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Original Article

Plasma diamine oxidase activity in asthmatic children

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ABSTRACT

Histamine plays an important role in the development of asthmatic symptoms. Diamine oxidase (DAO histaminase), which inactivates histamine, is located in the intestine and kidney and is released into plasma. Plasma DAO activity in asthmatic children was measured by a recently developed high performance liquid chromatographic method using histamine as the DAO substrate. Diamine oxidase activity was higher in severely asthmatic children than in those with mild asthma. A time course study during the acute exacerbation phase revealed that DAO activity rose during acute asthmatic attacks and then decreased gradually over several days. Although the mechanisms of plasma DAO activity increase during acute asthmatic attacks could not be explained, data showed that plasma DAO activity is an important index of histamine metabolism in asthmatics and may relate to some mechanisms of acute exacerbation of airway inflammation. Consequently, fluctuations in plasma DAO can be used as one of various indices of instability in management of asthma.

Key words: childhood asthma, diamine oxidase, histaminase, histamine

INTRODUCTION

It has recently become clear that asthma is a chronic eosinophilic inflammatory disease and many investigators are concentrating on the study of activation mechanisms of eosinophils and lymphocytes. They suggest that the late asthmatic response is more important than the immediate asthmatic response in generating symptoms of natural acute exacerbations of asthma. But the immediate asthmatic response almost always develops upon encountering the pathogenic allergens, resulting in histamine being released from mast cells. There are many reports about the role of histamine in asthmatics, including studies of histamine in plasma or urine and histamine metabolites in urine.[1–3] But, to the best of our knowledge, there are no reports about plasma (serum) histamine destroying enzyme in asthmatics, although there are some reports that serum DAO[4] or histamine destroying factor[5] increases after anaphylaxy in mammals. Therefore, by taking measurements of plasma DAO activity, we aimed to determine the histamine kinetics in asthmatic children.

METHODS

Materials

Fifteen asthmatic children (7 male, 8 female) were enrolled in the study to compare the severity of asthmatic attack with DAO activity. Heparinized venous blood was drawn during acute asthmatic attacks. Attack severity was categorized by Mitsui's symptom score: severe attack ≥ 4, mild attack ≤ 3.[6] Another 16 asthmatic children (8 male, 8 female) were enrolled in the study to compare DAO activity with severity of asthma where the severity of asthma was categorized by the Japanese Society of Allergology criteria. These criteria consist of a combination of attack severity and frequency. In this group, heparinized venous blood was drawn on non-attack days. Finally, seven asthmatic children (6 male, 1 female) were enrolled in the time-course study. Samples were taken during acute asthmatic attacks, then on the first day and on the seventh day of the convalescent period (no pulmonary additive sounds).

Plasma was separated from the heparinized blood immediately after blood sampling and kept at −25°C until DAO measurement.

Measurement of diamine oxidase

Plasma was mixed with one-nineteenth volume of 100 mmol/L phosphate potassium buffer (pH 7.0) including 0.01 mmol/L histamine (final concentration). Aminoguanidine (0.001 mmol/L; a

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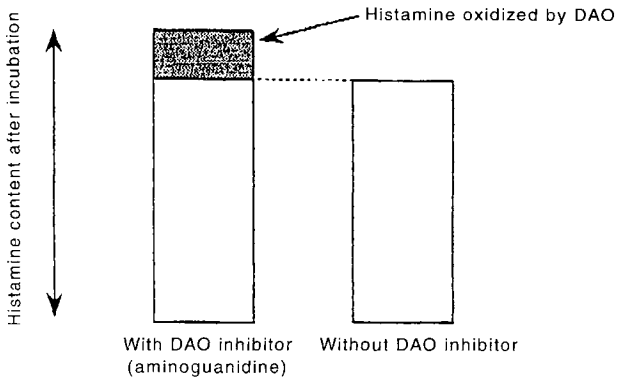

Fig. 1 Determination of diamine oxidase (DAO) activity by HPLC method.

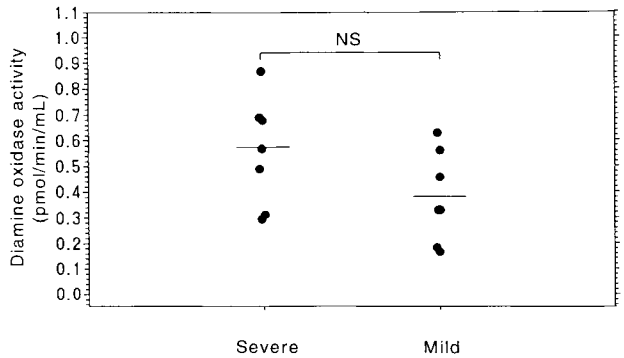

Fig. 2 Diamine oxidase (DAO) activity from asthmatics with acute attacks.

specific inhibitor against DAO) was added in the negative control assay. Perchloric acid (final concentration 3%) was added after incubation for 1000 min at 37°C and histamine was measured by high performance liquid chromatography.[7] The difference in histamine concentration between the sample and the negative control (Fig. 1) is the measure of DAO activity (pmol histamine destruction/min/mL plasma).

Statistical analysis

Student's *t*-test was used to examine differences. Paired *t*-test was used in the time-course study. Significance was determined at the 0.01 level.

