IN VITRO CHEMOTOXICITY OF ASPIRIN METABOLITES ON G6PD-NORMAL AND G6PD-DEFICIENT HUMAN ERYTHROCYTES

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

SUMMARY : The effects of aspirin metabolites on reduced glutathione (GSH), methemoglobin (MHb) and MHb reduction were studied in erythrocytes from glucose-6-phosphate dehydrogenase-normal (G6PD-normal) and G6PD-deficient volunteers. The aspirin metabolites used in various concentrations were sodium salts of salicylic acid (Na-SA), salicyluric acid (Na-SU) and gentisic acid (Na-GA). While Na-GA decreased GSH level appreciably, only slight lowering of GSH level was observed with Na-SA and Na-SU. Similarly, Na-GA, even at low concentration of 0.03 mM, showed effect on MHb formation and the effect was increased significantly with increasing concentration. But, no effect on MHb formation was observed with Na-SA and Na-SU. However, Na-SA and Na-SU showed significant inhibitory effects on the reduction of MHb which was far more prominent with Na-GA. These findings led to the notion that neither SA nor SU can produce any appreciable oxidative damage to Hb, while GA is the main metabolite to effect oxidation of Hb. However, SA and SU showed their ability to inhibit MHb reduction and can interfere with the regeneration of Hb. These effects of aspirin metabolites were more pronounced in G6PD-deficient erythrocytes as compared to G6PD-normal erythrocytes implicating the importance of G6PD in aspirin toxicity. Thus, the present study indicates that aspirin metabolites act at different sites of GSH and Hb metabolic pathways, ultimately producing increased erythrocyte fragility with consequent hemolysis. However, the fragility of cellular plasma membrane is dependent on the integrity of the physiological antioxidant systems. Therefore, further studies are warranted in these areas to understand fully the mechanisms of toxicity of aspirin metabolites in human erythrocytes.

Key Words : Aspirin, salicylate, salicylurate, gentisate, G6PD, erythrocyte.

INTRODUCTION

Aspirin is undoubtly effective in relieving pain and inflammation of rheumatic arthritis and other rheumatic disorders. Yet, use of aspirin is limited in many patients because of a high incidence of adverse effects, most notably gastrointestinal discomfort and bleeding, alterations in platelet function, hypersensitivity reactions and renal impairment (1,2). The most severe instances of aspirin toxicity among adults as well as children, however, result from therapeutic overdoses (3,4). Aspirin is rapidly hydrolyzed in the body to salicylic acid (SA). The SA conjugated with glycine to form salicyluric acid (SU) and with glucoronic acid to form salicylic acyl glucoronide (SAG) and salicylic phenolic glucorine (SPG). A small fraction of SA is further hydroxylated to gentisic acid (GA), and there may be several other minor metabolites (5,6). Accumulation of aspirin metabolites (SA, SU, GA) occur in patients with aspirin therapy, particularly with high doses (7). These metabolites of aspirin specially GA, even at low concentration, may lead to toxic manifestations (8). Aspirin, although classified as a mild hemolytic agent (9), has been reported on several occasions to give rise to severe hemolysis of erythrocytes even after small therapeutic doses (10-12). The hemolytic effects of aspirin has been shown to be

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

even greater in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals. Most of the drugs which cause hemolysis of G6PD-deficient erythrocytes have been reported to possess oxidative properties as mechanisms of lysis (9-12). This raises the question as to whether the aspirin metabolites (SA, SU, GA) also possess similar oxidative properties as probable mechanism for hemolysis. The present experiments were therefore, designed to investigate the in-vitro effects of sodium salt of aspirin metabolites [Na-salicylate (Na-SA), Na-salicylurate (Na-SU), Na-gentisate (Na-GA)], on reduced glutathione (GSH) and methemoglobin levels, and inhibition of methemoglobin reduction in G6PD-normal as well as in G6PD-deficient human erythrocytes.

MATERIALS AND METHODS

Blood specimens

Venous blood obtained from the anti-cubital vein of normal Caucasian volunteers (Number : 2; Age (yrs) : 25, 34, Sex : Male) with normal levels of erythrocyte G6PD activity were used as G6PD-normal specimens. The venous blood obtained from West African volunteers available locally with G6PD-deficiency (Type : GdA) (Number : 2; Age (yrs) : 21, 32, Sex : Male) were used as G6PD-deficient specimens. No subjects of Mediterranean origin with G6PD deficiency were available during the course of the study. A 20 ml aliquot of venous blood from each of G6PD-normal and G6PD-deficient volunteers were collected in acid-citrate-dextrose (ACD) solution, stored at 4°C and used within 12 hours.

