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SIDE EFFECTS OF STATINS, INCLUDING NEUROLOGICAL DISORDERS: NEW ADVANCES

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ABSTRACT

Statins are drugs that, through blocking hydroxy-methylglutaryl coenzyme A reductase, can decrease low-density lipoprotein (LDL) and triglyceride blood levels. With apparent efficacy, these drugs protect blood vessels and reduce the risk of cardiovascular events and strokes. However, like other drugs, statins can cause side effects, such as muscle pain, diabetes, gastrointestinal disorders, dementia and cognitive diseases, even if there is contradictory evidence that statins by lowering cholesterol levels may be protective in the brain. Here, we report some side effects of statins and their impact on the central nervous system (CNS).

KEYWORDS: *statin, side effect, neurological, CNS, dementia*

INTRODUCTION

Statins are a class of drugs that lower low-density lipoprotein (LDL) cholesterol in subjects with aortic, coronary, or carotid stenosis and protect against ischemic attacks, strokes, and cardiovascular events. These drugs have beneficial effects, reducing the frequency of heart attacks and lowering the frequency of mortality (1). Statins are hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors which the body tolerates very well. However, they cause side effects such as muscle symptoms, diabetes mellitus, and brain disorders. The risks of side effects due to these lipid-lowering drugs are less than the benefits of lowering cholesterol levels. Patients taking statins may present myalgia in 9-20% of cases and may develop, albeit rarely, autoimmune myopathy and rhabdomyolysis (2). In addition, antibodies to HMG-CoA reductase may be generated, which could cause the symptoms associated with statins. However, the diagnosis of statin side effects is complex, and if these effects do occur, switching treatment to other lipid-lowering drugs is advisable.

Rhabdomyolysis

Rhabdomyolysis can occur (with a frequency of less than 10%) or can be amplified in patients taking statins (3). It is a disease involving skeletal muscle tissue breakdown with symptoms of muscle weakness, myalgias, and reddish-brown urine. In this disease, there may be a nonspecific increase in creatine kinase (CK) levels with renal impairment, representing a diagnostic element. CK, also called creatine phosphokinase (CPK), is an enzyme produced by a striated muscle that catalyzes the reaction of creatinine into phosphocreatine with the consumption of ATP. CK is found in the bloodstream after muscular exertion or after taking statins (4). The increase in CK can occur after prolonged physical exercise in athletes who do not take statins. Elevated CK levels represent a sensitive laboratory index of muscle injury and often aid in diagnosing statin-associated symptoms. Therefore, the statin treatment of patients with high CK levels

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should always be considered. The appearance of rhabdomyolysis in subjects taking statins is very low and depends on the dose, advanced age, physical disability, a lower body mass index, and on the general physical condition of the patient (5). There is a correlation between dosage and symptoms; the higher the dose, the higher the frequency of statin-associated symptoms (2). Statins are mainly processed and catabolized in the liver by cytochrome P450 which transforms lipids into hydrophilic complexes that are subsequently eliminated (6) (Fig.1).

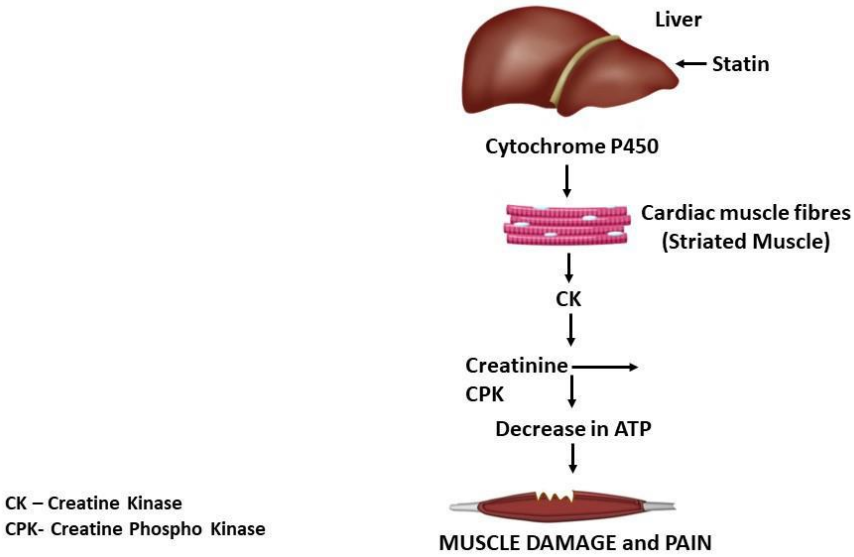

Fig. 1. *Statin is processed and catabolized through cytochrome P450 in the liver and increases creatine kinase, an enzyme produced by striated muscle which converts creatinine into phospho creatinine through the consumption of ATP. These reactions lead to muscle damage and pain.*

Diabetes mellitus

Additionally, it has been reported that subjects treated with statins may develop diabetes mellitus with blood glucose values above 100 mg/dl (7); this appears to have a higher incidence in older women than in men. The mechanism of action of statins on diabetes mellitus is not yet clear, but it is known that low blood cholesterol levels are associated with an increased risk of diabetes (8); this is because cholesterol could affect the pancreatic production of insulin with an alteration of glucose metabolism, although the risk is low and exceeded by the beneficial effects of statins (9). Furthermore, in addition to the benefits of statin therapy, their preventive use could prevent vascular events, including myocardial infarction and stroke, and interrupting therapy could cause brain and heart damage (10).

Neurological complications

Statins can also cause undesirable effects on the brain system and, in particular, on cognition. It is known that increased fat in the bloodstream correlates to the onset of some neurological symptoms, including dementia (11). In some cases, statin treatments reduce the incidence of these brain diseases and therefore protect the brain (12). However, statins appear to reduce cognitive dysfunction risk, which could be important in degenerative diseases such as Alzheimer's (13). Although there are not many studies on the effect of statins on the CNS, cholesterol inhibition could affect brain function, but this is quite unlikely since cholesterol has no active participation in the brain (14). In support of the thesis that statins protect the CNS, data shows that these cholesterol inhibitors can enhance neuron learning function, inhibit amyloid-beta, and reduce brain inflammation (15-17).

Furthermore, low cholesterol levels caused by statin treatment can cause a mild depressive syndrome, also related to the lowering of serotonin (18). However, all these CNS effects of statins still need to be confirmed. Furthermore, because statins can cause muscle problems, they could affect the myocardium, although there are currently no satisfactory scientific articles on the direct effect of these drugs on the myocardium (19). Therefore, it would be interesting to study the biological effects of statins on myocytes *in vitro* to improve their efficacy and possibly reduce the side effects, if any.

CONCLUSIONS

Here, in this short review, we can conclude that statins can increase CK, causing muscle pain, and may have effects on the brain that are not yet clear. However, it seems that they could be protective since the lowering of cholesterol leads to better cerebral blood circulation, resulting in the improvement of cognitive functions and the inhibition of inflammatory parameters (20).

Conflict of interest

The author declares that they have no conflict of interest.

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IMPACT OF INSULIN-LIKE GROWTH FACTORS 1 AND 2 IN THE INFLAMMATORY RESPONSE MEDIATED BY CYTOKINES

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ABSTRACT

In inflammatory disorders such as pancreatitis, immune cells are activated and produce pro-inflammatory cytokines and chemokines, including IL-1, IL-6, and tumor necrosis factor (TNF). These cytokines mediate the immune response while insulin-like growth factors (IGFs), hormones that promote physiological growth, also participate in the inflammatory response. Macrophages play an important role in this process since polarized M1 macrophages provoke acute inflammation, while the polarized M2 type is involved in the anti-inflammatory response and the development of tissue fibrosis. Neuroendocrine and metabolic responses are also present during the inflammatory process, and there is pituitary generation of IGF. A mitogen-activated protein kinase (MAPK) phosphorylation cascade is activated with the binding of IGF-1 to its receptor IGF-1R, which leads to gene expression and key biological responses such as cell proliferation. An increase of IGF-2 and its receptor IGF-2R is associated with diverse biological effects including hypertrophy and cardiac contraction, increases in cardiomyocytes and cardiomyocyte apoptosis, and decreased muscle regeneration and capillary remodeling, amongst others. IGF-2 also mediates chondrocyte hypertrophy with an increase in oxidative phosphorylation, as the inhibition of this process damages hypertrophic differentiation. In this article, we discuss the impact of IGFs in the inflammatory response that is mediated by cytokines.

