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Urinary Excretion of Acetylsalicylic Acid in Healthy Male Volunteers

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Abstract: Acetylsalicylic acid is a non-steroidal anti-inflammatory drug. Urinary excretion of ASA (as free salicylic acid) was investigated in twelve healthy male volunteers after an oral administration of 600 mg dose. Urine samples were collected upto 10 h. The concentration of drug was determined colorimetrically and mean ±SE value in urine was found to be 37.55±0.418 μg mL^{-1}. The values for diuresis and rate of excretion was 0.028±0.006 mL min^{-1} kg^{-1} and 0.849±0.226 μg min^{-1} kg^{-1}. The amount of free SA excreted was 5.40±1.15 mg. The % dose excreted was 0.92±0.19%. Cumulative amount and % cumulative dose excreted was found to be 17.74±2.91 mg and 5.47±0.78%. There is slight variations in urinary excretion data when compared with foreign investigated values.

Key words: Acetylsalicylic acid, urinary excretion male volunteers, salicylate, Diuresis, Rate of excretion

Introduction

Acetylsalicylic acid most commonly known as Aspirin is a member of salicylate group of compounds. It is a non-steroidal anti-inflammatory drug (NSAID) that possesses analgesic, anti-inflammatory and antipyretic properties. It is effective in relieving pain such as headache, myalgia, arthralgia and other pain arising from integumental structures rather than viscera. Because of its high efficacy, low toxicity and low cost, it is a standard with which all other non-steroidal anti-inflammatory drug are compared (Simon and Mills, 1980).

Aspirin is a potent cyclooxygenase (COX) inhibitor which irreversibly inhibits cyclooxygenase (COX), the enzyme responsible for the production of prostaglandins both peripherally and in central nervous system. It can also be used for the secondary prevention of myocardial infarction and stroke by inhibiting the platelets cyclooxygenase, so aspirin produces the anticoagulant effect by prolonging the bleeding time (Reynolds, 1989). The studies shows the qualitative and quantitative difference in metabolism in human volunteers. Male and female showed different metabolism and hence different rate of excretion (Low, 1998).

Aspirin is hydrolyzed in stomach and in blood to salicylic acid and acetic acid, the biological half life is therefore only 20 min (Levy and Tsuchiya, 1972).

Salicylates are excreted mainly by kidney as salicyluric acid (75%), free salicylic acid (10%), salicylic phenol (10%) and acyl (5%) glucuronides, and gentisic acid (<1%) when small doses (less than 250 mg in an adult) are ingested, all the pathways proceed by first order kinetics with an elimination half life of about 2-3 h. When higher doses are ingested (more than 4 g), the elimination half life becomes longer i.e. 15-30 h (Hartwig-Otto, 1983).

Dose and pH of urine have great effect on excretion rate. Excretion of salicylate increases four fold when pH of urine is $\geq$8.0. At this pH, drug is highly ionized due to acidic nature and can not readily diffuse from the tubular fluid (Wesley, 1990).

The biological, physiological and pharmacological influence of drug in local indigenous conditions has been found to be different in urinary excretion, metabolism and kinetic behaviour when compared with foreign investigated values (Nawaz, 1994) therefor, the present study was undertaken for comprehensive evaluation of drug under local environmental conditions.

Materials and Methods

Volunteers: A total of twelve healthy human male volunteers having mean age 21.4 years, mean body weight 66.3 kg and mean body height 174.63 cm selected for this study. A written consent was signed by all volunteers. Blood pressure and body temperature of each volunteer was also recorded before the start of experiment.

Study protocol

Drug Used: Dispirin (soluble aspirin) tablets (300 mg) containing acetylsalicylic acid manufactured by Rekit Benckiser Pakistan Ltd., Karachi was used for oral administration to volunteers.

Sample Collection and Handling: Sampling was done in the month of May 2002. After an overnight fasting, a drug free/control urine sample was collected from each volunteer before the administration of drug. Two tablets of Dispirin (soluble aspirin 2x300 mg = 600 mg) were given orally to each volunteer and then allowed to take a standard breakfast 2 h following the drug administration.

The urine samples were collected for 10 hours at 30, 60, 120, 180, 240 and 600 min following oral administration of drug. Total volume and pH of each urine sample voided by each volunteer were recorded. Then known volume of each urine sample was preserved in plastic bottles and store at -20°C till further analysis.

