PLASMA LEVELS OF ASPIRIN METABOLITES IN LIBYAN PATIENTS WITH RHEUMATOID ARTHRITIS AND RHEUMATIC FEVER

MUSTAFA M. ZIU*
A. S. M. GIASUDDIN*

SUMMARY: Plasma aspirin metabolite levels were studied in Libyan adult patients with rheumatoid arthritis (RA) (Number: 4, age: 37-50 years, sex: 2 males, 2 females) and in pediatric patients with rheumatic fever (RF) (Number: 6, age: 5-12 years, sex: 3 males, 3 females) given high doses of aspirin. Two hours after the daily dose of aspirin therapy, plasma salicylic acid (SA), salicyluric acid (SU) and gentisic (GA) were analyzed by high performance liquid chromatography system. In adult patients with RA, plasma levels of GA varied from 1.2 mg/L on day 1 to 2.7 mg/L on day 7, while SU levels varied from 4.9 mg/L on day 1 to 10.2 mg/L on day 7 and SA varied from 60.2 mg/L on day 1 to 305 mg/L on day 7. All adult patients with RA reached steady state for SA between 5th and 6th day of high aspirin doses. In pediatric patients with RF, plasma SA level varied from 130.2 mg/L to 610 mg/L, while SU level varied from 1.8 mg/L to 17.5 mg/L. Some pediatric patients showed very low levels of plasma GA, while others exhibited plasma GA levels as high as 4.1 mg/L. The females, in both groups of patients, had higher plasma SA levels than males. The female children with RF had higher GA levels than adult patients with RA (3.9 ± 0.1 vs $2.6\pm0.08 \rightarrow t=12.7$, $df = 5$, $p<0.01$). Thus this study had shown that there is a wide variation in the pharmacokinetics of aspirin metabolism. It is therefore suggested that plasma aspirin metabolite levels of patients on long term high dose salicylate therapy be monitored routinely to ascertain if therapeutic, rather than toxic, plasma levels are reached and being maintained.

Key Words: Aspirin, rheumatoid arthritis, rheumatic fever.

INTRODUCTION

Due to remarkable therapeutic versatility of acetyl salicylic acid (aspirin) and it's well known therapeutic action in rheumatic diseases, it is considered as the first drug of choice in the treatment of these diseases particularly rheumatic fever and rheumatoid arthritis (1,3,24,27). However, the pharmacokinetics of aspirin represents a major problem in the design of dosage regimens and may account for the wide differences in the recommended doses for infants and children (23). The problem is particularly serious in rheumatic fever and rheumatoid arthritis because effective aspirin therapy usually requires high doses approaching toxic levels (12). The most severe instances of aspirin toxicity among adults as well as children, however, results form therapeutic overdoses (2,9) and that many more children die of therapeutic rather than accidental aspirin poisoning (6). Aspirin is rapidly hydrolyzed in the body to salicylic acid (SA) which is conjugated in part with glycine to form salicyluric acid (SU) and with glucoronic acid to form salicylic acyl glucoromide (SAG) and salicylic phenolic glucoromides (SPG). A small fraction of SA is further

* From Department of Laboratory Medicine, Faculty of Medicine, Al-Arab Medical University, Benghazi, Libya.

hydroxylated to gentisic acid (GA), and there may be several other minor metabolites (19,21,35). At high dose levels accumulation of aspirin metabolites particularly GA occur, which even at low concentrations may lead to toxic manifestations (12). The incidence of rheumatic fever in the Arab countries including Libya is quite high and aspirin is the drug of choice for its treatment (11,26,34). Therefore as a preliminary, the present study of quantitating the plasma levels of aspirin metabolites (SA, SU, GA) in Libyan children as well as in adult patients with rheumatic fever and rheumatoid arthritis respectively was undertaken to ascertain whether the concentrations of aspirin metabolites were at therapeutic or toxic levels.