KEYWORDS: *insulin-like growth factor, inflammation, cytokine, myokine, IGF-1, IGF-2, macrophage, IL-1, TNF*

INTRODUCTION

Numerous inflammatory mediators, such as pro-inflammatory cytokines, are involved in pancreatitis where immune cells are activated, including macrophages that produce IL-1, tumor necrosis factor (TNF), and other pro-inflammatory cytokines and chemokines. Macrophages are known to play an important role in the pathogenesis of pancreatitis. They can be polarized as M1, that become protagonists of acute inflammation, while those that are polarized as M2 participate in the anti-inflammatory process and promote the development of pancreatic tissue fibrosis (1).

In addition, activated macrophages, in collaboration with IL-18 and IL-3, can stimulate mast cells (MCs) to produce IL-4 and IL-13 cytokines which increase the number of M2 macrophages (2) (Fig.1).

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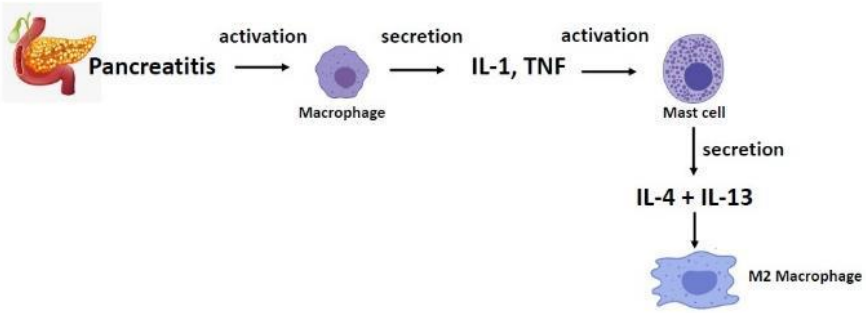

Fig. 1. *Diagram depicting the involvement of macrophages and mast cells in the pathogenesis of pancreatitis. Macrophages (M1) become activated and secrete IL-1 and tumor necrosis factor (TNF) amongst other pro-inflammatory cytokines and chemokines. These cytokines activate mast cells (MCs), which secrete IL-4 and IL-13, increasing the amount of M2 macrophages.*

Activated M2 macrophages are divided into M2a, M2b, M2c and M2d and are mainly involved in anti-inflammatory responses, while M1 macrophages are involved in pro-inflammatory ones (1). In acute pancreatitis, damaged pancreatic cells release molecules such as zymogen, trypsin, and other cell degradation compounds which activate and recruit macrophages that secrete pro-inflammatory cytokines such as IL-1, TNF, IL-6, interferon-γ (IFNγ), and IL- 18, which participate in the resulting damage (3). The cytokines that are released from the muscle are called myokines and regulate myogenesis and muscle hypertrophy. Myokines can have a systemic action on the immune system and on the adipose tissue that accumulates in the viscera following physical inactivity. The accumulation of adipose tissue results in low-grade inflammation mediated by the recruitment of monokine-releasing macrophages including TNF, IL-1, and IL-6 (4). On the other hand, physical activity activates muscles to produce myokines, such as the above cytokines, which participate in muscle growth and hypertrophy. Physical activity increases insulin sensitivity and fat oxidation leading to the reduction of the inflammatory state.

Insulin growth factor binding proteins (IGFBPs) bind insulin-like growth factor (IGF)-1 and IGF-2 with high affinity, causing biological effects (5). IGF is ubiquitous in all human and rodent fetal tissues and tends to decrease after birth, although, in humans, the serum and tissue levels of IGF-2 remain elevated (6).

IGF-1

IGF-1 is a growth factor of human muscles and bones that is synthesized in the liver and carries out its biological activity by binding to the IGF-1R receptor. IGF-1 mediates bone homeostasis and activates the mitogen-activated protein kinase (MAPK) pathway resulting in cell proliferation (7). MAPK is important in signal transduction and phosphorylation, crucial biological activities for cell signaling (8) (Fig.2).

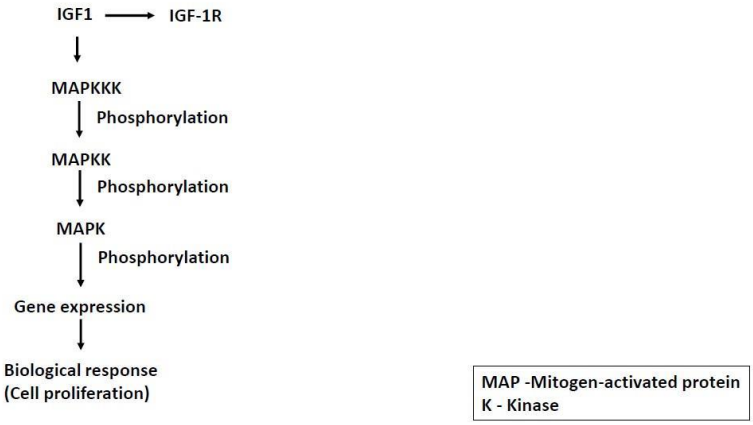

Fig. 2. *Insulin growth factor (IGF)-1 binds to the IGF-R1 receptor, which results in the activation of the mitogen-activated protein kinase (MAPK) pathway with protein kinase cascades; activation of a MAPK kinase kinase (MAPKKK) phosphorylates and activates the MAPKK, which, in turn, activates the MAPK that phosphorylates different substrates in the cytosol and nucleus, resulting in changes in gene expression that cause the biological response of cell proliferation.*

IGF-1 contributes to maintaining bone homeostasis and induces osteogenesis by acting on osteoclasts through receptor activator of nuclear factor kappa beta (RANKL) in *in vitro* experiments (9). In some experiments it has been observed that the overexposed IGF-1 in the osteoblasts leads to an elongation of the bones, while its inhibition reduces bone volume (10). It has been reported that IGF-1 is important for cell development such as chondrocytes and bone growth and plays a crucial role in metabolism by regulating energy function by acting on mitochondria (11). This effect would increase cell proliferation and differentiation, as well as protein synthesis (10). It appears that IGF-1 is instrumental in osteoblast differentiation since the lack of the IGF-1R receptor inhibits bone development. Therefore, IGF-1 is important for the correct development of the skeleton and is correlated with increased levels of this myokine in the muscle where it participates in its growth. In fact, a decrease of IGF-1 in muscle, where it is abundant, can lead to a reduction in bone development (12). Therefore, IGF-1 can be of ubiquitous circulating origin, and is particularly present in bone and muscle, playing a crucial role in bone metabolism.

IGF-2

IGF-2 is an endocrine hormone included in the family of three hormones that possess an insulin-like structure. In humans, the IGF-2 gene is located on chromosome 11 at position p15.5, while in mice, the gene is located on chromosome 7.This peptide has biological activity similar to insulin, with mitogenic and cell growth regulating activity (13). IGF-2 ispart of the IGF family made up of IGF-1 and IGF-2, whose biological activity is regulated by 6 proteins ranging from IGFBP-1 to 6 (14).

