Alpha₁-acid glycoprotein and the binding of lidocaine in children with congenital heart disease

Frederick A. Burrows MD FRCPC,
Jerrold Lerman MD FRCPC,
Kenneth M. LeDez MB, H. Andrew Strong PhD

The purpose of this study was to evaluate the effect of congenital heart disease (CHD) on the serum concentrations of alpha₁-acid glycoprotein (α₁-AGP) and the serum binding of lidocaine. Thirteen children with acyanotic CHD and 12 children with cyanotic CHD were studied and compared with 28 children without heart disease (control). The mean (±SD) serum concentration of α₁-AGP, as determined by radial immunodiffusion, did not differ significantly among the three groups. Five minutes after administration of 1.5 mg·kg⁻¹ intravenous lidocaine, the free fraction of lidocaine correlated inversely and linearly with the serum concentration of α₁-AGP in children with acyanotic CHD ($r^2 = 0.74$; $P < 0.001$) cyanotic CHD ($r^2 = 0.57$; $P < 0.005$), and control ($r^2 = 0.63$; $P < 0.001$). The slopes and intercepts of the linear regressions did not differ significantly among the three groups. We conclude that the serum concentration of α₁-AGP in children with CHD does not differ quantitatively or qualitatively from that in children without CHD.

Nous avons voulu mesurer l'association entre les malformations cardiaques congénitales (MCC), la concentration plasmatique de la glycoprotéine acide alpha-1 (α₁-AGP) et la liaison de la lidocaïne aux protéines plasmatiques. Nous disposons de 25 enfants avec MCC (dont 12 avec cyanose) et de 28 enfants sans

MCC. La concentration plasmatique d'α₁-AGP mesurée par immunodiffusion radiale était sensiblement la même chez les trois groupes. Nous avons observé que cinq minutes après l'injection intraveineuse de 1,5 mg·kg⁻¹ de lidocaïne, il existait une corrélation linéaire et inversement proportionnelle entre le taux de lidocaïne libre et la concentration plasmatique d'α₁-AGP chez les enfants avec MCC cyanogène ($r^2 = 0,57$; $P < 0,005$), avec MCC non-cyanogène ($r^2 = 0,74$; $P < 0,001$) et chez les enfants sans MCC ($r^2 = 0,63$; $P < 0,001$). La pente et l'ordonnée à l'origine de la droite de régression linéaire était virtuellement la même d'un groupe à l'autre. Donc, chez les enfants, la présence d'une MCC n'influence ni la concentration plasmatique ni la nature de α₁-AGP.

Key words

ANAESTHESIA: paediatric;
ANAESTHETICS, LOCAL: lidocaine;
BLOOD: albumin, alpha₁-acid glycoprotein;
HEART: congenital defects.

From the Departments of Anaesthesia and Clinical Pharmacology, and the Research Institute, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Supported with a grant from the Physicians' Services Incorporated, Toronto, Ontario, Canada.

Address correspondence to: Dr. Frederick A. Burrows, Department of Anaesthesia, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

The binding of drugs by serum proteins affects both their disposition and action. Changes in protein binding may alter drug effects by altering the free fraction of the drug.¹⁻⁸ Increases in protein binding decrease the availability of drug for distribution outside the plasma compartment by decreasing the free fraction and, correspondingly, the volume of distribution of the drug. The implications of this relationship apply in the case of lidocaine where serum protein binding is reduced in the neonate.^{5,9} This leads to a higher free fraction of the drug for a given dose per kilogram compared with an older infant or child and, therefore, a greater risk of toxicity.^{9,10}

Alpha₁-acid glycoprotein (α₁-AGP) is an acute phase plasma protein which increases in response to inflammation or body stress. Studies have shown that the serum concentration of α₁-AGP increases in patients with acute and chronic disease states.^{7,11-16} Alpha₁-AGP is important as it binds cationic drugs such as local anaesthetics (lidocaine), propranolol, verapamil and quinidine thereby reducing the free fraction of these drugs.^{14,17-20} There are no studies that examine either the quantitative or qualitative nature of plasma proteins such as α₁-AGP in children with congenital heart disease (CHD).