Urine Analysis: The standard curve was made by preparing the stock solution of salicylic acid (SA) 1000 mg L^{-1} of distilled water. From this stock solution of salicylic acid (SA) the working standards were prepared in drug free/control urine samples containing 50, 100, 150, 200, 250 and 300 μg mL^{-1} salicylic acid solution.

Improved colorimetric method (Farid *et al.*, 1975) was followed by taking 1ml of standard urine and 0.5 mL of 6 M HCl in an extracting tube. This mixture was extracted twice with 10 ml of carbon tetrachloride (CCl_4). Organic layer was separated and 5 ml of 1.7% freshly prepared $Fe(NO_3)_3$ reagent was added to this organic layer. After shaking for 5min a violet colour was appeared immediately. 3 ml of this coloured layer was centrifuged for 10 min, at 3000 rpm and after staying for 20 min, absorbance was noted at 530 nm with the help of spectrophotometer (Hitachi Model U-2001) and concentration of free SA was determined. For the determination of free salicylic acid concentration in urine samples same standard procedure of drug free/control urine was followed.

Calculations

From concentration of free salicylic acid other parameters were calculated by using these formulas.

Concentration (mg ml^{-1}) = Standard factor x absorbance

$$\text{Diuresis} = \frac{\text{Volume of urine in collection period (ml)}}{\text{Time (min) x body weight (kg)}} \quad (\text{ml min}^{-1}\text{kg}^{-1})$$

Amount of drug excreted (mg) = Uc x Uv

$$\text{Percentage of dose excreted} = \frac{\text{Amount of drug excreted (mg)}}{\text{Total dose (mg) given}} \times 100$$

$$\text{Percentage cumulative dose excreted} = \frac{\text{Cumulative amount excreted (mg)}}{\text{Total dose of (mg) given}} \times 100$$

$$\text{Rate of excretion} = \text{Diuresis (ml min}^{-1}\text{ kg}^{-1}\text{) x conc. Of salicylic acid excreted (μg mL}^{-1}\text{) (μgmin}^{-1}\text{kg}^{-1}\text{)}$$

Statistical analysis: The results were given as mean±SE by subjecting the data in tabulated form to statistical calculation and correlation between rate of excretion and pH of urine was determined by means of regression and correlation analysis (Steel and Torrie, 1992).

Results and Discussions

The mean ±SE values for pH of urine voided by male volunteers was 6.17±0.18 while pH of urine voided by female volunteers was 6.04±0.14 (Fatima, 2002).

The mean±SE values for urine concentration of ASA as free SA at various time intervals have been plotted in Fig. 1.

The urine concentration at 30 min was 30.4±4.08 μgmL^{-1} which increases with the passage of time due to availability of free drug. The mean maximum urine concentration 69.5±25.2 μg mL^{-1} was achieved at 60 min because that most free drug was found there at that time. After that it began to decrease and after 600 min. (10 h) there was mean value of 33.78±4.04 μg mL^{-1}. Present study showed mean±SE value for concentration of ASA as free SA in urine of 12 male volunteers was 37.55±0.418 μg mL^{-1}. While in female volunteers it was found to be 44.66±0.398 μg mL^{-1} (Fatima, 2002). Mays *et al.* (1984) reported that this value after 8h and at 975 mg dose was 85.0±1.1 μg mL^{-1}. The difference may be due to dose difference, sex and urine pH.

The rate of urine flow in a time period is called diuresis. The mean ±SE value for diuresis was 0.028±0.006 mL min^{-1} kg^{-1}. The slight difference may be due to body weight, sex, volume and pH of urine voided by volunteers. The rate of excretion of ASA as free SA was 0.849±0.226 μg min^{-1} kg^{-1}. Rate of excretion has direct relationship with pH of urine as it increases with increase in pH of urine due to acidic drug and vice versa.

The amount of ASA (mg) excreted as free SA in urine of male volunteers was 5.40±1.15 mg. Fatima (2002) calculated the amount of ASA as free SA in female volunteers and the value was 6.45±1.19 mg. Amick and Mason (1979) reported that this value with 650 mg dose was 46.3 mg at time of 0-10 h. While Farid *et al.* (1975) reported the amount was 34.7±0.4 mg after 579.7 mg dose of sodium salicylate which is equivalent to 500 mg of salicylic acid.

