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Renal Clearance of Acetylsalicylic Acid in Human Male Volunteers

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Abstract: Renal clearance of endogenous creatinine and acetylsalicylic acid was investigated in 12 healthy male volunteers. Blood and urine samples were collected at pre-determined time intervals. The concentration of acetylsalicylic acid (as salicylates) and creatinine in plasma and urine were determined colorimetrically. Mean \pm SE values were found to be, for diuresis $0.031 \pm 0.011 \text{ mL min}^{-1} \text{ kg}^{-1}$, Plasma concentration of creatinine and salicylic acid $15.11 \pm 0.166 \text{ } \mu\text{g mL}^{-1}$ and $29.38 \pm 0.12 \text{ } \mu\text{g mL}^{-1}$, urine concentration of creatinine and salicylic acid $855.73 \pm 1.53 \text{ } \mu\text{g mL}^{-1}$ and $38.30 \pm 0.43 \text{ } \mu\text{g mL}^{-1}$, renal clearance of creatinine and salicylic acid $1.52 \pm 0.08 \text{ mL min}^{-1} \text{ kg}^{-1}$ and $0.063 \pm 0.02 \text{ mL min}^{-1} \text{ kg}^{-1}$ and clearance ratio 0.054 ± 0.016 . The results indicated that there is less renal clearance of acetylsalicylic acid in local environment when compared with values given in literature.

Key words: Acetylsalicylic acid, renal clearance, male volunteers, creatinine, salicylates

Introduction

Aspirin formerly referred to generically as acetylsalicylic acid is a member of salicylate group of compounds. It is a synthetic drug. In pharmacological studies, aspirin has shown anti-inflammatory, analgesic and anti-pyretic activity. Because of its efficacy and low toxicity, it is the standard with which all other non-steroidal anti-inflammatory agents are compared (Simon and Mills, 1980). Aspirin shows antipyretic and analgesic effect by inhibition of synthesis of prostaglandins (Punnon, 1984). The primary precursor of prostaglandin is arachidonic acid which is present as conjugated component of the phospholipid of cell membrane and is released by the action of phospholipase enzyme (PLA_2) and prostaglandins and produced by oxidative metabolism of free arachidonic acid (Girdwood, 1979). It is also used against myalgia, myocardial infarction, stroke and rheumatoid arthritis because aspirin irreversibly inhibits the platelet cyclooxygenase by acetylation, hence diminishes the formation of thromboxane A_2 (Reynolds *et al.*, 1989).

Aspirin is hydrolyzed in stomach and in blood to salicylic acid and acetic acid, therefore its biological half life is 20 min (Done, 1960). After oral administration, 80-100% is absorbed in the stomach and small intestine. However, bioavailability is low because partial hydrolysis occurs during absorption (Borga *et al.*, 1976).

The studies conducted overall several years under indigenous conditions have revealed differences in kinetic behaviour, urinary excretion and metabolism when compared with values given in literature. Keeping in view

the above mentioned facts the present study was designed to investigate the renal clearance of acetylsalicylic acid in male volunteers under local condition.

Materials and Methods

Subject: 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 and drug administration: After an overnight fasting, control blood and urine samples were taken from twelve healthy male volunteers. Each volunteer was given two tablets of soluble aspirin (600 mg) direct orally (known as dispirin manufactured by Reckitt Benckiser of Pakistan Ltd., Karachi).

Sample collection: Blood samples were drawn after 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480 and 600 min in heparinized centrifuged tubes and urine samples were taken at 30, 60, 120, 180, 240 and 600 min after oral drug administration. pH of each sample was noted then the blood samples were centrifuged at 4000 rpm for 10 min to separate plasma. Plasma and urine samples were stored at -20°C till further analysis.

Analysis of creatinine: For the estimation of GFR, the endogenous creatinine renal clearance was measured in

plasma and urine sample colorimetrically by Bonsnes and Taussky (1945) method. To 0.5 ml of plasma, 4 ml of N/12 H₂SO₄ and 0.5 ml of 10% Na₂WO₄ was added and centrifuged at 4000 rpm for 15 min. To 3 ml of supernatant 2 ml of fresh alkaline picrate was added and absorbance was noted at 515 nm after 20 min.

Urine samples were diluted to 1:50 and 1:100. In 3 ml of each dilution 2 ml of freshly prepared alkaline picrate was added and absorbance was noted at 515 nm after 20 min.

