Form	Amount of salicylic acid in urine determined by HPLC at certain hours of time interval after introduction of dosage form. (mg)								
	0.5 hr.	**1 hr.**	**2 hrs.**	**3 hrs.**	**4 hrs.**	**5 hrs.**	**6 hrs.**	**7 hrs.**	**8 hrs.**
600mg Aspirin Capsules	2.08 ±0.61	5.44 ±0.78	11.27 ±1.42	15.48 ±1.21	18.94 ±0.88	14.20 ±0.77	8.63 ±0.55	5.30 ±1.20	4.25 ±1.18
600mg Aspirin Suppository of Base A	1.58 ±1.81	7.32 ±1.95	15.30 ±10.66	18.46 ±14.41	19.71 ±10.85	18.91 ±12.00	10.85 ±8.77	4.05 ±4.17	2.91 ±2.28
600mg Aspirin Suppository of Base B	2.07 ±0.58	7.95 ±3.24	19.43 ±12.46	23.63 ±4.50	21.19 ±3.40	15.60 ±6.76	13.65 ±7.37	8.40 ±2.02	4.08 ±0.78

Each data is obtained from three repeated tests and expressed as mean ± SD (n=12).

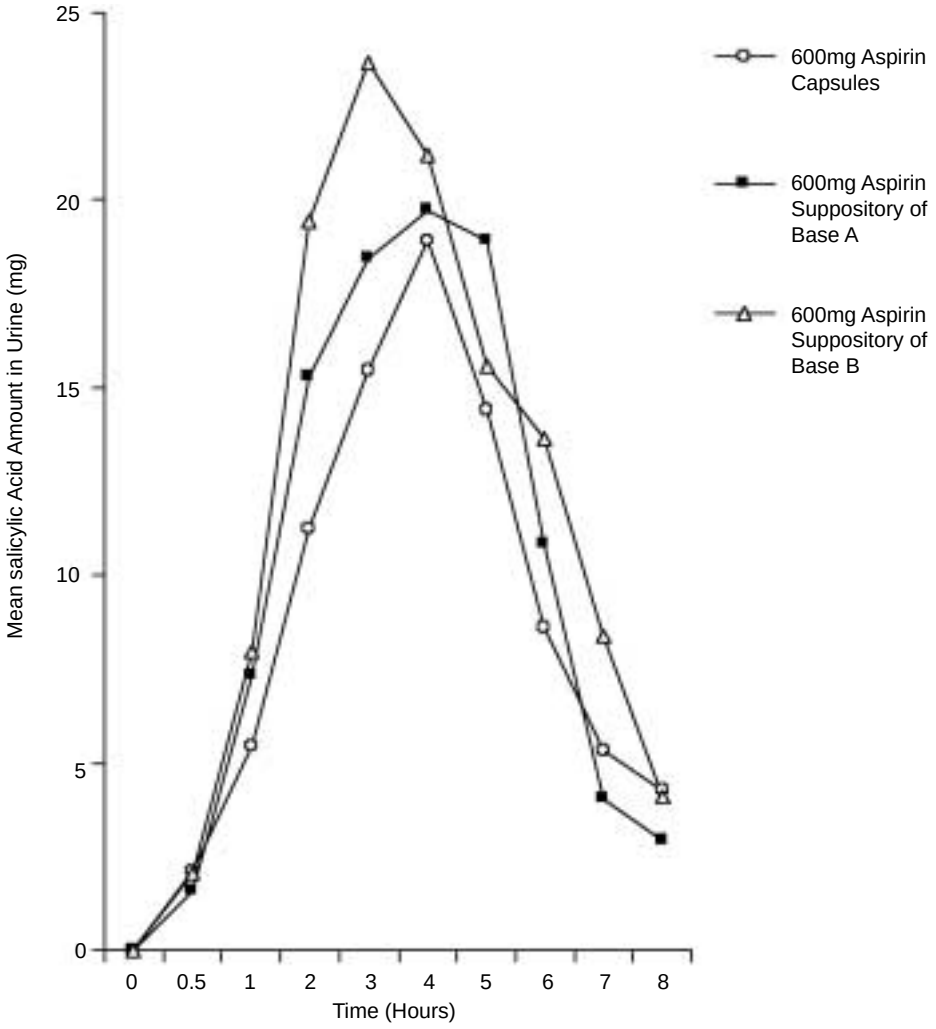

Figure 5. Comparison of plots of mean salicylic acid concentration in the urine against time for the oral capsule and the rectal suppositories made from the two selected palm kernel oil blends each preparation was tested on 12 different subjects (n=12) and the mean values of salicylic acid concentrations in urine were used to plot the curve

of the different suppository preparations relative to the oral dose, so the equation becomes:

$$\text{Relative availability} = \frac{[D_u]^{A\infty}}{[D_u]^{B\infty}} \quad \text{---------- Eq. 2}$$