The purpose of the present study was to quantitate the plasma proteins in children with CHD and to examine the

binding characteristics of α_1 -AGP in children with acyanotic CHD, cyanotic CHD, and to compare the results obtained with those from age-matched children without CHD.

Methods

With approval from the Human Subjects Review Committee written informed parental consent was obtained for 53 children and adolescents, ASA physical status I–III admitted for elective surgery. Exclusion criteria included the presence of any of the factors known to affect the serum concentration of α_1 -AGP including recent medications, surgery, infectious or inflammatory process, neoplasm, organ dysfunction, metabolic or endocrine disorder.

Patients were divided into three groups according to cardiologic diagnosis: acyanotic patients ($n = 13$), cyanotic patients ($n = 12$) and control patients with normal cardiac anatomy ($n = 28$). The patients with cardiac disease were classified according to physical, echocardiographic and/or angiographic assessments carried out by the Department of Cardiology.

All patients were unpremedicated. Anaesthesia was induced with sodium thiopentone ($5 \text{ mg} \cdot \text{kg}^{-1}$), atropine ($0.02 \text{ mg} \cdot \text{kg}^{-1}$) and succinylcholine ($1.5 \text{ mg} \cdot \text{kg}^{-1}$). The tracheas were intubated orally, and the lungs were ventilated to normocarbida ($\text{PaCO}_2 = 32\text{--}38 \text{ mmHg}$). Anaesthesia was maintained with a mixture of nitrous oxide and halothane in oxygen.

After induction of anaesthesia, 2 ml of venous blood were collected to determine the serum concentrations of α_1 -AGP, albumin, α , β and γ globulins and total proteins. Each blood sample was stored in a glass test tube that was sealed with plastic film. The clotted blood samples were centrifuged within one hour of collection and the serum was then pipetted into a second glass tube. These second tubes were sealed with plastic film and refrigerated at -20°C until the blood was analyzed.

Lidocaine ($1.5 \text{ mg} \cdot \text{kg}^{-1}$) was administered intravenously to each patient over a 30-second interval. Venous blood samples (2 ml) were obtained before administration of lidocaine and five minutes afterwards from a limb other than that in which lidocaine was administered. The blood was allowed to clot in a sealed glass test tube and was centrifuged within 30 min. A protein-free fraction of each sample was obtained by centrifuging a portion of the serum for 20 min at a centrifugal force of 1800 g in an ultrafiltration device (Centrifree[®], Amicon Corporation, Danvers, Massachusetts). The serum and protein-free fractions were stored in sealed glass test tubes at -20°C until analysed.

The total protein concentration was determined using an analysis based on the biuret reaction.²¹ A $10 \mu\text{l}$ sample

of serum was applied to a commercially available Kodak Ektachem 700[™] clinical chemistry slide. After incubation the amount of coloured complex was measured by reflected light at 540 nm. The serum protein profile was measured by electrophoresis. The equipment comprised a Gelman Semi Micro II Chamber, #51214, a Gelman Sepraphore III membrane, #5101, a Gelman Sepratek-8 Applicator, #51118, a Beckman Duostat D.C. Power Supply, Model RD-2 and a Gelman Compuscreen Densitometer, Profile 2.

The serum concentrations of α_1 -AGP were measured by radial immunodiffusion (RID).²² Five μl aliquots of serum were incubated for 72 hr in commercially prepared plates (Behring Inc.) containing rabbit antibodies to human α_1 -AGP. The diameter of the resultant precipitate was measured using an optical micrometer supplied by the assay manufacturer (Behring Inc.), and the serum concentration of α_1 -AGP was determined from standard tables. Each RID plate contained 12 wells: one or two wells were used to incubate the protein/standard solutions, and the remainder for patient serum. All of the plates were manufactured from the same batch of antibodies. Protein standard solutions were used both as standards and to confirm the accuracy of the standard tables. The standards ($90 \text{ mg} \cdot \text{dl}^{-1}$) were consistent throughout the study with a coefficient of variation ≤ 2 per cent. Because of the range of the serum concentrations of α_1 -AGP the relationship between the serum concentration of α_1 -AGP and the diameter of the precipitate was determined with the use of standard solutions between 4 and $90 \text{ mg} \cdot \text{dl}^{-1}$ α_1 -AGP. The standard solutions were prepared by dilution of the standard supplied by Behring Inc. with bovine serum albumin (5 per cent wt/vol). Each diluted standard was assayed in triplicate. The relationship between the concentration of α_1 -AGP and the diameter of the precipitate was linear ($r^2 = 0.98$).

