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The role of transferrin and citrate in cellular uptake of aluminium

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Summary. The ability of human erythroleukaemia K562 cells to take up aluminium from Al-transferrin and Al-citrate has been examined. Uptake from Al-transferrin was dose-dependent over the range 68–544 ng/ml of aluminium, and increased over a 12-day period. In contrast, uptake from Al-citrate was low even at an aluminium concentration of 6800 ng/ml and did not increase over time. Neither form of aluminium greatly affected cell growth. It is concluded that Al-transferrin, rather than Al-citrate, is the physiologically relevant form of this metal with respect to cellular uptake, but that any metabolic abnormalities induced by aluminium do not affect proliferation of this cell line.

Keywords: Aluminium – Transferrin – Citrate – Erythroleukaemia cells – Proliferation

Introduction

Aluminium overload in renal failure patients is associated with a number of pathological conditions, including bone disease (osteomalacia), anaemia and encephalopathy (Savory et al. 1985). In addition, localisation of aluminium in the brain may play a role in Alzheimer's disease (Ganrot 1986). However, the mechanisms involved in aluminium uptake and transport are poorly understood. Most aluminium in serum is bound to the iron-transport protein transferrin (Trapp 1983) and aluminium is known to interact with this protein's metal-binding sites (Tomimatsu and Donovan 1976; Cochran et al. 1984). Although the affinity of transferrin for aluminium is much lower than its affinity for iron (Martin et al. 1987), it is quite feasible for both Fe and aluminium to be bound by transferrin in vivo since it is normally only about 30% saturated with iron.

Recent studies have shown that iron status can influence aluminium uptake in an animal model and in cultured cells (Cannata et al. 1991), while in turn aluminium can interfere with cellular iron uptake (McGregor et al. 1990). However, this latter effect only occurred when aluminium was bound to transferrin, there being no effect when aluminium was present as Al-citrate. This suggests that uptake of aluminium by cells and tissues may vary according to the molecular form in which the metal is presented.

In this work we have compared the ability of Al-transferrin and Al-citrate to donate aluminium to the human erythroleukaemic cell line K562, and examined their effect on cell proliferation.

Materials and methods

Materials. Al-citrate was prepared by mixing freshly prepared $AlCl_3$ solution with a 20-fold excess of trisodium citrate. Al-transferrin (100% saturated) was prepared by incubating apotransferrin (Behringwerke, Hounslow, UK) with Al-citrate for 1 h at 4° C followed by dialysis to remove citrate and excess aluminium, as described previously (McGregor et al. 1990).

Cell culture. K562 cells were routinely cultured in RPMI 1640 medium (Flow, Rickmansworth, UK) containing 10% fetal calf serum (Flow).

Aluminium uptake. To determine aluminium uptake, cells were spun down, washed three times in RPMI 1640 (without serum) and resuspended at 2×10^5/ml in serum-free RPMI 1640 medium containing 1 mg/ml human serum albumin (Sigma, Poole, UK) and 50 μg/ml of human transferrin (Behringwerke, Hounslow, UK) saturated to 30% with iron using Fe-nitrilotriacetate (Graham and Bates 1976). Al-citrate or Al-transferrin were added as required. At 3-day intervals the cells were spun down, counted, and an aliquot subcultured at 2×10^5/ml in medium consisting of a 3:1 mixture of fresh medium (with appropriate additions) and the original medium (conditioned medium). The conditioned medium was included to ensure that uninterrupted growth was maintained. The remaining cells were washed twice in RPMI 1640 and lysed by a single cycle of freeze/thawing in 0.02% sodium dodecyl sulphate. The lysates were incubated for 30 min in a steam bath to solubilise DNA and thus produce a homogeneous solution, which was then analysed for aluminium content by atomic absorption spectroscopy. The aluminium content of the culture media was checked by atomic absorption spectroscopy and found to be very

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low (<4 ng/ml for medium containing fetal calf serum and <2 ng/ml for serum-free medium).

Reproducibility of results. Because relatively large numbers of cells were required in each aliquot to ensure reliable aluminium assays, it was not possible to perform replicate analyses on each sample at each time interval. Each experiment was repeated at least three times and results of representative individual experiments are shown.

Results and discussion

As shown in Fig. 1, when K562 cells were incubated with Al-transferrin there was a progressive increase in cell aluminium content over a 12-day incubation period; cell aluminium also showed a dose response in relation to the concentration of Al-transferrin in the medium, which ranged from 100 μg/ml (68 ng/ml of aluminium) to 800 μg/ml (544 ng/ml of aluminium). In contrast, uptake from aluminium citrate showed no dependence upon time and a 100-fold increase in aluminium concentration from 68 ng/ml to 6800 ng/ml produced no increase in cellular uptake. These results show that transferrin, rather than citrate, provides aluminium in a form that is readily acquired by cells. Since the medium contained 50 μg/ml of 30% iron-saturated transferrin (necessary to maintain cell growth), it seems likely that some of this modest uptake from Al-citrate may have been transferrin-mediated.

