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THE TOXIC EFFECT OF ALUMINIUM AND ITS SUPPOSABLE MECHANISM IN *DROSOPHILA*

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Abstract

During the early epoch of biological evolution, aluminium was not available for the primitive organisms because of the low solubility of aluminium compounds. This can explain why aluminium is toxic for the present-day living organisms. We studied the toxic effect of aluminium by feeding *Drosophila melanogaster* adults for 24 hrs. with a 1% sugar solution containing different concentrations of aluminium sulphate. The effect of magnesium chloride was also tested in combination with aluminium. After 24 hrs. we calculated the survival rate, and solubilised the flies in concentrated nitric acid in a microwave oven. The Al, Na, Mg and Zn content of the solubilised flies was measured with the ICP-AES method. We found no change in the Zn content while the Al and Mg content changed according to the treatment, and the Na content decreased significantly (20-30%) in all the cases. We suppose that the flies were mainly killed by the elevated sodium efflux resulting in a decreased intracellular pH. The cause of the elevated Na efflux could be the increased rigidity of the cell membrane caused by the aluminium. We discuss the experimental results and the supposable mechanism of toxicity.

Key words: Aluminium, magnesium, sodium, *Drosophila*, membrane rigidity.

INTRODUCTION

According to MATSUMOTO (1980) and BUTNARU (1996), the aluminium caused chromosome abnormalities (non-disjunction, chromosome breakage and rearrangement) which could have a deleterious effect in the offspring.

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The aluminium can inhibit the activity of some mitochondrial enzymes like hexokinase (LAI 1984). MITANI (1992) found that the Al caused a 25-60% increase of calcium concentration in certain cells of the central nervous system resulting in cell death. Aluminium can interfere with the energy production by inhibiting the ATPases (SIEGEL-HAUG 1983).

With respect to these deleterious effects, we studied the lethal effect of aluminium and its possible mechanism of action on adult *Drosophila melanogaster*.

MATERIALS AND METHODS

Adult flies of the Canton S wild type stock were used for the experiments. The flies were fed with 1% sugar solution containing different concentrations of aluminium sulphate, magnesium chloride and their combinations, as follows:

$Al_2(SO_4)_3$:	10 mM, 50 mM, 100 mM, 250 mM, 500 mM
$MgCl_2$:	100 mM, 250 mM, 500 mM
$Al_2(SO_4)_3 + MgCl_2$:	50 mM Al + 100 mM Mg

Twenty ml plastic vials were filled with the sugar solution and closed with wetted cotton plugs so that no air bubbles were trapped under the plugs. In this way, the plugs never dry out and the flies can continuously feed on them. The vials were put into 500 ml milk bottles closed with cotton plugs. 200 flies were weighed and added to each bottle. The treatments were made in three parallels each. After 24 hrs. we calculated the survival rate, pooled the flies of the three parallels and solubilised them (living and dead together) in cc.nitric acid in a microwave oven. The concentrations of Al, Mg, Na and Zn were determined with a Jobin Yvon 24 sequential atomic emission spectrometer (ICP-AES).

RESULTS AND DISCUSSION

Table 1 shows the effect on the survival and element content of feeding the flies with the different solutions. As it revealed, the different treatments had no effect on the Zn content of the flies, while the Al and Mg values changed according to the experimental conditions. Both the Al and the Mg treatments significantly decreased the Na content, although to different extents.

Table 1

Survival and the element contents of adult *Drosophila* after treatment with Mg and Al for 24 hours

Treatment, mM		Number of flies		Element content in flies, mg kg^{-1}			
$Al_2(SO_4)^3$	Mg SO_4	alive±SD	survival % ± SD	Al	Na	Mg	Zn
0	0	199 ± 2.0	100 ± 0.5	163	850	260	35.4
10	0	194 ± 4.0	97 ± 2.0	201	790	256	35.2
50	0	176 ± 9.5	88 ± 2	355	600	262	34.2
100	0	160 ± 7.5	80 ± 3.8	534	600	187	33.9
250	0	56 ± 15.0	28 ± 7.5	980	520	136	34.8
500	0	0	0	998	480	102	33.8
0	100	196 ± 4.4	98 ± 2.2	47	760	705	34.7
0	250	56 ± 14.9	28 ± 7.5	13	600	945	34.9
0	500	0	0	15	580	1089	35.2
50	100	166 ± 13.2	83 ± 6.6	430	619	310	34.6

The experimental results show that feeding with elevated concentrations of aluminium significantly increased the death rate of adult *Drosophila* already within a few hours. The intoxication with aluminium resulted in not only an increase of the Al content but a significant decrease of the Na content of the flies as well. Therefore, we think that the mechanism of the toxicity lies in these two effects. The treatment with Mg strongly reduced the Al content and, similarly to the treatment with Al, decreased the Na content albeit to a lesser extent. At higher concentrations (250–500 mM), the Mg strongly increased the death rate either.

Structural integrity and normal permeability of the cell membrane are of vital importance for its normal functioning and cell viability. The aluminium exerts a deleterious effect on both the cell membrane and important enzyme functions.

Already a low level (25 μM) of aluminium increased the rigidity of the cell membrane (DELERS 1986). In addition, in the presence of Al, the ion channels remained open for a longer time (DELHAIZE-RYAN 1995) which can result in the increased Na efflux (BITTAR et al. 1992). Because of the high Na efflux, the Na^+/H^+ balance is shifted leading to a decreased intracellular pH and an increased frequency of cell death (POYSSEGUR et al. 1984) We also found that treating the flies with aluminium decreased their Na content which offers a possible explanation for its toxicity. This is in parallel with the observation of HAVAS-LIKENS (1985) that in *Daphnia* the decrease of Na content is accompanied by their increased mortality.

Another possible factor in the mechanism of aluminium toxicity is the decreased activity of ATPase. According to SIEGEL and HAUG (1983), the Al binds to calmodulin, possibly causes the loss of the α-helix structure (KISS 1996), and changes the protein conformation. This would result in the loss of enzyme activity and can contribute to the increased mortality.

In the presence of aluminium, the blood platelets aggregate at a higher rate (NEAIVA et al. 1997): at 20 µM Al the rate of aggregation is 4%, at 50 µM it is 43% and at 100 µM it reaches 83%. The aggregation of proteins can further increase the death rate of *Drosophila*.

As Table 1 shows, the elevated Mg concentration above 100 mM can drastically increase the mortality. We also found that at higher Mg concentration the amount of Na in the flies decreased, probably due to the higher Na efflux which can result in the decrease of cytoplasmic pH in the cells. This is in line with the observation of GÜNZEL et al. (1996) that at higher Mg concentrations the Na efflux was increased.

The combined effect of Al and Mg (50 mM Al + 100 mM Mg, see Tab. 1) was not really additive. This can be explained by the antagonism of their biological effects (BALLA and KISS 1996).

In summary, we think that the decreased intracellular pH caused by the higher Na efflux, the lowered level of energy production due to the inactivation of ATPase, as well as the unfavorable changes in general protein conformation are important factors in the mechanism of aluminium toxicity.

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