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Chromosome instability in asthma

An antioxidant treatment as a possible pharmacological strategy?

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Abstract

Asthma is a disease with multiple phenotypes and different degrees of severity. Severe inflammation appears when oxidative stress (OS) overwhelms the antioxidant defense. Knowing the role of OS in the increase of chromosome instability (CI) and, consequently, in cancer predisposition, it is important to carry out studies to establish limits for harmful effects. The aim of this study was to evaluate OS-related CI in asthma patients and the possible protective effect of antioxidants. For that purpose, spontaneous and OS-induced lymphocyte cultures from patients with mild and severe asthma, and from normal controls, were performed. Antioxidant-enriched cultures from severe patients were posteriorly performed. A hundred metaphases per culture were scored in order to estimate the frequency of CI. Our results showed that lymphocytes from severe patients had increased CI, both in spontaneous and OS-induced cultures. However, in lymphocytes from mild patients there were no differences compared to controls. This suggests that in patients subjected to extreme OS conditions, a genotoxic effect may occur at the cellular level. When lymphocytes from these patients were treated with antioxidants, a decrease in CI was observed. Understanding how CI correlates to asthma patients' clinical situation may be pivotal to the design of future preventive measures.

Keywords: asthma; chromosome instability; oxidative stress

Aims of the study

The aim of this study was to provide a new insight into chromosome instability (CI) associated with asthma pathogenesis.

The specific aims are:

- To evaluate if in primary lymphocytes from asthma patients there are increased levels of CI;
- To study the efficacy of *in-vitro* administration of an antioxidant cocktail (NAC+ALA) in decreasing CI in primary lymphocytes.

Material and Methods

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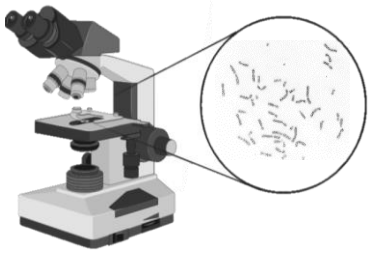
Subjects

- Controls (n=20) - healthy blood donors
- Severe asthma (n=15) - w/ frequent attacks and daily medication
- Mild asthma (n=4) – without an attack in the last couple of months and without a daily medication.

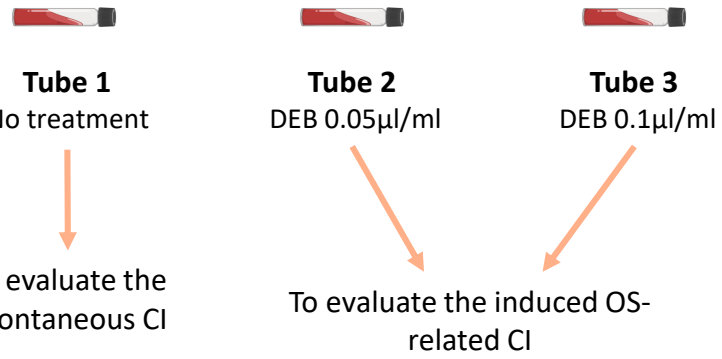
Cytogenetic Analysis

- In 100 metaphases per culture
- Chromosome instability (CI) parameters:
  - % of aberrant cells
  - Mean number of breaks per cell.