The total and free lidocaine concentrations were determined using a commercially prepared homogenous enzyme immunoassay (Emit Assay, Syva Corporation, Palo Alto, California).²³ The equipment comprised a Stasar III spectrophotometer (Gilford Instruments), an EMIT clinical Processor CP 5000 (Syva Corporation), and an EMIT Autocarousel (Syva Corporation). A minor modification of the EMIT technique was used to measure all concentrations less than $1.5 \mu\text{g} \cdot \text{ml}^{-1}$.²⁴ The reliable limit of detection (three times background) was $0.1 \mu\text{g} \cdot \text{ml}^{-1}$. The intra-assay coefficients of variation of a $1.0 \mu\text{g} \cdot \text{ml}^{-1}$ standard measured using the modified assay was less than 5 per cent ($n = 6$) while day to day variation was 7.7 per cent ($n = 16$).

All assays were performed in duplicate and the average value accepted.

To test for protein leakage during ultrafiltration and for

TABLE I Demographic data

	Age (mo)	Weight (kg)	Haematocrit (%)
Control	72.1	23.8	37.5
(n = 28)	± 53.8	± 18.3	± 3.7
Acyanotic	74.9	26.7	38.1
(n = 13)	± 51.3	± 18.2	± 2.2
Cyanotic	69.4	21.1	56.4*
(n = 12)	± 50.2	± 11.7	± 7.1

Data are mean ($\pm$ SD).

* $P < 0.05$ compared with control and acyanotic groups.

background lidocaine concentration, a control sample of blood was obtained from each patient (prior to the administration of lidocaine) and processed as described above.

Data analysis

Statistical significance was determined using one-way analysis of variance and the Tukey test for multiple pairwise comparisons for both the concentrations of α_1 -AGP and the remaining serum proteins and the per cent free lidocaine (i.e., the ratio of free lidocaine to total lidocaine concentrations).²⁵ The Tukey test was corrected for unequal replications per group.²⁶

The strength of the relationship between the concentration of serum proteins, α_1 -AGP and per cent free lidocaine for each group was determined using least squares linear regression and reported as the coefficient of determination (r^2). Correlation coefficients (r) for the three groups were compared using a two-tailed comparison of the Fisher Z transformed values.²⁶ To determine whether the linear relationships between the serum proteins and the per cent free lidocaine were significant, the slopes of the regression lines were compared with zero using Student's t test. The slopes and the intercepts of the linear relationships between α_1 -AGP and the per cent free lidocaine for the three groups were compared using analysis of covariance.²⁶

Statistical significance was accepted as $P \leq 0.05$.

Results

Patient demographic data is presented in Table I. The three groups did not differ significantly in age or weight. The haematocrit of the cyanotic patients was significantly greater than that of the acyanotic and control patients ($P < 0.05$).

The serum concentrations of α_1 -AGP, serum protein concentrations and per cent free lidocaine for the three groups are summarized in Table II. The serum concentrations of α_1 -AGP and other proteins, and the per cent free lidocaine did not differ significantly among the three groups.