MATERIALS AND METHODS

Patients (adults)

Four adult Libyan patients (age: 37-50 years; sex: 2 males, 2 females) admitted to the 7th April Hospital, Benghazi, Libya with rheumatoid arthritis were included in the study. The 2 male patients were receiving daily aspirin doses of 4.8 gm and 5.5 gm respectively and the 2 female patients were receiving daily aspirin doses of 4.0 gm and 4.8 gm respectively (65 mg per kg body weight and given in 3 divided doses at 8 hourly intervals) (Table 1). An aliquot of 10 ml venous blood was collected from each patient into heparinized tubes after 2 hours of the last dose of the day and for the 7 consecutive days.

Patients (children)

Six Libyan children (age: 5-12 years; sex: 3 males, 3 females) admitted at El-Fatah Children's Hospital, Benghazi, Libya with rheumatic fever and on a high dose aspirin therapy were studied. The period of aspirin therapy before the collection of blood varied from 8 to 28 days. The daily aspirin doses varied from 1.2 to 2.9 gm per day (60 mg per kg body weight in 3 divided doses at 8 hourly intervals) (Table 2). After 2 hours of the last daily dose, 5 ml aliquots of venous blood was collected in heparinized tubes from each patient.

The whole blood specimens from the patients (adults as well as children) were centrifuged at 1500 g for 15 minutes. The separated plasma samples were stored frozen in aliquots at -30°C until quantitative analysis for aspirin metabolites (SA, SU, GA) was made by high performance liquid chromatography (HPLC) system. Quantitative Analysis of Plasma Aspirin Metabolites By High Performance Liquid Chromatography (HPLC) System: The quantitative analysis of aspirin metabolites in plasma was carried out according to the method of Rumble *et al.* (31). The HPLC-system used was that supplied by Laboratory Data Control Ltd, Staffs, England (Model: Constrametric III, Spectro-Monitor III) and BDB multi-range recorder supplied by Enraf-Nonius Ltd, England. The column used was spherioserb S5 ODS (250 x 4.5 mm id) (Phase Separations Ltd, Clwyd, UK) with a mobile elution buffer consisting of acetonitrite (0.05%): phosphoric acid: water (15: 10: 75), pH: 2.3 and flow rate 2 ml per minute. The HPLC system was calibrated with standard metabolites (SA, SU, GA) and O-methoxybenzoic acid (O-MBA) as the internal standard (IS). The peak height ratios (%) of the standard metabolites (SA, SU, GA) compared to the internal standard (IS) were calculated (Figure 1) and were plotted graphically against concentrations of respective metabolites (Figure 2). Using the peak height ratios (%), the concentrations of the metabolites (SA, SU, GA) in the patient's plasma were then determined from the standard curves (Figure 2). For estimation of SU, 1 ml aliquot of each plasma specimen was hydrolyzed with 100 μl of β-glucoronidase enzyme for 18 hours at 37°C, extracted according to the method of Brodie *et al.* (4) and the extract was analyzed for SU content by the HPLC system as described.

All standards (salicylic, salicyluric, gentisic and O-methoxybenzoic acids) and β-glucoronidase were purchased from Sigma Chemical Co Ltd, England. The HPLC grade solvents, methanol and acetonitrite, were obtained from Fisons Chemicals Ltd, England or Rathburns Chemicals Ltd, Scotland. The HPLC eluents were ultrasonically degassed using an ultrasound bath (Decon FS 100) supplied by Decon Ultrasonics Ltd, England.

Figure 1: A representative chromatographic trace of blank plasma spiked with known concentrations of aspirin metabolites and internal standard (IS : O-MBA) (GA : 10 mg/L, SU : 20mg/L, SA : 40 mg/L and O-MBA : 20 mg/L) in HPLC-System.

