Eur J Clin Pharmacol (1984) 27: 459–463

European Journal of Clinical Pharmacology

Naloxone Protein Binding in Adult and Foetal Plasma

L. A. Asali and K. F. Brown[1]

[1]Department of Pharmacy, University of Sydney, Sydney, NSW, and Perinatal Pharmacology Laboratory, Foundation 41, Sydney, Australia

Summary. Binding of naloxone hydrochloride was determined at 37 °C, by equilibrium dialysis against 0.067 M phosphate buffer, pH 7.4, in plasma obtained from 18 healthy adults, and 18 samples of umbilical cord venous (foetal) plasma. The percentage free fraction (% free) in plasma was independent of naloxone concentration (9 ng/ml to 2.5 μg/ml). Percent free naloxone in adult ($\bar{x} = 54.0$) was lower ($p < 0.01$) than in foetal ($\bar{x} = 61.5$) plasma. In buffered solutions of purified HSA, %free naloxone ($\bar{x} = 68.7$) was independent of HSA concentration over the range 3.0 g/dl to 5.5 g/dl. Adult plasma concentrations of α_1-acid glycoprotein (α_1-AGP) and β-lipoprotein were higher ($p < 0.01$) than foetal concentrations. Furthermore %free naloxone in foetal plasma decreased with the in-vitro addition of purified α_1-AGP. It is suggested that qualitative differences in adult and foetal albumin and quantitative differences in plasma levels of α_1-AGP and perhaps β-lipoprotein are responsible for naloxone plasma binding differences between adults and the newborn.

Key words: naloxone, plasma protein binding; albumin, alpha$_1$-acid glycoprotein, beta-lipoprotein, adult plasma, foetal/umbilical plasma

Naloxone, a narcotic antagonist without agonist action, has become the drug of choice in the treatment of narcotic overdose [1], in the management of narcotic-induced depression in the newborn shortly after birth [2] and in adults post-anaesthesia [3]. More recently, higher doses (0.5 to 1.0 mg/kg) of naloxone have been administered in the management of shock [4] and neonatal apnoea of prematurity [5]. Despite the widespread use of naloxone in adults and the newborn, no information is available on the binding of naloxone to plasma proteins.

Albumin is the major binding protein for many acidic drugs. However, basic drugs may also exhibit high affinities for other plasma proteins including α_1-acid glycoprotein (α_1-AGP), lipoproteins and γ-globulin. A preliminary study [6] of naloxone binding to purified α_1-AGP (50 mg/dl), reported that less than 20% of the drug was bound. However the concentration of naloxone was not specified but was reported to be a realistic clinical level.

The fractions of various acidic and basic drugs bound to the proteins in umbilical cord plasma have often been reported to be less than in adult plasma [7, 8]. Thus, the objectives of this study were firstly, to determine the extent of naloxone binding in plasma of healthy adults and in foetal cord plasma and to establish the various plasma proteins responsible for variations in naloxone binding. Secondly, to establish whether the binding of naloxone is linear over a wide range of plasma concentrations.

Materials, Subjects and Methods

Naloxone hydrochloride and naltrexone hydrochloride were gifts from Endo Laboratory (Garden City, NY, USA). Aqueous standard solutions of these compounds were stored at 4 °C. Purified α_1-AGP and human serum albumin (HSA; Fraction V, electrophoretic purity, 100%) were purchased from Behringwerke AG (Marburg, FRG). All other chemicals were of analytical reagent grade.

Binding of naloxone in plasma was determined in 18 healthy adult volunteers comprising 7 males and 11 females (age range 22 to 42 years) and in a

further 3 parturient women (maternal plasma), at delivery. Binding of naloxone was also studied in the umbilical cord, venous plasma (foetal plasma) of 18 neonatal subjects.

Venous blood samples were collected, by single venipuncture of an antecubital vein from the adult volunteers and from the clamped umbilical cords, into plastic tubes (Disposable Products, Sydney, Australia) containing ammonium heparin, 125 IU/10 ml. Plasma was harvested after centrifugation for 5 min at 1000 *g* and stored frozen at −18 °C to −22 °C. Immediately prior to plasma binding determination, plasma was thawed at room temperature and centrifuged to remove fibrous material.

