# Effect of Aluminium Toxicity on Primary Cortical Astrocytes

M Abubaker, A Taylor, G Ferns

**Citation**

M Abubaker, A Taylor, G Ferns. *Effect of Aluminium Toxicity on Primary Cortical Astrocytes*. The Internet Journal of Toxicology. 2007 Volume 4 Number 2.

**Abstract**

The in vitro potential of aluminium to induce pro-oxidant or antioxidant effects, were studied in rat primary cortical astrocytes. These cells were exposed to aluminium (as aluminium sulphate) at different concentration. The results revealed that aluminium has a differential effect on the rat primary cortical astrocytes. The toxic effects was assessed using mitochondrial dehydrogenase activity, measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT); cell viability as revealed by Fluorescein diacetate-Propidium iodide (FDA-PI) staining; glutathione content; lipid peroxidation as determined by malondialdehyde production and reactive oxygen production (ROS). The data suggests that aluminium has both pro-oxidant and antioxidant effects.

## INTRODUCTION

Many in vitro and in vivo model systems have been employed to study aluminium toxicity ([1,2,3,4,5,6,7]). But despite this extensive effort the molecular/cellular mechanisms of Al toxicity are still yet to be clarified. Using isolated cell lines of primary culture and cells of neuronal origin, several workers have tried to justify their cell type in terms of responsiveness to acute /chronic exposure to chemical toxicity, particularly Al in these respects. However the most interesting features to note are where the compound is localised and what features are associated with it.

To date there is no ideal model for the study of aluminium neurotoxicity in vitro, probably due to its ubiquitous nature and difficulties in maintaining adult neurones in culture. In the present study primary cortical astrocytes from the brain of new-born rat was chosen as model systems for the in vitro testing of the effect of Al. These represent a classical monotypic culture of astrocytes. The choice of monotypic cultures of astrocytes from the brain of new-born rats was based on the fact that, in the mammalian brain cortex, cells with particular astrocytic morphology account for at least 30% in terms of volume although this varies with both region and species ([8]). Correspondingly astrocytes in rats are still dividing at the time of birth, unlike most neurones which are already postmotic. Furthermore astrocytes are more robust and continue to divide until they populate the entire dish, whereas in a suspension of brain cells, the neurones are more vulnerable and have lost ability to divide. These attributes have often made astrocyte to be regarded as cells expressing glial fibrillary acidic protein (GFAP) ([9, 10]), a similar protein found in brain cortex in vivo. Transport within GFAP has also been reported to be adversely affected following Al accumulation in aluminium toxicity.

Biochemical studies revealed that astrocytes are involved in production and metabolism of the amino acid transmiters glutamine and -amino-butyric acid (GABA), as well as in $K^+$ homeostasis at the cellular level, in addition to being metabolically active ([11]). Glial cells play a critical role in the development as well as physiological maintenance of the neurones ([12]) and may be the target and mediator of many insults to the CNS ([13]). Further studies have demonstrated that astrocytes are more resistant to ROS-mediated damage than the other neural cells. This may be related to the high GSH content ( ≈20 nmol/mg protein) ([14, 15]), though as high as 8 mM has been reported ([16]). Astrocytes survive in culture for as long as there is a source of glucose in the medium and die only when glucose is completely depleted followed by ATP depletion ([17]).

As mentioned earlier many in vivo studies ([18,19,20,21, 22]) are available that clearly report on the toxic effect of aluminium.

The above mentioned properties in addition to direct involvement of this cell type to chemical insult stimulated the interest of these cellular models to explore the likely

oxidative effects following Al exposure.

As described above, neurodegenerative disorders involving aluminium affect a large number of individuals, particularly the elderly and patients with chronic renal failure. In view of this, it is hoped that this research will to contribute towards alleviating the personal suffering and medical, financial and social burdens associated with aluminium exposure.

The aim of the present study was to explore in vitro model systems to assess and determine the mechanism of Al-induced neurotoxicity, focusing specifically on the potential effects of Al on oxidative stress.

## MATERIALS AND CHEMICALS

Aluminium sulphate was purchase from Alfa Johnson Matthey Compapany (Johnson Matthey GmbH, Zeppelinstra□e 7, Karlsruhe). Dubecco's modified eagle medium (DMEM), modified eagle medium (MEM), fetal calf serum (FCS) L-glutamate, gentamycin and L-15 media were obtained from GIBCO Co (Life Technology Ltd. Paisley, Scotland UK). Ransod and Ransel kits from Randox (Randox Laboratories, N. Ireland).While Papain and Ovomucoid are from Boehringer. Aluminium nitrate, Ethylenediaminetetra-acetic acid (EDTA), methanol, nickel nitrate, nitric acid, perchloric acid, potassium-sodium-tatrate, selenium nitrate, sodium selenite and sulphuric acid were BDH products (BDH Chemicals Ltd., Poole, UK). Copper (II) sulphate, sodium carbonate and trichloroacetic acid were purchase from Fisons(Fisons Sci. Equipment, Loughborough, UK). O-phthaldialdehyde, cysteine, DNase, reduced glutathione, trypsin solution and all other chemicals were from Sigma chemical Co. (Sigma Chemical Co. Poole, Dorset). Double-deionized distilled water (DDW) was used for preparation of solution.

Pye Unicam SP9 electrothermal atomic absorption spectrophotometer, Pye Unicam PU9200 atomic absorption spectrophotometer, Perkin Elmer LS50 Luminescence Spectrophotometer, iEMS microplate spectrophotometer (Labsystems Company), Cobasmira/Cobasbio automated analyser, fluoresence microscope and 95% $O_2$ and 5% $CO_2$ incubator.