IGF-2 is the ubiquitous ligand of the IGF-2R receptor and has a crucial role in the physiological development and differentiation of the mouse embryo mesoderm (15). In rodents, IGF-2 is a highly expressed embryonic growth factor, already present in the first days of embryonic development, while it is decreased after birth (16, 17). The binding of IGF-2 to its receptor IGF-2R could favor both tumor growth and onset and influence proliferative cellular pathologies (18). In various inflammatory diseases, such as muscle, rheumatic and cardiac diseases, it has been observed that the expression of IGF-2R is increased, favoring these pathological phenomena (19). Therefore, targeting the IGF-2R receptor could be a new therapeutic approach for proliferative cellular diseases.

The increase of IGF-2 has an important positive action on the development of apoptosis, a phenomenon that occurs in tumors and where IGF-2 and its receptor IGF-2R could have a tumor suppressor effect. Lack of expression of the IGF-2R gene delays the onset of breast cancer, but also the onset of other cancers, an effect that could be inconsistent with the activity of IGF-2 in cervical tumors or glioblastoma, where it acts as an oncogene (20). Inflammation and fibrosis can occur in dystrophic muscle with consequent overexpression of IGF-2 with action on fibroblasts, pericyte endothelial cells, myocytes, and cardiomyocytes (21, 22). In the repair process, immune cells such as macrophages, the complement system, MCs, lymphocytes and neutrophils are involved. The recruitment of these cells regulates the activation, proliferation, and differentiation of muscle cells (Table I).

Table I. *Some biological effects caused by an increase of IGF-2 after binding its receptor IGF-2R.*

Increase of:	• Hypertrophy and cardiac contraction • Cardiomyocyte apoptosis • Size of cardiomyocytes • EPC migration, adhesion, and invasion • Elasticity and adhesion of the vascular SHC • Development of tumors
Decrease of:	• Muscle regeneration • Capillary remodeling • Muscle force

IGF-2R performs an important function in suppressing IGF-1 signaling, an effect that could be useful against tumor development (23).

Compared to IGF-1, IGF-2 appears to be more effective against apoptosis (or programmed cell death) in the cells of the placenta. IGF-1 promotes the growth of muscle, which is a major storehouse of energy molecules required for

gluconeogenesis. IGF-1 is an important hormone for the energy system and the immune system, regulating the synthesis of some minerals (24). The factors responsible for the storage of energy-rich fuels include insulin IGF-1, testosterone, estrogens, vitamin D, and others, while the factors involved in their release include the renin-angiotensin-aldosterone system, the sympathetic nervous system, the hypothalamic-pituitary-thyroid axis, and the hypothalamic-pituitary-adrenal axis (25).

Somatotropin regulates IGF-2 during intrauterine life, promoting growth during gestation, in contrast to IGF-1, which is synthesized later in adult life. IGF-2 carries out its biological activity through binding to its receptor IGF-1R. During the maturation process of the follicles, the connective cells synthesize and secrete IGF-2, promoting follicular proliferation together with the hormone follicular stimulating hormone (FSH) (26). Subsequently, after ovulation, IGF-2 stimulates the secretion of the hormone progesterone which acts synergistically with the luteinizing hormone (LH) (27). IGF-2 has also been reported to promote survival of hippocampal neurons in newborns, a finding that may have therapeutic implications (28).

By acting on mitochondria, IGF-2 has anti-apoptotic properties, inhibiting cell death and improving the survival of cells such as islet transplantation and cartilage cells (29). It has been reported that physical activity allows for important physiological development through the muscle production of cytokines (also called myokines), including IGFs that perform an anti-inflammatory action (30).

The inflammatory process and the role of pro-inflammatory cytokines

During the inflammatory process, there are neuroendocrine and metabolic responses, and pituitary generation of IGF, which allows for cell survival. Cytokines have autocrine, paracrine, or endocrine effects and include IGF-1 and IGF-2 molecules, which are involved in enhancing vascular endothelial activity in ischemic vessels (31). IGF-1 and IGF-2 are found in various tissues, including muscle tissue, which acts in an endocrine way by targeting other organs including the kidneys, and therefore, could carry out a crosstalk between muscle and bone cells (32).

Indeed, it seems that IGF-1 does not protect against the pro-inflammatory and apoptotic action induced by cytokines. A protective effect occurs with IGF-2, which by binding to the IGF-1R receptor, induces IL-10, an anti-inflammatory cytokine which protects islet transplantation and improves survival (18).

In rheumatic diseases, the severity of the pathological state may depend on the balance between pro-inflammatory and anti-inflammatory cytokines. In rheumatoid arthritis (RA), the joint-inflamed site harbors many pro-inflammatory cytokines that belong to the IL-1 family such as IL-1, IL-18, IL-33, IL-36α, IL-36β and IL-36γ; but anti-inflammatory anti-receptors can also be found, such as the IL-1 receptor antagonist (IL-1Ra) and IL-36 receptor antagonist (IL-36Ra), and two cytokines, IL-37 and IL- 38, which inhibit innate immunity and inflammation. IL-37 acts as a suppressor by inhibiting mammalian target of rapamycin (mTOR) and increases AMP kinase activity. IL-38 performs its anti-inflammatory activity by binding to the IL-1R6 receptor, a complex that causes the recruitment of IL-1R9.

IGF-2 regulates bone development by acting on chondrocytes, osteoblasts, osteocytes, and osteoclasts, and is implicated in skeletal ageing (33). In fact, the serum levels of IGF-2 tend to decrease with the ageing of the bones, an action that takes place with the reduction of the mineral bone density (33).

IGFBP is involved in many biological activities including cartilage failure during osteoarthritis (OA) (34). In the synovial fluid and cartilage of OA patients, it was seen that IGFBP levels were increased (35), an effect that implicates IGF-1 and 2 as important factors in inflammation. IGFBP overexpression could influence the availability of IGFs with alteration of the chondrocyte vital pathway.

Chondrocyte hypertrophy is characterized by an increase in oxidative phosphorylation which is regulated by IGF-2. In a recent interesting article, Hollander JM et al., reported that the chondrocyte maturation during cartilage development indicates that the inhibition of oxidative phosphorylation in murine chondrocytes can damage hypertrophic differentiation (36). These authors reported that an IGF-2 deficiency can result in increased oxidative phosphorylation in hypertrophic chondrocytes. The results demonstrate that IGF-2 is important in evading excessive glucose metabolism and is determinant for bone development (36).

CONCLUSIONS

In conclusion, the inflammatory cytokines IL-1 and TNF mediate the immune response with the participation of IGFs. During inflammation, there are also neuroendocrine and metabolic responses with pituitary generation of IGF. Furthermore, IGF-1 and IGF-2 have different biological effects. IGF-1 mediates bone homeostasis and activates the MAPK pathway, resulting in cell proliferation. IGF-2 plays a crucial role in the physiological development and

differentiation of the mouse embryo mesoderm, favoring tumor growth and influencing cellular proliferative pathologies. A protective effect of IGF-2 is induced by the activation of IL-10, while IGF-1 does not protect against pro-inflammatory and apoptotic responses. Moreover, IGF-2 mediates chondrocyte hypertrophy with an increase in oxidative phosphorylation.

Conflict of interest

The authors declare that they have no conflict of interest.

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PILOT STUDY FOR THE ANALYSIS OF THE ORAL MICROBIOTA

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ABSTRACT

The human oral microbiota, comprising a complex community of microorganisms, plays a crucial role in oral and systemic health. This study aims to create a biobank and database to characterise the diversity of oral microbiota using the sequencing of bacterial DNA. The primary objective is to assess the heterogeneity of oral microbiota among individuals by recording bacterial species. The secondary objective is to analyse the interplay between bacterial species and their potential involvement in systemic and musculoskeletal pathologies. Inclusion criteria involve patients aged 18 and above with negative bleeding on probing (BOP) test and absence of gingival inflammation. Clinical assessments and oral swabs will be collected, followed by DNA extraction and library preparation for sequencing. The bioinformatics analysis will identify and classify bacterial species using the 16S rRNA gene and reference databases. We believe the study will shed light on the oral microbiota's biodiversity, facilitating a better understanding of its impact on health and disease through advanced sequencing and data analysis techniques. The establishment of a biobank will provide a valuable resource for future investigations in oral microbiota research.