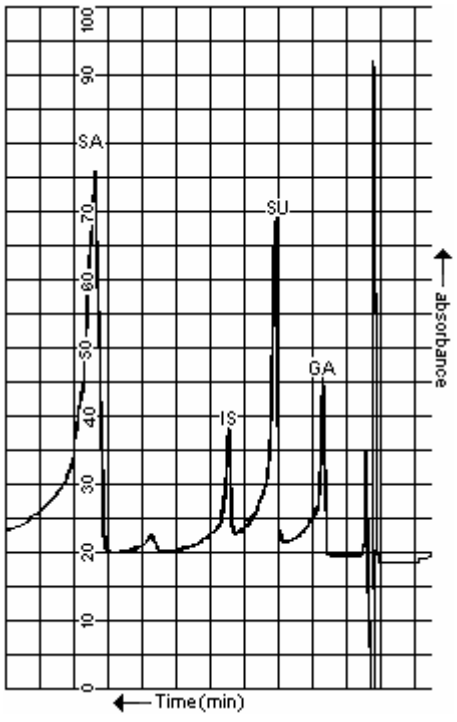


Table 1: Plasma levels of aspirin metabolites (SA, SU, GA; mg/L) two hours after the last daily dose in adult Libyan patients with RA and their statistical analysis.

Patients: Age (Yrs): Sex (F/M): B.Wt. (Kg): Dose (g/day):	FM 37 F 61 4.0	MF 42 F 74 4.8	Mean±SD[+] (Female)	MA 35 M 74 4.8	MM 50 M 85 5.5	Mean±SD[+] (Male)	Students t-test[++] (Female vs Male)	Mean±SD[+] (Male and Female)
*SA (mg/L)								
Day 1:	110	88	99±11	75	60	68±8	NS	84±18
Day 2:	229	174	202±28	162	142	152±10	NS	177±32
Day 3:	297	229	263±34	227	206	217±11	NS	240±34
Day 4:	313	259	286±27	248	220	234±14	NS	260±34
Day 5:	298	249	274±25	241	216	229±13	NS	251±30
Day 6:	304	249	277±28	245	210	228±18	NS	252±33
Day 7:	305	249	277±28	244	211	228±17	NS	253±34
*SU (mg/L)--								
Day 1:	4.9	7.5	6.2±1.3	6.7	7.5	7.1±0.4	NS	6.6±1.0
Day 2:	5.0	7.2	6.1±1.1	7.4	7.6	7.5±0.1	NS	6.8±1.1
Day 3:	7.5	6.5	7.0±0.5	7.0	7.5	7.2±0.3	NS	7.1±0.4
*Day 4:	6.0	6.5	6.2±0.3	7.8	9.1	8.5±0.6	NS	7.4±1.2
Day 5:	6.5	8.5	7.5±1.0	8.3	10.0	9.2±0.8	NS	8.3±1.2
*Day 6:	7.2	6.6	7.9±0.7	8.6	10.1	9.4±0.7	NS	8.6±1.0
Day 7:	8.5	8.6	8.6±0.1	9.2	10.2	9.7±0.5	NS	9.1±0.6
*GA (mg/L)								
Day 1:	1.2	1.8	1.5±0.3	1.6	1.8	1.7±0.1	NS	1.6±0.2
Day 2:	1.3	1.9	1.6±0.3	1.8	1.8	1.8±0.0	NS	1.7±0.2
Day 3:	1.9	2.5	2.2±0.3	1.9	1.8	1.9±0.05	NS	2.0±0.3
Day 4:	1.3	2.6	2.0±0.6	2.5	2.5	2.5±0.0	NS	2.2±0.5
Day 5:	2.5	2.5	2.5±0.0	2.6	2.5	2.6±0.05	NS	2.5±0.05
Day 6:	2.4	2.6	2.5±0.1	2.5	2.5	2.5±0.0	NS	2.5±0.07
Day 7:	2.5	2.7	2.6±0.1	2.5	2.6	2.6±0.05	NS	2.6±0.08

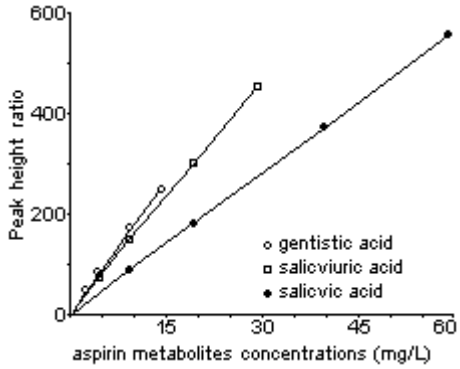
* Aspirin metabolites (SA: Salicylic acid; SU: Salicyluric acid; GA: Gentisic acid); [+]SD: Standard deviation; [++]S: Significant ($p<0.05$ or <0.02 or <0.01) and NS: Non significant ($p>0.05$).