Determination of packed cell volume

The packet cell volume (PCV) of erythrocytes was determined in heparinized capillary tubes using micro-hematocrit centrifuge and reading device (Hawkslay and Sons Ltd, London, UK) as described by Dacie and Lewis (13).

Preparations of erythrocytes

The 20 ml blood samples were centrifuged at 1500x g for 5 min, and the plasma and buffy layers of leucocytes were removed. The erythrocytes were made free of leucocytes contamination by washing twice with isotonic saline, and then reconstituted to their original PCV in appropriate buffer. These reconstituted erythrocyte suspensions, after being checked by Leishman stain for leukocyte contamination (13, 14), were assayed for G6PD activity (10,15) and then used in experiments as required.

Preparation of solutions of aspirin metabolites

Appropriate amounts of sodium salts of aspirin metabolites (Na-SA, Na-SU, Na-GA) were dissolved in isotonic saline. When 0.2 ml of these solutions were added to 2.0 ml of G6PD-normal or G6PD-deficient erythrocyte preparations, required final concentrations of the aspirin metabolites were obtained as described in the respective experiments.

Quantitation of reduced glutathione in erythrocytes

The reduced glutathione (GSH) contents in G6PD-normal and G6PD-deficient erythrocyte preparations were measured quantitatively by the method of Beutler et. al. (16, 17). The method is based on spectrophometric measurement at 412 nm of the yellow color developed by adding 5.5'-Dithio-bis (2-nitrobenzoic acid) to sulphahydryl compounds. The results were expressed as mg GSH per 100 ml PCV.

Estimation of total hemoglobin and methemoglobin

The total hemoglobin (THb) and methemoglobin (MHb) contents of erythrocytes were estimated by the modified method of Dacie and Lewis (13,14). The amounts of MHb contents were expressed as the percentage (%) of (THb.)

Effects of low concentrations of aspirin metabolites on GSH levels in G6PD-normal and G6PD-deficient erythrocytes

G6PD-normal and G6PD-deficient erythrocyte preparations were used in this experiment. Two ml aliquots in duplicate of each erythrocyte preparations were used with 0.2 mM, 0.4 mM, 0.6 mM and 0.8 mM final concentrations of each aspirin metabolite (Na-SA, Na-SU, Na-GA). An assay mixture containing 0.2 ml of isotonic saline, instead of 0.2 ml of the metabolite, served as the control. Before the incubation was started and as soon as the metabolites were added, 0.2 ml samples from each incubation mixture were taken to measure the zero time GSH level in erythrocytes. The remaining assay mixtures (2.0 ml each) were then quickly incubated at 37°C for up to 6 hours. From all the assay mixtures, 0.2 ml samples were taken at 2, 4 and 6 hrs of incubation at 37°C and GSH levels were determined as mentioned earlier. The results were expressed as mg GSH per 100 ml PCV.

Effects of higher concentrations of aspirin metabolites on GSH levels in G6PD-normal and G6PD-deficient erythrocytes

A further experiment was carried out with higher concentrations of each aspirin metabolite (Final concentrations: 1 mM, 5 mM, 10 mM and 20 mM). The assay procedure was the same as in the preceding experiment. The GSH levels were measured at 0, 2, 4 and 6 hrs of incubation at 37°C and the results were expressed as mg GSH/100 ml PCV.

Effects of aspirin metabolites on the methemoglobin levels in G6PD-normal and G6PD-deficient erythrocytes

Two ml aliquots in duplicate of G6PD-Deficient erythrocyte preparations were incubated with 0.03 mM, 0.06 mM, 0.15 mM and 0.3 mM concentrations of each metabolite (Na-SA, Na-SU, Na-GA) at 37°C (total volume of each assay mixture was 2.2 ml). An aliquot of 0.2 ml sample from each assay mixture was taken at 0, 30, 60 and 150 min of incubation and processed for THb and MHb estimation as stated before. The quantity of MHb was expressed as percentage of THb as stated earlier.