The per cent free lidocaine increased linearly as the serum concentration of α_1 -AGP decreased for each of the three groups. The least squares linear regression for the best-fit line between the per cent free lidocaine and the α_1 -AGP for each group and for all patients is presented in the Figure. The correlation coefficients for the regressions between α_1 -AGP and per cent free lidocaine for the acyanotic ($r = 0.86$), cyanotic ($r = 0.76$) and control ($r = 0.80$) did not differ significantly. The regressions between serum concentration of α_1 -AGP and per cent free lidocaine for the three groups and all of the patients together were significant ($P \leq 0.05$) and there was no significant difference among the slopes or the intercepts of the lines of regression of the three groups. There were no significant correlations between the per cent free lidocaine and the serum concentrations of albumin, α_1 , α_2 , β , γ globulin nor total protein.

Discussion

The profile of serum proteins of the children with CHD did not differ significantly from those without CHD (Table II). The latter values were consistent with previously published normal values for healthy children.^{4,20,27-29} The lack of significant correlations between the per cent free lidocaine and the serum concentrations of albumin, α_1 , α_2 , β , γ globulin and total proteins is not surprising when it is considered that cationic drugs such as lidocaine have a greater affinity for α_1 -AGP than for

TABLE II Serum concentrations of proteins and lidocaine in children with or without congenital heart disease

	α_1 -AGP (mg·dl ⁻¹)	Albumin (g·l ⁻¹)	α_1 -globulin (g·l ⁻¹)	α_2 -globulin (g·l ⁻¹)	β -globulin (g·l ⁻¹)	γ -globulin (g·l ⁻¹)	Total protein (g·l ⁻¹)	Total lidocaine (μ g·ml ⁻¹)*	Free lidocaine (%)*
Control	56.5	37.5	1.8	8.4	5.9	7.6	61.1	2.2	29.3
(n = 28)	± 15.3	± 2.6	± 0.5	± 1.1	± 1.0	± 2.6	± 4.2	± 0.6	± 4.7
Acyanotic	58.8	36.7	1.8	8.0	6.3	7.8	60.6	2.1	27.5
(n = 13)	± 14.8	± 2.5	± 0.4	± 0.7	± 1.1	± 2.7	± 5.7	± 0.7	± 7.6
Cyanotic	63.3	35.9	2.0	7.4	6.3	6.4	57.1	2.1	28.4
(n = 12)	± 15.4	± 2.8	± 0.1	± 2.3	± 0.8	± 1.6	± 5.1	± 0.6	± 5.2

Data are mean ($\pm$ SD).

*Obtained 5 minutes after 1.5 mg·kg⁻¹ intravenously.

FIGURE The regression lines for the control, acyanotic, cyanotic and combined data are presented. There were no significant differences in the slopes or intercepts of the lines among the three groups of patients or from the regression line of the combined data.

other proteins.¹⁷ Although these data demonstrate that the serum proteins in children with CHD do not differ quantitatively from those in healthy children, we cannot comment on possible qualitative differences in serum proteins, other than α_1 -AGP, since specific binding studies were not performed. The coefficients of variation in the serum concentrations of α_1 -AGP within each group are large but consistent with previously published normal values.⁹

Two of the factors that significantly affect the serum concentration of α_1 -AGP are the age of the patient and stress. Previous studies have demonstrated that serum concentration of α_1 -AGP increases during the first year after birth and reaches adult levels by one year.⁹ In order to minimize the effects of age in the present study, only patients older than one year were included. Although α_1 -AGP is an acute phase protein, that is the concentration of α_1 -AGP in serum increases within a short period after stress, previous studies have demonstrated that the serum concentration of α_1 -AGP in unanaesthetized patients is similar to that in anaesthetized patients²⁰ and that the serum concentration of α_1 -AGP in patients after surgery

or trauma does not increase until 6–8 hr after skin incision.^{7,11,30} This supports our contention that the serum concentration of α_1 -AGP measured immediately after induction of anaesthesia does represent the serum concentration of α_1 -AGP in the unstressed patient. Furthermore, these data suggest that the serum concentration of α_1 -AGP in the presence of acyanotic CHD is similar to that in cyanotic CHD.

