*****Optional chapter - discussion required whether required or not**

Exposure of newborn to silver via lactation

Virgin and lactating Sprague-Dawley rats were used to determine whether silver follows the same initial pathways as copper to tissues and milk (Hanson et al., 2001). Female rats were injected i.p. with tracer amounts of radioactive silver-110 (110Ag) in form of AgNO3 (doses were 206 or 343 µg/kg bw). Lactating rats were treated at days 2-3 post partum. Blood and tissues were examined at various times thereafter for total radioactivity and for incorporation into copper binding proteins in plasma and milk.

As with radioactive copper (67Cu), much of the 110Ag was rapidly incorporated into the liver. Skeletal muscle, spleen, mammary gland, ovaries, uterus, and adrenals also were significant initial accumulation sites, with or without lactation.

Lactation increased the proportion of radioactive silver in the mammary gland, uterus and plasma, and reduced that in liver and skeletal muscle. By 4h after injection, a large proportion of the silver tracer 110Ag (> 25%) was in the mammary gland of lactating rats, obviously preceding the peaking of 110Ag concentration in the milk (at 8h). This would be consistent with transfer of the silver from mammary epithelial cells to the milk, after its uptake from the plasma.

The incorporation of silver into copper binding proteins was determined by means of size exclusion chromatography of plasma components and milk samples from 110Ag injected rats. In plasma, most of the 110Ag was bound to a macroglobulin with an apparent molecular weight of about 800 kDa which is consistent with that of α1-macroglobulin composed of 4 subunits each, of 145 and 45 kDa. SDS-PAGE showed that pattern, and the single polypeptide chain of 145 kDa was identified by sequencing as α1-macroglobulin. Already after 1h, a small proportion of radioactivity was detected immunochemically in plasma ceruloplasmin. In contrast to radioactive copper, there was no association of 110Ag with transcuprein or albumin. Silver bound obviously to α1-macroglobulin via SH groups could not be substituted by copper ions (Cu2+).

With respect to 110Ag in milk, a larger proportion of the radioactivity in milk (about 15%) was associated with ceruloplasmin. This proportion seemed to be consistent at all times examined (from 1-24 h), suggesting that total silver and ceruloplasmin were entering the milk together.

Radioactive silver was also absorbed by pups suckling on 110Ag treated dams. Considerable radioactivity was detected in the GI tract of the pups, and smaller amounts were found in the livers and in the spleens.

In summary, it was shown that maternal i.p. exposure of lactating rats to tracer amounts of radioactive 110Ag went along with the occurrence of Ag-labelled ceruloplasmin in milk resulting in exposure of the newborn to silver.

To contribute to understanding possible mechanisms of adversity of silver-mediated effects available data on the involvement of ceruloplasmin in lactation are summed up briefly. The role of ceruloplasmin as a source of copper for the fetus (via placenta) and newborn (via milk) was investigated in few tracer studies with copper-64 (64Cu) or copper-67 (67Cu) in virgin and lactating rats and mice.

Intravenous infusion of pregnant Sprague-Dawley rats with radioactive copper-67 (67Cu) either bound to ceruloplasmin or to albumin and transcuprein (constituting the exchangeable plasma copper pool) showed that radioactivity in ceruloplasmin entered the placenta and the fetus much more rapidly than that from the exchangeable copper pool (Lee et al., 1993). Inhibition of the ceruloplasmin biosynthesis by cycloheximide reduced the appearance of radiotracer in plasma ceruloplasmin and was accompanied by a marked reduction in the 67Cu levels in placenta and the fetuses, further corroborating the importance of maternal ceruloplasmin for the fetus.

Tracer studies with 67Cu in female Sprague-Dawley rats injected i.p. with 67Cu(II)-NTA have shown that copper transport is particularly active in lactation, and that instead of first going to liver and kidney, a large proportion of the radiolabel goes directly to the mammary gland (comprising up to 50% of dose after 1h) and to milk, also entering milk ceruloplasmin (Donley et al., 2002).

Similar results were obtained for female C57BL/6 wild-type mice injected i.p. with tracer amounts of 64Cu(II)-NTA, but the uptake by the mammary gland amounted only 9%. Radioactive 64Cu also rapidly appeared in milk (Chu et al., 2012). Parallel studies in ceruloplasmin knockout C57BL/6 mice gave virtually identical results. Thus, knocking out ceruloplasmin made little difference to the initial distribution of copper that had entered the blood in ionic form, and the entry of exchangeable copper from the blood into the mammary gland was not affected. However, the milk of knockout dams contained less 64Cu radioactivity, and the copper concentration was less than 50% of that for milk from normal dams. Equally, copper content in liver of pups born to ceruloplasmin knockout dams also was half of that of pups from wild-type dams. Taken together, the availability of copper to the newborn through the milk and to the fetus is significantly decreased in the absence of ceruloplasmin supporting its significant role in perinatal copper transport.

Summary

Tracer studies in virgin and lactating female rats with radioactive silver (110Ag) have shown that the transport and distribution of silver resemble those for copper in some aspects, particularly with regard to its rapid accumulation in liver and lactating mammary gland, as well as in milk and in the ceruloplasmin of plasma and milk. With respect to competition with copper, transport of silver appears to be carried by α1-macroglobulin and not by the main copper transporters transcuprein and albumin. Given that ceruloplasmin is a significant copper binding component of milk it can be concluded that exposure of newborns to silver may occur as well through milk containing Ag-ceruloplasmin as evidenced by the detection of 110Ag radioactivity in pups.

References

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