Analysis of drug: The concentration of drug in the blood and urine was determined by following the process of Martens and Mayer (1995) and Farid *et al.* (1975) methods were used, to 0.5 ml of plasma sample added 0.5 ml of 6 N HCl and extracted twice with 6 ml of CHCl₃ and centrifuged for 15 min. Layer of CHCl₃ was separated two times, to this CHCl₃ layer added 3 ml of 0.1 N NaOH shaken and centrifuged then took 2 ml of supernatant, added 0.3 ml of 1 N HNO₃ and 0.2 ml of 1.7% Fe (NO₃)₃. Violet colour appeared and absorbance was noted at 530 nm after 20 min. For urine analysis 0.5 ml concentrated HCl was added to 1 ml of urine sample and extracted twice with 10 ml of CCl₄, shaken for 10 min. For further extraction added 5 ml of Fe (NO₃)₃ and shaken, violet colour appeared 3 ml of coloured layer was taken and centrifuged, after 20 min absorbance was noted at 530 nm with the help of spectrophotometer (Hitachi Model U-2001).

Calculations

$$\text{Diuresis (D)} = \frac{\text{Urine volume in a collection period (ml)}}{\text{Time (min)} \times \text{Body weight (kg)}}$$

Creatinine concentration = Absorbance x standard factor x dilution factor

Drug concentration = Absorbance x standard factor

$$\text{Renal clearance} = \frac{\text{Concentration in Urine (Uc)} \times \text{Diuresis (D)}}{\text{Concentration in plasma}}$$

$$\text{Clearance ratio} = \frac{\text{Renal clearance of drug}}{\text{Renal clearance of creatinine}}$$

Statistical analysis: The results obtained were presented at means±SE. The data was tabulated and subjected to regression/correlation analysis (Steel and Torrie, 1992).

Results and Discussion

The results showing average ±SE values for diuresis, pH of blood and urine samples, concentration of endogenous creatinine and salicylic acid in plasma and urine, their renal clearance and clearance ratio are given in Table 1 and Fig. 1-3. The average ±SE for the rate of urine flow was 0.03±0.01 ml min⁻¹kg⁻¹, while earlier studies average ±SE values were 0.032±0.006 (Rashid, 1998). The slight difference is only due to temperature difference because in hot climate evaporation reduces urine flow during summer (Nawaz and Shah, 1984).

Present study showed an average ±SE values for the pH of blood and urine which were 7.67±0.018 and 6.17±0.059 and comparable to blood pH 7.59±0.015 and urine pH 5.97±0.056 (Majeed, 2003). Beside the seasonal influence the urine pH is markedly influenced by type of food which is different in summer and winter season leading to lower urinary pH. Concentration of creatinine in plasma and urine was 15.11±0.166 and 855.37±1.52 µg mL⁻¹. But average±SE values are 14.16±0.55 and 493.22±1.18 µg mL⁻¹ for female volunteers (Majeed, 2003). This difference is only due to the difference in genome, age, temperature and weight. The mean ±SE values of salicylic acid in plasma and urine were 29.38±0.118 and 38.30±0.427 µg mL⁻¹ while in female average ±SE value is 26.64±0.63 µg mL⁻¹ in plasma (Ijaz, 2002) and 37.55±8.61 in urine (Fatima, 2002). The results were found to be statistically different which may be due to the difference in indigenous condition. The average ±SE values for renal clearance of creatinine and salicylic acid were 1.519±0.088 and 0.063±0.019 mL min⁻¹ kg⁻¹ and this value is less than 2 mL min⁻¹ kg⁻¹ (Ganong, 1997) which clearly indicates that GFR is lower in local species. The present study showed average ±SE clearance ratio which is 0.054±0.16 while in female is 0.0926±0.023 (Majeed, 2003). This value is less than 1. So, it indicates that reabsorption of drug take place.

A non significant positive correlation between diuresis and renal clearance of SA shows back diffusion. There is significant positive correlation between urine pH and SA clearance. So, pH of urine has significant influence on the renal clearance of drug. There is non significant positive correlation between diuresis and clearance ratio and also non significant positive correlation between pH of urine and clearance ratio. There are non-significant negative correlation's between plasma concentration of SA and clearance ratio and plasma concentration of SA and drug clearance. It is concluded that besides glomerular filtration, mechanism of back diffusion and reabsorption was also involved in SA reaction.