Effects of aspirin metabolites on the reduction of methemoglobin in G6PD-normal and G6PD-deficient erythrocytes

It was reported that the rate of MHb reduction in nitrite - treated red cells was inhibited in the presence of Na-SA (18). This experiment was therefore designed to investigate the inhibitory effects, if any, of aspirin metabolites (Na-SA, Na-SU, Na-GA) on MHb reduction in G6PD-normal and G6PD-deficient intact erythrocytes. The erythrocytes containing about 85% MHb were prepared by treatment with $Na\text{-}NO_2$ according to the modified technique of Worathumrong and Grimes (18). These erythrocytes, both normal and deficient, were resuspended to their original PCV in their respective plasma supplemented with glucose (99 ml plasma plus 1 ml isotonic saline containing 5% glucose). From these erythrocyte preparations, 2 ml aliquots in duplicate were

then incubated at 37°C with each of the aspirin metabolites at different final concentrations (Na-SA : 10 mM, 25 mM, 50 mM, 100 mM; Na-SU : 5 mM, 10 mM, 20 mM, 25 mM; Na-GA : 1 mM, 5 mM, 10 mM, 15 mM) in a final volume of 2.2 ml for each assay mixture. The incubation was carried out in a shaking water bath at 37°C and 0.2 ml samples were taken at 0, 1, 2, 3, 4, 5 and 6 hrs. for MHb estimation. The MHb concentrations were estimated and the results were expressed as % of THb as stated earlier.

Table 1: The effects of low concentrations of Na-GA on GSH levels in G6PD-normal and G6PD-deficient erythrocytes.

Concentration of aspirin metabolites**	mg GSH levels (% decrease) at different incubation periods							
	N	D	N	D	N	D	N	D
Control:	60.0 (0)	51.0 (0)	60.0 (0)	50.0(1.9)	62.3 (+3.3)	50.8 (0.4)	64.1 (+6.5)	50.9 (0.2)
0.2 mM	-	-	60.1 (+0.2)	49.5 (2.9)	62.4 (+4.0)	49.8 (2.3)	62.4 (+4.0)	48.9 (4.1)
0.4 mM	-	-	59.4 (1.0)	48.5 (4.9)	60.2 (+0.3)	48.3 (5.3)	60.2 (+0.3)	46.0 (9.8)++
0.6 mM	-	-	58.8 (2.0)	48.0 (5.9)	58.6 (2.3)	45.7 (10.4)++	57.7 (3.8)	43.3 (15.1)++
0.8 mM	-	-	57.0 (5.0)	46.0 (9.8)	56.1 (6.5)	44.8(12.2)++	54.5 (9.2)	38.4 (24.7)++

* GSH Conc:mg/100 ml PCV (Each value is the mean of 4 observations on 2 erythrocyte preparations in duplicate; N:G6PD-normal; D:G6PD-deficient.

** The same concentrations of Na-SA and NA-SU showed no effects on GSH levels in either G/PD-normal or G6PD-deficient erythrocytes.

++ Significantly different from the respective control values ($p<0.05$).

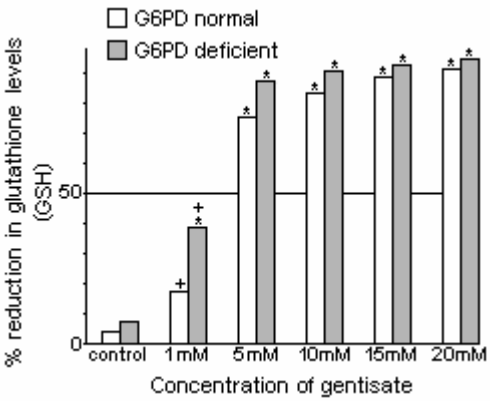
Table 2: The effects of high concentrations of aspirin metabolites (Na-SA, Na-SU, Na-GA) on GSH levels in G6PD-normal and G6PD-deficient erythrocytes.