## CELL CULTURE PREPARATION

Primary cortical astrocytes cell cultures were prepared as described by Booher & Sensebrenner, ($_{23}$). Briefly, 2-3 days-old Wister rat pup decapitated and the heads were dipped into 96% ethanol and then into cold MEM-HEPE'S (10 µM). The brain was removed, dissected to obtain cerebral cortex and the cortecies were removed from the MEM-HEPE'S, cut into pieces, added to papain solution and incubated at 37 C for 1 hr 15 min. After the incubation, papain inhibitor solution was added and cells were dissociated by passing the tissue through needles, filtration and centrifugation. Cell pellets were resuspended in primary medium DMEM containing 10% FCS v/v, 2 mM glutamine and 25µg/ml gentamycin (media). Cells were seeded onto 250 $cm^2$ flask with 2.5 pups/flask which approximately containing 2.5 x$10^5$ $cells/cm^2$ and incubated at 37 C in 5% CO2, 95% humidified atmospheric air. At 10 DIV flask were shaken and semi-pure culture of astrocytes were obtained.

Generally all the cells were continuously cultured and maintained in their respective media preparations until confluence was reached ( ≈5 x$10^5$ $cells/cm^2$ for astrocytes), with media changed every 3 days.

## CELL TRYPSINIZATION

Three days before cell plating, the media was removed by aspiration from the cells followed by addition of 5 ml trypsin solution (0.25%) in order to detach the cells from the flask. The trypsinized cells were then incubated at 37 C in 5% $CO_2$ humidified air incubator for 8 min. The trypsinization was stopped by the addition of an equal volume of FCS 10% v/v, the cells were centrifuged at 100 x g for 5 min and the resulting pellet suspended in 2 ml of the media. One ml of the suspension was then made up of to 25 ml of the media, swirled very well and 12.5 ml was transferred onto two separate 250 ml flasks and incubated until plating.

## 2.2.3 CELL PLATING

Cells were again trypsinized as mentioned above, but the resuspended pellet was made up to 10 ml with media and 25 µl from the suspension was used to count the cell number using a Neubauer double chambered haemocytometer. Cells were then diluted to 5,000 cell/100µl and plated onto either 96 well plate at 200 µl/well or 24 well plate at 500 µl/well. The plates were incubated at 37 C in a humidified atmosphere of 5% $CO_2$ and 95% $O_2$ for 24 hr before further treatment.

## ALUMINIUM TREATMENT

Twenty four hours after plating, cells were treated with different concentrations of aluminium sulphate for different durations of exposure. In order to construct a dose response curve, cells were exposed to a wide range of concentration of aluminium (0-3000 µM) for up to five days.

## CONTAMINATION

Stringent precautions were taken to prevent Al contamination from the reagents and materials used. Double-deionized distilled water (DDW)[Al]< 1 ng/ml assay was used to prepare all solutions and aluminium levels were measured in most reagents and were < 4 ng/ml. Containers used for sample collection and during analysis were generally polypropylene rather than glass and if so usually made Al-free by acid-washing technique ([24]).

## MEASUREMENTS

## DETERMINATION OF MITOCHONDRIAL ACTIVITY BY MTT ASSAY

Mitochondrial dehydrogenase activity was assessed based on the MTT colorimetric determination ([25,26, 27]). MTT was dissolved in PBS at 5 mg/ml and filtered through a sterile filter (0.2µm) to remove any amount of insoluble residue. MTT stock solution (5 mg/ml) of which 11 µl per 100µl medium was added to all the wells of an assay 96-well plate and incubated at 37 C in 95% $O_2$ and 5% $CO_2$ incubator for 1 hr. After one hour of formazan formation, the reaction was stopped by removing the MTT. The formazan crystals formed were subsequently dissolved in 150 µl DMSO/well, followed by addition of 25 µl glycine buffer (0.1 M glycine buffer, pH 10.5). To achieve complete solubilization of formazan crystals, plates were vigorously shaken on a microplate shaker at 200 rpm for 20 min. The absorbance was recorded directly, within 30 min, after the addition of DMSO using an iEMS microplate spectrophotometer (Labsystems Company) at a wavelength of 550 nm. Mitochondrial activity as a percentage of control (cytotoxicity or) was calculated relative to the calibration of the Al-free/untreated cells as follows:

**Figure 1**

$$\text{Mitochondrial activity} = \frac{[\text{OD Treated cell + MTT}] - [\text{OD Ave Blank-cell + MTT}]}{[\text{OD Control (untreated) cells + MTT}]} \times 100$$

These values were then used as index of cell viability.

## DETERMINATION OF CELL SURVIVAL USING FDA-PI STAINING

Cell survival or active cells was determined using a fluorescence method employing fluoreseein diacetate and propidium iodide ([28]). Cells were plated in a 24 well plate containing 5,000 cell/well in 500µl and exposured to 1 mM aluminium sulphate 24 hr after plating. The cells were exposed for a period of not more than five days, after which, the media was removed from the cell and replaced with equal volume of Kreb's-HEPE'S buffer pH 7.4. The Kreb's-HEPE'S buffer was removed from the wells and 150 µl FDA-PI mixture (60 µg/ml-20 µg/ml) was added, followed by incubation for 3 min at room temperature. After the incubation the cells were counted using a fluorescence microscope at an excitation of wavelength of 450-490 nm and emission wavelength of 510-520 nm.

