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Plenary article

Dynamics of oxidative stress and urinary excretion of melatonin and its metabolites during acute ischemic stroke

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ABSTRACT

Oxidative stress is a leading cause of neuronal damage in ischemic stroke. Melatonin may play a role in the antioxidant response. Melatonin and its metabolites may be involved in the modulation of oxidative stress in human acute stroke. No data are available in humans to establish this relationship. In this context, on the first and the fifth days post-stroke, we assessed serum total antioxidant capacity (TAC) and urine levels of melatonin, 6-sulfatoxymelatonin (aMT6S), and N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), the last compound being produced in the brain after reaction of melatonin with reactive oxygen species. Compared to controls' values, TAC and levels of melatonin and aMT6S were reduced, without difference between the first and the fifth days post-stroke, whereas AFMK levels remained in the normal range at both time points. Melatonin catabolism might be speeded up in acute ischemic stroke in order to increase the antioxidant response.

1. Introduction

Oxidative stress is an imbalance between free reactive oxygen species (ROS) and their neutralization by antioxidant defense systems. The ischemic cascade in stroke involves several mechanisms (excitotoxicity, inflammation, and mitochondrial dysfunction) and increased oxidative stress. In addition, defense against oxidative radicals is impaired [2]. Melatonin, an indolamine hormone mainly secreted by the pineal gland, can directly and indirectly detoxify free radicals [24] and contributes to the serum total antioxidant capacity (TAC) in humans [4]. Especially, a low affinity cytosolic binding site for the radiolabelled 2-[125I]-iodomelatonin designated as MT3 was identified as the same protein as human oxidoreductase 2 (QR2 protein) [6]. QR2 is also known as *N*-ribosyldihydronicotinamide: quinone oxidoreductase 2 (EC1.10.99.2) and is a detoxifying and antioxidant enzyme. Further, this hormone shows protective effects against ischemic damage in animals [19]. Melatonin treatment improved the survival rate and neural functioning of the mice by reduction of stroke-induced free radical production [9]. Nocturnal urine levels of melatonin and of 6-sulfatoxymelatonin (aMT6S), the main hepatic metabolite of melatonin, are both indexes of the hormone secretion [5,22]. N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) is generated from melatonin via several pathways including enzymatic, pseudo-enzymatic and interaction with a variety of ROS [26]. It displays an in vitro antioxidant capacity and is the last melatonin-related compound taking part in the process by which melatonin and its metabolites successively scavenge ROS, referred as the free radical scavenging cascade [27]. In order to counteract ROS overgeneration, melatonin catabolism might be increased after stroke injury and could lead to increased production of AFMK. In a previous study, we reported decreased melatonin and aMT6S excretion during the acute stage of ischemic stroke [25]. In this paper, we assessed kinetics of serum TAC and urine AFMK, melatonin and aMT6S levels in acute stroke.

2. Materials and methods

2.1. Patients and controls

Consecutive patients with a first hemispheric ischemic stroke were prospectively included. Exclusion criteria were a past medical history of stroke, renal or hepatic failure, or acute hemorrhagic stroke. The neurological status of the patients was evaluated using the National Institute of Health Stroke Scale (NIHSS) at baseline

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(Day 0), on day 1 (D1) and day 5 (D5). Acute ischemic stroke was documented by multimodal brain MRI (DWI, gradient echo, FLAIR, and MR angiography). The study was approved by the Local Ethical Board and all patients gave written consent before enrolment. The control groups consisted of healthy volunteers with an age range of 60–70 years for the study of melatonin levels ($n = 50$), aMT6S levels ($n = 81$), and AFMK levels ($n = 10$) and an age range of 50–70 years for the TAC study ($n = 49$).

2.2. Melatonin-related compound assays

Urine samples were collected from 8 pm to 8 am during the first and the fifth nights after stroke (N1 and N5). Light intensity during urine collection was maintained below 50 lx in order to limit inhibition of melatonin secretion. Melatonin, aMT6S, and AFMK levels were determined by radioimmunoassay as described previously [7,16,17]. AFMK assay included a previous extraction followed by partition chromatography on celite column, in order to improve specificity of radioimmunoassay. Results are expressed as ng/h, which integrates the volume and time span of urine collection.