Concentration of aspirin metabolites**	% GSH levels (% decrease) in erythrocytes at different incubation periods*							
	0 hrs.		2 hrs		4 hrs		6 hrs	
	N	D	N	D	N	D	N	D
Control:								
C_{SA}	100 (0)	100 (0)	99 (1)	100 (0)	99 (1)	100 (0)	99 (1)	100 (0)
C_{SU}	100 (0)	100 (0)	99 (1)	100 (0)	100 (1)	99 (1)	100 (0)	100 (0)
C_{GA}	100 (0)	100 (0)	98 (2)	98 (2)	97 (3)	95 (5)	96 (4)	93 (7)
1 mM Conc.:								
SA_1	-	-	98 (2)	100 (0)	98 (2)	100 (0)	98 (2)	100 (0)
SU_1	-	-	100 (0)	98 (2)	100 (0)	100 (0)	100 (0)	101 (+1)
GA_1	-	-	96 (4)	86 (14)	89 (11)	72 (28)+	82 (18)++	60 (40)++
5mM Conc.:								
SA_2	-	-	98 (2)	100 (0)	98 (2)	98 (2)	98 (2)	96 (4)
SU_2	-	-	100 (0)	99 (1)	100 (0)	98 (2)	101 (+1)	101 (+1)
GA_2	-	-	68 (32)++	67 (33)++	44 (56)+	31 (69)++	23 (77)+	11 (89)+
10 mM Conc.:								
SA_3	-	-	97 (3)	98 (2)	97 (3)	96 (4)	94 (6)	92 (8)
SU_3	-	-	99 (1)	99 (1)	100 (0)	97 (3)	101 (+1)	100 (0)
GA_3	-	-	41(59)++	40 (60)++	27 (73)++	19 (81)++	14 (84)++	8 (93)++
15 mM Conc.:								
SA_4	-	-	97 (3)	98 (2)	95 (5)	95 (5)	90 (10)	86 (14)
SU_4	-	-	98 (2)	98 (2)	100 (0)	96 (4)	100 (0)	97 (3)
GA_4	-	-	24 (76)++	24 (76)++	16 (84)++	11 (89)	80 (92)	4 (96)++
20mM Conc.:								
SA_5	-	-	95 (5)	96 (4)	93 (7)	90 (10)	87 (13)	80 (20)
SU_5	-	-	100 (0)	97 (3)	101 (+1)	94 (6)	101 (+1)	95 (5)
GA_5	-	-	15 (85)++	15 (85)++	10 (90)++	7 (93)++	5 (95)++	2 (98)++

C_{SA}: 100% GSH=61.0 mg/100 ml PCV for N, 100% GSH=50 mg/100 ml PCV for D; C_{SU}: 100% GSH=61.0 mg/100 ml PCV for N, 100% GSH=51 mg/100 ml PCV for D; C_{GA}: 100% GSH=63 mg/100 ml PCV for N, 100% GSH=55 mg/100 ml PCV for D; N: G6PD-normal α+D: G6PD-deficient erythrocytes;

** Significantly different from the respective control values ($p<0.01$).

Figure 1: Percentage decrease in GSH levels in G6PD-normal and G6PD-deficient erythrocytes, after 6 hrs incubation with high concentrations (1 mM-20 mM) of Na-GA. (*Significantly different from the control, p=0.05; +Significant different between G6PD-normal and G6PD-deficient erythrocytes, p 0.05).



Statistical analysis

The statistical evaluation of the results were carried out by Student's t-test (19).

RESULTS

Table 1 shows the effects of low concentrations of Na-GA (0.2 mM-0.8 mM) on GSH levels in G6PD-normal (N) and G6PD-deficient (D) erythrocytes. Na-GA started to show effect at a concentration of 0.4 mM with deficient erythrocytes. When the concentration of Na-GA was increased to 0.8 mM, both G6PD-normal and G6PD-deficient erythrocytes were affected by 9.2% and 24.7% respectively at 6 hrs of incubation. However, no effects were observed in either N or D erythrocytes with the same concentrations of Na-SA and Na-SU under similar experimental conditions.

The effects of higher concentrations of aspirin metabolites (1 mM-20 mM) on GSH levels in G6PD-normal (N) and G6PD-deficient (D) erythrocytes are seen in Table 2. The control with Na-GA showed little change at 6 hrs. of incubation, while with 1 mM Na-GA the GSH levels were lowered to 82% and 60% for G6PD-normal and G6PD-deficient erythrocytes, respectively. When Na-GA concentration was increased to 5 mM, only 23% and 11% GSH remained in normal and deficient cells, respectively. Above this concentration Heinz body formation became evident, erythrocytes became very fragile and heavy hemolysis occurred after 6 hrs. of incubation. With 20 mM Na-GA, the levels of GSH were decreased by 95% and 98% in G6PD-normal and G6PD-deficient erythrocytes. This prominent effect of decreasing GSH levels in erythrocytes by Na-GA are shown graphically in Figure 1. With similar concentrations of Na-SA and Na-SU and under similar experimental conditions, only slight lowering of GSH levels were observed. Na-SA began to lower GSH levels at 10 mM, and with 20 mM at 6 hrs of incubation GSH levels were reduced in normal and deficient cells by only 13% and 20% respectively. With Na-SU no significant decrease in GSH levels were observed even at 20 mM concentration at 6 hrs. with G6PD-normal erythrocytes, while the GSH levels was reduced by only 5% in G6PD-deficient erythrocytes.