## PROTEIN DETERMINATION

The total protein content was determined according to the method of Lowry et al. ([29]).

The media was removed from the wells and cell sample were washed with 150µl PBS, the removal of the PBS was followed by the addition of 50µl of 0.1M NaOH and the plate was frozen at -20 C overnight to allow adequate solubilization.

Tissue supernatant were appropriately diluted and thereafter the amount of protein determined colorimetrically at 750 nm using BSA (0-250 µg/ml) as standard and amount in the unknown sample calculated from the standard curve of BSA. The concentrations of protein in the aliquots of samples were determined by employing the above method.

## ALUMINIUM DETERMINATION

## METHODS OF DETERMINATIONS

Cells pellets were washed twice with 50 mM Tris-HCl buffer pH 7.4 to remove any traces of the media. The residue were suspended (to a suitable volume) in about 1-2 % (v/v) $HNO_3$ and transferred immediately to a plastic sterile Al-free tubes. Aluminium content in cell pellet was determined by ETAAS as described by Taylor and Walker ([30]).

## DETERMINATION OF REDUCED GLUTATHIONE

The determination of GSH levels in biological samples requires that oxidation be minimised and the -glutamyltranspeptidase be inhibited. The method of Hissin and Hilf ([31]) was employed where GSH forms a fluoresent adduct with o-phthaldialdehyde (OPT).

Treated cells were washed twice with cold PBS and then re-suspended in acidified condition with 50 µl PCA. The samples were frozen at -20 C until required for analysis. 50 µl of unknown sample or standard were transferred to 1.5 ml of phosphate-EDTA buffer, followed by the addition of 50 µl OPT to all the samples. The mixtures were mixed well, protected from light and then allowed to stand at room temperature, in the dark for 30 min. Thereafter the fluorescence intensity was measured as stated above and the

amount in the unknown sample calculated from the standard curve.

## MEASUREMENT OF LIPID PEROXIDATION USING THE THIOBARBITURIC ACID REACTION (TBARS)

Treated cell samples were washed twice with cold PBS and then resuspended in 50 mM Tri-HCl buffer pH 7.4. The samples were frozen at -20 C until required.

50 µl of cell samples were prepared and made up to 500 µl with DDW. 250 µl of 1.34% TBA was added to all the tubes followed by an addition of equal volume of 40% TCA. The mixture was shaken and incubated for at least 30 min. in a hot boiling water bath with a temperature > 90 C. Tubes were allowed to cool down after the incubation and the absorbances were read at 532 nm using zero concentration as blank. The amount of malondialdehyde formed in nmoles by the unknown samples was calculated from the standard curve ([32], [33]).

## DETERMINATION OF SUPEROXIDE DISMUTASE ACTIVITY

Superoxide dismutase was determined as described by Marklund ([34], [35]).

## ANALYSIS OF RESULTS

Data obtained in this research are expressed as mean ± standard error of the mean (SEM) and results tested for statistical significance (P<0.05 to P<0.001) of the differences between means by using simple student t-test or one way analysis of variance (ANOVA).

## RESULTS

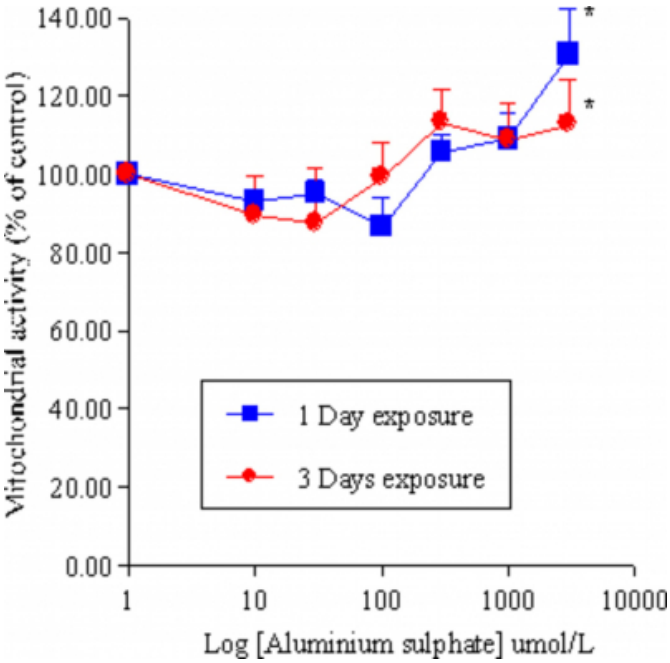
## CELL VIABILITY

## MITOCHONDRIAL ACTIVITY IN PRIMARY CORTICAL ASTROCYTES

Primary cortical astrocytes revealed a mitochondrial activity to both 1 and 3-days exposure of cell to aluminium sulphate (300-3000 µmol/L). There was however no significant effect at Al concentration up to 300 µM but a significant effect was observed at the highest dose for both day 1 and 3 as shown in figure 1.

**Figure 2**

Figure 1: Mitochondrial activity in astrocytes after a duration of exposure at different concentration (0-3000 Âµmol/L) of aluminium sulphate. Values are mean Â± SEM (n=8) and values considered significance at *P<0.05 compared to control using paired ANOVA and post hoc t-tests.