Previous work has also suggested that qualitative differences in α_1 -AGP can reduce the affinity of cationic drugs for α_1 -AGP.⁹ We hypothesized that if qualitative differences in α_1 -AGP were produced by chronic hypoxaemia, then a similar reduction in the affinity of α_1 -AGP to bind cationic drugs may be evident in comparing age-matched children with and without cyanotic CHD. The results of this study indicate that chronic hypoxaemia has no measurable effect on the binding affinity of α_1 -AGP for lidocaine (Figure).

It has been suggested that the pharmacokinetic behaviour of cationic drugs may vary with the type of anaesthetic administered.³¹ In the present study, halothane and nitrous oxide were administered to all children.

Although halothane is lipid-soluble and is moderately soluble in blood (blood/gas partition coefficient of 2.39 in children),³² we have shown previously that α_1 -AGP does not affect the blood/gas partition coefficient of halothane to any measurable extent.³³ There are no data available on the effect of α_1 -AGP on the solubility of nitrous oxide in blood. This study demonstrates that the presence of CHD in children affects neither the serum concentration of α_1 -AGP nor the binding affinity of α_1 -AGP for lidocaine. We are currently investigating the pharmacokinetic behaviour of the free fraction of lidocaine and total lidocaine in children with acyanotic CHD and cyanotic CHD to determine whether the type of CHD lesion affects the pharmacokinetics of lidocaine.

References

- 1 Shand DG. α_1 -acid glycoprotein and plasma lidocaine binding. *Clin Pharmacokinet* 1982; 9(Suppl 1): 27–31.
- 2 McNamara PJ, Slaughter RL, Pieper JA, Wyman MG, Lalka D. Factors influencing serum protein binding of lidocaine in humans. *Anesth Analg* 1981; 60: 395–400.
- 3 Kurz H, Mauser-Ganshorn A, Stickel AA. Differences in the binding of drugs to plasma proteins from the newborn and adult man. *Eur J Clin Pharmacol* 1977; 11: 463–7.
- 4 Wood M, Wood AJJ. Changes in plasma drug binding and α_1 -acid glycoprotein in mother and newborn infant. *Clin Pharmacol Ther* 1981; 29: 522–6.
- 5 Piafsky KM, Woolner EA. The binding of basic drugs to α_1 -acid glycoprotein in cord serum. *J Pediatr* 1982; 100: 820–2.
- 6 Pieper JA, Wyman MG, Goldreyer BN, Cannon DS, Slaughter RL, Lalka D. Lidocaine toxicity: effects of total versus free lidocaine concentrations. *Circulation* 1980; 62(Suppl 3): 181.
- 7 Edwards DJ, Lalka D, Cerra F, Slaughter RL. Alpha₁-acid glycoprotein concentration and protein binding in trauma. *Clin Pharmacol Ther* 1982; 31: 62–7.
- 8 Piafsky KM. Disease-induced changes in the plasma binding of basic drugs. *Clin Pharmacokinet* 1980; 5: 246–62.
- 9 Lerman J, Strong HA, LeDez KM, Swartz J, Rieder MJ, Burrows FA. Effects of age on the serum concentration of α_1 -acid glycoprotein and the binding of lidocaine in pediatric patients. *Clin Pharmacol Ther* 1989; 46: 219–25.
- 10 Garner L, Stirt JA, Finholt DA. Heart block after intravenous lidocaine in an infant. *Can Anaesth Soc J* 1985; 32: 425–8.
- 11 Aronsen KF, Ekelund G, Kindmark CO, Laurell CB. Sequential changes of plasma proteins after surgical trauma. *Scand J Clin Lab Invest* 1972; 29 (suppl 124): 127–36.
- 12 Chio LF, Oon CJ. Changes in serum alpha₁ antitrypsin, alpha₁ acid glycoprotein and beta₂ glycoprotein in patients with malignant hepatocellular carcinoma. *Cancer* 1979; 43: 596–604.
- 13 Johansson BG, Kindmark CO, Trell EY, Wollheim FA. Sequential changes of plasma proteins after myocardial infarction. *Scand J Clin Lab Invest* 1972; 29(suppl 124): 117–26.
- 14 Piafsky KM, Borga O, Odar-Cederlof I, Johansson C, Sjoqvist F. Increased plasma protein binding of propranolol and chlorpromazine mediated by disease induced elevations of plasma α_1 -acid glycoprotein. *N Engl J Med* 1978; 299: 1435–9.
- 15 Schmidt K. α_1 -Acid glycoprotein. In: Putnam FM (Ed.). *The Plasma Proteins*. Volume 1. New York: Academic Press, 1975; 317–21.
- 16 Fukui T, Hameroff SR, Gandolfi AJ. Alpha-1-acid glycoprotein and beta-endorphin alterations in chronic pain patients. *Anesthesiology* 1984; 60: 494–6.
- 17 Pike E, Skuterud B, Kierulf P, Fremstad D, Abel Sayed SM, Lunde PRM. Binding and displacement of basic, acidic and neutral drugs in normal and orosomucoid-deficient plasma. *Clin Pharmacokinet* 1981; 6: 367–74.
- 18 Routledge PA, Barchowsky A, Bjornsson TD, Kitchell BB, Shand DG. Lidocaine protein binding. *Clin Pharmacol Ther* 1980; 27: 347–51.
- 19 McGowan FX, Reiter MJ, Pritchett ELC, Shand DG. Verapamil plasma binding: relationship to α_1 -acid glycoprotein and drug efficacy. *Clin Pharmacol Ther* 1983; 33: 485–90.
- 20 Routledge PA, Stargel WW, Kitchell BB, Barchowsky A, Shank DG. Sex-related differences in the plasma protein binding of lignocaine and diazepam. *Br J Clin Pharmacol* 1981; 11: 245–50.
- 21 Kingsley GR. The direct biuret method for determination of serum proteins as applied to photoelectric and visual colorimetry. *J Lab Clin Med* 1942; 27: 840–5.
- 22 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 1965; 2: 235–54.
- 23 Walberg CB. Lidocaine by enzyme immunoassay. *J Anal Toxicol* 1978; 2: 121–3.
- 24 Jain S, Johnston A. The measurement of lignocaine at low concentrations in plasma, a comparison of gas liquid chromatography with enzyme assay. *Br J Clin Pharmacol* 1979; 8: 598–9.
- 25 Wilkinson L. SYSTAT: the system for statistics. Evanston, IL: SYSTAT Inc. 1987; STAT 1–7.
- 26 Zar JH. *Biostatistical Analysis*, 2nd ed. Englewood Cliffs: Prentice-Hall Publishers Inc, 1984; 185–90, 268–72, 300–4, 313–4.
- 27 Piafsky KM, Borga O. Plasma protein binding of basic drugs. II. Importance of α_1 -acid glycoprotein for

