

The binding of drugs to major human milk whey proteins

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The binding of nine drugs of diverse physicochemical characteristics to major human milk whey proteins is reported. This group included acids, bases and neutral drugs. No drug bound to α -lactalbumin, which is the protein present in greatest concentrations in mature milk. Four drugs, diclofenac, phenytoin, prednisolone and warfarin, bound to albumin but to a much lesser extent than in plasma, consistent with quantitatively less albumin in milk. None of the basic drugs studied bound to albumin. Five drugs, atenolol, diclofenac, prednisolone, propranolol and warfarin, bound to lactoferrin though the extent was minimal except for diclofenac. This group included acids, bases and neutral drugs.

Keywords protein binding milk proteins ultrafiltration human milk

Introduction

The dose of drug ingested in milk by a breast-feeding infant is determined by the composition of milk and the physicochemical characteristics of the drug (Wilson, 1983). The protein content of milk changes during the time course of lactation, mainly due to a decline in the concentration of immunoglobulins and the milk-specific proteins lactoferrin and α -lactalbumin (Lonnerdal *et al.*, 1976a), while the albumin content, is relatively constant (about 0.4 g l^{-1}) (Lonnerdal *et al.*, 1976a). Depending on the extent of binding of drugs to the specific milk proteins which vary in concentration during lactation, the 'dose' ingested by a suckling infant might also vary. The extent of binding of drugs to total milk proteins has been reported (Atkinson & Begg, 1988; Syverson & Ratjke, 1985) but not to individual milk proteins. The aim of this study was to examine the binding of drugs with different acid-base characteristics to the major individual milk whey proteins, albumin, α -lactalbumin and lactoferrin. Since acidic and basic drugs bind to different proteins in plasma (Piafsky & Borga, 1977) the same might apply to milk.

Methods

Materials

The following drugs were used: [^{14}C]-atenolol ($4.6 \text{ mCi mmol}^{-1}$) and atenolol (ICI, England);

[^{14}C]-diclofenac ($6.7 \text{ mCi mmol}^{-1}$) and diclofenac (Ciba-Geigy, Switzerland); [^3H]-digoxin (New England Nuclear (NEN), USA, 18 Ci mmol^{-1}), digoxin (Wellcome, England), [^3H]-flunitrazepam (NEN, $78.9 \text{ Ci mmol}^{-1}$), flunitrazepam (Roche, Switzerland); [^3H]-ketotifen (60 Ci mmol^{-1}) and ketotifen (Sandoz, Switzerland); [^3H]-phenytoin (NEN, 46 Ci mmol^{-1}), phenytoin (Parke-Davis, England), [^3H]-prednisolone (Amersham, Australia, $67.4 \text{ Ci mmol}^{-1}$), prednisolone (Sigma, USA); [^3H]-propranolol (NEN, $18.5 \text{ Ci mmol}^{-1}$), propranolol (ICI, England); [^{14}C]-warfarin (NEN, 46 Ci mmol^{-1}) and warfarin (Sigma). The radiochemical purity of the drugs was stated to be 97-99%. The proteins albumin, α -lactalbumin and lactoferrin were obtained from Sigma, USA.

Protein binding

Mature human milk was collected from five lactating volunteers. After centrifugation ($10,000 \text{ rev min}^{-1}$, 15 min) and removal of the lipid layer the samples were pooled to form a milk bank and milk ultrafiltrate prepared as described by Atkinson & Begg (1988). Individual milk proteins were then dissolved in milk ultrafiltrate and adjusted to milk pH 7.2, (Ansell *et al.*, 1977; Harrison & Peat, 1972), using 5% carbon dioxide in nitrogen gas bubbled through the solution.

Protein concentrations represented the maximum likely concentrations in mature milk; albumin (0.4 g l^{-1}), α -lactalbumin (3.3 g l^{-1}), and lactoferrin (1.9 g l^{-1}), (Lonnerdal *et al.*, 1976a).

Unbound drug concentrations were determined by ultrafiltration as described by Atkinson & Begg (1988). The studies were performed at the lowest drug concentrations in skim milk which might occur during therapy (Table 1). In this way, falsely low values due to saturable protein binding would be avoided.

Retention by the YM10 ultrafiltration membranes (MW cut off 10,000) of specific milk proteins albumin (MW 66,000, Lentner, 1984a) and α -lactalbumin (MW 23,000, Malpress and Hytten, 1964) was greater than 99.9%, and 99.7% \pm 0.07%, respectively (N=5 in each case), as assessed by a microprotein assay (Bradford, 1976). Lactoferrin (MW 75,000) (Querinyean *et al.*, 1977) was not studied because it would be expected to be retained to at least the extent of albumin.

Nine drugs of diverse structure and acid/base characteristics were studied: diclofenac, phenytoin and warfarin (acidic); atenolol, flunitrazepam, ketotifen and propranolol (basic); digoxin and prednisolone (neutral).