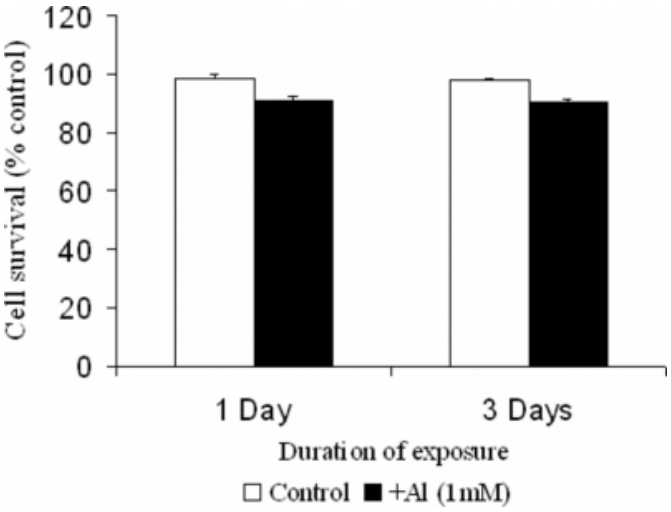



## EFFECTS OF ALUMINIUM ON ASTROCYTES VIABILITY USING FDA-PI STAINING

The number of surviving untreated primary cortical astrocytes cells was found to be greater than 95% regardless of the duration of experiment, while the number of Al treated (1000µM) cells were found to be 10% less than the untreated (Figure 2).

**Figure 3**

Figure 2: Effects to 1 mmol/L aluminium sulphate of primary rat cortical astrocytes . Values are means ± SEM (n=5).



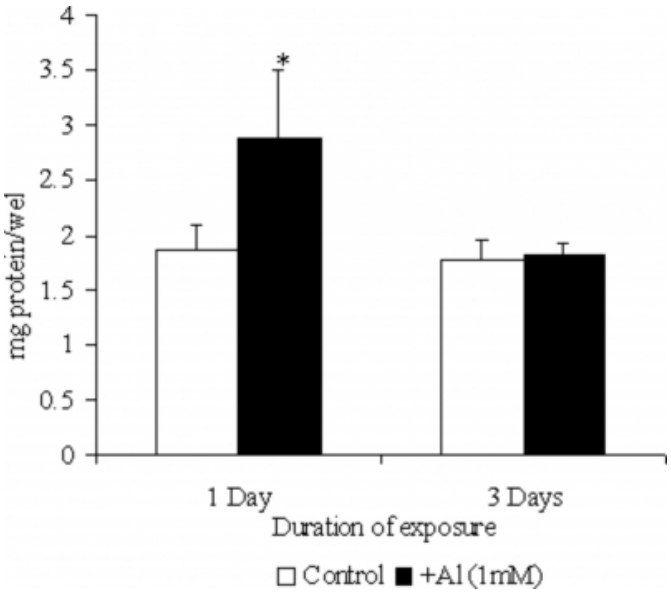

## EFFECTS OF ALUMINIUM TREATMENT ON CELL PROTEIN CONTENT

### PROTEIN CONTENT OF ASTROCYTES

The amount of protein in cells treated with 1 mmol/L aluminium sulphate showed an increase after 1-day exposure compared to non treated cells, with no significant change after 3-days exposure (Figure 3). This is either due to the increased number of cells per well or the protein content per well.

**Figure 4**

Figure 3: Protein content in astrocytes after 1 mmol/L aluminium sulphate exposure. Values are means ± SEM (n=5), and values considered significant at P



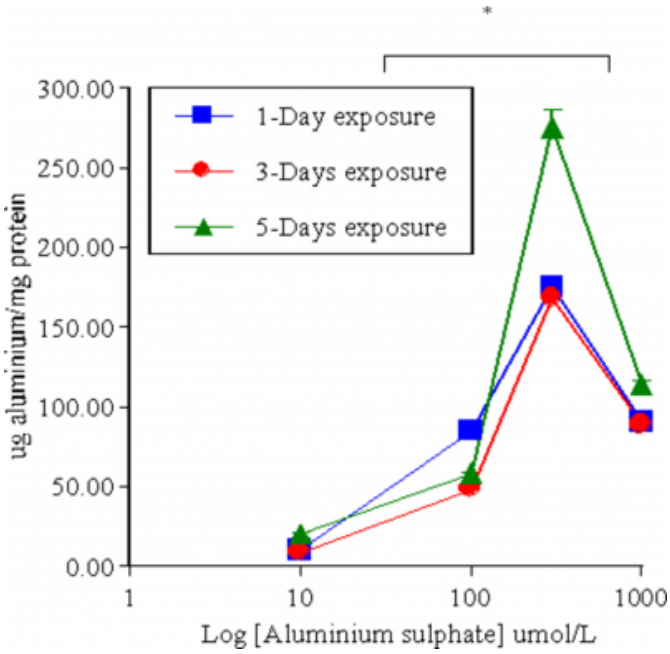

## EFFECTS OF ALUMINIUM ON ALUMINIUM CONTENT

### ALUMINIUM CONTENT OF ASTROCYTES

Figure 4, shows the cell associated Al accumulation in astrocytes as determined by ETAAS following Al treatment of the cells. The results indicate a response with maximum cell uptake at a dose of 300 µmol/L irrespective of duration of exposure. At a high dose (1000 µmol/L), there were few cells/well. The results were found to be significant at 100-300 µmol/L using parametric ANOVA where P value <0.001. However, a comparison between the duration of exposure was not significantly different (P>0.05).

**Figure 5**

Figure 4: Aluminium content of cortical astrocytes after exposure to different concentration (0-1000 Âµmol/L) of aluminium sulphate. Values are means Â± SEM (n=3) and values considered significance at *P<0.001 compared to control using paired ANOVA and post hoc t-tests.



