SAR Journal of Psychiatry and Neuroscience

Abbreviated Key Title: *SAR J Psychiatry Neurosci*
Home page: https://sarpublication.com/journal/sarjpn/home
DOI: https://doi.org/10.36346/sarjpn.2024.v05i04.002

OPEN ACCESS

ISSN 2707-7764 (P)
ISSN 2709-6939 (O)

Original Research Article

Role of Sphingosine-1-Phosphate (S1P) Signaling in Diabetes-Induced Cognitive Decline in Wistar Rats

Jean Idrice Aymar Keletela[1,5], Ghislain Loubano-Voumbi[1,3], Landry Martial Miguel[1], Didier G. Tchatchouang Njilo[1], Archange Michel Emmanuel Mboungou Malonga[1], Freddy Saturnin Pouki[1,5,6,7*], Aladin Atandi Batchy[1,8], Luc Magloire Anicet Boumba[1,2,10], Ange Antoine Abena[1,9]

[1]Faculty of Health Sciences, Marien Ngouabi University, BP: 69 Brazzaville, Congo
[2]Pointe-Noire Research Zone, Institut National de Recherche en Sciences de la Santé (IRSSA), Congo
[3]Medical Analysis Laboratory, Hôpital Général de Dolisie, Congo
[4]Laboratory Department, Hôpital Général Adolph Sicé, Pointe-Noire, Congo
[5]Louise Michel Private Clinic, Pointe-Noire, Congo
[6]TiéTié Reference Hospital, Pointe-Noire, Congo
[7]Clinique Médicale Guénin, Pointe-Noire, Congo
[8]Endocrinology and Metabolic Diseases Department, Hôpital Général Adolph Sicé, Pointe-Noire, Congo
[9]President of Université Denis Sassou Nguesso Brazzaville, Congo
[10]Molecular Biology Laboratory, Fondation Médicale Marie Gomez, Pointe-Noire, Congo

***Corresponding Author:** Freddy Saturnin Pouki
Faculty of Health Sciences, Marien Ngouabi University, BP: 69 Brazzaville, Congo

Article History: | Received: 17.10.2024 | Accepted: 22.11.2024 | Published: 23.11.2024 |

Abstract: ***Background and objective***: The study of the S1P signaling pathway in the context of diabetes offers a novel perspective for understanding the mechanisms underlying cognitive decline and developing new therapeutic strategies. ***Material and methods***: The study involved 24 Wistar rats, divided into three groups: group 1 was given distilled water and served as a control group; group 2 was made up of rats made diabetic and given distilled water; group 3 was made up of rats made diabetic and treated with D-erythro-dihydrosphingosine (SPK1 and SPK2 inhibitor). Behavioral tests included object recognition and maze tests, and Rt-PCR was used to assess sphingosine-1-phosphate expression. ***Results***: The study revealed significant differences in weight between rats, with a notable decrease observed from day 7 onwards. It also revealed significant differences in the time spent exploring objects and the mental influence of diabetes on the acquisition of spatial knowledge. The study also revealed that diabetes affects working memory and inhibits S1P expression in the hippocampal region. ***Conclusion***: The study reveals significant differences in exploratory behavior and memory capacity between diabetic and normal rats, highlighting the negative effects of diabetes on memory.

Keywords: Cognitive deficit, diabetes, rats, sphingosine-1-phosphate.

1-INTRODUCTION

Chronic disorders such as diabetes, cognitive dysfunction and dementia are very common in individuals over the age of 65. Decreased cognitive capacity may be linked to reduced ability to self-manage diabetes and increased dependence on care (Sinclair AJ *et al.*, 2000). The number of people affected by these diseases is estimated to be high, and is set to rise sharply in the coming decades (Saeedi P *et al*., 2019; Ferri CP *et al.*, 2005). Indeed, over the last few decades, a wealth of clinical and experimental epidemiological evidence has accumulated to support the negative role of low-level inflammation in adipose tissue in the development of diabetes and numerous cognitive disorders, such as anxiety and memory problems. Currently, diabetic retinopathy, peripheral neuropathy, nephropathy and cardiovascular disease are common complications on which diabetes treatment focuses. Similarly, numerous studies have shown that untreated chronic diabetes leads to the formation of inter-neuronal amyloid plaques in the brain, leading to the neuropathological manifestations of Alzheimer's disease (AD) (Maceyka *et al*., 2012).