- interindividual variation. *Clin Pharmacol Ther* 1977; 22: 545-9.
- 28 *Piafsky KM, Mpamugo L.* Dependence of neonatal drug binding on α_1 -acid glycoprotein concentration. *Clin Pharmacol Ther* 1981; B-17, 272.
- 29 *Abernethy DR, Kerzner L.* Age effects on alpha-1-acid glycoprotein concentration and imipramine plasma protein binding. *J Am Geriatr Soc* 1984; 32: 705-8.
- 30 *Colley CM, Fleck A, Goode AW, Muller BR, Myers MA.* Early time course of the acute phase protein response in man. *J Clin Pathol* 1983; 36: 203-7.
- 31 *Wood M, Whelan E, Shay S, Wood AJJ.* Acute effect of halothane on drug distribution. *Anesthesiology* 1989; 71: A259.
- 32 *Lerman J, Schmitt-Bantel BI, Gregory GA, Willis MM, Eger EI II.* Age and solubility of volatile anesthetics in blood. *Anesthesiology* 1984; 61: 139-43.
- 33 *Sinclair L, Strong HA, Lerman J.* Effects of AAGP, local anaesthetics and pH on the partition coefficients of halothane, enflurane and sevoflurane in blood and buffered saline. *Can J Anaesth* 1988; 35: S99.