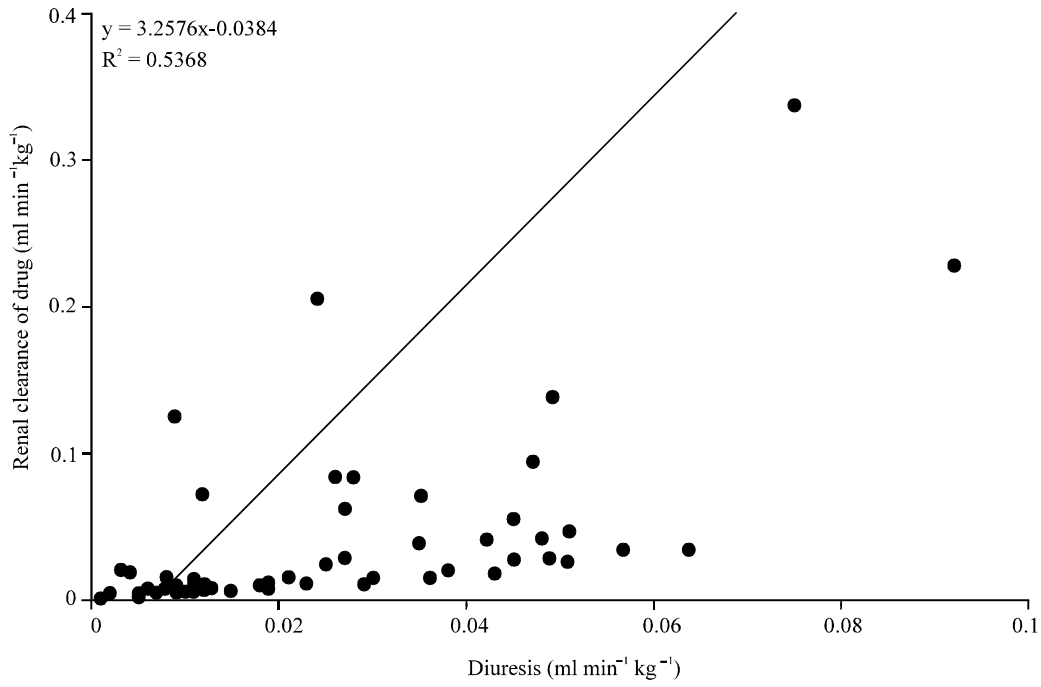


Fig. 1: Relationship of diuresis with renal clearance of salicylic acid in 12 healthy male volunteers

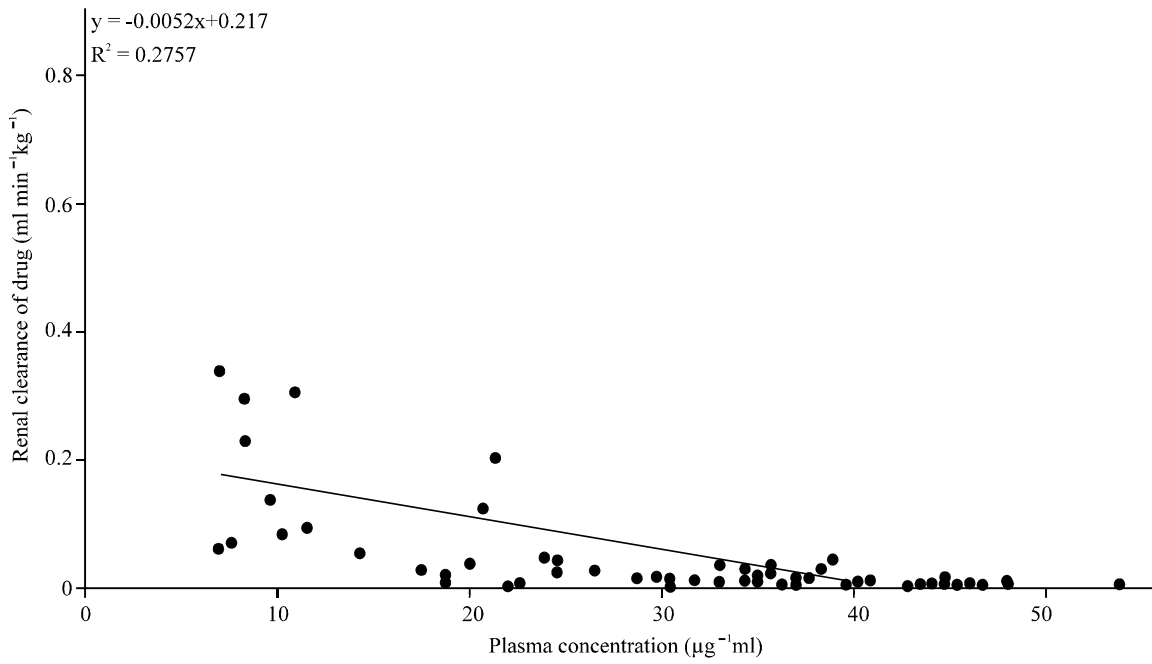


Fig. Relationship of Plasma concentration of salicylic acid and its renal clearance in 12 healthy male volunteers