Table 2: Plasma levels of aspirin metabolites (SA, SU, GA; mg/L**) two hours after the last daily dose in Libyan (pediatric) patients with RF and their statistical analysis.

Patients	Age (Yrs)	Sex (F/M)	Body Wt(Kg)	Period[+] (Days)	Dose of aspirin (gm/day)[++]	Plasma aspirin metabolites (mg/L)			
							SA	SU	GA
AS	9	F	25	15	1.5		410.1	7.5	3.8
KG	12	F	45	27	2.7		610.0	1.8	4.1
NG	8	F	20	8	1.2		383.3	6.2	4.0
						Mean±SD	467.8±101.1	5.2±2.4	3.9±0.1
*MM	8	M	20	15	1.2		130.2	6.1	1.0
*SG	5	M	20	28	1.2		226.7	4.0	0.0
GS	8	M	30	9	1.8		295.4	4.5	4.0
						Mean±SD	217.4±67.7	4.9±0.8	1.7±1.6
Statistical analysis by Students's t-test (Female vs Male)							P < 0.05	P > 0.1	P < 0.01

** SA: Salicylic acid; SU: Salicyluric acid; GA: Gentisic acid; * Patients taking antacids; [+] Period of aspirin therapy before collection of blood specimens; [++] 60 mg/kg/day in divided doses taken 6 hourly $p>0.05$: Non significant; $p<0.05$ or $p<0.01$: Significant.

Figure 2: The calibration curves for aspirin metabolites (SA, SU and GA) obtained by plotting the peak height ratios for each metabolite against concentrations (mg/L).



Statistical analysis

Student's t-test was used to evaluate the statistical significance of the results.

RESULTS

Figure 1 shows a representative chromatographic trace of HPLC analyzed blank plasma with known concentrations of the aspirin metabolites and internal standard. GA was eluted at 3.0 min, SU at 4.4 min, IS (O-MBA) at 5.7 min and SA at 10.0 min under the experimental conditions.

Figure 2 illustrates the calibration curves for SA, SU and GA obtained by plotting the peak height ratios (Mean ± standard deviation of 4 observations) for each metabolite against concentration (mg/L). The coefficients of variation were 1.9-4.7% for SA, 2.7-6.3% for SU and 3.6-7.9% for GA for highest and lowest concentrations respectively.

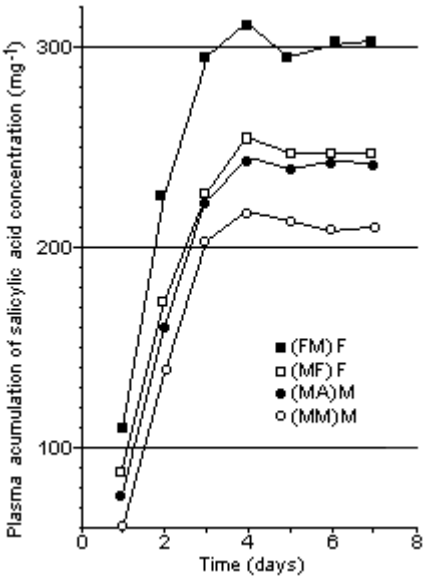
Table 1 shows plasma levels of SA, SU and GA in specimens taken 2 hours after the last daily dose from the 4 Libyan adult patients on 7 consecutive days. The levels of GA varied from 1.2 mg/L on day 1 to 2.7 mg/L on day 7, while SU levels varied from 4.9 mg/L on day 1 to10.2 mg/L on day 7. The levels of SA were however, much higher and varied from 60.0 mg/L on day 1 to 305.0 mg/L on day 7. The female patients had much higher plasma SA levels than the male patients, although statistically not significant ($p>0.05$). Figure 3 illustrates the plasma profiles for SA for the 4 Libyan adult patients. All 4 profiles showed peak levels at 4 days. One patient (MF) reached steady state on the 5th day, while the other patients reached steady state by the 6th day of dosage. All the patients had steadily accumulated SA in their plasma during the first 4 days.