Where D_u is the cumulative amount of aspirin excreted in the urine. In order to ensure that this equation is valid, we had taken into consideration normal weight with minimal variation when selecting the subjects for our study. Using this consideration, we can safely assume that the volume distribution and elimination of aspirin and absorption are likely to be the same. This will allow us to take the cumulative amount of salicylic acid in the urine from zero hour to eight hours as the relative availability for the particular preparation. Table 2 depicts the relative availability values obtained by comparing the preparation of suppositories from each of the five selected blends with the oral preparation. The aspirin excretion from the urine data collected reflects the absorption of aspirin in the oral and rectal dosage forms. In addition, the different suppository bases proposed in this study were also compared. Tables 2, 3 and 4 depict these results. Based on Shargel and Yu (8), and Gibaldi and Perier (10), we adopted the following pharmacokinetics formula which was suitable in our case where we only had urine data:

$$\frac{dD_u}{dt} = K_e D_B \quad \text{---------- Eq. 3}$$

where ke is the renal excretion rate constant, D_u is the amount of drug excreted in the urine and D_B is the amount of drug in the body at time t. The following equation can be derived taking D_B to be in logarithmic interval:

$$\frac{dD_u}{dt} = k_e D_B^0 e^{-kt} \quad \text{---------- Eq. 4}$$

From the above equation, taking the natural logarithm of both sides and then transforming to common logarithms, the following equation is obtained:

$$\text{Log } \frac{dD_u}{dt} = \frac{-kt}{2.3} + k_e D_B^0 \quad \text{---------- Eq. 5}$$

From the above equation, plotting the excretion rate with the mid-point of urine sampling interval, we determined the elimination constant, k, from the gradient and from here we calculated the half-life, t1/2, of salicylic acid excreted in the urine. Where:

$$t^1/_2 = 0.693k \quad \text{---------- Eq. 6}$$

Table 3 and 4 depict the overall results for these pharmacokinetic data obtained from the graphs (plotted on semi log paper) of the rate of excretion and the midpoint of time intervals.

Table 3. Relative bioavailability of aspirin for each suppository preparation compared to the oral preparation

Suppository of Different Base	Cumulative Amount of Salicylic Acid Excreted in 8 hours (mg)	Cumulative Amount of Salicylic Acid Excreted for oral preparation in 8 hours (mg)	Relative availability of suppository preparation relative to the oral preparation
600mg Aspirin Suppository of Base A	99.09	85.63	1.16
600mg Aspirin Suppository of Base B	118.00	85.63	1.38

Table 4. Elimination rate constant and the salicylic acid half-life calculated for each preparation.

Dosage form	Gradient (-k/2.3)	Elimination Rate Constant (k)	Half-life of Salicylic acid (t1/2=0.693/k) (hr)
600mg Aspirin Suppository of Base A	-0.208	0.479	1.45
600mg Aspirin Suppository of Base B	-0.189	0.435	1.59
600mg Aspirin Capsule	-0.191	0.438	1.58

