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Urinary Excretion of Acetylsalicylic Acid in Female Volunteers

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Abstract: Urinary excretion of acetylsalicylic acid (aspirin) as free Salicylic Acid (SA) was observed in 12 healthy young female volunteers. After oral administration of 600 mg of drug (ASA) urine samples were collected at predetermined time intervals. Mean±SE value for pH of urine in this study was 6.04±0.14, diuresis was 0.029±0.005 $mLmin^{-1} kg^{-1}$, concentration of free Salicylic Acid (SA) was 44.66±0.389 $\mu g\ mL^{-1}$. Mean ±SE value for rate of excretion was 1.087±0.247 $\mu g\ min^{-1} kg^{-1}$, amount excreted was 6.445±1.188 mg and percentage dose excreted was 1.076±0.197 percent. The mean ±SE value for the cumulative amount excreted was 20.85±2.708 mg and percent cumulative amount excreted was 6.45±0.64 percent. The results indicated that there was low urinary excretion under indigenous conditions.

Key words: Acetylsalicylic acid, urinary excretion, salicylates, cumulative excretion

Introduction

Acetylsalicylic acid (aspirin) is common household non-steroidal, anti-inflammatory drug (NSAID) having analgesic, antipyretic and anti-inflammatory effect (Stoker, 2001). It irreversibly inhibits the activity of enzyme ±Cyclooxygenuse± which catalyses the conversion of free arachidonic acid into endoperoxide compounds (Payan, 1992). It is also used in ischemic heart diseases and strocks (Babu and Salvi, 2000). Acidic NSAIDs react with antacids which increases urinary pH >7 (Brouwers and Desmet, 1994). They are used for painful and inflammatory disorders such as postoperative pain, dental surgery, headache, acute and chronic musculoskeltal pain (Walker, 1995).

After hydrolysis acetylsalicylic acid (aspirin) is converted into salicylic acid (SA) and acetic acid. Salicylic acid is pharmacologically active metabolite of aspirin which is oxidized to produce gentisic acid (GA), conjugates with glycine to form salicyluric acid (SUA) and with glucuronide forms ester and ether conjugates. Half life of aspirin is 20-30 minutes with acute administration it is 3-6 h, while with chronic administration it is 15-30 h (Wesley, 1990). Excretion of free salicylic acid is extremely variable and depends upon dose of drug and pH value of urine. In alkaline urine more than 30% of ingested drug may be eliminated as free salicylic acid where as in acidic urine this may be as low as 2% (Insel, 1996).

The clearance of salicylates is increased 4 folds when pH of urine is >8. At this pH it is highly ionized and cannot readily back diffuse from tubaular fluid (Wesley, 1990).Many factors such as age, specie, seasons, sex variation and enzyme inhibition effect the metabolism of drug. Rabbits and mice do not show the sex difference in drug metabolism. However, in humans male and female individuals show the difference in metabolism of nicotine and aspirin (Low, 1998).

Genetic and environmental factors play an important role in inter and intra-individual variability in drug metabolism. A series of indigenous studies in human beings and animals have clearly indicated that metabolism and urinary excretion of investigated drugs were different under indigenous condition when compared with values given in literature (Nawaz, 1994, Rashid *et al*., 2001). Keeping in view the indigenous condition the present project was designed to study urinary excretion of acetylsalicylic acid in female volunteers from this study we can also study the drug toxicity problem.

Materials and Methods

Subjects: Twelve healthy young female volunteers having mean age 21.08 years, mean body weight 57.67 kg and mean height 159.39 cm selected from Chemistry Department (University of Agriculture, Faisalabad, Pakistan). All volunteers were apprised of the study protocol and a written consent was signed by each subject.

Study design: Sampling was done in month of May. Drug free urine sample was collected by each volunteer before the experimentation. After overnight fastening each volunteer received 600 mg Dispirin (soluble aspirin) by Rekitt Benckiser Pakistan Ltd. orally. Volunteers did not receive any medication seven days prior to and during course of study. All subjects were allowed to take breakfast two h after following the oral dose.

Collection of urine samples: Urine samples were collected at 30, 60, 120, 180, 240 and 600 minutes after following oral

dose. Total volume of urine voided during this time was noted. The pH of all urine samples was recorded and known volume of each sample was preserved in small plastic bottles at -20°C in freezer for further laboratory analysis.