Figure 2a: The inhibitory effects of Na-SA on MHb reduction in G6PD-normal erythrocytes. (a:Significantly different from the respective control values, p≤0.05).

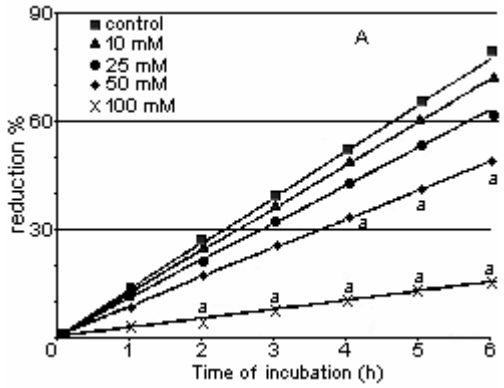


Figure 2b: The inhibitory effects of Na-SA on MHb reduction in G6PD-deficient erythrocytes. (a:Significantly different from the respective control values, p≤0.05).

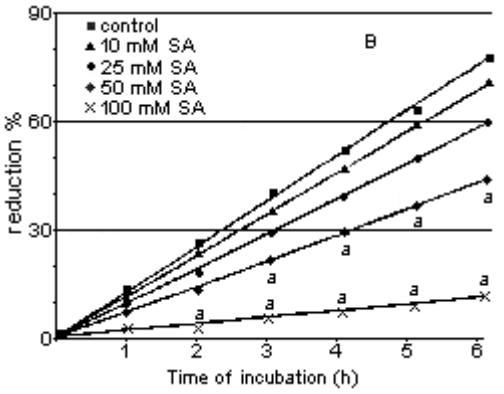


Table 3: The effects of low concentrations of Na-GA (0.03-0.3 mM) on MHb levels in G6PD-normal (N) and G6PD-deficient (D) erythrocytes.

Concentration of Na-GA	gm MHb per 100 gm THb at various incubation periods *							
	0 hr		1/2 hrs		1,1/2 hrs		2,1/2 hrs	
	N	D	N	D	N	D	N	D
Control:	1.1	1.2	1.1	1.3	1.2	1.7	1.7	2.0
0.03 mM	-	-	1.5	2.2	2.5	3.3	4.4	4.8
0.06 mM	-	-	1.3	2.1	4.1	4.2	4.5	6.2
0.15 mM	-	-	4.6	4.9	8.6++	9.9++	13.5++	15.7++
0.03 mM	-	-	10.5++	10.6++	16.9++	21.0++	24.5++	30.9++

* MHb as % of THb; Each value is the mean of duplicate observations on erythrocytes prepared from two volunteers of each type (Nor D); The experiments carried out with the same concentrations of Na-SA and Na-SU showed no changes in the levels of MHb from the control values;
++ Significantly different from the respective control values ($p<0.05$).

Figure 3a: The inhibitory effects of Na-SU on MHb reduction in G6PD-normal erythrocytes. [a:Significantly different from the respective control values ($p\leq0.05$); b:Significantly different from the respective values for G6PD-deficient erythrocytes. ($p\leq0.05$) as shown in figure 3b.