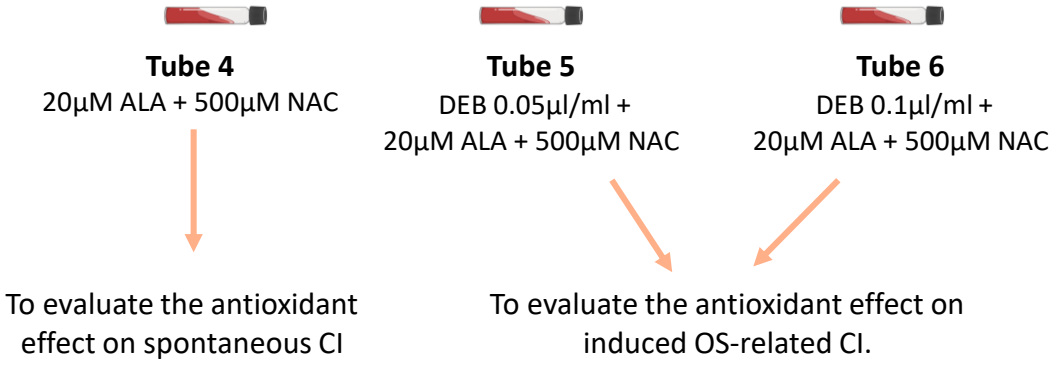


Experimental planning

1. Initially, three sets of lymphocyte cultures were performed with cells from all individuals:

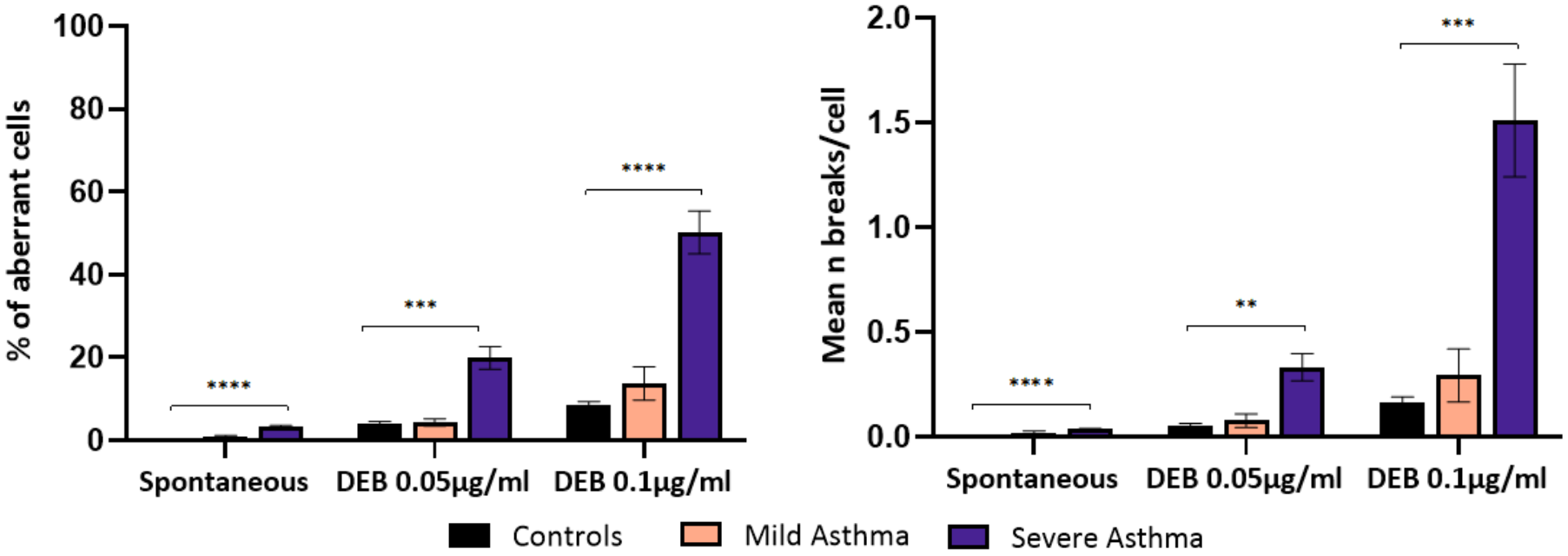


2. Then, three new sets of lymphocyte cultures were performed with cells from individuals presenting increased levels of CI:



Results

CI evaluation in lymphocyte cultures from asthma patients



CI evaluation:

Severe asthma	>	Controls
Mild asthma	=	Controls

Comparative analysis between the levels of CI in in cultured lymphocytes from controls, mild and severe asthma patients. Data are expressed as means ± SEM. Differences between groups (Mild asthma vs Controls and Severe asthma vs Controls) were evaluated by Two-factor mixed-design ANOVA followed by a multicomparison Turkey's test (α= 0.05): ****p< 0.0001, ***p< 0.001, **p< 0.01, *p< 0.05.