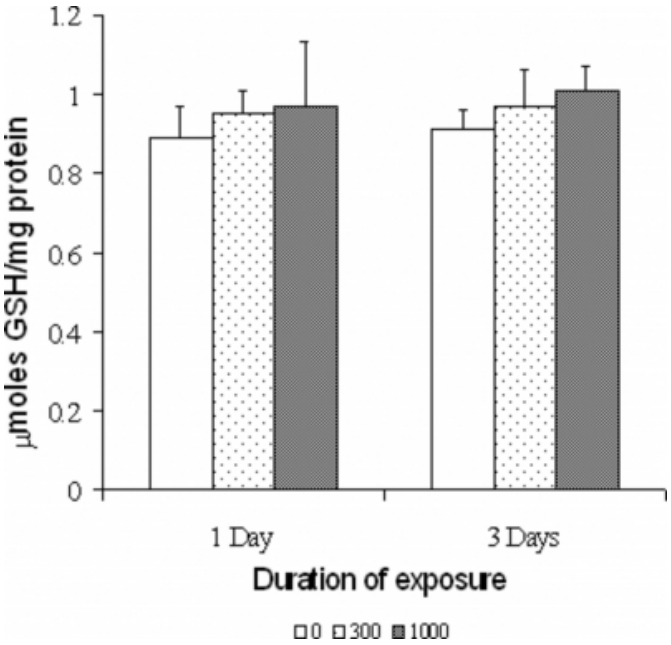

## EFFECTS OF ALUMINIUM TREATMENT ON CELL GLUTATHIONE CONTENT

### GLUTATHIONE CONTENT OF ASTROCYTES

The GSH content in astrocytes (Figure 5) revealed no significant changes at all levels of exposure to aluminium despite different duration of exposure.

**Figure 6**

Figure 5: Glutathione content in primary cortical astrocytes after 1 mmol/L aluminium sulphate exposure. Values are means Â± SEM (n=5), and values considered not significant at P<0.05 compared to control using paired ANOVA and post hoc t-tests.



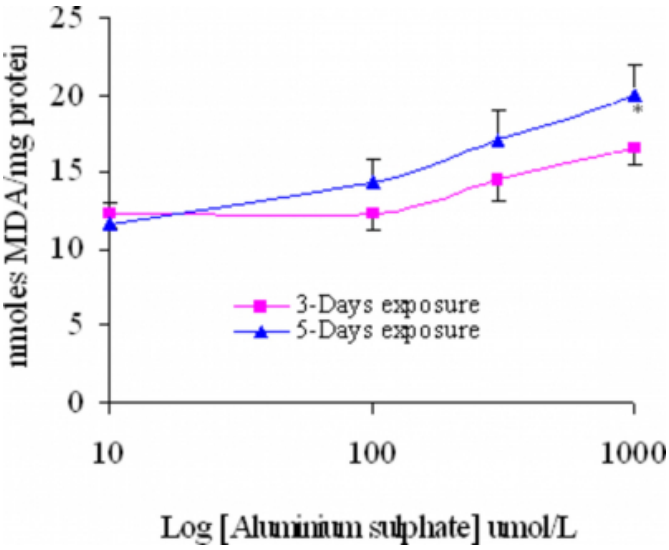

## EFFECTS OF ALUMINIUM TREATMENT ON MEASURES OF CELLULAR LIPID PEROXIDATION

### MALONDIALDEHYDE PRODUCTION IN ASTROCYTES

Aluminium treatment was associated with significant increase in the production of MDA (Figure 6) a measure of lipid peroxidation.

**Figure 7**

Figure 6: Malondialdehyde production in astrocytes, after exposure to aluminium sulphate. Values are mean ± SEM (n=3), *P

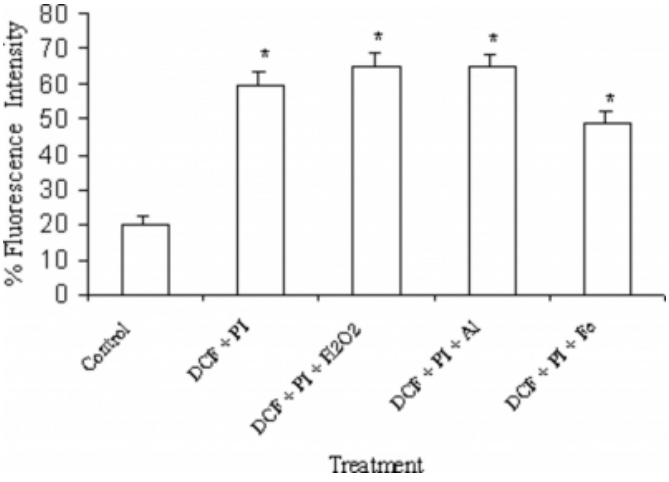



## FLOW CYTOMETRIC ANALYSIS OF DCFH-DA TREATED CELLS

The results for astrocytes (Figure 7) interesting responses that utilises only DCFH-DA alone. The astrocytes revealed consistent fluorescence intensity similar to the data obtained from microscopic viability test that uses FDA-PI.

**Figure 8**

Figure 7: FACSan intensity of oxidative stress in astrocytes, after treatment with aluminium (1000 Âµmol/L) and iron (100 Âµmol/L). Values are mean Â± SEM (n=5) and values are considered significance at *P<0.01 compared to control. Comparisons were assessed for significance using one way ANOVA and posthoc-test of Bonferroni multiple comparison.