The association between diabetes and cognitive decline is well established. Various pathophysiological mechanisms, including chronic hyperglycemia insulin resistance and systemic inflammation, may explain this link. Sphingosine 1-phosphate (S1P), produced from sphingosine by sphingosine kinases (SK1 and 2), is a bioactive lipid that regulates fundamental cellular processes, such as cell proliferation, survival or differentiation. S1P is involved in modulating the inflammatory response in the central nervous system, which may influence cognitive decline. Sphingosine 1-phosphate (S1P) plays a role in signal transduction both outside and inside cells. Inside the cell, S1P produced by sphingosine kinase (Sphk) is released by the ABC family of cellular transporters, enabling it to act autocrine or paracrine (Maceyka *et al*., 2012). The biological effects of S1P are mainly linked to the activation of specific G protein-coupled receptors (S1PRs) present on the cell surface. Modulation of this pathway could offer new prospects for mitigating the adverse effects of diabetes on cognitive function. Hence the interest of this study to verify whether sphingosine 1 phosphate (S1P) can be used as a signaling pathway leading to cognitive dysfunction in rats with diabetes.

2-MATERIALS AND METHODS

2-1-Animals and Treatments

Our study was carried out in the laboratory of the Marien Ngouabi Faculty of Health Sciences, in the experimental neuropathology unit. Male Wistar rats aged between seven (07) and ten (10) weeks from the Faculty of Health Sciences animal house were purchased and used. They were housed in polystyrene cages and maintained under optimal temperature and humidity conditions (21 ± 1◦C and 55 ± 2% humidity) under a 12 h light/dark cycle and free access to food and water. Animals were acclimatized to laboratory conditions for 2 weeks prior to the start of the experiment. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

The animals were divided into three (03) groups of 08 rats each, and treated as follows:

- **Group 1:** Received distilled water and served as a control group (GT);
- **Group 2:** Diabetic rats given distilled water (DTN);
- **Group 3:** Diabetic rats treated with D-erythro-dihydrosphingosine: SPK1 and SPK2 inhibitor (DEDHS) (DTT).

The products (distilled water and DEDHS) were administered orally for 28 days.

2-2-Diabetes Induction and Blood Glucose Measurement

Diabetes was induced by a single intraperitoneal administration of alloxane monohydrate at a dose of 150 mg/kg body weight. Animals were placed in individual cages, with free access to food and 5% glucose solution, to avoid hypoglycemic shock. Three days after alloxane monohydrate administration, diabetes was confirmed by measuring blood glucose levels using a glucometer (*On Call Plus II*). A drop of blood obtained through a small incision at the tip of the tail was used to measure blood glucose levels on days 1, 7, 14, 21 and 28. Only rats with blood glucose levels above 180 mg/dl were selected for the experiment.

Weight Evolution:

Selected rats were weighed on an electronic scale from day one before the onset of diabetes (start of manipulation) and then throughout the days (7, 14, 21 and 28).

2-3-Evaluation of Cognitive Deficit

Two behavioral tests were set up to assess the decline in cognitive ability:

Object Recognition Test

This test is based on the rodents' attachment to exploring objects, particularly an unfamiliar one. It assesses short-term memory capacity. It is based on rodents' natural exploration behavior. The test is performed in three different stages: introduction to the context (familiarization), acquisition and regular recall. After familiarization with the arena, rats are treated and placed in the arena in the presence of two similar objects (training phase). Each object is recorded in terms of the time spent exploring it. During the test phase, the animals face one known object (discovered during the training phase) and one unknown object.

- Familiarization stage

The animal was first introduced to the device without an object and allowed to explore the environment for 10 minutes. This step reduced the anxiety-provoking aspect of the environment. The rat was then locked in its cage for 15 minutes.

- Acquisition stage

In the second phase, two identical objects were presented to the animal. Rats were positioned on opposite sides of the object in the apparatus and left to explore two identical objects for a period of 10 minutes.

- Recall stage

After 24 hours, one of the two objects was replaced by a second. The animal was introduced into the device and allowed to observe both objects, the familiar one and the new one, for 10 minutes. The type of object and its position (right or left) were randomly alternated for each animal. The time invested in exploring the new object was evaluated as a percentage of the total time spent exploring the two objects. Time spent touching, sniffing or being very close to the object was considered as exploration. We then distinguished between the time allocated to the familiar object (TF) and the time allocated to the new object (TN).

