Molecular activity of human milk xanthine oxidase varies with time after parturition

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Xanthine oxidase is a widely distributed molybdoenzyme with a generally accepted role in purine catabolism, catalysing the oxidation of hypoxanthine to xanthine and of xanthine to uric acid [1]. It can also act as a source of reactive oxygen species, passing electrons to oxygen to generate superoxide anion and hydrogen peroxide [1]. This facility has been implicated in anti-microbial defence [2] and as a destructive factor in ischaemia-reperfusion injury [3]. Although the latter implication has led to a great deal of clinically targeted research, human xanthine oxidase has only recently been characterised [4]. This enzyme, purified from breast milk has surprisingly low activity towards hypoxanthine or xanthine, suggesting the presence of inactive isoforms [4]. Successive preparations indicated that the specific activity of pure enzyme might vary, depending on the milk sample; being higher in the early days post partum. Because variation and possible control of human xanthine oxidase molecular activity could have important implications in both pathological and normal physiological contexts, these indications were further examined in the present study. Serial samples (approx 1ml) of breast milk were donated from 14 individual mothers, taken over periods of 4-58 days, and starting as soon post partum as practicable. Xanthine oxidase activity in whole milk, whether assayed radiometrically by oxidation of hypoxanthine [5] or fluorimetrically, by oxidation of pterin [6], varied greatly with time post partum, but was always one to two orders of magnitude less than levels similarly determined in fresh bovine milk [80± 5.0 mU/ml (mean ± SD, n=3)]. A general pattern emerged, whereby xanthine oxidase activity in breast milk rose to a peak within the first days after birth. Activities remained relatively high for several days before falling, by as much as 98%, to basal levels (approx 0.1 mU/ml), which were usually achieved by day 10. In several cases, the first samples measured showed the maximum activities, possibly because, for practical reasons, earlier samples were not available.

In contrast to these wide variations in enzyme activity, levels of xanthine oxidase protein remained relatively constant. Xanthine oxidase protein in each sample of breast milk was determined by densitometric comparison of the 150 KD band, obtained by running extracts on SDS-PAGE, with the corresponding bands from pure human enzyme of known concentrations. Xanthine oxidase protein generally varied less than 20% from mean levels of approx 0.1 mg/ml, levels which are very similar to those similarly determined on fresh bovine milk [0.1 ± 0.03 mg/ml (mean ± SEM, n=5)].

These time dependent variations in xanthine oxidase activity, together with relative constancy of xanthine oxidase protein levels, combine to show quite dramatic activation - deactivation cycles, during the 8-10 day course of which the molecular activity of xanthine oxidase changes by as much as 50 fold. Similar molecular activation has been described in the liver xanthine dehydrogenase of chickens fed a high protein diet [7] and was attributed to changes in content of non-functional 'desulpho' enzyme. As mentioned above, xanthine oxidase purified from human milk has properties consistent with a very high content of inactive, possibly 'desulpho', forms of the enzyme [4], and it may well be that the presently reported activation-deactivation cycles reflect systematic variations in that content. Xanthine oxidase from human heart appears to have properties similar to those of the human milk enzyme [8] and the potential for regulation of human xanthine oxidase, not only in milk but in other tissues, raises the intriguing possibility of corresponding control of the generation of reactive oxygen species with pathogenic and even normal signal transduction roles.