Table 2 shows plasma levels of aspirin metabolites (SA, SU, GA) 2 hours after the last daily dose in the Libyan children with rheumatic fever. The lowest SU concentration was 1.8 mg/L (patient KG) and the highest concentration was 7.5 mg/L (patient AS). Some patients showed small amounts of GA (patient SG), the highest level being 4.1 mg/L (patient KG). This patient (KG) also had the highest SA level of 610 mg/L), the lowest SA concentration being 130.2 mg/L in patient NM. Some children with rheumatic fever, especially females, had higher GA levels than adults [3.9±0.1 vs 2.6±0.08 (Day 7): $t=12.7$, $df=5$, $p<0.01$] (Tables 1 and 2).

DISCUSSION

In the present study, steady state (plateau) for salicylate was reached in Libyan adult patients within 5-7 days of commencing chronic aspirin administration which is in agreement with some previous reports (6,19). Graham *et al.* (15) reported that after aspirin doses of 65 mg/kg body weight, plasma concentrations of salicylate ranging from 50 to 290 mg/L were obtained. The levels of salicylate obtained in our adult patients were in agreement with this report (Table 1). For our patients, the plateau was reached

Figure 3: The plasma profiles of salicylic acid (SA) for the 4 Libyan adult patients with rheumatoid arthritis on high dose aspirin therapy.



at a level ranging from 210 to 303 mg/L which was within the requirement of more than 150 mg/L for clinically significant anti-inflammatory activity (15). Graham *et al.* (15) also reported that daily doses of aspirin rarely exceeds 4.8 g per day. However, one of our patients who was receiving 5.5 g daily had the lowest plasma salicylate level of the whole group even though he was receiving such a high dose of aspirin. Although statistically not significant, the two females in the adult patient group produced higher plasma salicylate levels than the two males ($p>0.1$). Graham *et al.* (15) also observed high levels of plasma salicylate in females to be frequent. The high salicylate (SA) levels in females, both adults and children, in the present study were probably due to lower albumin levels (7, 36).

Furst *et al.* (13) reported that volunteers exposed to high doses of aspirin had enhanced formation of salicylic acid. Montgomery and Sitar (28) found that serum concentrations of salicylic acid and gentisic acid increased in older patients (>65 years) receiving chronic aspirin therapy which was attributed to decreased renal excretion. In the present study, our patients were middle aged and the oldest was only 55 years. Day *et al.* (8) studied volunteers receiving 75 mg aspirin kg/day for 14 years and reported that during this period the salicylic acid formation was increased. This increase was explained being possibly due to availability of more enzymes for conjugation of salicylic acid with glycine. This could be due to either increased synthesis of the enzyme protein or inhibition of degradation of the enzyme. However, an increase in salicyluric acid was not observed in our study.

Among the pediatric patients, the three female children had high plasma levels of salicylate as compared to male children ($p<0.05$) (Table 2). One of them had a plasma salicylate level of over 610 mg/L which is at the toxic level (12). It is not unusual to find children on high doses of aspirin suffering from toxic effects especially in a hospital where plasma salicylate levels are not monitored. Some children accumulated slightly higher gentisic acid levels in their plasma than adult patients ($p<0.01$). This concentration of gentisic acid was about 4.1 mg/L which had been reported to cause toxic effects on red blood cells (12). This could be one of the reasons why aspirin is more toxic in children than adults. Also, it has been reported that gentisic acid enhances the toxicity of salicylic acid in red blood cells by formation of methemoglobin (32). Patients who produce relatively high levels of gentisic acid may have enhanced ability for the hydroxylation of salicylic acid to gentisic acid. In our study, however, some children did not form gentisic acid at all which may be due to reduced ability for hydroxylation of salicylic acid to gentisic acid (25). Two of the children (males) with rheumatic fever showed appreciable reduction of serum salicylate concentrations. This might be due to antacid (aluminum hydroxide) given with the dose of aspirin as reported by Levy *et al.* (20). The antacid increases urinary pH causing an increase in renal clearance of salicylate. Levy *et al.* (20) suggested the possibility of a chemical interaction between aspirin and aluminum ions released from the antacid resulting in decreased aspirin bioavailability and hence lower plasma salicylate levels. Also, it is well known that the presence of food can affect the absorption, solubility and hence bioavailability of many drugs including aspirin (16). Secondly, lower plasma salicylate level may also lead to induction of salicylate metabolism rather than poor compliance (8,13,14, 31).

