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Behavioural effects of gestational exposure to aluminium

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Summary. - The involvement of aluminium in the aetiology of a number of human pathological diseases has altered its status from being a nontoxic, nonabsorbable, harmless element. This maybe of particular concern to the developing foetus which is more susceptible to agents and at lower levels than the adult. Little attention has been given to aluminium's potential reproductive toxicity until recently and further research is required for a full evaluation of its toxicity. Our preliminary results demonstrate behavioural and neurochemical alterations in the offspring of mice exposed to aluminium during gestation. Further, the effects of such exposure are also present in the adult animal suggesting persistent changes in behaviour following prenatal exposure.

Key words: aluminium, prenatal exposure, mouse.

Riassunto *(Effetti comportamentali della esposizione ad alluminio sulla vita fetale).* - Le recenti acquisizioni sul ruolo svolto dall'alluminio nell'eziologia di alcune patologie umane hanno fatto sì che questo non venisse più considerato un elemento di difficile assorbimento, non nocivo e non tossico. Questo ha stimolato una maggiore attenzione sugli effetti delle esposizioni ad alluminio nelle prime fasi di vita fetale. Tale periodo è infatti caratterizzato da un'estrema sensibilità a vari agenti chimici anche a concentrazioni molto inferiori a quelle efficaci nell'animale adulto. Tuttavia, scarsa attenzione è stata finora rivolta alla potenziale azione teratogenica dell'alluminio, e sono quindi necessari ulteriori studi per una più approfondita valutazione delle proprietà neurotossiche di questo elemento. I presenti risultati preliminari dimostrano la presenza di alterazioni a carattere neurochimico e comportamentale nella prole di femmine esposte all'alluminio durante la gestazione. Gli effetti a lungo termine di tale esposizione prenatale riscontrati nell'animale adulto suggeriscono che le alterazioni comportamentali osservate siano a carattere permanente.

Parole chiave: alluminio, esposizione prenatale, topo.

Introduction

Human exposure to aluminium (Al) arises from several sources; it is present in food and used in the food processing industry, in cookware, in pharmaceutical preparations and as a flocculant in water treatment. In addition there are growing amounts of Al entering the environment, particularly in the area of Al smelters, and as a result of leaching from soils by acid rain [1]. From these diverse routes of contact there is an increased likelihood of unknowingly being exposed to Al at levels higher than is recommended.

For a long time it had been presumed that exposure to Al was without toxic effect. However in contrast to this assumed harmlessness, Al has been implicated as a causative factor in several human pathological diseases in recent years; dialysis encephalopathy and osteomalacia [2], senile dementia of the Alzheimer's type (SDAT) [3], amyotrophic lateral sclerosis and Parkinson's disease of Guam [4]. Although its exact role in these diseases is still a subject of discussion, this implication has led to an abundant literature concerning possible mechanisms by which Al exerts its neurotoxicity in adult animals and to tentative animal models for its effects on human beings.

The lack of concern over the effects of Al ingestion resulted from the assumption that absorption from the gastrointestinal tract was minimal. It is now known that this is not the case and that aluminium may be absorbed e.g from aluminium containing antacids [5]. Moreover, the absorption of Al is increased by the presence of parathyroid hormone [6] and by certain dietary factors, particularly citrate [7].

Of greater relevance to behavioural teratology is the accumulating clinical evidence which points to Al loading in infants with renal dysfunction not receiving dialysis but exposed to Al from the oral ingestion of Al-containing antacids. The levels of Al in plasma, serum and bone were elevated compared to controls [8, 9]. Moreover, Sedman *et al.* [10] have shown that the intravenous feeding of premature infants resulted in a 10 fold increase of Al in bone. Further, urinary Al concentration did not reach the control level until several weeks after parenteral feeding was stopped, suggesting an accumulation of Al within body tissues. In addition, cases have been reported of infants with Al intoxication when the only possible source of exposure was from infant formula [11].

In the only documented study of accidental exposure of pregnant women to high levels of oral Al, Golding *et al.* [12] found no effects on the occurrence of perinatal

death, on body weight at birth, before term delivery or congenital malformations. However, there was an increased incidence of talipes among exposed infants (4.4% of cases).

In contrast to the extensive literature on the neurotoxic effects of Al, few studies have considered the consequences of exposure to Al compounds during pregnancy and the early postnatal period. In recent years some attention has been given to such effects on the developing foetus, but a full evaluation of risk effects has not been undertaken. This is of particular concern as exposure of the developing nervous system to insults may have very different consequences than those resulting from exposure of the adult nervous system. Furthermore, the developing foetus is more susceptible to and affected at doses far lower than those required to produce changes in the adult central nervous system [13].

