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FUNCTIONAL-EICOSANOID-TEST (FET) AND DISEASE

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Eicosanoids are involved in most cellular activities. Measurement of their levels in tissue or blood renders information about the function of activated cells. An extended analysis will improve the conclusions regarding eicosanoid-related diseases. Peripheral white blood cells (WBC) were used for the test. Stimulating or inhibiting substances to influence the generation and the metabolism of eicosanoids were separately added to the samples. Prostaglandins (PG) and leukotrienes (LT) were measured after incubation in culture medium for 20 minutes at room temperature. Healthy controls rendered normal data. Patients with intolerance to acetylsalicylic acid (ASS) showed an elevated output of PG and LT upon stimulation. Addition of ASS shifted from PG to LT. An altered pattern of eicosanoids also was found in patients suffering from gastroduodenal ulcer and in intestinal malignancy. The sensitivity regarding the ASS-intolerance is >80% and the specificity in the same group >70%. We concluded that the FET is a suitable test for the demonstration and verification of intolerance to ASS. It also detects an imbalance of the eicosanoids in intestinal malignancy. This makes the FET a helpful tool for diagnosis and for the elucidation of pathogenic mechanisms.

Key words: eicosanoids, prostaglandins, leukotrienes, intolerance, imbalance

INTRODUCTION

The Functional Eicosanoid Test (FET) initially has been established for diagnostic use in allergy. This was done in analogy to a former test basing upon histamine-release. The evaluation relying on the non-specific components of the allergic immune-response offered an adaptation to detect also non-allergic reactions. In contrast to histamine which is physiologically active only in one form eicosanoids have different active agents. Therefore the FET is able to detect

a variety of deviations no matter they are caused by substances influencing the generation and metabolism or by a systemic inborn special feature.

Table 1 summarizes disorders detectable by the FET.

Table 1. Eicosanoid-related diseases detectable by the FET

EICOSANOID - DEVIATION

- **Exogenous factors**
 - COX-inhibition
 - Generating lipids
- **Endogenous factors**
 - Enzymatic capacity
 - Inborn irritability
 - Concomitant diseases

The general role of the eicosanoids in the organism

The term eicosanoids means 20 (εικοζ = twenty in Greek) C-atomes within one molecule. This group derives originally from phospholipids located within the membranes of almost every cell. Phospholipases set free arachidonic acid which is the initial compound metabolized - shortly - by Lipoxygenase (LOX) or Cyclooxygenase (COX) to LT, PG and thromboxans (TX). Like mediators they participate in substantial activities of the cells. This fact explains their ubiquitous importance for the organism (1). Therefore any abnormality places the seed for disorders and diseases which will precipitate in the patient and manifestate in different organs according to the general individual situation and to the particular local conditions.

The specific role of the eicosanoids

LT, PG and TX are metabolized to a wide range of compounds with the affix called A,B,C,D,E and 1,2,3,4. Their effects are roughly elucidated. However, the most important products are characterized. So PGE₂ is responsible for inflammatory symptoms due to cellular activation and causing damage of tissue and pain, while LT CDE₄ is a complex most efficient regarding especially the constriction of the smooth muscle of the bronch (2).

MATERIAL AND METHODS

The design of the FET

The test elucidates the action and reaction of living cells under selected conditions. This is done in short-time cultures. The evaluation is based on the measurement of eicosanoids using commercial available ELISA (Spibio; Paris; France). Stimulation and inhibition the generation and metabolism of the eicosanoids reveals a shift of the formation of PG and LT. The FET therefore is analyzing any alteration under conditions assessed according to the patients disease. Singular cells or biopsy-

specimens can be used likewise. Since the response of living cells upon external factors is measured the test is a functional one.

The spectrum of the FET

The yielded informations comprise at least the absolute quantity of different groups of the eicosanoids and additionally the relation in between. Therefore data of at least two groups have to be available. With respect to the importance for the pathogenetic effects prostaglandins (PG) and leukotrienes (LT) are preferred. Under conditions aligned with the assumed pathogenic mechanism the FET is able to find patterns pertinent with specific or non-specific hypersensitivity like allergy or intolerance. Regardless of this kind of diseases it reveals endogenous, probably inborn abnormal relations of singular eicosanoids responsible for other diseases like malignancy. In contrast to intolerance it is a separate imbalance of its own.