Naloxone HCl (200 ng/10 µl, equivalent to 180 ng/10 µl naloxone base) was dissolved in Sorenson's phosphate buffer, 0.067 M, pH 7.4, containing 3.5 g/dl HSA. 10 µl of this solution were added to 0.99 ml of HSA solution or plasma resulting in a final concentration of 180 ng/ml naloxone base. Binding of naloxone (180 ng/ml) in solutions containing a range of concentrations of purified HSA (0.5 to 5.5 g/dl) in Sorenson's buffer was determined. In addition, binding of a range of naloxone concentrations (18 ng/ml to 3.6 µg/ml) to HSA (3.5 g/dl) was also determined. Furthermore, binding of naloxone to adult and foetal whole plasma samples was determined using similar methodology.

Binding of naloxone was determined by equilibrium dialysis in 1 ml, PTFE half dialysis cells (Spectrum Medical Industries, Inc., California, USA). One ml aliquots of HSA solution or plasma were dialysed across a semipermeable membrane (Spectrapor II, Spectrum Medical Industries, Inc., California, USA) against an equal volume of Sorenson's phosphate buffer at 37 °C by rotating for 1 h. Preliminary studies had confirmed that equilibrium was achieved within that period. At equilibrium, naloxone concentrations in aliquots of the protein containing compartment (300 µl) and buffer containing compartment (750 µl), were determined by the HPLC method of Asali et al. [9] using naltrexone HCl (50 ng/50 µl) as internal standard. The percentage of free naloxone (% free) was calculated as the ratio of naloxone concentrations in the buffer and protein containing compartments. The reproducibility of the binding methodology was established using HSA 3.5 g/dl and found to be good (C.V. = 3.8%, $n = 8$, mean %free = 68.8%).

Total protein and albumin concentrations in adult and foetal plasma were measured by Rapid Stat Kit (Pierce, Rockford, Ill, USA). Concentrations of α_1-AGP and β-lipoprotein were measured by radial immunodiffusion (M-partigen, Behringwerke AG, Marburg, FRG).

Statistical Analysis

Unless otherwise stated, data are expressed as mean ± SD. Statistical comparisons between the binding in plasma samples from adult and foetal groups were made using the Mann-Whitney U Test [10]. Correlations between endogenous plasma constituents and the plasma binding of naloxone were performed by linear, partial and multiple regression analyses [11].

Results

Influence of Variation in HSA Concentration

The influence of HSA concentration on the binding of naloxone (180 ng/ml) was determined (Fig. 1). The %free naloxone remained constant at 68.7%, independent of HSA concentrations between 3.0 to 5.5 g/dl. At 0.5 g/dl and 1.5 g/dl HSA, the %free were higher (94.6% and 84.5% respectively) as expected. The constancy of %free with increasing HSA concentration above 3.0 g/dl reflects the fact that, at fixed total drug concentration the free concentration diminishes as the number of available sites increases.

Influence of Variation in Naloxone Concentration

The %free naloxone in 3.5 g/dl HSA solution (68.7 ± 0.865%), in plasma from an adult (55.9 ± 0.748%) and a sample of umbilical venous plasma

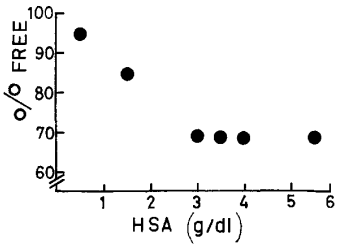

Fig. 1. Variation in %free naloxone with HSA concentration

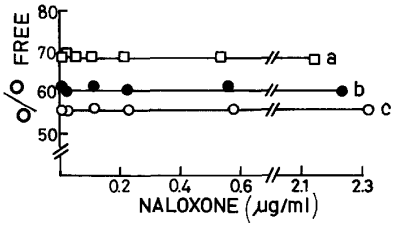

Fig. 2. %Free naloxone as a function of naloxone total concentration in (□), 3.5 g/dl HSA; (●), foetal plasma; and (○), adult plasma

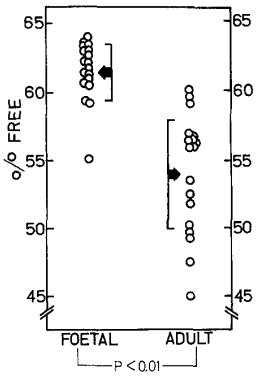

Fig. 3. %Free naloxone in foetal (umbilical venous) and adult plasma, spiked with an initial concentration of 180 ng/ml naloxone. Arrows and bars indicate means and standard deviations

Table 1. Plasma protein concentration in foetal, adult and maternal plasmas and naloxone %free