## DISCUSSION

Aluminium has been implicated in the etiology of many

diseases, such as dialysis dementia, microcytic anemia with/without iron deficiency, in addition to the most controversial and a possible role it plays in Alzheimer's disease ([36], [37]). It is well known that aluminium can bind to phosphates and any other oxygen donating ligands leading to stable complexes, the consequence of which may be to disrupt cellular enzyme activity. One site of action may be the mitochondria thereby affecting the electron transport chain which may give rise to increased production of reactive oxygen species which eventually leads to oxidative injury ([38]). The exact mechanisms via which Al might exert its neurotoxic effect are yet to be substantiated. But there are several experimental studies supporting the potential of Al to promote the pro-oxidant properties of iron and other transitional elements ([39],[40],[41],[42]).

## CELL VIABILITY AND MITOCHONDRIAL DEHYDROGENASE ACTIVITY

In the present in vitro studies, isolated cell line of rat primary cortical astrocytes was utilized in order to assess the possible antioxidant/pro-oxidant effect to acute exposure to aluminium sulphate. The results of this study are in agreement with the previous report of Oteiza et al. ([42]), where Al was found to exhibit a dual effect. From the MTT results (figures 1-3) it is seen that aluminium initially induces an increase in the mitochondrial dehydrogenase activity, which may be due to an increase in activity of viable cells. At higher concentration (3000 µM) there was simply a general trend of increased mitochondrial activity. This may be due to cell proliferation as compensatory responses to the toxic effect of Al. But what is not known is whether the increase in activity is due to proliferation of the cells or by an increase in protein synthesis. In order to answer this question, the amount of protein was determined in untreated and Al-treated (1000 µM) cells, and these did not change significantly. Thus, it was established that, the increase in mitochondrial activity observed was not due to change in protein content and this is similar to what was earlier observed by Campbell et al, ([4]). Secondly, an attempt to assess clearly cell survival rate, investigations were carried out employing the simultaneous double staining method of FDA-PI ([29]). The results of primary cortical astrocytes show that living viable cells (which are stained green by the method) were less than 10% different in Al-untreated and Al-treated. Hence, the characteristic increase in mitochodrial dehydrogenase activity observed in the different cell types showed their different susceptibility and also confirmed possible cell damage via radical species leading to the increase activity as a result of compensatory response to toxic agent. It should be noted that under normal conditions mitochondrial respiration results in about 3% of the oxygen consumed being converted to hydrogen peroxide ([43]).

## OXIDANT STATUS ASSESSMENT

However, to determine whether the increased mitochondrial activity observed due to Al exposure is indeed as a result of reactive oxygen species generation, certain enzyme markers of oxidative stress were assessed. It is well established that electron leakage from the respiratory chain can lead to formation of excess reactive oxygen species ([38]). Oxidative stress generated via ROS with the loss of neurones, occurs during the progression of neurodegenerative diseases particularly the ALS, AD and Parkinson's diseases ([45],[46],[47]). Glutathione is one of the first natural intracellular defences against oxidant events. A biphasic effect of Al was observed on the GSH level primary cortical astrocytes. At lower Al concentration there seemed to be an increase in the GSH level and a decrease at high doses. One might argue on the observed effect, but it is not surprising as astrocytes have been reported to contain high levels of GSH with a range between 16 nmol/mg protein ([15]) and 50 nmol/mg proteins ([48]). Relatively, the cytosolic GSH level of astroglial cultures can even reach 8 mM ([17]). These can however be modulated by a variety of condition and highly inhibited by the availability of glutamine ([17]).

The effect of Al exposure on this cell type, mitochondrial dehydrogenases, GSH thiol status and lipid peroxidation as assessed by increase malondialdehyde production was also accompanied by about 10-15% increase of superoxide dismutase.

## ALUMINIUM UPTAKE BY THE CELL

These studies show that the rat primary cortical astrocytes cells are able to accumulate Al against a concentration gradient. This is supported by the report of Guy et al. ([1]).

## CELL MORPHOLOGY

Morphologically, using FDA-PI staining, the fluorescence photographs (Picture not shown), In Al treated, primary cortical astrocytes appeared less extended, disintegrated, and spread neurites were fewer and thinner, i.e. abnormal clustering and aggregation plus loose adhesion, but the number of live cells did not change (<10%) in comparison to untreated cells. The interesting point about this finding is that aggregation of cell bodies and fasciculation of processes have been previously reported ([49]) in cultured cortical

neurons following long-term exposures to Al. Moreover, both neuritic processes and aggregated cells could be confidently stained using an antibody to microtubule-associated protein tau, which is one of the main components of neurofibrillary tangles seen in Alzheimer's disease ([49]).

## CONCLUSION

The potential role of Al involvement in oxidation or pro-oxidant activities remains controversial to-date. Evidence for ([23], [42], [50]) and against ([41], [42]) in its potential involvement have both been reported. The present work contributes to the existing controversies of Al antioxidant at low concentration and pro-oxidant at high concentration. Similarly, suggesting that Al might facilitate membrane peroxidation via alteration in membrane rheology by increasing their susceptibility to free radical induced damage ([51], [52]) as a compensatory effect due to toxic insult.

The dual or biphasic effect of Al exposure observed on this cell type is probably due to direct interaction of Al with cell components, rather than to reaction with oxidative-reactive species. This is not surprising at all because biphasic effect of Al has been reported in diverse cell system including phosphorylation of neurofilament sub-units ([53]). The effect may be due to the formation of reversible/irreversible Al complexes. Therefore, the present effects may be due to an indirect effect of Al on free radical scavenging enzymes and glutathione.