The paucity of studies aimed at assessing this aspect of Al's biology may have resulted from the lack of detailed information on the distribution of Al following exposure and from the lack of adverse effects revealed by initial studies [14]. Within the studies which have addressed this question, it is difficult to generalise the effects resulting from exposure to Al. This is because studies have used different Al salts, the solubility of which vary greatly [15], different doses and experimental animals.

In the present paper we briefly report some preliminary findings from our studies on gestational exposure to aluminium sulphate ($Al_2(SO_4)_3$) in mice and compare these with results in other studies. In the frame of our studies aimed at investigating the role of the genotype in individual sensitivity to drugs or toxicants, we have chosen the mouse as our experimental model. This altricial rodent has a similar placentation to that of humans and has been reported to show similarities in behavioural responses following exposure to known human behavioural teratogens [16]. Moreover, the development of inbred strains of mice has led to the introduction of almost isogenic individuals within a strain.

Pregnant CBA mice were exposed to $Al_2(SO_4)_3$ at a dose of 200 mg/kg body weight and injected intraperitoneally during days 10 to 13 of gestation. The dose range and gestational period of exposure have been selected on the basis of previous experiments [17, 18]. We have used a variety of ethological measures, which have been shown to be sensitive indicators of toxicants, with the aim of assessing subtle behavioural effects on the mother and the behavioural development of pups [19].

The use of a fostering procedure in behavioural teratology studies, to differentiate between direct effects of prenatal exposure and those arising from alterations in maternal behaviour or physiology, has been recommended by a number of authors (e.g [20]). For this reason, all litters were cross-fostered on the day after birth (postnatal day 1) so that each mother reared two control and two treated pups. This gave 4 treatment groups; control pups fostered to control mothers (Cc), control pups fostered to treated mothers (Ct), treated pups fostered to treated mothers (Tt) and treated pups reared by control mothers (Tc).

Effects of prenatal exposure

Maternal weight

In our experiments gestational weight gain of CBA females was reduced during the treatment period (gestational days 10-13). This decrease in maternal body weight was transient as all mothers increased their weight following the termination of treatment. Futher, there was no difference in body weight between the treatment groups during the preweaning period. A decrease in maternal body weight following exposure to oral and injected Al has been observed by several authors [21, 22].

Maternal behaviour

Alterations in the behaviour of the mother are known to affect infant development and several drugs have been shown to disrupt elements of maternal behaviour [23]. Thus, any disturbance to maternal care or the delicate mother-pup relationship may explain differential patterns of behaviour in the offspring rather than direct effects of prenatal exposure to a toxicant. The results of a pilot study suggested that differences exist in the pattern of maternal behaviour displayed by control and Al-exposed mothers. Control mothers spent more time involved in the pup-directed behaviours of nursing and licking and less time in nest-building during the first two postnatal weeks than dams treated with Al during gestation.

To further characterise any differences between Al-treated and control mothers we employed a pup retrieval test. Treated mothers had a longer latency to retrieve on postnatal day 3 (Pd3), although this difference did not reach significance.

Only one previous study has included simultaneous recording of maternal behaviour after prenatal exposure to Al, showing no significant differences between treatment groups in nest-building, retrieval of pups to the nest or time spent with the young following exposure to $AlCl_3$ in the dam's diet from day 8 of gestation [24].

Thus it will be necessary to quantify further the maternal behaviour of mothers rearing control and Al-treated pups, an investigation currently being undertaken in our laboratory.

Pup weight

Prenatal exposure of pregnant female CBA mice to $Al_2(SO_3)_4$ had no significant effect upon breeding performance; the length of gestation, litter size and sex

ratio were unaffected by prenatal Al. Pup mortality, as a result of infanticide or neglect by the mother, occurred in both treatment groups at birth, thus this cannot be attributed to Al. The mean birth weight was significantly lower (by 6%) among Al-exposed offspring.

Similarly, the offspring of BALB/c mice had a reduced birth weight after i.p. exposure to AlCl3 during gestation days 7 to 16, and an increased incidence of foetal resorptions was also reported [25]. Such effects on Al-treated offspring were also found following oral exposure of pregnant Sprague-Dawley rats to Al by gavage [26].

The lower birth weight of CBA treated pups persisted for those pups reared by treated mothers only, reflecting the significant effect foster mother treatment had on body weight. Thus, when treated pups are given adequate maternal care it is possible to overcome the weight impairment. As maternal food and water intake did not differ during the preweaning period, the diminished body weights cannot be accounted for in terms of nutritional deficiency.