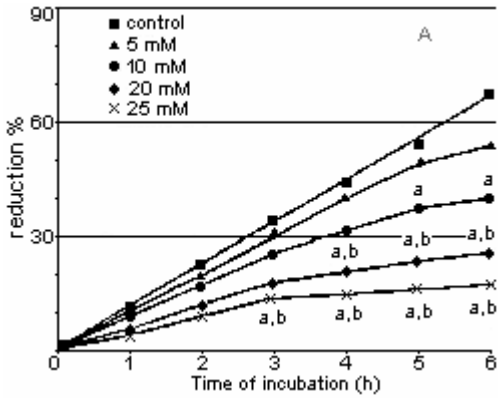
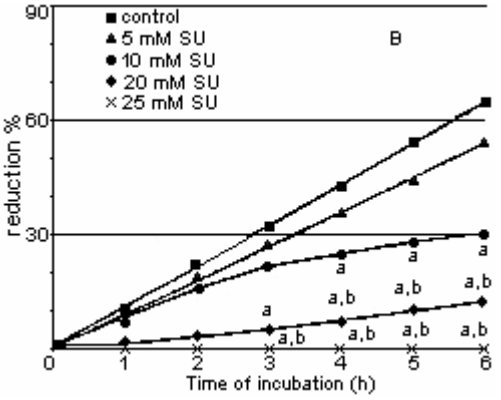


Figure 3b: The inhibitory effects of Na-SU on MHb reduction in G6PD-deficient erythrocytes. [a:Significantly different from the respective control values ($p\leq0.05$); b:Significantly different from the respective values for G6PD-normal erythrocytes. ($p\leq0.05$) as shown in figure 3a.



Regarding the influence of aspirin metabolites on the oxidation of hemoglobin, no effects on MHb formation in either normal or deficient cells were observed with 0.03 mM-0.3 mM concentrations of Na-SA and Na-SU, but Na-GA showed a slight effect even at the low concentration of 0.03 mM with both normal and deficient erythrocytes. The formation of MHb was increased with increasing concentration of Na-GA reaching 25% and 31% of THb at 2.5 hrs for normal and deficient cells respectively (Table 3).

The effects on MHb reduction with increasing concentrations of aspirin metabolites are graphically presented in Figures 2 (Na-SA), Figure 3 (Na-SU) and Figure 4 (Na-GA). There was almost a linear relationship of the inhibition of MHb reduction with time of incubation and concentration for Na-SA and Na-SU. It can be seen from Figures 2a and 2b that at all concentrations of Na-SA, there was little difference in effect on the two types of erythrocytes. For example, with 100 mM Na-SA at 6 hrs of incubation the MHb reduction was only 19.0% and 14.0% in normal and deficient erythrocytes respectively. However, Na-SU was observed to be more inhibitory with deficient erythrocytes where % reduction of MHb was almost zero with 25 mM concentration as compared to 5-19% reduction of MHb with 25 mM Na-SA (Figures 3a and 3b). With Na-Ga, inhibition of MHb reduction was observed even at the lowest concentration of 1 mM. At the highest concentration of 10 mM Na-GA, inhibition of MHb reduction was complete and at no time there was any reductive activity present (Figures 4a and 4b). As with Na-SU, but unlike Na-SA, Na-GA showed more inhibitory effects with G6PD-deficient than G6PD-normal erythrocytes.

The intra-assay and inter-assay coefficients of variation were <3.0%. Since the standard deviations were quite small, they are omitted from the tables and diagrams for

Figure 4a: The inhibitory effects of Na-GA on MHb reduction in G6PD-normal erythrocytes. [a: Significantly different from the respective control values for G6PD-normal erythrocytes ($p \leq 0.05$); b: Significantly different from the respective values for G6PD-deficient erythrocytes ($p \leq 0.05$) as shown in Figure 4b].

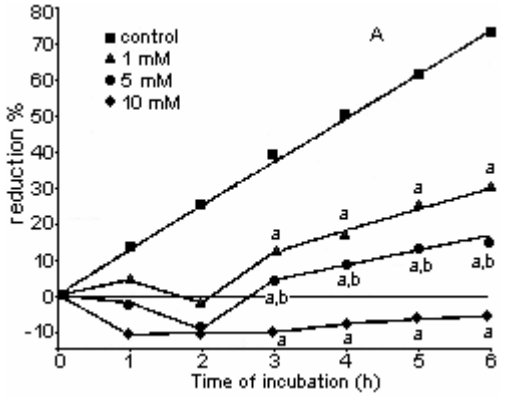
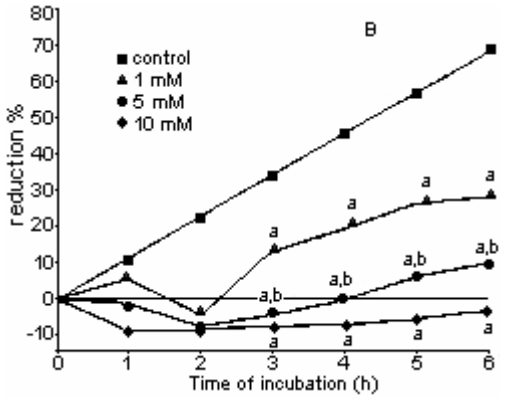