However, under physiological and biochemical conditions, the antioxidant effect observed with Al could only occur in the early stages of its accumulation thereby given an excellent condition to Al slow process of intoxication, concomitant to the fact that Al binds to negatively charged membranes more avidly than most divalent cations ([54]), in addition to the presence of iron in most cell compartments. Therefore, this suggests that the pro-oxidant effect will be more likely to be exerted in vivo. Hence the presented results support the notion that changes in ratio of free radical in response to toxic insult due to Al leading to membrane changes (damage) may contribute to the phathophysiology of Al neurotoxicity.

### References

1. Guy SP, Seabright P, Day JP, and Itzhaki RF, (1990) Uptake of aluminium by human neuroblastoma cells. J. Trace Elem. Electrolytes Health Dis. 4(3): 183-187.
2. Zatta PF, Zambenedetti P, Bruna V, Filippi B (1994). Activation of acetylcholinesterase by aluminium (III): The relevance metal species. NeuroReport 5: 1777-1780.
3. Zatta PF, Nicosia V, Zambenedetti P (1998). Evaluation of MAO activities on murine neuroblastoma cells upon acute or chronic treatment with aluminium (III) or tacrine. Neurochem Int. 32(3): 273-279.
4. Campbell A, Prasad KN, and Bondy SC, (1999). Aluminum-induced oxidative events in cell lines: glioma are more responsive than neuroblastoma. Free Radic. Biol. Med. 26(9-10): 1166-1671.
5. Suarez-Fernandez MB, Soldado AB, Sanz-Medel A, Vega JA, Novelli A, Fernandez-Sanchez MT (1999). Aluminum-induced degeneration of astrocytes occurs via apoptosis and results in neuronal death. Brain Res. 835(2): 125-136.
6. Golub MS, Han B, Keen CL (1999). Aluminum uptake and effects on transferrin mediated iron uptake in primary cultures of rat neurons, astrocytes and oligodendrocytes. Neurotoxicology 20(6): 961-970.
7. Cucarella C, Montoliu C, Hermenegildo C, Saez R, Manzo L, Minana MD, Felipo V (1998). Chronic exposure to aluminum impairs neuronal glutamate-nitric oxide-cyclic GMP pathway. J. Neurochem. 70(4): 1609-1614.
8. Pope A (1978). In dynamic properties of Glia Cells: Schoffeniels, E., Franck, G., Hertz, L., and Tower, D. B. Eds., Pergamon Press, Oxford. Pp. 13-20.
9. Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B (1971). An acidic protein isolated from fibrous astrocytes. Brain Res. 28(2):351-354.
10. Bignami A, Dahl D (1977). Specificity of the glial fibrillary acidic protein for astroglia. J. Histochem. Cytochem. 25(6): 466-469.
11. Hertz L, Peng L, Lai JC (1998). Functional studies in cultured astrocytes. Methods 16(3):293-310.
12. Hertz L (1993). Metabolic interactions between neurons and astrocytes. In: Fedoroff S, Juurlink BHJ, Doucette R, eds. Biology and Pathology of Astrocytes- Neuron Interactions. New York, Plenum Press. pp 1-13.
13. Aschner M, LoPachin RM Jr, (1993). Astrocytes: targets and mediators of chemical-induced CNS injury. J. Toxicol. Environ. Health 38(3): 329-342.
14. Raps SP, Lai JC, Hertz L, Cooper AJ (1989). Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. Brain Res 493(2): 398-401.
15. Sagara JI, Miura K, Bannai S (1993). Maintenance of neuronal glutathione by glial cells. J. Neurochem. 61(5): 1672-1676.
16. Dringen R, Hamprecht B (1998). Glutathione restoration as indicator for cellular metabolism of astroglial cells. Dev. Neurosci. 20(4-5): 401-407.
17. Sochocka E, Juurlink BH, Code WE, Hertz V, Peng L, Hertz L (1994). Cell death in primary cultures of mouse neurons and astrocytes during exposure to and 'recovery' from hypoxia, substrate deprivation and simulated ischemia.Brain Res. 638(1-2): 21-28.
18. Abubakar MG, Taylor A. and Ferns GA, (2004A). Regional accumulation of Aluminium in the Rat Brain is affected by dietary Vitamin E. J. Trac. Elem. Biol. 18: 53-59.
19. Abubakar MG, Taylor A. and Ferns GA, (2004B).. The effects of Aluminium and Selenium Supplementation on Brain and Liver Antioxidant Status in the Rat. Afri J. Biotech. 3 (1): 88-89.
20. Abubakar MG, Taylor A. and Ferns GA, (2003). Aluminium administration is associated with enhanced hepatic oxidant stress that may be offset by dietary vitamin E in the rat. Int. J. Expt. Path. 84: 49 - 54.
21. Abubakar MG, Taylor A. and Ferns GA, (2002). Regional Distribution of Aluminium in the Rat Brain: Influence of Vitamin E. Metals. Ions Biol. Med. 7: 217 - 221.
22. Exley C (1999). A molecular mechanism of aluminium-