This postnatal maternal influence may have resulted from retention of Al within the mother's body allowing continued exposure via the dam's milk after termination of treatment. Yokel and McNamara [27] injected lactating rabbits with aluminium lactate (AlLact) and found 12% of the total injected Al still present in the area of the injection site seven days after the last injection. This postnatal effect seems likely as in our experiment control pups, which had no prenatal contact with Al, fostered to treated mothers (Ct) had reduced body weights compared to control pups reared by control mothers (Cc). Yokel [21] also found this to be the case; offspring of mothers exposed to 400 μmol/Al/kg in utero and control offspring fostered to these does, gained less weight.

Impairment in offspring body weight gain during the preweaning period is the most consistent observation following prenatal exposure to Al, irrespective of the route of administration or the chemical form of Al used [21, 22, 24, 26]. However, the extent of present research with gestational Al does not permit the exclusion of a maternal factor accounting for this weight impairment.

Neurobehavioural development

We have employed a modified version of the Fox battery of tests to measure sensory-motor development in control and Al-exposed offspring [28]. We found that treated offspring differed in their attainment of a mature response for forelimb grasping and pole grasping on Pd15. A maternal effect appeared also in the results for pole grasping as few Ct or Tt pups had reached a mature response by Pd15. Similarly, a significant difference between treated and controls was found for slow righting on Pd6, cliff aversion on Pd12 and screen climbing on Pd18. The impairments in attainment of these reflexes were transitory as all pups eventually achieved the adult response.

It could be argued that the delays in neurobehavioural development are secondary to the reduction in physical maturation. However, we did not find a correlation between the performance in the particular Fox measure and body weight on the same day. Bernuzzi *et al.* [24] found that by Pd9, when delays in the response to negative geotaxis were present, no further treatment group differences in body weight existed. In accordance with our observations, Golub *et al.* [22], using a combined score to estimate performance in a battery of neurobehavioural tests, observed a delay in prenatally Al-treated offspring on Pd14 and 16.

Thus, Al treatment in utero affects maturation of certain sensory-motor skills but not all.

Pup behaviours

In our study with CBA pups, the overall expression of the behavioural repertoire was unaffected by exposure to Al. However, the appearance of certain behaviours were delayed in Al-exposed offspring. Pup locomotor activity during postpartum days 9-18 showed a trend towards diminished activity levels in pups reared by treated mothers. Control pups spent more of their time involved in crawling compared to treated pups, as was the case for pups fostered to control mothers. In line with this, pups fostered to treated mothers exhibited a greater frequency of bouts of being still. On Pd18 treated pups exhibited a greater number of bouts of rearing which were of longer duration than that of controls.

Ultrasonic vocalisations

Rodent neonates emit ultrasonic vocalisations with a reliable and stable pattern from a few days after birth. The developmental and physical characteristics of such calls depend not only on the species under investigation but also the strain. As pointed out by Zbinden [29], this response may function as a sensitive indicator of subtle behavioural effects caused by prenatal or postnatal drug treatments. Several authors have shown consistent alterations in the production of these calls following drug exposure which have led them to recommend their incorporation in any battery of tests aimed at assessing behavioural teratogenicity [30].

In a very recent study we found that prenatal treatment with Al produced a striking reduction in the total number of ultrasonic vocalisations emitted by exposed CBA pups compared to controls on removal from the nest [31]. This difference was especially pronounced on days 3 and 4 after birth. In addition, treated pup calling did not reach a maximum until a day later than control offspring. In combination with the results of the sensory-motor tests, in which pups exposed to Al prenatally were slower to mature in certain tests than controls, this suggests that the mechanisms responsible for sound production matured

later in Al-treated offspring. An alternative explanation is that in utero exposure to Al alters the ability of the neonate to detect or respond to a reduced temperature.

It is not known if the ultrasonic calls of Al-exposed pups differed in other acoustic properties e.g. sound pressure, duration etc. as such measures were not undertaken. However analysis of such characteristics, to further reveal any treatment effects, are currently under investigation.

Activity at weaning

The effect of in utero exposure to Al on CBA activity scores in an openfield at weaning (Pd21) was sex dependent. Treated females had lower activity scores during the 5 min test than control females. Conversely, Al-exposed male pups crossed more squares than controls. Although treated pups took less time to approach a novel object placed in the centre of the openfield at the end of the activity test, this difference was only marginally significant.

In a test of locomotor coordination, in which the subject was placed in water and could reach a platform by climbing a metal rod, Wistar rat pups from mothers treated orally with AlLact during gestation required more time to complete the task [32].