Our results agree with the recent studies of Shi and Haug (1990), who showed that citrate interferes with aluminium uptake by a neuroblastoma cell line, and of Roskams and Connor (1990) who have implicated transferrin as the carrier of aluminium into rat brain tissue. This contrasts with the proposal of Farrar et al. (1990) who suggested that Al-citrate rather than Al-transferrin is the important form of the metal for transport across the blood-brain barrier, and that an abnormality in transferrin binding of aluminium in Alzheimer's patients could account for increased uptake into the brain. Our results do not support this hypothesis,

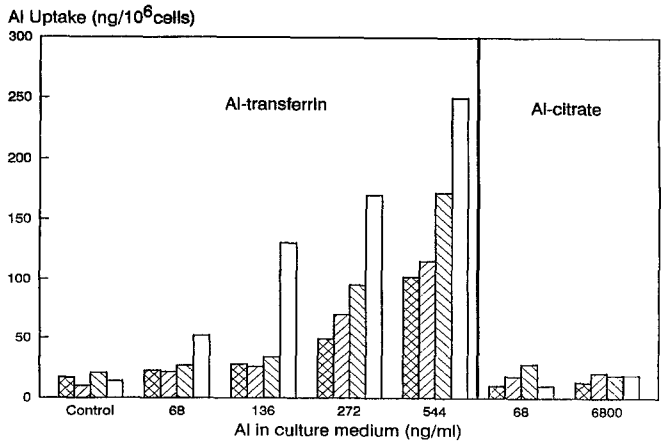

Fig. 1. Uptake of aluminium by K562 cells after incubation for various periods with Al-transferrin or Al-citrate. The control contained no added aluminium. ▨ = 3 days, ▨ = 6 days, ▨ = 9 days, □ = 12 days

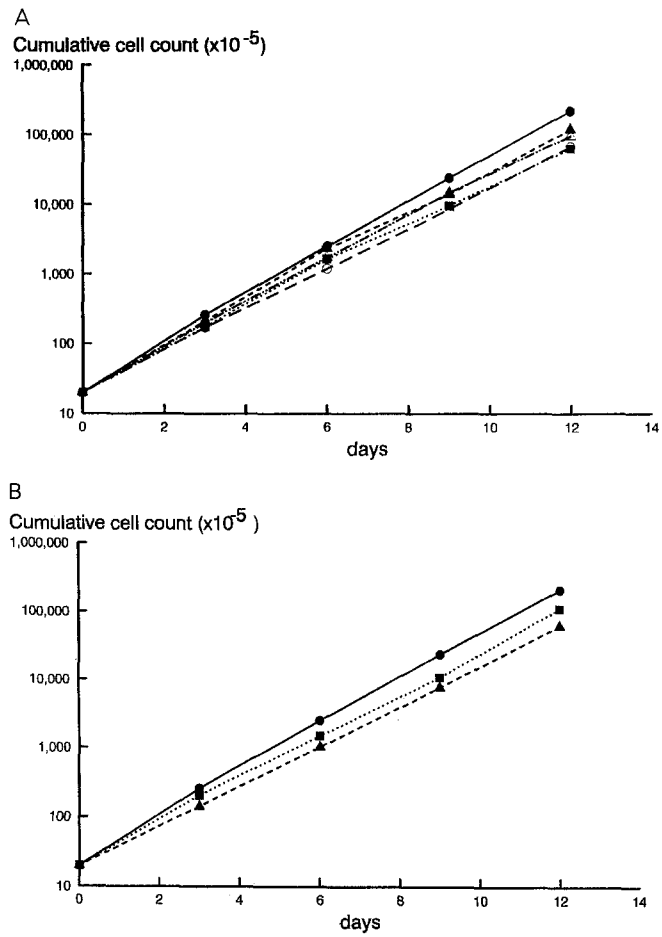

Fig. 2. Cumulative cell count of K562 cells cultured in the presence of aluminim. (A) Al-transferrin: Al concentration: 68 ng/ml (▲); 136 ng/ml (■); 272 ng/ml (○); 544 ng/ml (△); control, no added Al (●). (B) Al-citrate: Al concentration: 68 ng/ml (▲); 6800 ng/ml (■); control, no added Al (●)

and indeed would indicate the reverse, i.e. that decreasing binding of aluminium by transferrin would lead to reduced transmembrane transport. Martin (1986) has reported that at neutral pH only charged Al-citrate complexes are present, which are unlikely to be able to traverse the cell membrane, and neutral complexes capable of such transport are only found at around pH 5.

From this work and our previous studies showing that Al-transferrin interferes with transferrin-mediated uptake of iron (McGregor et al. 1990), it seems reasonable to assume that uptake of aluminium from transferrin occurs by the receptor-mediated endocytic process responsible for iron uptake. The fact that uptake from transferrin showed dose-dependency over a wide range of concentrations undoubtedly reflects the fact that Al-transferrin binds to the transferrin receptor with a much lower affinity than Fe-transferrin so that saturation of receptors occurs only at a high concentration of Al-transferrin (McGregor et al. 1990).

Aluminium did not greatly affect cell proliferation (Fig. 2). A small reduction in cell number was noted, but this was not dose-related and occurred in the presence of both Al-transferrin and Al-citrate. None of the

treatments significantly affected cell viability, which was always >95%. This suggests that any metabolic abnormalities resulting from aluminium uptake do not greatly affect proliferation of K562 cells. However, other cells may be more susceptible to aluminium, as we have found that Al-transferrin inhibits proliferation of mitogen-stimulated human lymphocytes (unpublished results).

We thus conclude that transferrin is an important mediator of aluminium uptake by cells and that citrate, while possibly important in enhancing gastrointestinal absorption of aluminium (Froment et al. 1989), is largely irrelevant to cellular uptake or transcellular transport at physiological pH.

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