Figure 4b: The inhibitory effects of Na-GA on MHb reduction in G6PD-deficient erythrocytes. [a:Significantly different from the respective control values ($p \leq 0.05$); b: Significantly different from the respective values for G6PD-normal erythrocytes. ($p \leq 0.05$) as shown in figure 4a.



the sake of clarity. Some of the significant statistical values are shown in the respective tables and figures.

DISCUSSION

The most significant findings of the present study were concerned with large oxidation of GSH and the high formation of MHb induced by Na-GA in G6PD-normal as well as G6PD-deficient erythrocytes, although the effects were more pronounced in the G6PD-deficient cells (Tables 1, 2 and 3). However, Na-SA and Na-SU appeared to show very little oxidative effect on GSH in erythrocytes even with 20 mM concentration at 6 hrs. of incubation (Table 2). Also, Na-SA and Na-SU did not appear to be capable of oxidizing Hb to MHb in both types of erythrocytes. These observations, supported by the findings of Shahidi and Westring (20), led to the notion that neither SA nor SU can perhaps induce any appreciable oxidative damage to Hb. Since GA alone was found to cause a significant decrease in the in vivo survival of G6PD-deficient red cells, Shahidi and Westring (20) proposed that GA acts both as an inhibitor of G6PD-activity as well as an oxidizing agent. Considering these observations and our findings of interest in the present study, GA seems to be the main metabolite of aspirin with oxidative properties. The role of other minor metabolites that may exist can not, however, be ruled out (21).

The other findings of interest in the present study inhibitory effects of aspirin metabolites on MHb reduction. Inhibition of MHb reduction was readily detected for Na-SA, Na-SU and Na-GA at concentrations $^{3}10$ mM, $^{3}5$ mM and $^{3}1$ mM, respectively (Figures 2, 3, 4), which agreed with the results of Worathumrong and Grimes (18). The inhibition of MHb reduction was always more pronounced in the G6PD-deficient cells, presumably because of an overall lack of reducing activity. Among the metabolites, GA was reported to produce H_2O_2 from MHb and decrease the activity of GSH-peroxidase leading to markedly increased erythrocyte fragility (22). The formation of MHb in small quantity (usually <1%) has been suggested as a normal physiological process as there are enzyme systems within erythrocytes (e.g. NADH-MHb reductase, NADH-MHb reductase) which reduce MHb back to Hb. It appears, therefore, that aspirin metabolites act at different sites of the GSH and Hb metabolic pathways ultimately producing increased erythrocyte fragility with consequent hemolysis. Of the metabolites, GA may act at multi-points while SA and SU may act at more restricted points of the GSH and Hb metabolism to produce their toxic effects in human erythrocytes.

The fragility of the cellular plasma membrane is, however, dependent on the integrity of the physiological antioxidant systems, e.g. vitamin E and selenium (23-25), GSH-reductase and GSH-peroxidase (26, 27), superoxide dismutase (27) and Vitamin C (28, 29). The nutritional status and the integrity of the physiological antioxidant systems were unknown in our volunteers. Secondly, the activity of the drug metabolizing enzyme systems (liver

microsomal cytochrome P 450 system) had been reported to be dependent on the antioxidants like alpha-tocopherol and selenium (30). Therefore, further studies are warranted in these areas to understand fully the mechanism of toxicity of aspirin metabolites in human erythrocytes. We have made some studies on the effects of aspirin metabolites on NADH-MHb reductase (Diaphorase) activity in normal human erythrocytes and the results will most likely be reported in the future issue of this journal.

ACKNOWLEDGEMENTS

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

REFERENCES

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

2. Lee TH : Mechanism of aspirin sensitivity. Am Rev Respir Dis, 145:534-536, 1992.

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

4. Craig JO, Fergusan IC and Syme J : Infants, toddlers and aspirin. Br Med J, 1:757-760, 1976.

5. Levy G : Pharmacokinetics of salicylate elimination in man. J Pharm Sci, 54:959-957, 1965.

6. Levy G and Leonards JE : 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.

7. Ziu MM and Giasuddin ASM : Plasma levels of aspirin metabolites in Libyan patients with rheumatoid arthritis and rheumatic fever. Submitted to J Islam Acad Sci, 1993, submitted.