RESULTS

Laboratory findings in eicosanoid-dependent diseases

There are no typical findings when applying the usual tests. Blood cell count, proteins, electrolytes and enzymes are within the normal range. An increase of eosinophilic granulocytes often is found but not mandatory.

Tests to detect a specific immunoreactivity render negative results. Activated lymphocytes or antibody to mediators are absent since intolerance is no allergy.

The only way to demonstrate intolerance or imbalance depends on the objectivation of alterations typical for deviations of the involved system. The FET fulfils this requirement and is suited to cover the complete spectrum of pathogenesis concerning eicosanoid-related diseases.

Classical diseases associated with a shift of the eicosanoids

Diseases caused by a shift of eicosanoids have been described first time by Hirschberg (3) who realized Aspirin^R to be causative for asthma and rash without knowing the mechanism. Later on Widal (4) studied those diseases followed by Samter (5) who accumulated more information. The actual pathophysiology has been investigated by Szczeklik (6). The generation and metabolism of the eicosanoids was elucidated by Samuelson (3) and by Vane (7). All investigations were initiated by epidemiological observations.

Rhinitis, polyposis of the nose and of associated sinus as well asthma is due to an altered local synthesis and distribution of PG and LT (8). The complete pathogenetic line of singular events on the molecular level is not yet elucidated. Since these diseases are the common consequence they are called classical.

FET in intolerance to analgetics

The common indication for the FET are diseases correlated with an intolerance to COX-inhibitors. They are mostly located within the respiratory

tract. The typical manifestation is rhinitis, polyposis of the nose and asthma separately or in any combination.

The FET can be performed taking affected tissue or blood-cells. Several samples have to be tested separately. Generally speaking, one sample renders the data of the basic status, another sample the data after stimulation, another sample the data upon COX-inhibition. Sometimes additional samples are needed for the exposition to pharmacological substances like corticosteroids to check their influence on the eicosanoid-pattern.

For the demonstration of the influence on the eicosanoids at least two lines have to be measured, mostly PG and LT. For that purpose an adapted ELISA is used which detects PGE₂ or LT CDE₄. The absolute amount as well as the relation of LT and PG normalized to the basic data is the basis for the calculation which results in a scale yielding arbitrary units. The normal grouping when investigating healthy individuals is "0" whereas patients suffering from intolerance are assigned to group "1", "2" or "3" corresponding with the degree of deviation.

In a great number of results comprising nearly 1000 individuals healthy controls showed group 0 in more than 90%. *Viceversa*, patients were classified group 1, 2 or 3 in more than 90% when using biopsy specimens of affected tissue. Taking blood of patients suffering from classical eicosanoid-associated diseases abnormal eicosanoid patterns were found in more than 80%. This means that, on the one hand, some patients render normal results probably pointing to another pathogenic background and that, on the other hand, some healthy individuals render abnormal results probably anticipating a disease associated with this situation.

These data show that an abnormal laboratory result is indicative for a deviating pattern in the organism. It means a disposition for the disease. Generally speaking, additional factors are responsible for the manifestation. Strictly speaking, particular cofactors are responsible for the localization of the affection. Presently there is no clearly discriminating difference regarding the results when investigating the mentioned parameters to distinguish patients suffering from rhinitis, from polyposis or from asthma.

FET in gastroduodenal ulcer

The frequency of GD-disorders caused by ASS which is within the same range as in the respiratory tract prompted to apply the FET also in this disease. Moreover, Konturek (9) already pointed at the role of eicosanoids in this field. Using blood, an abnormal eicosanoid pattern was found in about 60% of the patients (10). It must be mentioned that in most cases also *Helicobacter pylori* (Hp) has been detected within the affected tissue. However, it had been successfully eradicated before the test was performed. This shows that the altered eicosanoid pattern is not due to an actual infection with Hp. So it has to be assumed that Hp may be invasive especially in individuals which have an abnormal pattern.