	Foetal[1]	Adult[1]	Maternal
%Free	61.5 (± 2.13)[2]	54.1 (± 4.40)	53.2
Albumin [g/dl]	3.83 (± 0.361)	4.60 (± 0.699)	3.32
α_1-AGP [mg/dl]	15.4 (± 7.75)	49.8 (±14.8)	56.6
β-Lipoprotein [mg/dl]	85.6 (±25.9)	248 (±46.6)	223
Total protein [g/dl]	5.70 (± 0.595)	6.56 (± 1.09)	6.31
n	18	18	3

[1] All differences between adult and foetal mean values are significant ($p<0.01$); [2] figures in parentheses are SD

(61.1 ± 1.38%) was constant, independent of naloxone concentration over the range of initial concentrations from 18 ng/ml to 3.6 µg/ml (Fig. 2). The range of equilibrium, naloxone concentrations (9 ng/ml to 2.25 µg/ml) is typical of the plasma levels encountered after high doses of naloxone (of the order of 0.5–1.0 mg/kg) are administered to neonates for the treatment of apnoea of prematurity (unpublished data).

Binding of Naloxone to Adult and Foetal Plasma

Naloxone binding was greater ($p<0.01$) in both an adult and a single foetal plasma sample, than in HSA; indicating that significant non-albumin binding of naloxone occurs in whole plasma (Fig. 2).

Individual data for the binding of naloxone in adult and foetal plasma are compared in Fig. 3. The mean values of %free were higher ($p<0.01$, Table 1) in the foetal plasma samples (61.5 ± 2.13%) than in the adult samples (54.0 ± 4.40%).

Plasma concentrations of albumin, α_1-AGP, β-lipoprotein and total protein in adult samples were consistently higher ($p<0.01$) than in the samples of cord plasma (Table 1).

A weak bivariate correlation was observed between the values of %free naloxone and pooled values of plasma albumin concentration ($r=-0.465$, $p<0.01$, $n=39$) including adult, foetal and 3 maternal samples collected at delivery (Fig. 4). However stronger correlations (Figs. 5 and 6) were observed between %free naloxone and values of plasma α_1-AGP concentration ($r=-0.762$, $p<0.01$, $n=39$), and plasma β-lipoprotein concentration ($r=-0.731$,

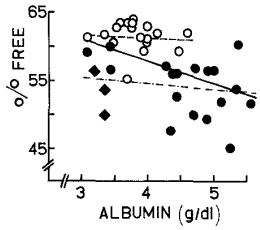

Fig. 4. Relationship between %free naloxone and plasma albumin concentrations in foetal (○), adult (●) and maternal samples (◆). Solid line, pooled data; $Y=-3.4207X+71.53$; $r=0.4651$; $p<0.01$. ----, foetal plasma; $Y=-0.5108X+63.46$; $r=-0.0866$; $p>0.05$. -·-·-·-, adult and maternal plasmas; $Y=-1.3504X+59.77$; $p>0.05$

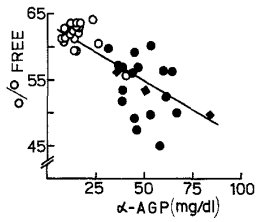

Fig. 5. Relationship between %free naloxone and pooled plasma α_1-AGP concentrations in foetal (○), adult (●) and maternal (◆) samples. $Y=-0.1815X+63.63$; $r=-0.8089$; $p<0.01$.

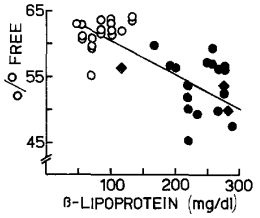

Fig. 6. Relationship between %free naloxone and pooled plasma-lipoprotein concentrations in foetal (○), adult (●) and maternal (◆) samples. $Y=-0.0441X+64.67$; $r=-0.7310$; $p<0.01$

Table 2. Percentage of free naloxone in umbilical cord plasma before and after the in-vitro addition of purified α_1-AGP ($Y_{\%free} = -0.1937\ X_{\alpha_1}\text{-AGP} + 64.02$; $r = -0.9394$)

Cord plasma	Plasma α_1-AGP concentration [mg/dl]		%Free naloxone	
	Initial	Final	Initial	Final
1	28.0	57.4	59.6	49.7
2	18.5	50.0	60.7	54.0
	18.5	67.8	60.7	49.8
	18.5	70.5	60.7	51.9
	18.5	73.2	60.7	51.7
	18.5	84.6	60.7	47.6

$p < 0.01$, $n = 38$, one female subject was excluded from the adult group as an outlier).