KEYWORDS: *microbiota, microorganisms, bacteria, DNA, oral cavity*

INTRODUCTION

Human oral microbiota is the ecological community of commensal, symbiotic, and pathogenic microorganisms in the oral cavity (1). The oral microbiome is an ensemble of more than 1,000 different microorganism genomes in the oral cavity (2, 3).

It is widely accepted that oral microorganisms are responsible for various diseases, mainly by a synergistic or cooperative manner, and the interspecies interactions within the oral community play a crucial role in determining whether oral microbiota elicit diseases or not (4-6). The oral microbiota is also associated with several systemic diseases, namely cardiovascular disease, pneumonia, heart disease, metabolic syndrome, rheumatoid arthritis, pancreatic cancer, colorectal cancer, esophageal cancer, and stroke (1, 4).

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For this study, we have designed a study protocol with the aim of creating a biobank to record the different microorganisms of the oral cavity using modern metagenomics. The primary goal of the study is to create a database with the names of the bacterial species to evaluate the oral microbiota heterogeneity between different individuals. The secondary objective of the study will be to evaluate and highlight the presence of a network between the different bacterial species present in these patients and to analyze their dynamics in the development of systemic and musculoskeletal pathologies.

MATERIALS AND METHODS

The inclusion criteria of patients enrolled in this study are as follows: age over 18, negative BOP (bleeding on probing), absence of clinical signs of gingival inflammation (enlarged gingival profiles due to edema or fibrosis, chromatic transition towards a red and/or bluish red hue, increased gingival exudate), absence of plaque and tartar deposits, patients undergoing regular IOP, signature of informed consent for the analysis of the oral microbiome and data processing.

Patients who do not agree to participate in the study will be excluded from this study.

Clinical protocol

A specialist dental check-up will be performed according to the normal clinical practice of the complex operating unit of dentistry at Verona University.

Following an explanation of the purpose of the study and the signing of informed consent, patients will receive, in addition to the dental specialist visit and routine diagnostic radiographs (intraoral radiographs, orthopantomography of the dental arches and CT scan of the dental arches), an oral swab (Swab Collection and DNA Preservation System, Norgen Biotek Corp.) of the oral cavity to sample the microbiota (7).

Sequencing

All bacterial DNA will be purified from the sampling swabs (Microbiome DNA Isolation Kit - Norgen Biotek). We will prepare sequencing libraries (QIAseq 16S/ITS - Qiagen kit) of the 9 hypervariable regions of the gene for the 16S subunit of bacterial ribosomal RNA. Using an Illumina NGS sequencing platform (MiSeqDX), and following bioinformatics and statistical analysis, it will be possible to identify the individual bacterial species in the initial sample (8).

The swabs will be performed directly at the control visit. After being collected, the samples will be analysed in the biology and genetics section of the Department of Neuroscience, Biomedicine, and Movement at the University of Verona.

RESULTS AND DISCUSSION

Bioinformatic analysis

The bioinformatics analysis involves the V3-V4 hypervariable regions of the 16S rRNA gene. We will conduct our analysis working with the Amplicon Sequence Variants (ASVs), inferring DNA sequences within a sample. Each of these sequences belongs to a possible different bacterial species in the human oral cavity.

A taxonomic classification will be assigned to each ASV according to the known reference sequences available in public databases, reaching, if possible, the 7 taxonomic levels (kingdom, phylum, class, order, family, genus, species). The Divisive Amplicon Denoising Algorithm (DADA2) and the pre-trained classifier provided by the Human Oral Microbiome Database (HOMD) will be used to identify microorganisms in the oral cavity (9).

CONCLUSIONS

This study will provide a first glimpse of the extent of the oral microbiota's biodiversity, which has been limited until today. With the creation of a biobank, we could identify a greater number of microorganisms, which would provide effective statistical power for understanding the biological mechanisms underlying the state of health and disease of the oral cavity using modern sequencing and data analysis techniques.

Statement of Ethics

Ethics approval was given by the Verona University Ethics Committee (Prog.3032-CESC).

Conflict of interest

The authors declare that they have no conflict of interest.

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OROFACIAL MOVEMENT DISORDER CAUSED BY PRAMIPEXOLE ABUSE. A CASE REPORT.

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ABSTRACT

Orofacial movement disorders are a group of hyperkinetic extrapyramidal movements presenting dysfunctional activities on the masticatory, facial mimic, or tongue musculatures. The most common cause of acquired orofacial movement disorders is drug-induced dyskinesias. Our report describes a rare case of pramipexole-induced orofacial movement disorders in a patient with restless legs syndrome.

KEYWORDS: *dyskinesia, pramipexole, movement disorder, restless leg syndrome*

INTRODUCTION

Orofacial movement disorders (OMD) are a group of hyperkinetic extrapyramidal movements presenting as isolated or combined dysfunctional activation of the masticatory, facial mimic, or tongue muscles (1). The aetiology of OMD may be genetic, idiopathic, or acquired (2). However, the most common cause of acquired OMD is acute or tardive drug-induced dyskinesias, mainly caused by antidepressants, antiemetics, neuroleptics, or levodopa (3).

The main treatment of dyskinetic/dystonic symptoms is the slow tapering of the offending drug. Pharmacological treatment is mostly empirically based on using tetrabenazine, clonazepam, amantadine, or piracetam. In addition, anticholinergic drugs are useful for associated dystonic symptoms (4). Besides conventional therapies, levodopa-induced dyskinesias (LID) may also benefit from non-ergot dopamine-agonist (DA), clozapine, or antiepileptics (5).

Case presentation

Here we report a case of acute OMD caused by chronic DA abuse in a non-Parkinson's disease (PD) patient under treatment with pramipexole for restless legs syndrome (RLS). A 72-year-old woman was admitted to the emergency room for the appearance, during the last week, of intermittent, involuntary muscle contractions, causing repetitive eye movements, grimacing, pursing of the mouth and lips, and writhing of the tongue with stereotyped vocalizations and sustained neck dystonia.

Neurological examination was otherwise normal. The patient's medical history included RLS, low back pain, diabetes mellitus type 2, and surgical removal of a foot acral melanoma with inguinal lymphadenectomy. Her medical therapy consisted of 1,000 mg metformin daily, 150-200 mg tapentadol daily, and pramipexole prescribed at a 0.18 mg daily dose

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to control RLS, but arbitrarily taken at 0.9 mg daily dose for 2 months due to a self-assessed lack of efficacy. No past or current exposure to other relevant drugs was reported.

The patient underwent a computed tomography scan of the brain and a dopamine transporter single photon emission tomography (DAT SPECT) scan, with normal results. In addition, the patient was prescribed a reduction of pramipexole to 0.18 mg daily, which resulted in the complete resolution of OMD in 4 days.

DISCUSSION

Our report describes a rare case of DA-induced OMD in a patient with RLS due to drug abuse. The mechanism underlying LID is attributed to a specific enhancement of the direct striatopallidal pathway and the inhibition of the indirect striatopallidal pathway, which may be found in PD patients (6). The resulting decreased output of the internal globus pallidus may lead to increased activity in the motor nuclei of the thalamus (7).