Urine analysis: Quantitative determination of acetylsalicylic acid as a free salicylic acid (SA) was carried out by a validated calorimetric method of Levy and Proknal as modified by Farid *et al.* (1975). It was based on the selective extraction of free salicylic acid (SA) from 1 mL of urine by two 10 ml portions of CCl_4 after acidification with HCl. The reextraction was made by adding 5 mL of 0.17% of $Fe(NO_3)_3$ solution (a 10-fold dilution of 17 gm of $Fe(NO_3)_3 . 9H_2O$ in 1 L of 70 $mmoleL^{-1}$ HNO_3). The colored aqueous phase was centrifuged at 3000 rpm and absorbance was noted by spectrophotometer (Hitachi Model U-2001) at 530 nm. To calibrate the instrument run blank by adding 1 mL of distilled H_2O + 5mL of 0.17% $Fe(NO_3)_3$ reagent.

Urinary excretion: Excretion is a process by which drug or its metabolites are eliminated from the body without chemical change.

Urinary excretion of drug was studied by calculating the following parameters:

Concentration of drug (μgmL^{-1}) = standard factor x absorbance

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

Urinary Excretion

Amount of drug excreted = Uc x Uv

Uc = Concentration of drug in urine (mg)

Uv = Total volume of urine voided (mL)

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

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

Statistical analysis: Results were tabulated and subjected to statistical analysis according to Mean±SE values (Steel and Torrie, 1992).

Results and Discussion

Metabolism and excretion constitute the elimination phenomenon. Human body is composed of physiologically and biochemically different characteristics on the basis of age, body weight, sex, kidney functions which play an important role in drug elimination. Urinary excretion of drug depends upon pH of urine, dose of drug given and diuresis i.e. rate of urine flow.

Results have shown the mean ±SE value for pH of urine, diuresis ($mLmin^{-1}\ kg^{-1}$), concentration of free salicylic acid excreted (μgmL^{-1}). Amount excreted in (mg). Rate of excretion of drug $\mu gmin^{-1}\ kg^{-1}$, percentage dose excreted cumulative amount excreted (mg) and percentage cumulative amount excreted as mean±SE values are discussed.

In this study mean±SE value for pH of urine in 12 female volunteers was 6.04±0.14 which is comparable to urine pH 6.5±0.7 for male and 6.3±0.5 for the female volunteers after giving 900 mg oral dose of drug as reported by Hutt *et al.* (1986). The difference was due to dose of drug used, sex variation nutritional ingredients used and geonatical difference.

Mean ±SE value for the concentration of free salicylic acid in female volunteer was 44.66±0.398 μgmL^{-1} while after 10 h the value was 58.93±0.44 μgmL^{-1} as shown in Table 1. The plot of concentration of free SA versus time is shown in Fig. 1. Concentration of drug depends upon the pH of fluid and pka value of drug. pH is an important parameter which differs among local and foreign species (Nawaz, 1994). Weak acids are excreted more rapidly in alkaline medium primarily because they are more ionized and passive reabsorption is decreased and vise versa.

In this study mean±SE value for diuresis was 0.029±0.005 $mLmin^{-1}\ kg^{-1}$ in 12 female volunteers. The value is comparable to earlier reported value 0.037±0.017 $mLmin^{-1}\ kg^{-1}$ is male volunteers (Fauzia, 1998). In summer season diuresis was less because of sweating. Diuresis value may also vary due to body weight, volume of urine and gender variation.

Mean ±SE value for rate of excretion in this study was 1.087±0.247 $\mu gmin^{-1}\ kg^{-1}$. Amount excreted as free salicylic acid was 6.445±1.188 mg. While Amick and Mason (1979) reported amount of free salicylic acid 46.3 mg 10 h after following oral dose of 650 mg of drug. However, value is also in comparison with 34.7±0.4 mg after 579.7 mg dose of sodium salicylate as reported by Farid *et al.* (1975). The difference among local and foreign literature was due to dose of drug used and pH of urine. The mean ±SE value for percentage dose excreted as free salicylic acid in urine of 12 female volunteers in this study was 1.076±0.197%. However, after 1000 mg of dose 3.6% free salicylic acid was excreted as reported by Levy (1963). After 900 mg dose of both the plain and entericcoated tablets percentage dose as free salicylic was 9.4±3.5% and 6.4±3.1% respectively (Montgomary and Sitar, 1986). After 900 mg of oral dose during 0-12 h percentage dose of free salicylic acid excreted was 8.4±5.3% in female while