8. Dromgoole SH and Furste ED : Applied pharmacokinetics, principles and therapeutic drug monitoring : Salicylates; Ed by WE Evans, JJ Schentag and WJ Jusko ; San Francisco : Applied Therapeutic Inc, 1981.

9. Kellermeyer RW, Tarlor AR, Brewer GJ, Garson PE and Alving AS : Hemolytic effect of therapeutic drugs, clinical considerations of the primaquine-type hemolysis. J Am Med Assoc, 180:388-393, 1962.

10. Szeiberg A, Kellerman J, Adam A, Sheba C and Ramot B : Hemolytic jaundice following aspirin administration in a patient with a deficiency of glucose-6-phosphate dehydrogenase in erythrocytes. Acta Hematol, 23:58-62,1960.

11. Westring DW and Pisciotta AV : Anemia, cataracts and seizures in patients with glucose-6-phosphate dehydrogenase deficiency. Arch Intern Med, 118:385-391, 1966.

12. Colonna P : Aspirin and glucose-6-phosphate dehydrogenase deficiency. Br Med J, 283:1189-1194, 1981.

13. Dacie JW and Lewis SW : Practical Hematology, London, Churchill Livingstone, 1975.

14. Dacie JW and Lewis SW : Practical Hematology, London, Churchill Livingstone, 7th edition, 1991.

15. Solem E : Glucose-6-phosphate dehydrogenase deficiency : An easy and sensitive quantitative assay for the detection of female heterogygotes in red blood cells. Clin Chem Acta, 142:153-160, 1984.

16. Beutler E, Darn BJ and Alving AS : The hemolytic effect of primaquine. VI : An in vitro test for sensitivity of erythrocytes to primaquine. J Lab Clin Med, 45:50-59, 1955.

17. Beutler E, Duron O and Kelly BH : Improved method for the determination of blood glutathione. J Lab Clin Med, 61:882-888, 1963.

18. Worathumrong N and Grimes AJ : Inhibition of methemoglobin reduction by sodium salicylate. Br J Hematol, 23:323-328, 1972.

19. Kirkwood BR : Essentials of Medical Statistics. Oxford : Blackwell Scientific Publications, 1989.

20. Shahidi NT and Westring DW : Acetylsalicylic acid induced hemolysis and its mechanism. J Clin Invest, 49:1334-1341, 1970.

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

22. Hopkins J and Tudhope GR : The effects of drugs on erythrocytes in vitro : Heinz bodies formation, glutathione peroxidase inhibition and changes in mechanical fragility. Br J Clin Pharmacol, 1:191-198, 1974.

23. Diplock AT and Lucy JA : The biochemical mode of action of Vitamin E and selenium : Hypothesis. FEBS Letts, 29:205-210, 1973.

24. Diplock AT, Machlin IJ, Packer L and Pryor WA (eds) : Vitamin E : Biochemistry and Health Implications. Ann NY Acad Sci, 570:1-555, 1989.

25. Giasuddin ASM and Diplock AT : The influence of Vitamin E on membrane lipids of mouse fibroblast in culture. Arch Biochem Biophys, 210:346-362, 1981.

26. Rotruck JT, Pope AL, Ganther HE, et. al. : Selenium : Biochemical role as a component of glutathione peroxidase. Science, 179:588-591, 1973.

27. Ceballos-Picot I, Triver JM, Nicole A, Sinet RM and Thevenim M : Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. Clin Chem, 38:66-70, 1992.

28. Chen LH : Interaction of vitamin E and ascorbic acid (review). In Vivo, 3:199-209, 1989.

29. Gotoh N and Niki E : Rates of interaction of superoxide with vitamin E, vitamin C and related compounds as measured by chemiluminescense. Biochem Biophys Acta, 1115:201-207, 1992.

30. Giasuddin ASM, Caygill CPJ, Diplock AT and Jeffery EH : The dependence on vitamin E and selenium of drug demethylation in rat liver microsomal fractions. Biochem J, 146:339-350, 1975.

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