A chronically increased dopaminergic tone in RLS may induce a postsynaptic receptor down-regulation, mainly of the indirect pathway (8). Thus, in RLS patients, the relative impairment of dopamine transmission becomes clinically evident in the late hours since circadian dopamine activity is physiologically lower in the evening. Accordingly, a low dose of DA at bedtime is the first-line therapy for RLS (9).

RLS association with PD did not receive sufficient evidence in the literature (10), and our patient did not show any PD-related motor or non-motor symptoms, as often happens in RLS cases (10). Nevertheless, after excessive intake of DA, she developed symptoms that resembled LID, a typical PD complication.

CONCLUSIONS

This report is the first on an OMD following excessive intake of DA in non-PD patients. Follow-up of this patient will address the possible future development of Parkinson's disease.

Statement of Ethics

The authors confirm that the approval of an institutional review board was not required for this work. Written informed consent was obtained from the patient's caregiver to publish this case report. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author Contributions

Conceptualization: DC and LB; methodology: LB, CC, and GN; writing-original draft preparation: DC and MADR; writing-review and editing: DC, MADR, and LB; visualization: DD and LB; supervision: MO, FC, and LB.

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Letter to the Editor

IMMUNITY AND CANCER: IS THE VACCINATION READY FOR USE?

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INTRODUCTION

Cancer is a global disease and a leading cause of death worldwide, second only to cardiovascular disease in Western countries (1). Tumor cells replicate irregularly and form metastasis which tend to invade surrounding and distant tissues. In recent years, there has been a continuous evolution in the field of immunotherapy against tumors. This is also due to new diagnostic techniques and investigations such as positron emission tomography (PET), computed tomography, and magnetic resonance imaging (MRI). Immunotherapy is a therapeutic route often used by researchers that utilizes molecular investigations both for the diagnosis and treatment of tumors.

Immunotherapy makes use of the knowledge of natural killer cells (NK), CD3, CD8, and of the study of Chimeric Antigen Receptor (CAR) T cells, a type of immune cell (now the focus of many laboratories) which utilizes a patient's T lymphocytes which are then genetically modified in a laboratory to allow them bind to cancer cells, attacking the cancer (2). For example, it has been seen that some types of oncolytic viruses destroy tumor cells without killing healthy tissue cells, inducing anti-tumor immune responses with a promising therapeutic strategy (3). The anti-cancer vaccination is making its way in small steps and is based on the use of target antigens, even if these appear very weak and of low immunogenicity. This makes it difficult to induce clinical responses and address the problem of therapeutic tumor vaccination.

Researchers are working to develop therapeutic vaccines against cancer. The use of vaccine vectors, both biological and synthetic, can improve the responses of the immune system regarding T cells, as well as B-lymphocytes and antigen-presenting cells (APC) such as macrophages and dendritic cells (4). These therapeutic strategies act on the mechanisms used by tumor cells to evade and inhibit the immune system. To date, several types of vaccines against tumors have been developed, such as antibodies that attack tumor cells, proteins, peptides, RNA, DNA, and antigens, however these are still under investigation (5). In the wake of the mRNA vaccine used against COVID-19, vaccines of this type have also been used as a new method against tumors (6). These vaccines are based on the inoculation of mRNA which encodes the production of specific tumor antigens by the host with triggering of the immune response (7). By isolating tumor mRNA, this therapy can be adapted to each type of cancer. But before these vaccines can be employed in the clinic, many challenges must be overcome, such as identifying the route of administration, mRNA efficacy, specificity, and side

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effects. Vaccine immunotherapy against tumors has proved to be encouraging, promising, and safe, but phase III results are still needed for the application of these vaccines in clinical oncology.

DISCUSSION

Lethal brain tumors have poor immune responses and are difficult to treat as they produce substances that escape the immune system (8,9). Targeting tumor antigens could reduce the development of the tumor and decrease immunosuppression. Microglia and infiltrating cells such as monocytes and lymphocytes can fuel tumor cell replication by producing pro-inflammatory and immunosuppressive cytokines (10). Targeting the tissue that provides nourishment and support of a malignant tumor, such as vascularized connective tissue and infiltrated immune cells, could be of therapeutic help. However, this stromal system, on one hand, constitutes a defense reaction of the organism to fight cancer; while on the other, it allows the tumor to develop by supplying it with the elements necessary for growth (11). Non-immune cells also contribute to the immunosuppressive environment by producing different compounds. The development of therapeutic vaccines against cancer are engaging many researchers around the world. Although cancer cells evade and suppress the immune system, it is hoped that in the future cancer vaccinations can create effective and side-effect-free anti-tumor immunity.

To date, many types of cancer vaccines have been tested which target a specific type of antigen, but so far none have been completely satisfactory (12). For example, astrocytes are cells that produce factors that feed the growth of tumor cells and favor metastasis, while neurons favor the proliferation of tumor cells by generating molecules such as neuroligin-3, a protein encoded by the NLGN3 gene that can act as a specific ligand of junction site for beta-neurexins and may be involved in central nervous system (CNS) synapses.

In recent decades, cancer therapies have had a significant improvement, also in terms of survival (13). However, despite new treatments, this effect does not apply to brain tumor therapies, which statistically, do not improve survival rates. Neoantigens are specific to the tumor and are generated by expressed gene mutations of the tumor cells (14). Therefore, personalized neoantigen vaccines can be created for therapy against certain types of tumors (15). Neoantigens can generate immune responses causing tumor rejection. In addition, neoantigenic (multi-epitope) vaccination is used for some cancers, including glioblastoma which usually has a relatively low number of mutations. This type of vaccination can stimulate neoantigen-specific CD4+ and CD8+ T lymphocytes, positively modifying the immune system of patients with glioblastoma (16). This method marks a new, encouraging step towards the immunotherapy of brain tumors.

CONCLUSIONS

In conclusion, the progress in the field of tumor immunotherapy and in vaccines against tumor antigens, is a promising new frontier in the battle against cancer. However, the results obtained thus far are not satisfactory and therefore more studies are needed to improve outcomes for this important disease which afflicts the worldwide population.

Conflict of interest

The author declares that they have no conflict of interest.

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OLFACTORY DYSFUNCTION AS A PREDICTOR OF THE FUTURE DEVELOPMENT OF PARKINSONISM IN COVID-19 PATIENTS: A 18F-FDOPA PET STUDY

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ABSTRACT

Background: Among the various clinical manifestations of COVID-19, olfactory dysfunction is reported in 68% of patients, and is persistent in 10%. When inhaled, the virus travels along the olfactory pathway, infects the olfactory bulb and can retrogradely attack, through a direct nigro-olfactory connection, the Substantia Nigra. The evidence of cases of parkinsonism after SARS-CoV-2 infection that are preceded by olfactory dysfunction highlights a possible link between SARS-CoV-2 infection and the subsequent development of parkinsonism. Here, we report two cases out of 4 patients with long-term olfactory dysfunction, persistent at 4-9 months after SARS- CoV-2 infection, who presented with dopaminergic imaging abnormalities. Methods: The patients underwent nasal endoscopy, olfactory smart threshold test, and olfactory event-related potentials, were examined by movement disorder specialists for parkinsonian signs (with UPDRS part III), and underwent positron emission tomography (PET) imaging with 3,4-Dihydroxy-6-(18F)fluoro-L-phenylalanine (18F-FDOPA). Results: Nasal endoscopy was normal. Both patients resulted anosmic at both the olfactory smart threshold test and the olfactory event-related potentials, and showed a minimal reduction in tracer concentration in the posterior portion of the putamen bilaterally in absence of clinical parkinsonism. Conclusions: 18F-FDOPA PET showed damage to the basal ganglia in patients with persistent olfactory dysfunction after SARS-Cov2 infection, highlighting the presence of an olfactory-nigral dysfunction, possibly representing a pre-clinical signature of parkinsonism. Follow-up and longitudinal studies should verify this hypothesis.