Table 1: Concentration (μgmL^{-1}) of salicylic acid excreted in urine of healthy female volunteers following oral dose (2×300 mg) of aspirin

	Time (min)					
Volunteers	30	60	120	180	240	600
1	21.66	22.99	30.50	26.08	31.83	64.00
2	23.87	31.83	22.99	22.54	22.10	79.13
3	34.92	80.01	36.69	23.43	22.54	57.03
4	37.13	39.34	38.02	31.83	68.96	129.08
5	24.75	149.86	42.88	38.02	23.87	80.89
6	23.87	26.62	47.30	30.06	24.31	62.33
7	51.72	93.72	98.58	53.93	38.02	42.38
8	31.83	35.36	28.29	26.52	21.22	38.46
9	38.02	29.62	25.64	26.08	26.96	30.94
10	81.34	198.05	71.62	57.03	42.88	45.09
11	40.67	55.26	33.59	38.46	32.71	50.39
12	22.10	24.31	36.25	23.87	23.43	27.41
Mean	35.99	65.58	42.70	33.15	31.57	58.93
+SE	0.34	0.63	0.39	0.28	0.31	0.44

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

	Time (min)					
Volunteers	30	60	120	180	240	600
1	0.32	1.28	2.14	2.62	3.41	5.81
2	1.19	2.31	3.26	4.02	5.01	6.33
3	0.87	4.21	5.31	6.09	6.84	7.79
4	0.77	2.34	4.11	5.70	8.29	8.83
5	0.21	0.58	1.08	1.84	2.28	3.63
6	0.40	0.99	3.35	4.36	5.01	5.60
7	0.28	0.35	2.98	4.06	5.96	6.96
8	0.21	0.45	0.64	1.70	2.93	3.89
9	0.25	1.19	2.37	3.56	4.91	6.20
10	0.54	1.17	1.65	4.26	6.76	8.45
11	1.53	4.29	5.55	6.99	8.89	10.57
12	0.83	1.10	1.54	2.54	3.03	3.37
Mean	0.62	1.69	2.83	3.98	5.28	6.45
± SE	0.12	0.39	0.46	0.48	0.62	0.64

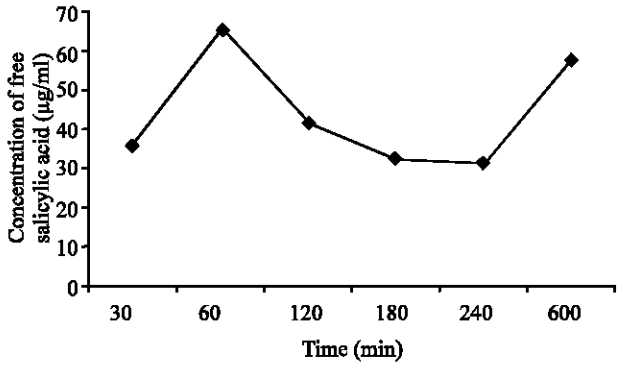

Fig. 1: Plot of urine concentration of Salicylic acid ($\mu g\ mL^{-1}$) excreted versus time (min) in female volunteers following oral dose of 2×300 mg aspirin

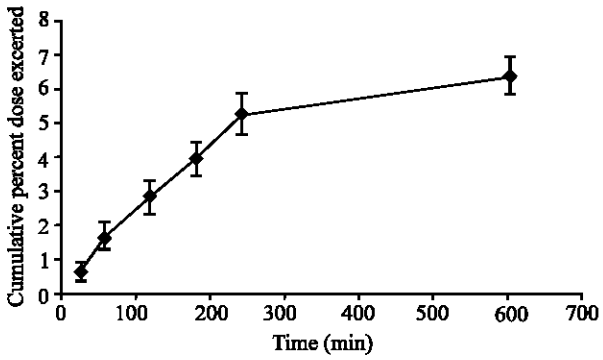

Fig. 2: Plot of percentage cumulative amount of Salicylic acid excreted in urine of female volunteers versus time following oral dose of 2×300 mg aspirin

in male it was 8.1±6.5% while total study shows the mean±SE value 8.3±5.9% (Hutt *et al*., 1986). The difference was due to chemical nature of two formulations, dose of drug given and other variation in environment. In this study since re-absorption of drug take place so we can also study the drug toxicity problems.