Results

Plasma DAO activity during acute asthmatic attacks was slightly higher in severe attacks (0.57 ± 0.20 pmol/min/mL) than in mild ones (0.38 ± 0.18 pmol/min/mL), but this difference was not significant ($P<0.10$; Fig. 2). Figure 3 shows DAO activity at the remission phase in 16 asthmatic patients. Six patients had mild asthma and 10 patients had moderate or severe asthma. Diamine oxidase activity was clearly higher in moderately or severely asthmatic children (0.15 ± 0.05 pmol/min/mL) than in

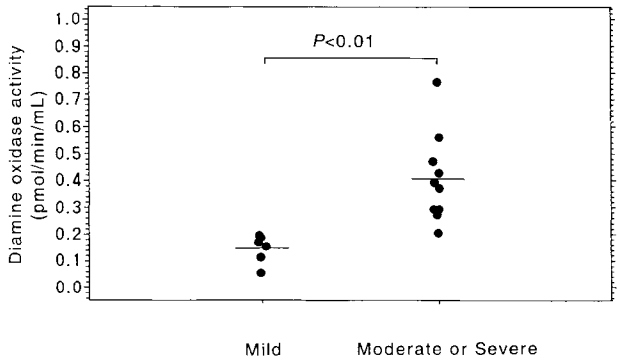

Fig. 3 Diamine oxidase (DAO) activity from asthmatics without acute attacks.

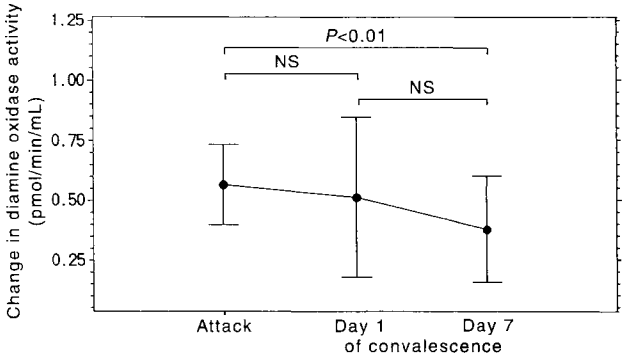

Fig. 4 Change in diamine oxidase (DAO) activity from acute attacks phase through convalescence.

those having mild cases (0.41 ± 0.16 pmol/min/mL; $P<0.01$). $\%FEV_1$ and $FEV_1\%$ was found to be 101.8 ± 13.9 and 86.8 ± 5.7% in mild asthmatics, and 96.4 ± 17.8 and 76.9 ± 8.9% in moderate and severe asthmatics, respectively. The time-course of DAO activity from the attack phase through to recovery is shown in Fig. 4. Diamine oxidase activity decreased gradually from the time of the acute attacks to the seventh day of convalescence. A statistically significant difference ($P<0.01$) in DAO activity was found to exist between the acute attacks and the seventh day. Measurements of DAO activity were found to be 0.56 ± 17 pmol/min/mL during acute attacks, and 0.51 ± 0.33 and 0.38 ± 0.22 pmol/min/mL on the first and seventh day of convalescence, respectively.

Discussion

Histamine is the first chemical mediator to be incriminated as a bronchoconstrictor substance in asthma. Its importance in immediate asthmatic response is clear, but its importance in late asthmatic response is controversial.[8-10] It has become clear that other chemical mediators such as leukotrienes, prostaglandins, thromboxanes and platelet activating factor also play an important role in acute asthmatic attacks. They probably operate in a complex network.

The new antihistamines that block histamine at the H1-receptor level again demonstrated the importance of histamine in acute asthmatic attacks.[11,12] Histamine is metabolized by histamine *N*-methyltransferase (HMT) or DAO and excreted into the urine in the form of *N*-methyl histamine, *N*-methyl imidazole acetic acid, imidazole acetic acid and imidazole acetic acide riboside.[13] The studies of these urinary metabolites are important to determine the histamine kinetics. However, by studying enzyme activity, we intended to determine the histamine kinetics in asthmatics. The relative roles of HMT and DAO in the metabolism of endogenous histamine have not yet been established.[14] Although it has been generally accepted in the past that HMT works predominantly in the central nervous system and DAO in the peripheral organs,[15] some recent reports showed the importance of HMT in the airways.[16,17] Further, Löwhagen *et al.* reported that urinary excretion of methyl histamine and 1-methyl-4-imidazole acetic acid, HMT metabolites were increased during acute asthma in intrinsic adult asthmatics.[18] On the other hand, HMT can work only in cells with co-factor, S-adenosylmethionine, but DAO can work in both cells and plasma; and we suppose that this plays an important role in histamine catabolism in asthmatics. In this context, we first took measurements of plasma DAO activity both during acute asthmatic attacks and in non-attack periods in asthmatic children in order to study histamine kinetics in asthmatics.

Although we have no data about plasma DAO activity in healthy children, the normal levels for adults and infants are 0.262 ± 0.211 pmol/min/mL plasma (mean $\pm$ SD, $n=178$) and 0.145 ± 0.120 pmol/min/mL plasma (mean $\pm$ SD, $n=10$), respectively. Diamine oxidase activity in mildly asthmatic children in the non-attack phase was within the normal range. However, DAO activity in moderately or severely asthmatic children in the non-attack phase was higher. Diamine oxidase activity during acute asthmatic attacks was higher than normal and higher during severe attacks than during mild ones. The time-course study of DAO activity for acute asthmatic attacks through to convalescence showed that DAO activity decreased gradually after recovery of about 1 week.

At present, we do not know the mechanism by which the plasma DAO activity increases during asthmatic attacks, but we believe there are two possibilities: (i) heparin released from bronchial mucosal mast cells with histamine induces the release of DAO from the small intestine; or (ii) activated eosinophils (and probably neutrophils) increase in blood during acute asthmatic attacks and release DAO, as it has been reported that DAO activity in eosinophils increases in active asthma.[19] Although we must study DAO together with plasma histamine, urinary histamine metabolites, HMT and histidine decarboxylase to clarify the histamine kinetics in asthmatic children, our preliminary data show that plasma DAO activity is one of the important factors in histamine metabolism and that the stability of asthma management can be assessed by plasma DAO activity levels.

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