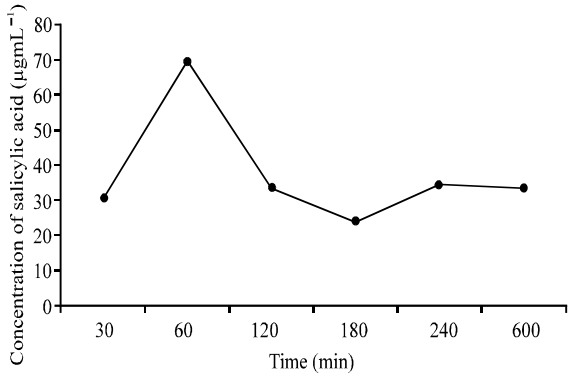

Fig. 1: Plot of urine concentration of salicylic acid (μgml^{-1}) excreated versus time (min) in male volunteers following oral dose of 2x300 mg aspirin

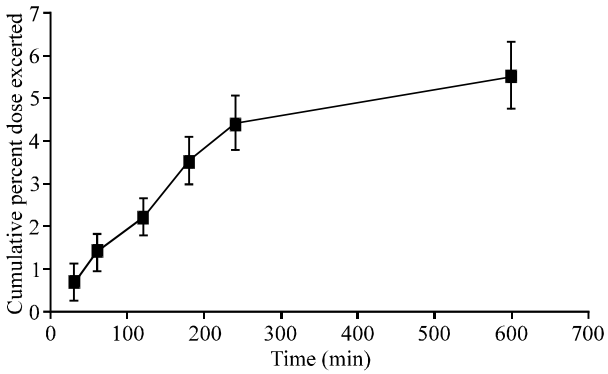

Fig. 2: Plot of percentage cumulative amount of salicylic acid excreted in urine of male volunteers versus time following oral dose of 2x300 mg aspirin

Table 1: Cumulative percentage amount of acetylsalicylic acid (mg) excreted as free salicylic acid in urine of male volunteers following oral administration of (2 x 300 mg) aspirin

	Time (min)					
Volunteers	30	60	120	180	240	600
1	0.82	1.35	2.33	3.69	4.51	5.07
2	0.14	0.46	1.05	2.11	2.32	2.88
3	0.19	0.32	0.35	0.73	1.41	1.90
4	0.71	0.80	1.39	3.15	4.23	5.42
5	0.28	0.41	1.73	3.97	4.96	7.11
6	2.69	3.46	5.02	7.14	8.47	10.11
7	0.40	1.12	1.62	2.15	2.97	3.26
8	1.11	1.51	3.13	6.44	8.29	10.32
9	0.26	0.74	1.20	1.98	2.50	3.33
10	0.46	3.51	4.03	5.06	5.66	6.47
11	0.81	2.71	3.50	3.92	4.63	5.56
12	0.20	0.45	1.05	2.30	3.01	4.24
Mean	0.67	1.40	2.20	3.55	4.41	5.47
±SE	0.20	0.34	0.41	0.55	0.64	0.78

The present dose excreted as free salicylic acid in this study was 0.92±0.19% while in females the % dose excreted was 1.07±0.20%. Levy (1963) reported that percent dose excreted as free SA was 3.6% in urine after taking 100 mg of dose. Hutt *et al.* (1986) reported that after 900 mg orally dose in 0-12 h, percent dose of free SA excreted in both males and females was 8.1±6.5% and 8.4±5.3% respectively.

Cumulative amount of ASA as free SA was 17.74±2.91 mg in male volunteers whereas in female it was 20.85±2.71 mg (Fatima, 2002). Cumulative percent of dose excreted as free salicylic acid was given in Table 1 and cumulative percent of dose excreted with the passage of time was given in Fig. 2.

The present study showed that this amount was found to be 5.47±0.778%. While Fatima (2002) calculated the cumulative percent of dose of same drug in females and the value was 6.45±0.64%.

The results of this study indicated that urinary excretion of acetylsalicylic acid as a free salicylic acid is found to be slightly different in local subjects (male and female). Moreover urinary excretion data also differed when compared with foreign literature. Therefore the present project was designed to evaluate the dosage regimen of aspirin in our local environment.

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