Results

α -lactalbumin did not account for significant binding to any of the drugs. Binding to albumin was significant for diclofenac (32.9%), warfarin (31.6%), prednisolone (5%) and phenytoin (2.4%). Binding to lactoferrin was significant for diclofenac (30.6%), prednisolone (6.3%), propranolol (5.3%), warfarin (3.6%) and atenolol (1.7%). Three of the drugs, flunitrazepam, ketotifen and digoxin did not bind significantly to any of the proteins (Table 1).

Discussion

Whey proteins account for 70–80% of milk proteins (Lonnerdal *et al.*, 1976b, 1977) and include albumin (0.4 g l^{-1}), α -lactalbumin ($2.7\text{--}3.3 \text{ g l}^{-1}$), lactoferrin ($1.4\text{--}1.9 \text{ g l}^{-1}$), IgA ($1\text{--}3 \text{ g l}^{-1}$) and lysozyme (0.4 g l^{-1}). Casein, a heterogeneous mixture of proteins, accounts for the remainder (Jelliffe & Jelliffe, 1977). Concentrations of α -lactalbumin, lactoferrin and IgA vary considerably, decreasing from a maximum in colostrum to values of mature milk (2–3 weeks postpartum) after which a gradual decline is evident (Lonnerdal *et al.*, 1977). In addition, protein content varies within a feed, with hindmilk containing 1.5 times more protein than foremilk (Hall, 1975).

Total protein concentration in milk, $0.8\text{--}0.9 \text{ g l}^{-1}$ (Lonnerdal *et al.*, 1976a) is lower than in plasma, 74.6 g l^{-1} (Lentner, 1984b), so less binding would be expected and is observed (Atkinson & Begg, 1988). Binding to specific proteins is therefore comparatively low. Binding was studied at the lowest likely therapeutic concentration and is therefore likely to represent the maximum binding, although the possibility of saturable binding does exist. Diclofenac and warfarin, both highly bound to albumin in plasma, were also bound relatively highly to albumin in milk (32%). None of the bases bound to albumin. There was no pattern in terms of acid-base characteristics evident in the binding to lactoferrin. Because the binding to lactoferrin for most drugs is likely to be low it is unlikely that the alterations in the concentration of this protein during lactation would alter the total content of the drug in milk enough to alter decisions about the 'dose' to which the infant might be exposed.

For diclofenac and prednisolone, the aggregate binding to albumin and lactoferrin was greater than total binding measured in skim milk (Atkin-

Table 1 Binding of drugs to major mature milk whey proteins

Drug	Skim milk (% bound)	Drug concentration ($\mu\text{g l}^{-1}$)	Albumin (0.4 g l^{-1}) (% bound)	α -lactalbumin (3.3 g l^{-1}) (% bound)	Lactoferrin (1.9 g l^{-1}) (% bound)
Diclofenac	48 ¹	470	32.9	0	30.6
Phenytoin	10 ²	1300	2.4	0	0
Warfarin	20–50 ²	4.0	31.6	0	3.6
Atenolol	4.6 ¹	600	0	0	1.7
Flunitrazepam	–	4.0	0	0	0
Ketotifen	–	750	0	0	0
Propranolol	14.8 ¹	9.0	0	0	5.3
Digoxin	5.3 ¹	0.5	0	0	0
Prednisolone	10.2 ¹	1.1	5	0	6.3

¹ Atkinson & Begg (1988)

² Sverson & Ratjke (1985)

son & Begg, 1988). This is because the concentration of lactoferrin used was the maximum which would be expected in mature milk.

Where a small amount of binding in mature milk was not accounted for, the difference would be expected to be associated with casein or other whey proteins which were not individually measured, IgA ($1-3 \text{ g l}^{-1}$), IgG (0.014 g l^{-1}), IgM (0.016 g l^{-1}) and lysosyme (0.4 g l^{-1}), (Lonnerdal *et al.*, 1976a,b, 1977). The concentrations of immunoglobulins are, except for IgA, much greater in plasma than in milk; IgA ($0.7-3.6 \text{ g l}^{-1}$), IgG ($6.6-18.4 \text{ g l}^{-1}$), IgM ($0.4-2.6 \text{ g l}^{-1}$) (Lentner, 1984c). Since binding to immunoglobulins is less than 1% of total binding in plasma (Albengres, 1987), binding to immunoglobulins in human milk is unlikely to be significant. α_1 -acid glycoprotein which accounts for

significant binding of basic drugs in plasma (Piafsky & Borga, 1977) is not reported as being present in significant amounts in human milk (Lonnerdal *et al.*, 1976a,b, 1977).

In summary, albumin and lactoferrin appear to be the major specific drug binding proteins in human milk. Because total protein binding in milk is low, and lactoferrin only a part of this, the changes in lactoferrin concentration are unlikely to have a major effect on the dose of drugs ingested by a breastfeeding infant during the course of lactation.

The authors would like to thank the members of the La Leche League who donated milk, the Canterbury Medical Research Foundation for financial support, and Ciba-Geigy, ICI, Parke-Davis, Roche, Sandoz and Wellcome for gifts of drugs.

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(Received 9 December 1987,
accepted 22 February 1988)