The present study demonstrated wide variations in plasma levels of aspirin metabolites in Libyan patients with RA and RF on long term aspirin therapy. In the hospitals, in Libya, therapeutic monitoring is not routinely practiced. As a result, it is not known whether patients on chronic aspirin therapy reach toxic levels. The chemical analysis of drugs and poisons in body fluids has had a long history and as a result of recent important advances in analytical technology, the rapid specific identification and quantitation of most drugs and common poisons have become possible at reasonable costs (30). From the findings of our present study, it is therefore imperative and important that the facilities for therapeutic drug monitoring and clinical toxicology be made available in Libyan hospitals as well. This would help to monitor therapeutic efficacy as well as therapeutic poisoning particularly salicylism (5,10,17,29,33).

This report is the first of its kind in Libyan patients. Further studies are in progress on the kinetics of aspirin metabolism on a larger series of patients to substantiate our present observations.

ACKNOWLEDGEMENT

The authors would like to thank Mr. Gener R. Ronquillo for typing the manuscript.

REFERENCES

1. Altman RD : Salicaylates in the treatment of arthritic disease. Postgrad Med, 84:206-210, 1988.

2. Anderson RJ, Potts DE, Carbow PA, Rumack BH, Schrier RW : Unrecognized adult salicylate intoxication. Ann Intern Med, 75:745-749, 1976.

3. April PA, Abeles M, Baraf HSB : Does the acetyl group of aspirin contribute to the inflammatory efficacy of salicylic acid in the treatment of rheumatoid arthritis? J Rheumatol, 16:321-327, 1989.

4. Brodie BB, Underfriend S, Coburn AF : The determination of salicylic acid in plasma. J Pharmacol Exp Therap, 80:114-117, 1944.

5. Buchanan N and Rabinowitz L : Infantile salicylism: A reappraisal. J Pediatr, 84:391-394, 1974.

6. Craig JO, Ferguson IC, Syme J : Infants, toddlers and aspirin. Br Med J, 1:757-760, 1966.

7. Dales LG, Friedman GD, Siegelaub AB, Seltzer CC : Cigarette smoking and serum chemistry tests. J Chron Dis, 27:293-296, 1974.

8. Day RO, Shen DD, Azarnoff DL : Induction of salicyluric acid formation in rheumatoid arthritis patients treated with salicylate. Clin Pharmacokinetics, 8:263-267, 1983.

9. Done AK : Ontogenic analysis of salicylate intoxication, in Salicylate, An international Symposium, ed by Dixon, Martin, Smithand Wood; London, Churchill, 1963.

10. Done AK : Aspirin overdose, incidence, diagnosis and management. Pediatrics, 62:890-893, 1968.

11. Drah MM : Rheumatic fever and rheumatic heart disease in male children Al-Jamahiria (Libya). An assay for diploma in tropical medicine, Liverpool School of tropical Medicine, Liverpool, England, UK, 1984.

12. Dromgoole SH and Furst ED : Applied Pharmacokinetics, Principles of therapeutic drug monitoring. Salicylates, ed by Evans, Schentag, Jusko; San Francisco: Applied Therapeutic Inc,1981.

13. Furst DE, Gupta N, Plus HE : Salicylate Metabolism in twins. Evidence Suggesting a genetic influence and induction of salicylurate formation. J Clin Invest, 60:32-36, 1977.