Mean ±SE value of cumulative amount (mg) excreted in female volunteer was 20.85±2.708 mg. While percentage cumulative amount excreted was 6.45±0.64% after 10 h of oral drug administration as represented in Table 2. The plot of cumulative percent amount excreted in female volunteers versus time is also shown in Fig. 2.

The study supports the comprehensive evaluation of the drug under indigenous conditions to obtain the useful information on which the rational dose regimens of drug could be based.

References

Amick, E.N. and W.D. Mason, 1979. Determination of Aspirin, Salicylic Acid, Salicyluric Acid and Gentisic Acid in Human Plasma and Urine by High Pressure Liquid Chromatography. J. Anal. Letters, 12: 629-640.

Babu, K.S. and S.S. Salvi, 2000. Aspirin and Asthma (Review). Chest, 118: 1470-1476.

Brouwers, J.R.B.J. and P.A.G.M. Desmet, 1994. Pharmacokinetic and Pharmacodynamic Interaction with Non-steroidal Anti-inflammatory Drugs. Clin. Pharmacokientic, 27: 462-485.

Farid, N.A., G.S. Born, W.V. Kessler, S.M. Shaw and W.E. Lang, 1975. Improved Colorimetric Determination of Salicylic Acid and its Metabolites in Urine. Clin. Chem., 21: 1167-1168.

Fouzia, A.C., 1998. Urinary Excretion of Aspirin in Male volunteers. M.Sc. Thesis, Univ. Agric., Faisalabad.

Hutt, A.J., J. Caldwell and R.L. Smith, 1986. The Metabolism of Aspirin in man: a Population Study. Xenobiotica, 16: 239-249.

Insel, A.P., 1996. Analgesic-Antipyretic and Anti-inflammatory Agents and Drug Employed in the Treatment of Gout. In: The Pharmacological Basis of Therapeutics. 9th Ed. McGraw Hill company. Inc. New York, pp: 625.

Levy, G., 1963. Pharmacokinetics of Salicylate Elimination in Man. J. Pharmaceutical Services, 7: 959-967.

Low, L.K., 1998. Metabolic changes of drugs and related organic compounds: In. Text Book of Organic Medicinal and Pharmaceutical Chemistry. 10th Edition Lippincott-Raven Publishers. Philadelphia, New York, pp: 110-111.

Montgomery, P.R. and D.S. Sitar, 1986. Acetylsalicylic Acid Metabolites in Blood and Urine After Plain and Enteric Coated Tablets. Biopharm. Drug Dispos., 7: 21-25.

Nawaz, M., 1994. Geonotical factors affecting biodisposition of drugs. Canadian. J. Physiol and Pharmacol., 72: 307.

Payan, D.G., 1992. Nonsteroidal Anti-inflammatory Drugs. Non opioid Analgesic Drugs Used in Gout. In: Basic and Clinical Pharm. 5th Ed., pp: 491-508.

Rashid, A., M.Z. Chaudry and S. Bakhash, 2001. Pharmacokinetics of Sulphamethaxazole in Male Human Volunteers. JOPAS, 20: 37-51.

Steel, R.G.D and J.H. Torrie, 1992 Principles and Procedures of Statistics. McGraw Hill book Co. Inc., New York.

Stoker, S.H., 2001. Organic and Biological Chemistry 2nd ed. Houghton company. New York, pp: 159.

Walker, J.S., 1995. NSAID-An update on Their Analgesic Effects. Clin and Exp. Pharmacol and Physiol., 22: 855-860.

Wesley, G.C., D.Q. Craig, M.D. Brater and A.R. Johnson, 1990. Goth's Medical Pharmacology. Asian Student Edition. Galgotia Publication Ltd., pp: 371-372.