2.3. Oxidative stress assay

We measured the TAC in serum of blood sampled at 7 am on D1 and D5. A subgroup of patients was assessed on D0 before administration of recombinant human tissue plasminogen activator [r-tPA; 0.9 mg/kg intravenous, starting 0–3 h (average 2 h 40 min) after stroke]. The principle of the measurement is to analyze the speed of neutralization of secondary reactive oxygen species and/or peroxides (SOS) induced by photodynamically induced singlet oxygens ($^1O_2$) (produced by addition of a final concentration of 5 μg/ml of rose Bengal to 5% serum and irradiation at 514 nm at 20 J/cm^2) by measuring the fluorescent product DCF generated from DCFH. Activated DCFH was added to each sample immediately after the end of light delivery and the area under the curve (AUC) for the change in DCF fluorescence (excitation 488 nm, emission 525 nm) over time measured [21]. Values were corrected for hemolysis (measured at 413 nm) and baseline absorbance (at 650 nm), then the corrected value was divided by the DCF fluorescence AUC value for a serum pool from 75 healthy donors used as a reference. A ratio higher than 1 indicates a greater AUC fluorescence than the reference pool used and thus a lower capacity of the given serum to neutralize $^1O_2$ and SOS as a function of time, whereas a lower value indicated a higher capacity.

2.4. Statistical analysis

As the data were not normally distributed (Shapiro test), the results are expressed as the median and interquartile range. Wilcoxon and Friedmann tests were used to compare medians. Statistical significance was set at $p < 0.05$. All tests were performed using R v.2.13.0. (R development core team, Vienna, Austria, http://www.r-project.org/).

3. Results

3.1. Patients

Between May 2009 and December 2010, 75 patients were enrolled in the study, of which 33 were excluded (19 because of a lack of a urine sample, 12 were discharged before D5, and 2 died before D5), leaving 42 who completed the protocol; these consisted of 15 women and 27 men (age range: 27.7–88.5 years; median age = 73.1 years). Vascular risk factors included hyperlipemia ($n = 20$), hypertension ($n = 16$), and diabetes mellitus ($n = 6$). NIHSS scores on D0, D1, and D5 were, respectively, 12.00 [7.25–17.00], 8.00 [3.00–16.75], and 6.00 [2.25–13.75]. Each patient was classified according to TOAST criteria [1]: stroke was related to a cardioembolic source in 20, large-artery atherosclerosis in 16, small-vessel occlusion in 1, or a rare cause of stroke in 2, or was

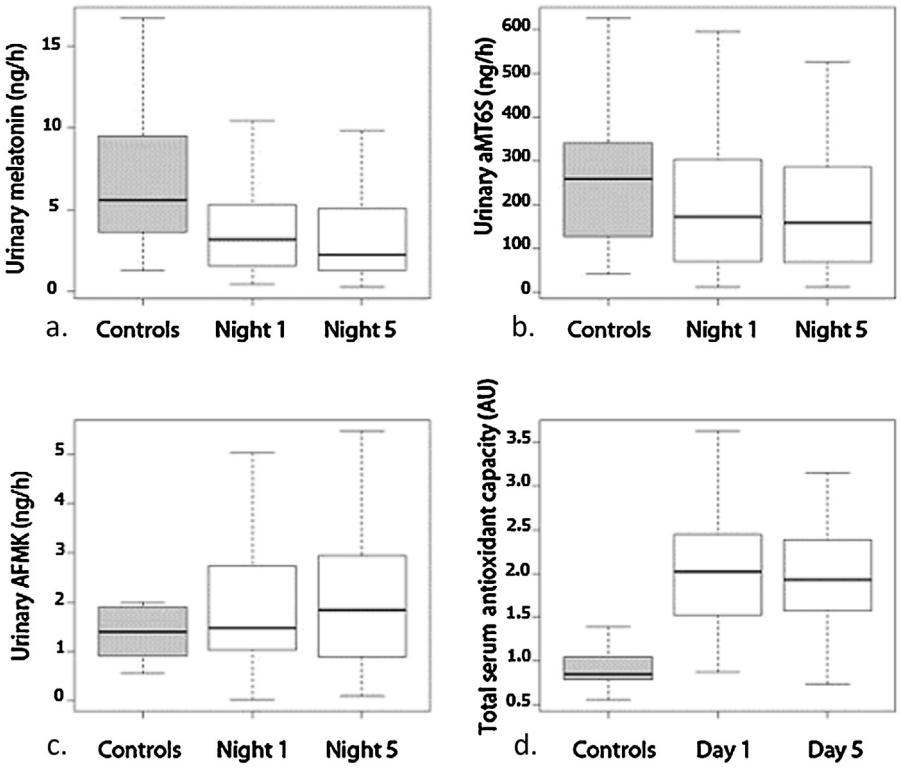

Fig. 1. Urinary excretion of melatonin (a), aMT6S (b), or AFMK (c) and the TAC (d) in controls and patients on day 1 and day 5. AUC ratio higher than 1 indicates a lower TAC.

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