Results

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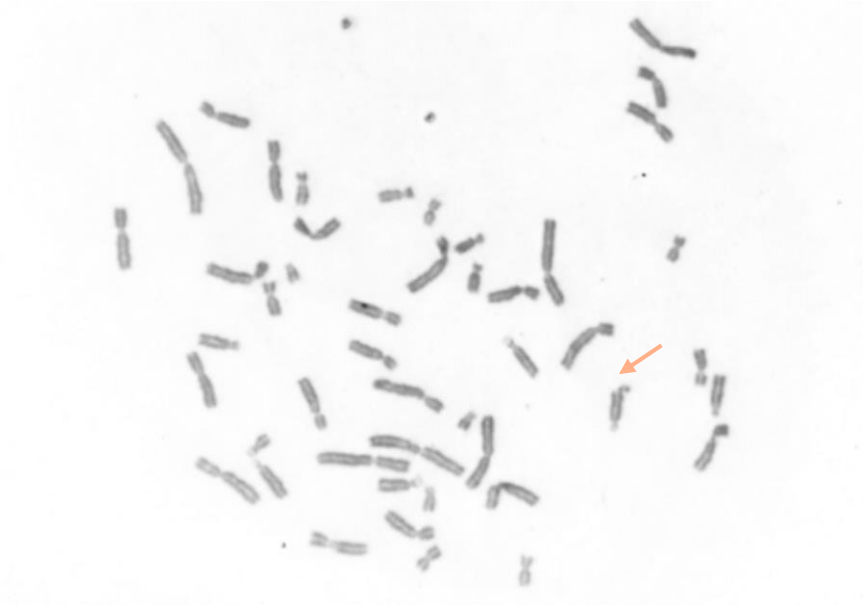
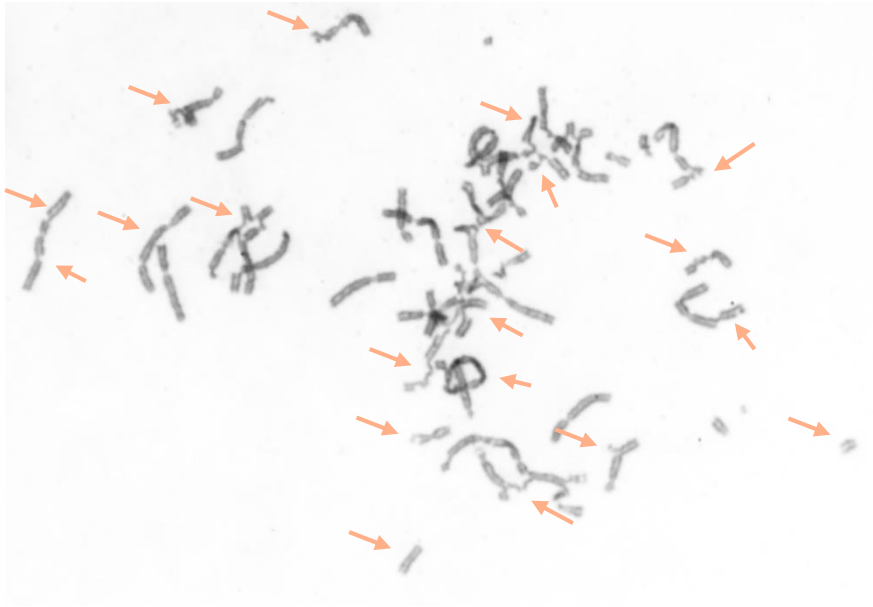
Images of CI pattern in selected metaphases from DEB-induced (0.1µg/ml) lymphocyte cultures

