Abstract

As proinflammatory cytokines released during ischaemia are detrimental to the brain, the study aimed to evaluate serum interleukin-18 (IL-18) levels in stroke patients and to investigate the relation between these and epidemiological and clinical data. The study comprised 23 ischaemic stroke patients and 15 controls. Blood sampling for IL-18 determination and for chemistry, and brain CT were performed within 24 h of stroke, while neurological stroke severity and functional disability were estimated, respectively, with the Scandinavian stroke scale (SSS) and Barthel index (BI) within the same interval and two weeks later. There were higher serum IL-18 levels in stroke patients. These correlated with erythrocyte sedimentation rate (ESR), brain CT hypodense area volumes, and SSS and BI scores calculated at both studied times. Moreover, IL-18 levels were higher in patients with non-lacunar stroke subtype than in those with lacunar strokes. The results suggest that IL-18 is involved in stroke-induced inflammation and that initial serum IL-18 levels may be predictive of stroke outcome.

Keywords

Cytokines • Interleukin-18 • Stroke

Introduction

Proinflammatory cytokines released following ischaemia by activated brain resident and peripheral blood cells are important mediators of immunological and inflammatory responses, the processes exacerbating cerebral damage with regional influx of leukocytes [1, 2]. Several studies have reported elevations of proinflammatory cytokines in peripheral blood of acute ischaemic stroke patients [2, 3]. Moreover, the magnitude of the cytokine response in human studies correlates with the brain infarct size and with stroke severity and outcome [4, 5].

Interleukin-18 (IL-18), initially described as interferon-gamma inducing factor, is a novel proinflammatory cytokine of the IL-1 family and is able to induce gene expression and synthesis of proinflammatory cytokines, CC/CXC chemokines, adhesion molecules, and Fas ligand [6], as well as to activate neutrophils [7], monocytes [8], macrophages, and T and NK cells [9]. IL-18 is synthesised in the peripheral immune system by macrophage cells and human peripheral blood mononuclear cells or by the first line of immune defense of central nervous system, i.e. microglia [9, 10]. Regarding brain cells, IL-18 may also be synthesised by astrocytes [11], neurons and ependymal cells [12].

It is therefore intriguing to consider that IL-18, like other proinflammatory cytokines, may be involved in the pathophysiology of acute cerebral ischaemia. In this paper we present IL-18 levels in the serum of ischaemic stroke patients within 24 h after the onset of the disease and compare the results with those of a control group. Moreover, we demonstrate a relation between serum IL-18 levels in stroke patients and the epidemiological and clinical data, including stroke risk factors, peripheral indices of inflammation, volume of early CT signs of brain infarction, stroke severity, its outcome and stroke subtypes.
Material and methods

Patients

We studied 23 patients with first-ever acute ischaemic stroke and 15 controls diagnosed with tension headache. Stroke patients were admitted between 6 and 20 h (median, 12 h) after the onset of symptoms. After admission, blood samples were obtained from each stroke patient within the next 30 min, brain computed tomography (CT) was performed within the next 30 min, and the evaluation of stroke severity and disability were performed within the next 60 min.

All patients had complete ischaemic stroke defined as clinical symptoms persisting for >24 h [13] and confined to the carotid artery territory. To avoid the enrollment of patients with concurrent diseases or conditions interfering with the expression of inflammatory mediators, the following exclusion criteria were applied: presence of infections, other inflammatory, autoimmune, haematological or malignant diseases, severe renal or liver failure, tissue injury-related conditions (e.g. myocardial infarction or surgical interventions) within the last year, immunosuppression and treatment with anti-inflammatory drugs within the last six months, deep vein thrombosis, psychiatric disorders, malnutrition, intoxication or addiction. The same exclusion criteria were applied to the controls in which, additionally, any medication within the last six months was not permitted. As acute stroke patients are prone to infections, especially of the chest and urinary tract [14], we included only normothermic (normothermia range, 36.6°–37.5° C [15]) patients with no signs of infections on physical examination, on chest radiography and urine tests performed after admission and repeated during the observational interval of two weeks after stroke onset. There were also no inflammatory changes in other tests performed, if appropriate (e.g. paranasal sinuses radiography or echocardiography) and no further increases in erythrocyte sedimentation rate (ESR) or in leukocyte counts as compared to the initial data.

Laboratory procedures

Blood samples from stroke patients were collected within 24 h of onset of disease symptoms. Blood samples were also obtained from tension headache patients. The samples obtained by the antecubital venipuncture were allowed to clot at room temperature for 30 min, and after being centrifuged for 10 min, the serum was immediately frozen and stored at -80°C. IL-18 levels in serum samples were quantified by ELISA (MBL, Naka-ku Nagoya, Japan) according to the manufacturer’s instructions. The sensitivity of the method was 12.5 pg/ml. Serum samples from each individual were analysed twice.

Parts of the blood samples served for the determination of ESR measurement (by the Westergren method) and leukocyte count (by an automated haematology analyser).

Evaluation of the volume of early brain CT hypodense areas

Early hemispheric brain CT hypodense areas evidenced within 24 h after the onset of stroke represent both the post-ischaemic oedema [16] as well as irreversibly damaged tissue [17] and they are considered early CT signs of hemispheric brain infarction [17]. CT brain scans were obtained parallel to the orbitomeatal line using 10 mm (supratentorial) and 5 mm (infratentorial) slice thicknesses. The volume (in cm³) of early CT hypodense areas was calculated according to the formula based on length x depth x height (in mm) [18]. CT scans were reviewed by a neuroradiologist blinded to the clinical and laboratory data. Each ischaemic stroke patient (except one with radiologically invisible changes) presented anatomically relevant single early CT hypodense area localised in cerebral hemisphere. There were no other early CT hypodense changes, CT signs of old lesions, silent infarcts, and white matter disease. The measurements of the hypodense areas and calculations of their volume were performed twice with differences not exceeding 5%. In all control patients brain CT was also performed and there were no pathological changes.

Evaluation of neurological stroke severity and stroke-related functional disability

The neurological stroke severity was determined with the Scandinavian stroke scale (SSS) [19] within 24 h after the disease onset and two weeks after the beginning of symptoms, with the latter determination being considered neurological stroke outcome. SSS has a score ranging from 58 (normal neurological status) to 0 (maximal neurological deficit). The functional disability of stroke patients was determined with the Barthel index (BI) [20] within 24 h after the disease onset and two weeks after the beginning of symptoms, with the latter determination being considered functional stroke outcome. BI has a score ranging from 100 (no disability) to 0 (complete dependence in activities of daily life).

Determination of ischaemic stroke subtype

We determine the ischaemic stroke subtype according to TOAST definitions [21]: 12 strokes (52%) were identified as lacunar, 9 (39%) as atherosclerotic, and 2 (9%) as cardioembolic. Because in our study the number of patients with the cardioembolic subtype of stroke was relatively small and in view of previous evidence indicating the low frequency of any cardiac sources of embolism in lacunar infarcts [22], stroke subtype was dichotomized as lacunar (n=12; 52%) and non-lacunar (n=11; 48%). After blood sampling, brain CT, and estimation of initial neurological stroke severity and functional disability, antiplatelet drugs were administered to patients with atherosclerotic and lacunar infarcts and while anticoagulants were given to those with cardioembolic infarcts.

The study was conducted according to the principles established in the Declaration of Helsinki and was approved by the Ethics Committee of the University School of Medicine in Poznan. Both stroke and control patients gave informed consent prior to their inclusion into the study.

Statistical analysis

For a comparison of the data in Table 1, Student’s t or Mann-Whitney U test was used as appropriate, except for comparisons of