KEYWORDS: *SARS-CoV-2, COVID, parkinsonism, olfactory dysfunction, 18F-FDOPA, olfactory-nigral pathway*

INTRODUCTION

Among the various clinical manifestations of SARS-CoV-2 disease (COVID-19), olfactory dysfunction (OD) is reported in 68% of patients and results are persistent in 10%. OD can depend on the neuroinvasive properties of the virus,

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which could reach the olfactory bulb (OB) through the olfactory mucosa and neurons (1). SARS-CoV-2 also induces an inflammatory response in the Central Nervous System (CNS) that can reduce the sense of smell. SARS-CoV-2 shares characteristics with the influenza virus H1N1, that was responsible for the 1918 Spanish flu outbreak, and the subsequent Encephalitis Lethargica (EL), which was followed by an increased incidence of post-encephalitic parkinsonism (2, 3). Destruction of the Substantia Nigra (SN), responsible for the pathogenesis of parkinsonism, was demonstrated in EL. The etiology of post-encephalitic parkinsonism has been recently linked to the olfactory vector hypothesis (2). When inhaled, the virus travels along the olfactory pathway and infects the OB to attack the SN (1) retrogradely. Degeneration of this nigro-olfactory connection may be involved in parkinsonism, where hyposmia is a prodromal symptom. Other Coronaviruses have been demonstrated to cause CNS neuronal death through the nasal cavity, and basal ganglia lesions have been discovered related to thromboembolic encephalopathies (4). Additionally, a case of acute parkinsonism associated with COVID-19 infection has been previously reported (5). The evidence of cases of parkinsonism after COVID-19, that are preceded by OD, highlights a possible link between SARS-CoV-2 infection and the subsequent development of parkinsonism.

Here we report two cases of persistent OD due to SARS-CoV-2 infection that presented with dopaminergic imaging abnormalities.

MATERIALS AND METHODS

Four patients, healed from a previous SARS-CoV-2 infection, were recruited by the COVID-19 Unit of the SS Annunziata Hospital of Chieti. A detailed medical history was collected for all patients. Nasal endoscopy was performed to exclude nasal causes of anosmia. The patients were tested with an olfactory smart threshold test (OST), which is useful for fast preliminary screening of the olfactory function, that works based on four disposable items based on the Connecticut Chemosensory Clinical Research Center threshold test and the Italian population age phenotype threshold test (7).

Olfactory event-related potentials (OERPs) were also performed. OERPs consist of a negative component, the N1, followed by two positive components, P2 and P3. The early component (N1) reflects the exogenous cortical activity related to sensory input detection and primary sensory processing. In contrast, the later olfactory OERP components (P2 and P3) reflect endogenous cortical activity related to secondary cognitive processing (8). Latency (range of 530 to 800 ms after stimulus onset), amplitude (approximately between 4 and 20 μV), and shape are the main parameters of OERP components (8). OERPs are the results of a grand average of 10 stimulations recorded by EEG power lab equipment (AD-Instruments) following standard procedure, and are only detectable in the periphery at high concentrations (8, 9). The patients were subsequently examined by movement disorder specialists and clinical evaluations included a neurological examination with the Unified Parkinson's Disease Rating Scale part III (UPDRS-III) (6).

Finally, patients underwent positron emission tomography (PET) imaging with 3,4-Dihydroxy-6-(18F) fluoro-L-phenylalanine, (18F-FDOPA). The patients, who fasted for at least 6 h, underwent an 18F-FDOPA brain scan using aPET-CT Discovery MI DR tomograph (GE Healthcare). PET-CT 15 minutes static image acquisition was performed 90min after administration of 185MBq of 18F-FDOPA. No premedication with carbidopa was performed. A low-dose CTscan was performed for anatomical localization and for attenuation correction of PET images. PET-CT images wereassessed qualitatively (10). Neurophysiological, clinical, and imaging assessments were conducted blindly for the resultsof all the tests.

RESULTS

The four patients (2 males, aged 55 and 62 years old, and 2 females, aged 49 and 75 years old) healed from COVID-19 without remarkable sequelae besides anosmia and had a normal nasal endoscopy. Anosmia was confirmed by OST and OERPs. Clinical evaluation resulted as substantially normal in all the patients (except for mild rigidity in the 75 year old patient, detailed in the case report). Among them, two patients showed abnormal uptake in the putamina at the 18F-FDOPA brain scan. Here we report these two cases.

Case 1

Patient 1 is a 49-year-old woman, with no relevant medical or pharmacological history. Her family history was negative for neurological diseases (i.e., parkinsonism). She was infected with SARS-CoV-2 during the first pandemic wave, in April 2020, without relevant respiratory symptoms. She was treated at home for flu-like symptoms, and she reported early anosmia and ageusia. She tested negative for SARS-CoV-2 at day 14 after the first positive swab, but OD persisted. Nasal endoscopy did not show nasal obstruction on the roof of the nasal fossa or turbinate hypertrophy. Anosmia

was tested by the OST test (negative score to maximum concentration of n-butanol) and recognized as incomplete anosmia by OERPs (persistence of anomalous shape and duration trigeminal cross-modal perception, at 4 months after the acute disease) (Fig.1a). Her neurological examination was normal; her UPDRS-III score was 0. A 18F-FDOPA-PET was performed to address the possible presence of olfactory-nigral involvement in the persistence of OD and showed minimal reduction in tracer concentration in the posterior portion of the putamen bilaterally (Fig.1b).

Case 2

Patient 2 is a 75year old woman with hypertension with no history of neurological disorders. She had COVID-19 in May 2021 and was treated at home with corticosteroids and cholecalciferol for cephalalgia, sinusitis, and conjunctivitis. She had severe hyposmia. After recovery from the acute symptoms, OD persisted. Nasal endoscopy resulted normal. OD was confirmed by the OST test (negative score to a maximum concentration of n-butanol) and confirmed by OERPs performed at 9 months after the acute SARS-CoV-2 infection (Fig.1c). She underwent neurological examination which was negative. Her UPDRS-III score was 6 due to mild rigidity of the neck and limbs. She underwent 18F-FDOPA-PET which showed a minimal reduction in tracer concentration in the posterior portion of the putamen bilaterally (Fig.1d).