14. Gerstein HR, Gray RM, Ward JR : Patients compliance with in the context of seeking medical care for arthritis. J Chron Dis, 26:689-694, 1973.

15. Graham GG, Champion GX, Day RO, Paul PD : Patterns of plasma concentrations and urinary excretion of salicylate in rheumatoid arthritis. Clin Pharm Ther, 22:410-415, 1977.

16. Harrison LI, Riedel DJ, Armstrong KE, Goldlust MB, Ekholm BP : Effect of food on salicylate absorption. Ther Drug Monit, 14:87-91, 1992.

17. Karliner JS : Non-carcinogenic forms of pulmonary edema. Circulation, 46:212-216, 1972.

18. Koch-Weser : Serum drug concentrations as therapeutic guides. N Engl J Med, 287:227-231, 1972.

19. Levy G : Pharmacokinetics of salicylate elimination in man. J Pharm Sci, 54:959-967, 1965.

20. Levy G, Lampman T, Kamath BL, Garrettson LK : Decreased serum salicylate concentrations in children with rheumatic fever treated with aspirin. N Engl J Med, 14:323-327, 1975.

21. Levy G and Leonards JR : Absorption metabolism and excretion of salicylates. In: The Salicylates: A critical bibliographic review, ed by MJH Smith and PK Smith, New York, John Wiley and Sons, pp 5-48, 1966.

22. Levy G and Tsuchiya T : Salicylate accumulation kinetics in man. N Engl J Med, 287:430-436, 1972.

23. Levy G and Yaffe SJ : The study of salicylate pharmacokinetics in intoxicated infants and children. Clin Toxicol, 1:409-413, 1968.

24. Lim RK : Salicylate metabolism. In: The salicylate : A critical Biographic Review, ed by MJH Smith and PK Smith, New York, John Wiley and Sons, pp 155-202, 1966.

25. Mahgoub A, JR Idle, LG Dring, LG Lancaster, RL Smith : Polymorphic hydroxylation of debrisoquine in man. Lancet, 2:584-587, 1977.

26. Majeed HA, Doudin K, Lubani M, Shaltout A, Doussary L, Soliman MA : Acute rheumatic fever in Kuwait: The decline incidence. Ann Saudi Med, 13:56-59, 1993.

27. Markowitz M and Gordis L : Treatment of acute rheumatic fever. In: Rheumatic fever, 2nd edition, Vol-II in the series Major Problems in Clinical Pediatrics. Philadelphia, WB Saunders, pp 120-132, 1977.

28. Montgomery PR and Sitar DS : Increased serum salicylate metabolites with age in patients receiving chronic acetylsalicylic acid therapy. Gerontology, 27:329-334, 1981.

29. Pierce RP, Gazewood J, Blake RL (Jr) : Salicylate poisoning from enteric-coated aspirin. Delayed absorption may complicate management. Postgrad Med, 89:61-64, 1991.

30. Prescott LF : Clinical drug toxicology. Ther Drug Monit, 14:85-86, 1992.

31. Rumble RH, Robert MS, Wanwlmolruk S : Determination of aspirin and its major metabolites in plasma by high performance liquid chromatography without solvent extraction. J Chromat, 225:252-257, 1981.

32. Shahidi NT and Westring DW : Acetylsalicylic acid induced hemolysis and its mechanism. J Clin Invest, 49:1334-1339, 1970.

33. Snodgrass W and Rumack BH : Salicylate toxicity following therapeutic doses in young children. Clin Toxicol, 18:247-251, 1981.

34. Strasser T : Report on duty travel trip to Libya. Cardiovascular disease unit, World Health Organization, Geneva, pp 8-28, 1978.

35. Williams RT : Detoxication mechanisms, second ed, London, Chapman and Hall, p 359, 1959.

36. Yacobi A, Stoll RG, Disanto AR, Levy G : Inter-subject variation of warfarin binding protein in serum of normal subjects. Res Comm In Chem Path Pharm, 14:743-747, 1976.

Correspondence:
M. M. Ziu
Department of Laboratory Medicine,
Al-Arab Medical University,
P.O. Box 1036,Benghazi, LIBYA.