To establish whether or not the correlation between %free naloxone and α_1-AGP is real, concentration of α_1-AGP in two samples of cord plasma were increased by in-vitro addition of the purified protein to produce final concentrations equivalent to adult levels (Table 2). The percentage of free naloxone decreased consistently with progressive increases in α_1-AGP concentration ($Y_{\%free} = -0.194\ X_{\alpha_1-AGP} + 64.02$; $r = -0.939$).

The simultaneous influences of the concentrations of plasma α_1-AGP, albumin and β-lipoprotein were examined by multiple linear regression analyses. When the combined effects of variation in α_1-AGP and β-lipoprotein concentrations, on %free naloxone were examined, a value for multiple R^2 of 0.5592 was obtained. When the additional influence of variation in albumin was included with the two variables, multiple R^2 increased only marginally ($R^2 = 0.591$).

Discussion

Although the plasma protein binding of naloxone is relatively weak, the %free in adult plasma (54.0%) was lower than that in cord plasma (61.5%). At HSA concentrations ranging from 3.0 g/dl to 5.5 g/dl, %free naloxone was 68.7% (Fig. 1). Thus, it is obvious that plasma albumin is the major binding constituent in plasma but that binding also occurs to plasma constituents other than albumin.

Despite the fact that albumin concentrations in adult plasma are higher than in foetal plasma, the %free naloxone in HSA solutions was independent of variation in HSA concentration over the range 3.0 g/dl to 5.5 g/dl. This implies that the difference between foetal and adult plasma binding is unrelated to variation in albumin concentration. The weak bivariate correlation between %free naloxone and plasma albumin concentration ($r = -0.465$) of foetal, adult and maternal samples is therefore probably fortuitous. The relationship between %free naloxone and plasma albumin was further examined using partial correlation analysis where α_1-AGP was held constant. Under these conditions, the linear correlation between %free naloxone and plasma albumin disappears ($r = +0.228$, $p > 0.05$, $n = 39$). This confirmed that the correlation between %free and plasma albumin concentration is spurious and results from the interdependence of albumin and α_1-AGP concentrations, which were correlated linearly ($r = 0.455$, $p < 0.01$, $n = 39$).

It has been reported that albumin isolated from human cord blood (Alb-F) is different in several physicochemical respects [12] and in amino acid sequence and content [13] from albumin isolated from adults (Alb-A). Both Alb-F and Alb-A were shown to coexist in roughly equal amounts in the serum of cord blood [14]. Foetal albumin is gradually replaced by Alb-A during ontogenic development during the first 4 to 5 months after birth. Thus, consistent with the findings for diazepam plasma binding [14] it would appear that there are qualitative differences between adult and foetal albumin with respect to naloxone plasma binding.

Nation [15] reported that there was no significant difference between plasma albumin concentration in cord plasma and maternal plasma collected at delivery. This is supported in this study in that albumin concentrations determined in 3 samples of maternal plasma, collected at delivery (Table 1) were similar in magnitude to the cord levels and lower than the concentrations in the 18 healthy adults. Notwithstanding the lower maternal albumin concentrations compared with the adult plasma, the binding of naloxone was similar in both maternal and healthy adult plasma; and higher than in the cord plasma (Fig. 3) which had albumin concentrations of similar magnitude (Fig. 4). These findings would appear to provide strong evidence that the lower binding of naloxone in umbilical cord plasma relative to either maternal or healthy adult plasma is partly attributable to the existence of qualitative differences between adult and foetal albumins as suggested previously [12–14]. Thus the apparent weak correlation, described above, between %free naloxone with albumin concentration data, pooled from both adult and cord plasmas, is not valid; since the cord and adult albumins belong to different populations. As expected correlations could not be demonstrated between %free naloxone and albumin concentration data (Fig. 3) from either cord plasma ($r = -0.0866$; $p > 0.05$, $n = 18$) or adult and maternal plasmas ($r = -0.263$, $p > 0.05$, $n = 21$).

The finding of a positive correlation between %free naloxone and the acute phase protein α_1-AGP ($r = -0.762$) suggests that α_1-AGP is involved in naloxone binding as reported previously [6]. In proof of these proposals, elevation of the concentration of α_1-AGP in 2 samples of cord plasma to adult concentration levels by in-vitro addition of purified α_1-AGP increased naloxone binding (Table 2) to a value within the adult range. Thus, α_1-AGP concentration is an important determinant of naloxone plasma binding, and is partially responsible for the fact that cord plasma binds naloxone to a lesser extent than adult plasma. Partial regression analyses, correcting for the effects of albumin and β-lipoprotein, indicated that plasma α_1-AGP accounted for the largest proportion ($R^2 = 0.248$) of the overall variability in %free naloxone.