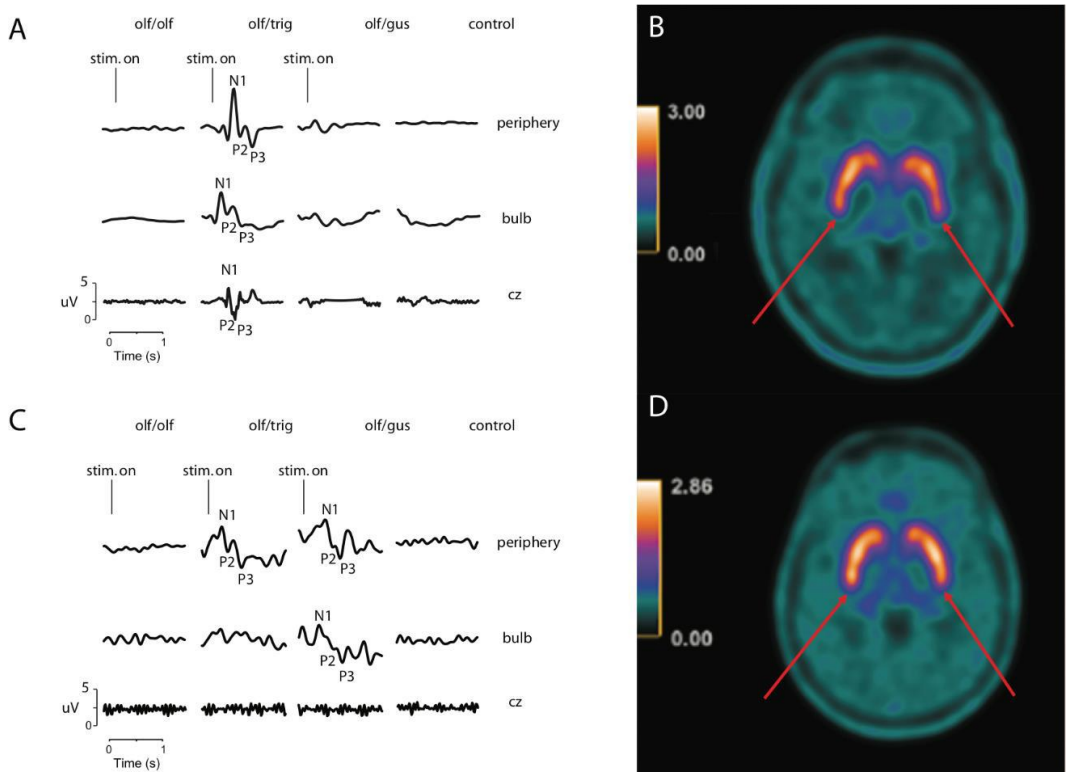

Fig. 1. A): *Olfactory event-related potential (OERPs) of case 1: Electrophysiological recordings from the olfactory periphery, main olfactory bulb and vertex with unimodal olfactory stimulation (olf/olf, pure olfactory stimulation with orange odour, produced by citral) and cross-modal stimulations (olf/trig, olfactory/trigeminal with mint odour, produced by R-(-)-carvon and olf/gus olfactory/gustative with mushroom odour produced by n-octen-3-ol) compared to the unstimulated basal control recording. The patient shows OERP exclusively for the olfactory/trigeminal cross-modal stimulation, no signal is detectable for the other stimulations. The patient is to be considered "incomplete anosmic". Trigeminal olfactory perception is however impaired because the signal is abnormal in shape, amplitude and duration;* **B):** *^{18}F-FDOPA-PET images of case 1 which show minimal reduction in tracer concentration in the posterior portion of the putamen bilaterally (red arrows);* **C):** *Olfactory event-related potential (OERPs) of case 2: Electrophysiological recordings from the olfactory periphery, main olfactory bulb and vertex with unimodal olfactory stimulation (olf/olf, pure olfactory stimulation with orange odour, produced by citral) and cross-modal stimulations (olf/trig, olfactory/trigeminal with mint odour, produced by R-(-)-carvon and olf/gus olfactory/gustative with mushroom odour produced by n-octen-3-ol) compared to the unstimulated basal control recording. The patient shows OERP in the olfactory periphery for cross-modal stimulations, of which only the olfactory/gustatory persists in the main olfactory bulb. In any case, no signal is detectable for any modalities in cz, so the patient is considered anosmic due to a deficit of the central electrophysiological signal;* **D):** *^{18}F-FDOPA-PET images of case 2 which show minimal reduction in tracer concentration in the posterior portion of the putamen bilaterally (red arrows).*

CONCLUSIONS

Our two patients with persistent OD after COVID presented with putaminal damage demonstrated by 18F-FDOPA-PET, likely due to an olfactory-nigral dysfunction caused by SARS-CoV2 infection. Even though the patients did not show clinical features of parkinsonism as assessed by UPDRS, they nevertheless showed the presence of putaminal involvement. This damage might be a prodromal sign of the possible future development of parkinsonism, as happened after the Spanish flu with the appearance of postencephalitic parkinsonism. The real epidemiological impact of such an association should be addressed by follow-up and longitudinal studies.

Statement of ethics
Written informed consent was obtained from the patients for publication of this case series and any accompanying images. The paper is exempt from ethical committee approval because it is not necessary for the publication of the case report. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of interest
The authors declare that they have no conflict of interest.

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Data availability statement
Data is available from the corresponding author upon reasonable request.

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Letter to the Editor

HISTORY OF CYTOKINES: MY CONTRIBUTION

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INTRODUCTION

Professor Pio Conti began scientific research in London in 1977 where he worked in the laboratory of Prof. D.A. Willoughby, studying the mechanisms of chronic inflammation. From 1981-1983, he worked in the USA in Washington D.C. at the Immunology Center at Georgetown University, directed by Prof. J.A. Bellanti. In this lab, he studied the eicosanoids and the effect of lymphotoxin on neutrophils in vitro, in collaboration with Prof. Peter W. Ramwell and Dr. Terry W. Williams, the collaborator of G.A. Granger (University of California), the discoverer of lymphotoxin, which was later named tumor necrosis factor (TNF). In 1985, P. Conti and T.W. Williams published an interesting article highlighting that lymphotoxin damages human neutrophils, causing vacuolization and increasing thromboxane. Later, this discovery proved to be the basis for myocardial infarction. In 1984, P. Conti was invited to Boston (USA) to carry out research on the cytokine IL-1 in the laboratory of Prof. Charles A. Dinarello, the purifier and cloner of IL-1 and the discoverer of various cytokines (IL-18, IL-33, IL-37, IL-38, IL-1RA). His work here led to the publishing of a pioneering article on the effects of IL-1 on natural killer cells and tumor killing with J.W. Mier (who discovered IL-2 with Robert Gallo from NIH). From 1985-86, Prof. Conti worked at Harvard Medical School in Boston, where he collaborated with Dr. C.N. Serhan (collaborator of Prof. Bengt I. Samuelsson, Nobel Prize winner), the discoverer of Lipoxins A and B, and published an original paper on the stimulation of lipoxin A on the release of thromboxane by neutrophils. From 1986-2022, he studied the pathophysiology of mast cells at the Molecular Pharmacology and Drug Discovery Laboratory at Tufts University in Boston, directed by Prof. T.C. Theoharides. The studies done in this research center led to the publication of a significant number of articles in the best international scientific journals. From 2009 to today, he has collaborated with Dr. Susan E. Leeman (former Nobel Prize candidate), discoverer of the neuropeptide neurotensin and purifier of substance P. In 2020, during the pandemic, Professor Conti published an article on the damage effects of cytokines released in COVID-19 which obtained many citations (1,744).

In 1969, the first lymphokines were isolated in *in vitro* lymphocyte cultures, which were also called interleukins due to their ability to act between cells (1). Subsequently, monokines, which derive from monocytes, were discovered and these proteins together with lymphokines were called cytokines. Cytokines are low molecular weight immunoregulatory proteins that can act at very low concentrations (nanograms). They modulate our body's immune responses, but when their levels exceed physiological limits, they can mediate both acute and chronic inflammation. Here, we report some

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articles published in the past 40 years by Professor Pio Conti concerning the activity of cytokines in acute and chronic inflammation. In conducting the experiments reported here, Prof. Conti collaborated with internationally renowned scientists who played a decisive role in the discovery and function of cytokines.

DISCUSSION

Prof. Conti began studying chronic inflammation in the laboratory of Professor D.A. Willoughby at Saint Bartholomew Hospital in London in the year 1977. Wistar rats were injected subcutaneously with 0.2 ml of saturated water solution of potassium permanganate crystals diluted at a ratio of 1:40. After a week, calcium granulomas indicating chronic inflammation were obtained. These experiments allowed to study at the molecular level both some anti-inflammatory pharmacological compounds and some inflammatory cytokines/chemokines. In 1981, Prof. Conti started attending Prof. Peter Ramwell's laboratory at Georgetown University Medical Center in Washington D.C., where he studied the arachidonic acid cascade in neutrophil granulocytes isolated *in vitro*. Subsequently, from 1982-83, at the Georgetown Immunology Center directed by Prof. J.A. Bellanti (author of the famous textbook: "Immunology"), Prof. Conti collaborated with Dr. Terry Williams, the collaborator of Prof. G.A. Granger who discovered the cytokine lymphotoxin, which was later named tumor necrosis factor (TNF). The experiments conducted at the Immunology Center highlighted for the first time the damage, in terms of vacuolization and cell death, of lymphotoxin on human granulocytes *in vitro* (2).