induced Alzheimer's disease?. J. Inorg Biochem. 76(2): 133-340.
23. Booher, KR, Jr., Schmidtknecht, TM (1977). Cerebellar medulloblastoma with skeletal metastases: case report and review of the literature. J Bone Joint Surg Am. 59(5):684-686.
24. Moody JR, Lindstrom RM (1977). Selection and cleaning of plastic containers for stroge of trace element samples. Anal. Chem. 49: 2264-2267.
25. Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65(1-2): 55-63.
26. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res. 47(4): 936-942.
27. van de Loosdrecht AA, Nennie E, Ossenkoppele GJ, Beelen RH, Langenhuijsen MM (1991). Cell mediated cytotoxicity against U 937 cells by human monocytes and macrophages in a modified colorimetric MTT assay. A methodological study. J Immunol Methods . 141(1): 15-22.
28. Jones KH, Senft JA (1985). An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. J Histochem Cytochem. 33(1): 77-79.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265-275.
30. Taylor A, Walker AW (1992). Measurement of aluminium in clinical samples Ann. Clin. Biochem. 29 (Pt 4): 377-389.
31. Hissin PJ, Hilf R (1976). Afluorometric method for determintion of oxidised and reduced glutathione in tissues. Anal. Biochem 74: 214-226.
32. Gutteridge JM, Quinlan GJ (1983). Malondialdehyde formation from lipid peroxides in the thiobarbituric acid test: the role of lipid radicals, iron salts, and metal chelators. J. Appl. Biochem. 5(4-5): 293-299.
33. Hartman PE (1983). Putative mutagens and carcinogens in foods. IV. Malonaldehyde (malondialdehyde). Environ. Mutagen. 5(4):603-607.
34. Marklund SL (1976). Spectrophotometric study of spontaneous disproportionation of superoxide anion radical sensitive direct assay for superoxide dismutase. J. Biol. Chem. 251: 7504-7507.
35. Marklund SL (1985). Direct assay of superoxide dismutase with potassiumsuperoxide. In: h book of Methods for OxygenRadical Research. Greenewald, R.A ed. Boca Ranto, Fla: CRC Press Inc. 249-255pp.
36. Alfrey AC, Le Gendre GR, Kaehny WD (1976). The dialysis encephalopathy syndrome: possible aluminium intoxication. N. Engl. J. Med. 294: 184-187.
37. Crapper DR, Krishnan SS, Dalton AJ (1973). Brain aluminium distribution in Alzheimer's disease and especially neurofibrillary degeneration. Science 180: 511-513.
38. Halliwell B (1992). Reactive oxygen species the central nervous system. J. Neurochem. 59: 1609-1623.
39. Bondy SC, Ali SF, Guo-Ross S (1998). Aluminum but not iron treatment induces pro-oxidant events in the rat brain. Mol. Chem. Neuropathol. 34(2-3): 219-232.
40. Bondy SC, Kirstein S (1996). The promotion of iron-induced generation of reactive oxygen species in nerve tissue by aluminum. Mol Chem Neuropathol. 27(2): 185-194.
41. Xie CX, Mattson MP, Lovell MA, Yokel RA (1996). Intraneuronal aluminum potentiates iron-induced oxidative stress in cultured rat hippocampal neurons. Brain Res . 743(1-2):271- 277.
42. Oteiza PI, Fraga C, Keen CL (1993). Aluminum Has Both Oxidant and Antioxidant Effects in Mouse Brain Membranes. Arch. Biochem. Biophysics. 300: (1) 517-521.
43. Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian organs. Physiol Rev. 59(3):527-605.
44. Bains JS, Shaw CA (1997). Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Res Brain Res Rev. 25(3): 335-358.
45. Cadet JL, Brannock C, (1998). Free radicals and the pathobiology of brain dopamine systems. Neurochem Int. 32(2): 117-131.
46. Reiter RJ (1998). Oxidative damage in the central nervous system: protection by melatonin. Prog Neurobiol . 56(3): 359-384.
47. Sun AY, Chen YM (1998). Oxidative stress and neurodegenerative disorders. J Biomed Sci. 5(6):401-414.
48. Devesa A, O'Connor JE, Garcia C, Puertes IR, Vina JR (1993). Glutathione metabolism in primary astrocyte cultures: flow cytometric evidence of heterogeneous distribution of GSH content. Brain Res . 618(2):181-189.
49. Kawahara M, Muramoto K, Kobayashi K, Kuroda Y (1992). Functional and morphological changes in cultured neurons of rat cerebral cortex induced by long-term application of aluminum. Biochem Biophys Res Commun. 189(3):1317-1322.
50. Evans P (1993). Free radicals in brain metabolism and pathology. Brt. Med. Bull. 49: 577-587.
51. van Rensburg SJ, Daniels WM, Potocnik FC, van Zyl JM, Taljaard JJ, Emsley RA (1997). A new model for the pathophysiology of Alzheimer's disease. Aluminium toxicity is exacerbated by hydrogen peroxide and attenuated by an amyloid protein fragment melatonin. S Afr Med J. 87(9): 1111-1115.
52. Ohyashiki T, Suzuki S, Satoh E, Uemori Y (1998). A marked stimulation of Fe2+-initiated lipid peroxidation in phospholipid liposomes by a lipophilic aluminum complex, aluminum acetylacetonate. Biochem. Biophys Acta.1389 (2): 141-149.
53. Leterrier JF, Langui D, Probst A, Ulrich J (1992 ). A molecular mechanism for the induction of neurofilament bundling by aluminum ions. J. Neurochem. 58(6):2060-70.
54. Deleers M (1985). Cationic atmosphere cation competition binding at negatively charged membranes: pathological implications of aluminum. Res. Commun. Chem. Pathol. Pharmacol. 49(2): 277-294.

## Author Information

**M. G. Abubaker, PhD**
Department of Biochemistry, Usmanu Danfodiyo University

**A. Taylor, PhD**
Associate Professor, School of Biomedical & Life Sciences, University of Surrey

**G. A. Ferns, MD, DSc (Med Sci)**
Professor, School of Biomedical & Life Sciences, University of Surrey