(a) Selected metaphase from a control



One chromatid break

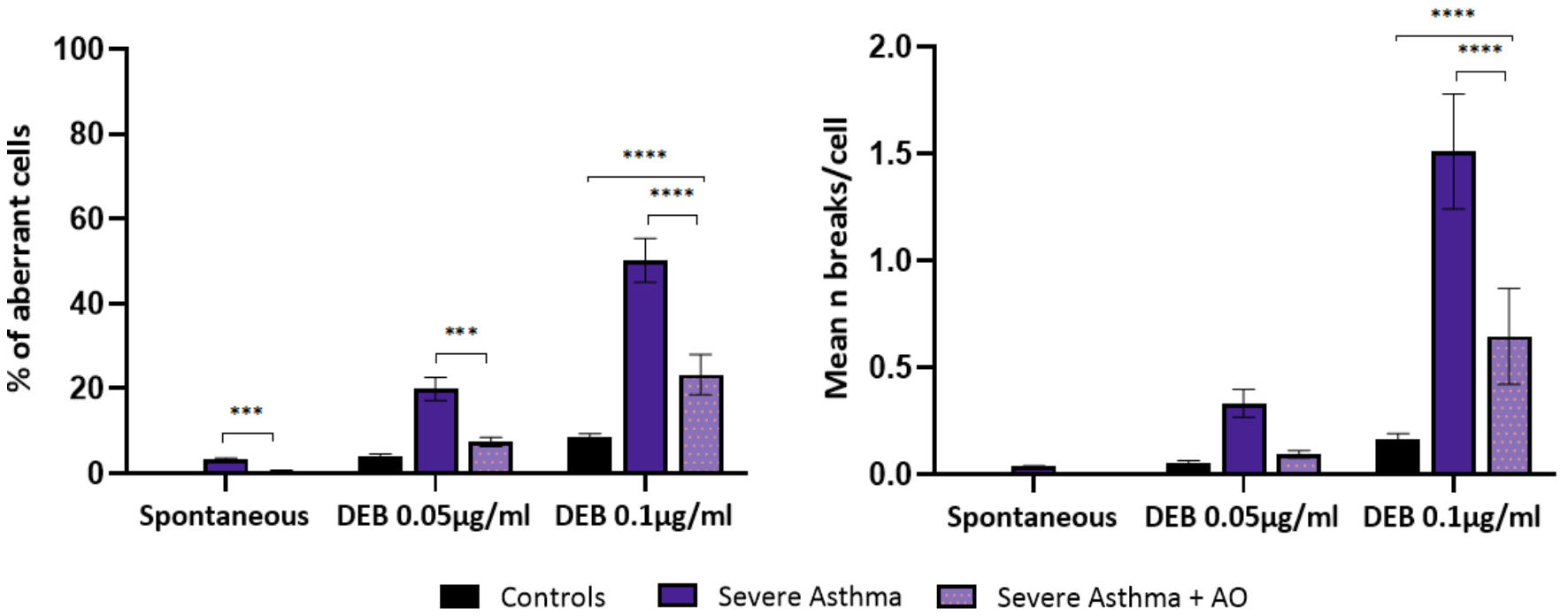
(b) Selected metaphase from a severe asthma patient.



High level of chromosome instability, with multiple breaks and rearrangements.

Results

Antioxidant effect against CI in lymphocyte cultures from severe asthma patients



CI evaluation:

Severe asthma > Severe asthma + AO

Severe asthma + AO = Controls

Effect of NAC+ALA in CI in cultured lymphocytes from severe asthma patients. Data are expressed as means ± SEM. Differences between groups were evaluated by Two-factor mixed-design ANOVA followed by a multicomparison Turkey's test (α= 0.05): ****p< 0.0001, ***p< 0.001, **p< 0.01, *p< 0.05.

Discussion

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	Already described in the literature	Described in our study	
Asthma attack	Characterized by increased levels of OS at the cellular level.	↑ spontaneous CI in severe asthma	This result is in agreement with the pathological feature already described
		↑ OS-related CI in severe asthma	This result suggests that these patients might have a higher sensitivity to the **cumulative** genotoxic effect of OS
Effect of Antioxidants (AO)	The cocktail NAC+ALA was previously proven to effectively reduce CI in *in vitro* studies with cells from patients with increased OS-related CI.	↓ spontaneous CI in severe asthma + AO	These results suggest a putative protective effect of the cocktail NAC+ALA at cellular level
		↓ OS-related CI in severe asthma + AO	

Conclusions

In conclusion, the present study provides an important and novel finding that may have a clinical applicability. Understanding how CI due to exposure to OS correlates to asthma patients' clinical phenotype may be pivotal not only to the design of preventive measures, in order to avoid the cumulative OS genotoxic effect, but also to design patient-specific treatments, like the prophylactic use of antioxidants.

! Further studies are still required in order to understand why these particular antioxidants were effective in the reduction of CI in these cells, namely studies of ROS characterization and mitochondrial function.

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