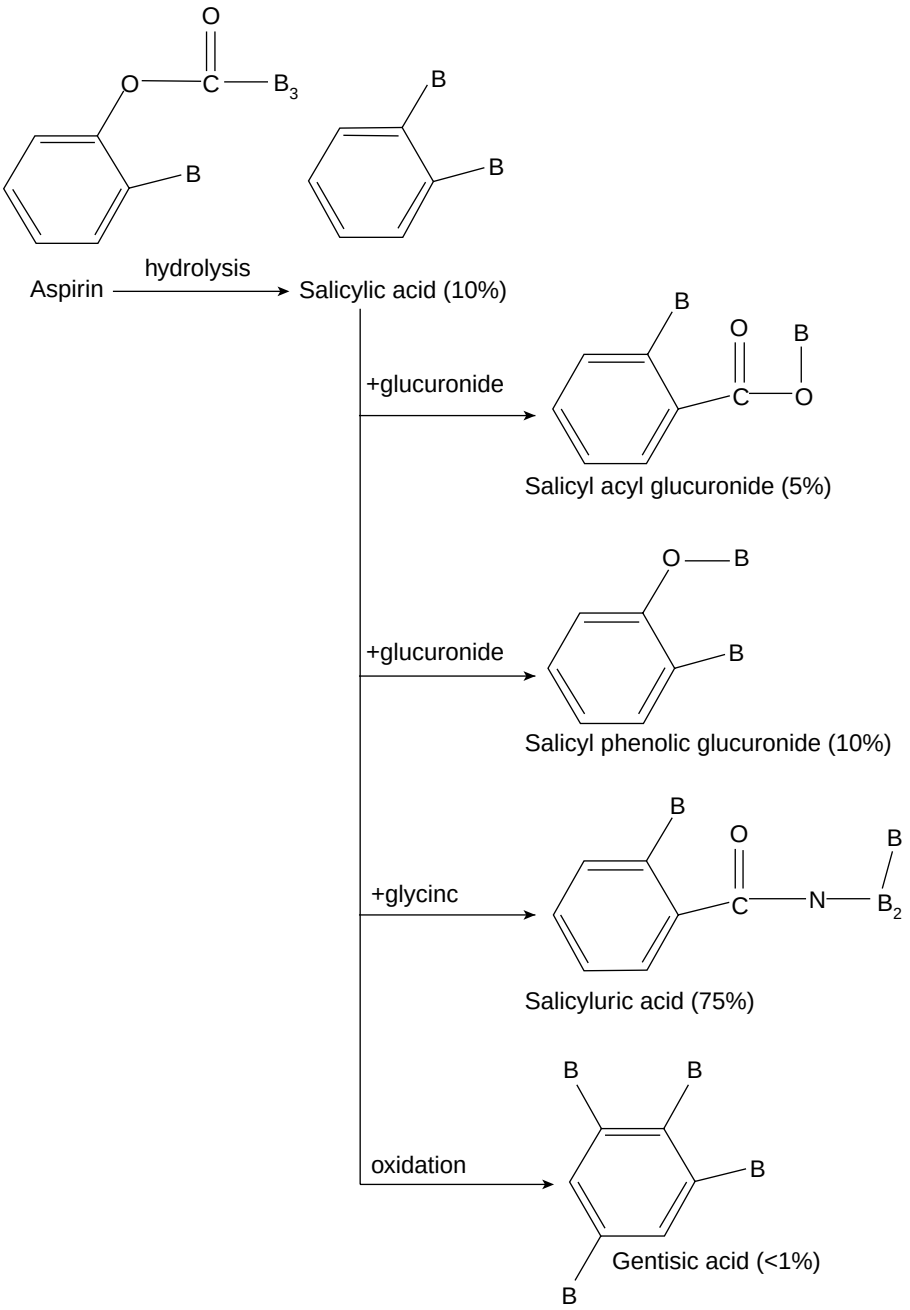

Figure 6. The percentage amount stated for each metabolite was adopted from Insel (II)

The fact that all the graphs plotted show the rate of excretion steadily decreasing linearly in the latter portion shows that excretion followed first order kinetics where as the metabolite's concentration in the body falls, the rate of excretion also falls. This is in agreement with the study by Gibaldi and Perrier (10).

Due to some limitations, we could not determine the full pharmacokinetic profile for each of our preparations. We could only determine the release of aspirin in the form of salicylic acid in the urine, and since we did not have data such as the IV bolus and the plasma level of the drug, we were unable to do a full pharmacokinetic study. But this study indirectly shows that aspirin was released from the proposed suppository bases and was absorbed into the body with steady elimination rates. Table 3 shows the relative availability of all rectal aspirin preparation relative to the oral preparations. The relative availability for each rectal preparation was found to be better compared to the oral preparation. This may be due to the rectal anatomy and its physiological conditions. Drug that is given rectally may be transported by the inferior and middle haemorrhoidal veins and bypass the liver. As such the availability of drug will be different and higher than the oral route.

If we calculate the percentage of salicylic acid released in the urine for the oral and rectal preparations we found that it ranges from 13.7% to 17.1%. The percentage of salicylic acid excreted in urine after ingestion of aspirin is extremely variable and depend upon the dose and the urine pH and the amount can be as low as 0.5% to as high as 30% (11,12,13,14). Figure 6 shows the various aspirin metabolites that can be found in the urine with their reported percentages.

Table 4 depicts the half-life of salicylic acid calculated from the urine data. The half-life of aspirin preparation in the body is very well established as it is not a new drug. Aspirin had been documented to have very short half-life which is less than thirty minutes but the salicylic acid normally shows longer half-life of more than two hours (15). The half-life of salicylic acid in this study was found to range from 1.45-1.58 hours and they are lower than the reported values. This may be due to the small population of subjects where in this study the number were only twelve.

Conclusion

The excretion of aspirin in the urine of twelve subjects taking suppositories made from the two different blends indirectly reflects the drug release ability of these preparations. The bioavailability of the two aspirin suppositories were found to be better than the oral route of administration.

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