Bickel [16] showed that the binding affinity and capacity of lipoproteins for basic drugs including chlorpromazine and imipramine was at least as high as that of albumin, while Nilson [17] demonstrated that approximately 31% of quinidine plasma binding was associated with lipoproteins. Thus, the linear correlation observed between naloxone %free and plasma β-lipoprotein concentration ($r = -0.731$) in this study suggests that the lower concentrations of β-lipoprotein observed in the foetal group samples might also partially explain the lower binding of naloxone in cord plasma compared with that in the adult group. Partial regression analysis between %free naloxone and plasma β-lipoprotein concentration, with plasma albumin and α_1-AGP concentrations held fixed, indicate that variation in β-lipoprotein concentration accounts for only 14% of the variability in naloxone %free.

Using multiple regression analysis it was shown that quantitative differences in both α_1-AGP and β-lipoprotein together account for only 55.9% of the overall variability in naloxone binding. Thus, it seems likely that qualitative differences in the binding affinities between adult and foetal albumins and perhaps other variables, including the globulin fraction [18] may account for the remaining proportion of the variability in naloxone binding.

Acknowledgements. The authors gratefully acknowledge the receipt of a University of Sydney Postgraduate Award for LAA. The assistance of J. French in collecting blood samples is gratefully acknowledged.

References

1. Simons PS (1973) The treatment of methadone poisoning with naloxone. J Pediatr 83: 846
2. Wiener PC, Hogg MIJ, Rosen M (1977) Effect of naloxone on pethidine induced neonatal depression. Br Med J 2: 228–231
3. Davis GK, Tolhurst-Cleaver CL, James TL (1980) Respiratory depression after intrathecal narcotics. Anaesthesia 35: 1080–1083
4. Amir S (1982) Opiate antagonists improve survival in anaphylactic shock. Eur J Pharmacol 80 [1]: 161
5. Burnard ED, John E, Henderson-Smart D, Todd DA (1983) Naloxone and recurrent apnoea of prematurity. Intensive Care Newborn 4: 127–142
6. Romach MK, Piafsky KM, Abel JG, Khouw V, Sellers EM (1981) Methadone binding ro orosomucoid (α_1-acid glycoprotein): determinant of free fraction in plasma. Clin Pharmacol Ther 29 [2]: 211–217
7. Kurz H, Mauser-Ganshorn A, Stickel HH (1977) Differences in the binding of drugs to plasma proteins from newborn and adult man I. Europ J Clin Pharmacol 11: 463–467
8. Piafsky KM, Woolner EA (1982) The binding of basic drugs to α_1-acid glycoprotein in cord serum. J Pediatr 100 [5]: 820–822
9. Asali LA, Nation RL, Brown KF (1983) Determination of naloxone in blood by high-performance-liquid-chromatography. J Chromatogr 278: 329–335
10. Freund JE (1974) Modern elementary statistics. Prentice-Hall International: London
11. Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH (1975) Statistical package for the social sciences. Mc Gregor & Werner, New York, USA
12. Miyoshi K, Saijo K, Kotani Y, Kashiwagi T, Kawai H (1966) Characteristic properties of fetal human albumin (Alb F) in isomerization equilibrium. Tokushima J Exp Med 13: 121–128
13. Wallace S (1977) Altered plasma albumin in the newborn infant. Br J Clin Pharmacol 4: 82–84
14. Ridd MJ, Brown KF, Nation RL, Collier CB (1983) Differential transplacental binding of diazepam: causes and implications. Eur J Clin Pharmacol 24: 595–601
15. Nation RL (1981) Meperidine binding in maternal and fetal plasma. Clin Pharmacol Ther 29 [4]: 472–479
16. Bickel MH (1975) Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and blood components. J Pharm Pharmacol 27: 733–738
17. Nilson OG, Storstein L, Jacobsen S (1977) Effect of heparin and fatty acids on the binding of quinidine and warfarin in plasma. Biochem Pharmacol 26: 229–235
18. Kurz H, Michels H, Stickel HH (1977) Differences in the binding of drugs to plasma proteins from newborn and adult man II. Eur J Clin Pharmacol 11: 469–472

Received: May 18, 1984
accepted: August 24, 1984

Kenneth F. Brown, Ph. D.
The University of Sydney
Department of Pharmacy
NSW 2006, Sydney, Australia