In 1984, Prof. Conti was invited by Prof. Charles A. Dinarello to work in his laboratory of Infection Diseases at Tufts University Medical School in Boston, MA. In his experiments, Prof. Dinarello had just demonstrated that the endogenous leukocyte pyrogen (ELP) of fever was the same molecule as interleukin-1 (IL-1) and was generated by macrophage cells (3). This was a revolutionary concept in medicine that clarified the etiopathogenesis of fever and showed that IL-1 was not a molecule produced by neutrophil granulocytes, a concept reported in many textbooks. The experiments conducted in Prof. Dinarello's laboratory led to the publication of an interesting article authored by C.A. Dinarello, P. Conti and J.W. Mier, in which IL-1 was shown to have an effect on natural killer (NK) cells in tumor activity (4). This was the first evidence of the involvement of IL-1 in tumor pathology. Dr. J.W. Mier was a junior researcher at NIH in Bethesda, who had already been highlighted for his role as a collaborator with Dr. R.C. Gallo in the characterization and purification of T-cell growth factor (TGF), subsequently named IL-2.

Until then, IL-1 was used in purified form, but after a few months, Prof. Dinarello cloned IL-1, an important work published in the journal *The Proceedings of the National Academy of Sciences* (PNAS) (5). Following this discovery, Prof. Conti used recombinant human IL-1 in his experiments on polymorphonuclear leukocytes, highlighting the ability of this cytokine to stimulate the production of thromboxane A2 (TxA2) detected as TxB2 (6).

This was an important finding as IL-1 was implicated in cardiovascular disease where platelet aggregation is mediated by TxA2. The study of the arachidonic acid cascade was one of the main themes in the career of Prof. Conti. In fact, these studies continued from 1985-86 at Harvard, Boston, at the laboratory of Dr. C.N. Serhan, collaborator of Prof. Bengt I. Samuelsson, Nobel Prize winner for his studies on eicosanoids. At Harvard, Prof. Conti began to conduct experiments with lipoxin, a new molecule discovered by Dr. Serhan. Experiments show that lipoxin A (LxA) augments the release of thromboxane from human polymorphonuclear leukocyte suspensions (7). It was also reported that LxA regulates TNF-directed neutrophil action *in vitro* and stimulates the secretion of IL-4 in immune responses (8).

From 1986 until today, Prof. Conti has been studying the pathophysiology of mast cells (MCs) at the Molecular Pharmacology and Drug Discovery Laboratory at Tufts University in Boston, MA, under the direction of Prof. T.C. Theoharides. This long period of work and study has led to the publication of a large number of interesting articles (9-16).

In 1995, at Tufts, he found that monocyte chemotactic protein-1 (MCP-1) and MCP-3, pro-inflammatory chemokines that attract leukocytes, cause clump formations on MCs, a phenomenon absent in untreated cells (controls). These results confirm the chemo-attraction capacity of these chemokines and, when analyzed under an electron microscope, demonstrate communication between the cell membranes and the cytoplasm of adjacent MCs (17). In 1997, also at Tufts, Conti et al. found that the chemokine regulated upon activation normal T expressed and secreted (RANTES) attracted basophilic cells in a dose-dependent manner. When this chemokine was injected into rat skin, there was an increase in histidine decarboxylase (HDC) mRNA, demonstrating that histidine genes were activated, leading to histamine synthesis (18). In the same year, still on the study of MCs, Conti et al. found for the first time that RANTES and MCP-1 injected subcutaneously in rats caused the recruitment of MCs and the stimulation of HDC. These studies were published in important international journals and aroused much interest in the scientific community (9-11,17).

In 2002, Prof. Conti, with the group of Dr. Theoharides, published that IL-6, a crucial cytokine for MC maturation, is important for the maturation of CD34+ cells, human umbilical cord stem cells, in the presence of stem cell factor (SCF) (19). In the 2000s, the study of IL-10 emerged in the scientific community, as it was highlighted that this cytokine is anti-inflammatory and is involved in immunosuppression. In this respect, Conti et al. published an interesting article showing that IL-10 inhibits some inflammatory cytokines, Th2, and natural killer (NK) cells, but not tryptase from MCs (20). The studies done by professors Conti and Theoharides led to the elucidation of the role of MCs in tumor growth, as many articles were contradictory on this topic. In a review on this subject published in the journal *Trends in Immunology*, Conti and Theoharides highlighted that by producing certain compounds, MCs could promote tumor and metastatic growth, but by generating other compounds, they could inhibit tumor development (21). These concepts were highly appreciated and cited by the scientific community. Moreover, in the journal *Trends in Pharmacological Sciences*, Theoharides and Conti reported that the corticotropin-releasing factor (CRF), which leads to the selective release of cytokines and other pro-inflammatory mediators, increases following stress (22).

The studies at Tufts University were very prolific in terms of publication and highlighted various aspects of immunology and MC biology. In 2007, Prof. Conti, together with the group of Dr Theoharides, summarized a part of these studies in an important review which highlighted the mechanisms of MC activation and the relevance of their inhibition (23). Studying the central nervous system (CNS) at Tufts, Prof. Conti performed *in vitro* experiments on MCs in the CNS. This work had a notable development thanks to the collaboration with Nobel Prize candidate Dr. S.E. Leeman, the scientist who had discovered the neurotransmitter neurotensin and purified substance P (24,25).

In the first decade of the 2000s, the group of Prof. Theoharides, including Prof. Conti, started to study autism spectrum disorders (ASD). In 2008, they published an article showing that MCs can also be activated in a non-allergic way and could be involved in the pathogenesis and therapy of ASD (26). In 2010, Conti, Leeman, and Theoharides et al. reported in the journal PNAS that the interaction between SP, IL-33, and MCs leading to VEGF release, contributes to the inflammation involved in psoriasis, a nonallergic neurogenic hyperproliferative skin disease (12). In 2017, Conti et al. reported that SP and IL-33 together markedly enhance TNF synthesis and secretion in human MCs mediated by the interaction of their receptors, highlighting an amplification process of TNF synthesis and secretion, an effect inhibited by methoxyluteolin (15). In light of these studies, in 2018, this research group reported that Substance P and IL-33 administered together stimulate a marked secretion of IL-1β from human MCs, a scientific basis that could lead to new therapies (16).

Meanwhile, studies on autism continued and led to the publication of an article in 2020 which was entitled "IL-38 inhibits microglial inflammatory mediators and is decreased in amygdala of children with autism spectrum disorder". This paper reported the effect of IL- 38, a new anti-inflammatory cytokine that suppresses the IL-1 produced by microglia. The inhibitory effect of IL-38 was more potent than that of IL-37, opening new avenues for ASD therapy (14).

The year 2019 marked the start of the global health crisis of the COVID-19 pandemic caused by SARS-CoV-2. In this period, many elderly patients were hospitalized for lung inflammation and there were many deaths, mainly resulting from respiratory failure. Prof. Conti was one of the first scientists to point out that inflammatory cytokines play an important role in this lung pathology. The article published in a ‘small’ journal was of great scientific interest and the basis of study for other authors, so much so that to date it has received 1,744 citations in the best international journals (27).

CONCLUSIONS

With this article, Prof. Pio Conti takes the opportunity to thank all his collaborators, (in particular, Dr. T.C. Theoharides and Dr. C.A. Dinarello) and the university of Tufts, Boston, and the "G. D'Annunzio" University of Chieti-Pescara, Italy.

Conflict of interest

The author declares that they have no conflict of interest.

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