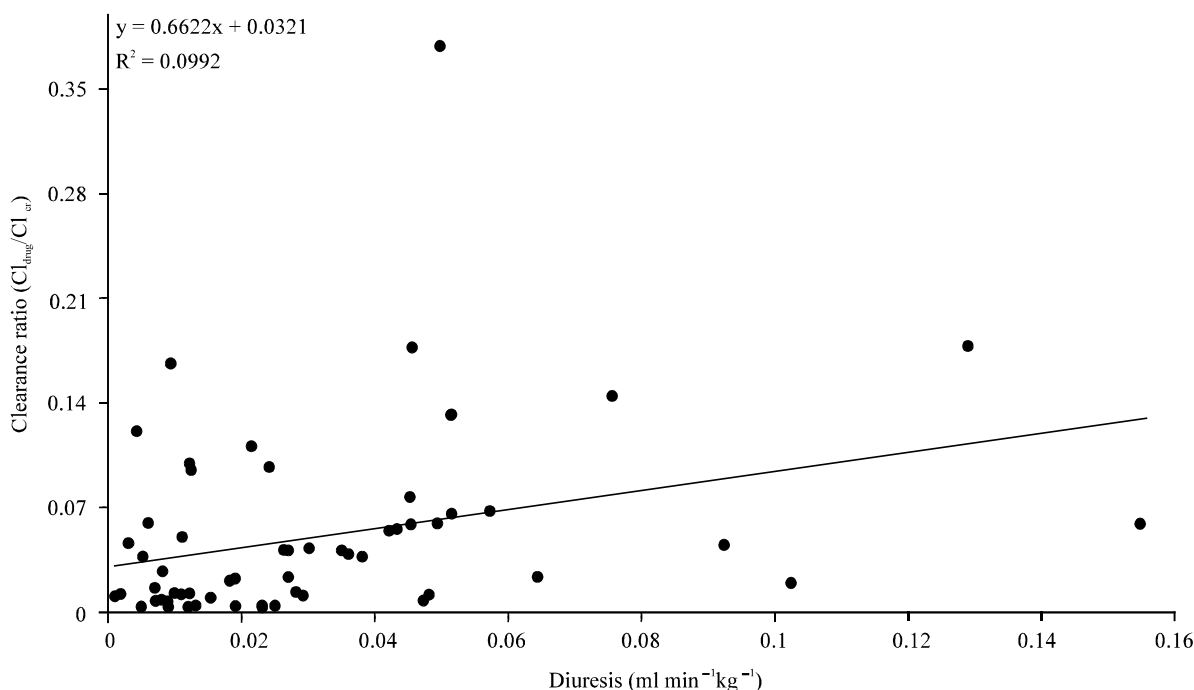


Fig. 3: Relationship between diuresis and Clearance ratio in 12 healthy male volunteers

Table 1: Average data of renal clearance of endogenous creatinine and aspirin in male volunteers after oral dose of 2x 300 mg tablets

Volunteers	Body weight (kg)	Diuresis ml min ⁻¹ kg ⁻¹	pH		Concentration (µg ml ⁻¹)		Concentration (µg ml ⁻¹)		Renal clearance ml min ⁻¹ kg ⁻¹		Clearance ratio Cl _{asp} /Cl _{cr}
			Urine	Blood	Creatinine plasma	Creatinine urine	Drug plasma	Drug urine	Creatinine	Drug	
A	65	0.06	5.82	7.64	17.25	460.58	25.87	16.79	1.44	0.08	0.05
B	62	0.03	6.89	7.65	15.84	677.68	27.05	16.79	0.87	0.03	0.03
C	67	0.01	5.57	7.64	22.85	1551.57	27.56	16.70	0.88	0.02	0.02
D	72	0.03	5.63	7.69	13.95	1265.53	29.01	32.01	1.54	0.06	0.03
E	65	0.03	5.96	7.63	18.83	544.90	30.57	31.65	0.52	0.03	0.14
F	65	0.06	6.42	7.62	17.09	584.69	30.71	36.86	1.53	0.20	0.09
G	70	0.03	6.62	7.63	14.10	762.39	32.02	22.89	2.20	0.03	0.04
H	65	0.05	6.22	7.67	8.78	994.85	31.23	40.85	4.51	0.08	0.02
I	59	0.02	6.77	7.67	10.35	909.35	31.49	22.10	2.29	0.03	0.02
J	68	0.02	5.56	7.77	10.75	732.05	30.84	60.12	1.33	0.07	0.04
K	67	0.02	6.81	7.72	13.90	588.24	28.09	107.59	0.72	0.10	0.10
L	70	0.01	5.86	7.71	17.68	1196.97	28.09	55.28	0.42	0.03	0.07
Average	66.250	0.03	6.18	7.67	15.11	855.73	29.38	38.30	1.52	0.06	0.05
SD	3.596	0.02	0.51	0.05	3.99	336.33	2.02	26.20	1.12	0.05	0.04
±SE	0.158	0.01	0.06	0.02	0.17	1.53	0.12	0.43	0.09	0.02	0.02

The result of this study indicated that renal clearance is found to be slightly different in local subjects (male and female). Moreover, renal handling data also different when compared with foreign values. Study supports the need for comprehensive evaluation of drug under our own environmental conditions to obtain clearance ratio on which rational dose regimen of drug could be based.

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