FET in inflammatory bowel disease

Chronic inflammatory bowel disease has been supposed to be caused in certain cases by intolerance of analgetics. This is the case when patients experience an exaggeration upon sulfasalazin which is a first-line drug to reduce the inflammation. Moreover, adverse effects of food containing considerable amounts of salicylate support this assumption. Finally, absence of specific parameters like antibody is another argument (11).

By application of the FET about 6% of the patients suffering from inflammatory bowel disease had an abnormal eicosanoid pattern. Individuals meeting the mentioned symptoms showed more often an abnormal result.

FET in intestinal malignancy

Epidemiological studies discovered the protective effects of COX-inhibitors (12). This was first suggested when patients taking acetylic salicylic acid or other NSAID were identified to benefit from medication in this concern. Corresponding to this observation an increase of COX-inhibitors has been demonstrated in the area of the malignant transformation (13).

This occasioned to investigate the eicosanoid pattern of patients who had been successfully treated. It has to be mentioned that investigations were performed using blood which was drawn during complete remission. Of course, the patients were free of affections associated with eicosanoid-abnormality. Since their eicosanoid pattern of the blood was atypical in about 60% compared to less than 10% in healthy individuals this finding is pertinent to a special feature also implemented in cells of the peripheral blood (14).

Factors influencing the FET

Familiar accumulation

Little has been investigated regarding factors influencing the FET. There are few data showing an altered pattern of eicosanoids also in the blood of the patient's children. This finding points to some genetic determination.

Physical activity

Only few data also are known with respect to physical activity which obviously is shifting the relation towards patterns typical of diseases. This had to be expected because of the general stress with high levels of adrenaline and cortisol.

Age

With respect to the age it is well known that intolerance is manifesting mostly in adults. Since analysis of the blood of individuals with and without

diseases shows the same pattern over years it has to be suggested that this peculiarity was already in the patient before the manifestation.

Infection

There is some evidence that systemic infection with fever influences the FET. However, there are no consistent data regarding type or length of the infectious diseases.

Other diseases

Metabolic diseases or functional disorders of liver or kidney are candidates to change the eicosanoid-pattern. However, no study has been performed to answer this question. The same is true regarding autoimmunopathy.

Medication

All drugs influencing the generation and the metabolism of arachidonic acid change the evaluation of the FET.

Food

The generation of eicosanoids and the distribution of its metabolites are influenced by factors inhibiting or stimulating the appropriate enzymes. In a similar way it also depends on the availability of essential materials. So food containing omega-fatty-acids reduce the capability to transform compounds to eicosanoids. A second effect is the content of salicylic acid in plants. Both reduce the biologic power of the eicosanoids or singular groups. This is pertinent to the observation that patients benefit to some degree from diet (15). Of course, this corresponds with the extent of the deviation regarding their eicosanoid-pattern. Therefore it is understandable that this effect has been reported in diseases of the skin (16), of the joints (17) and of the gut (18). Interestingly, also a benefit has been found in patients suffering from multiple sclerosis (19).

The range of factors influencing the results of the FET is summarized in *Table 2*.

Table 2. Range of influencing factors

FACTORS INFLUENCING THE FET

- **Exogenous**
 - Physical activity
 - Stress
 - Food
 - Medication
- **Endogenous**
 - Infection
 - Concomitant diseases
 - Age

CONCLUSION

FET for diagnostic help

Different situations like vague suspicious complaints or diseases at risk for exposition or provocation need diagnostic help. Presently there is only one modification of the FET adapted to intolerance to ASS.

Basis of pathophysiological considerations

The FET was developed for diagnostic use of diseases which were identified by epidemiological means. Initially, tissue of the affected organ has been investigated. After the detection of corresponding results when blood of the patient was used it was possible to generate data of a great number of individuals suffering from different diseases. Meanwhile a lot of data have been accumulated by application of the FET. Still lacking are data *e.g.* of patients suffering from systemic inflammatory diseases like HELLP-syndrome or algodystrophy whereas investigations concerning sepsis/SIRS render first results.

After all it has to be discussed whether one test is representative for a singular pathophysiological event. If it is so all diseases with identical results using the same test are akin. Therefore the FET might contribute to define and assign diseases of unknown origin.

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