Long-term effects of early exposure

Since subtle behavioural changes are not always immediately apparent, the attribution of such an effect to a substance taken by the mother during pregnancy is difficult. Thus, in experimental models of behavioural teratology it is vital to test subjects at different ages throughout life to assess whether effects from exposure are transient and may be recovered from, persist into adulthood or are present as delayed effects which only arise late in development. To date, few studies have considered long-term effects of early Al exposure on the adult animal.

In our experiments, 12-week old male subjects were tested in an 8-arm radial maze to assess learning ability. Results suggested an effect of Al on performance in the maze. More control animals reached the criterion of eight correct arm choices in the first 8 entries on 2 consecutive days (77%) than subjects previously exposed to Al in utero (55%). Also, the number of days required to reach this criterion was greater for treated mice.

Tsujii and Hoshishima [33] observed deficits in learning ability of 5- to 7-week old mice exposed prenatally to repeated injections of Al and raised in standard laboratory cages. Young rabbits exposed during gestation to Al and tested for a classically conditioned response task at 11 weeks of age, showed dose dependent effects; animals in the low dose group performed better whilst the high dose group showed impaired acquisition and retention [21]. Muller *et al.* [32] found that the performance of rats treated prenatally with AlLact in an operant conditioning test, carried out 65 days after birth, was behind that of controls.

In an activity test performed at 22 weeks of age, CBA female mice reared by treated mothers (Ct and Tt subjects) tended towards lower activity scores.

In our experiments, the impairment in growth of offspring reared by treated mothers persisted into adulthood. As with performance in a classical conditioning learning task, Yokel [21] found a biphasic effect of gestational exposure to subcutaneously injected AlLact on the offspring's weight gain after weaning. Although the results were not significant, exposure to low levels of Al increased weight gain whereas weight loss was recorded at high exposure levels.

Neurochemical changes

The most consistent neurochemical finding in postmortem brains of patients with, for example, SDAT, has been alterations to the cholinergic system, the extent of which varies with brain region [34]. Because of the suggested association between the incidence of these diseases and exposure to Al, we have measured the levels of choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acethylcholine, in a number of brain regions and at different ages.

Although some inconsistencies existed in the levels of ChAT activity at each age and between each brain region, the direction of change in the cerebral cortex, cerebellum and hippocampus (the areas affected in SDAT) between control and treated subjects was the same at each time point; treated brains exhibited a reduced level of enzyme activity compared to controls. For example, the amount of ChAT activity measured at 34 weeks of age in the cerebral cortex of treated animals was 44% less than that in controls.

Discussion

It is clear that there is a lack of information on a number of behavioural measures following gestational exposure to Al which limits a more comprehensive evaluation of aluminium's behavioural teratogenicity. The results presented in this brief report suggest that prenatal exposure to Al causes alterations to body growth, ultrasound production and sensory-motor development. Moreover, exposure to Al during gestation may result in behavioural deficits during early postnatal development which may persist into adulthood.

The foetus may be directly exposed to Al in utero via placental transfer. The extent of any persistence of Al in the body is not fully known. However, the data presented herein suggests that Al is available to distribute into the dam's milk resulting in continued exposure of the neonate over time.

Further work is required to investigate the consequences of Al exposure on the mother-infant interaction. For example, as ultrasonic calls function to elicit maternal care [35], exposure to Al during gestation may have affected the ability of treated mothers to respond adequately to the ultrasonic calls of their fostered pups.

Although the exact neural mechanisms responsible for eliciting ultrasonic vocalizations are unknown, the involvement of a number of different neurotransmitter systems has been proposed. These include the dopaminergic [36], GABAergic [37], cholinergic [38] and the opioid system [39]. We have shown that prenatal exposure to Al resulted in decreased levels of ChAT during adulthood which leads to the tentative suggestion that the cholinergic system may play a role in the production of these calls.

From a methodological point of view the results we report herein certainly suggest that ultrasound recording should be included in any battery of tests aimed at assessing possible behavioural teratogenicity.

In particular, the presence of a maternal influence in our results emphasis the importance of including a fostering design in teratology studies to ensure adequate interpretation of results. To date only one study has done so [21].

Al is present in the environment in a number of different chemical forms. The extent of the solubility of Al in the body is dependent on the Al salt to which exposure results, thus the need to evaluate inter- and intraspecies differences in the bioavailability of Al and its different salts is great. As susceptibility to a number of drugs depends on the genotype, further studies of gestational exposure to Al with a second inbred strain of mouse are being carried out.

Aluminium may be poorly absorbed under normal conditions from the gastrointestinal tract. However in the developing organism such protective barriers are immature which may render it more susceptible to any toxic effects.

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