- **Radial Arm Maze**

According to Olton and Samuelson (1976), the concept of the eight (8)-arm maze is widely used to assess memory and spatial information management abilities in different species of animals. The maze consists ~~of~~ a center from which eight branches emerge, arranged like the spokes of a wheel. A food pellet (45 mg) was placed at the end of each branch and was not replaced during the test, allowing for a maximum of 8 rewards. Firstly, with the diet, the body weight of these animals was reduced to 85% of its initial values, and this weight was maintained throughout the study. In the center of the platform, the animals were arranged facing the same branch for each trial and for each rat (the maze branches were numbered from 1 to 8). The animal continued to get lost in the maze until it encountered eight branches (four legs had to reach the entrance edge). Errors made during branch visits were recorded in chronological order. The different groups of animals were tested after each week, with an interval period of seven (07) days.

2-4-Collection of Behavioral Test Parameters

Each stage of the behavioral tests was recorded using a camera in order to make better use of the various parameters used as variables in each test.

2-4-1-Rat Sacrifice and Seahorse Sampling

Once treatment had been completed after 28 days, rats in three (03) groups were euthanized by cervical dislocation, then decapitated. Once the brains had been removed from the skull, the brain structure, in particular the hippocampus, was rapidly harvested and cleaned with phosphate-buffered saline. These samples were then stored in sterile vials and immediately frozen at -80°C for use in molecular analysis.

2-5-Molecular Analysis of the Sphingosine 1 Phosphate (S1P) Signaling Gene

2-5-1- DNA Extraction from Rat Hippocampal Tissue

DNA was extracted using the "ReliaPrepTM gDNA Tissue (Promega)" kit, in accordance with the manufacturer's instructions. The amount of DNA in each sample was assessed using Qubit 3.0 fluorescence technology (Qubit® 3.0 Fluorometer, Life Technology). This assay enabled us to evaluate the amount of DNA in ng/µL.

2-5-2-Amplification by RT-PCR

Extracted DNA underwent PCR using the Fast-track diagnostics kit.

- **Mode opératoire:**

Step 1: Preparing the Mix

Ingrédients	Volume for one sample
Buffer (Tampon)	12,5 µl
Ppmix (SONDE)	1,5 µl
Enzyme	1 µl
Amorce sens	0,5 µl
Amorce antisens	0,5 µl
ADN	9 µl

The sequences of sphingosine 1 phosphate (S1P) and β2-microglobulin (eurofins®, France) primers used are listed in Table I.

Table I: Sequence of primers used

Target	Forward Sequence 5'—3'	Reverse Sequence 5'—3'	T° de fusion
S1P	CTTGAGCGAGGCTGCTGT	GACGGTGGTGCAGAAGAGAA	60 °C
B2-µGLOBIN	TCGCAACCTCAGGAACAGAC	CAGGAAAGGGGGCTTAGTGG	60 °C

Second step: Mic (thermocycler) programming

Step	Times	Temperature
Initial denaturation	3 min	94°C
Denaturation	30 seconds	94°C
Hybridization	8 seconds	94°C
Final Extension	34 seconds	60°C
cycles number	40	

Step 3: Expression of sphingosine 1 phosphate (S1P) signaling gene

We evaluated this expression using Livak's method with the formula

Rq = 2^-(ΔΔCt). A positive value of relative quantification (Rq) corresponds to overexpression and a negative value to under expression. S1P expression in each sample was performed in duplicate and the level normalized to β2-microglobulin.

2-6-Statistical Analysis

The mean of the data was expressed ± SD. Comparison between two groups was performed using Student's t-tests. Results were examined using ANOVA for multiple comparisons. Figures and graphs were created using GraphPad Prism 5 software. A value of $P < 0.05$ was considered statistically significant.

3-RESULTS

3-1-Body weight of Wistar rats

The weights of rats in the various groups were recorded from D 1 to D 28. After statistical analysis with

the Kruskal-Wallis test, there was a decrease in weight from day 7, with a significant difference. (P =0.027, n =8 for each group). These results confirm the idea that hyperglycemia reduces body weight (Fig 1).

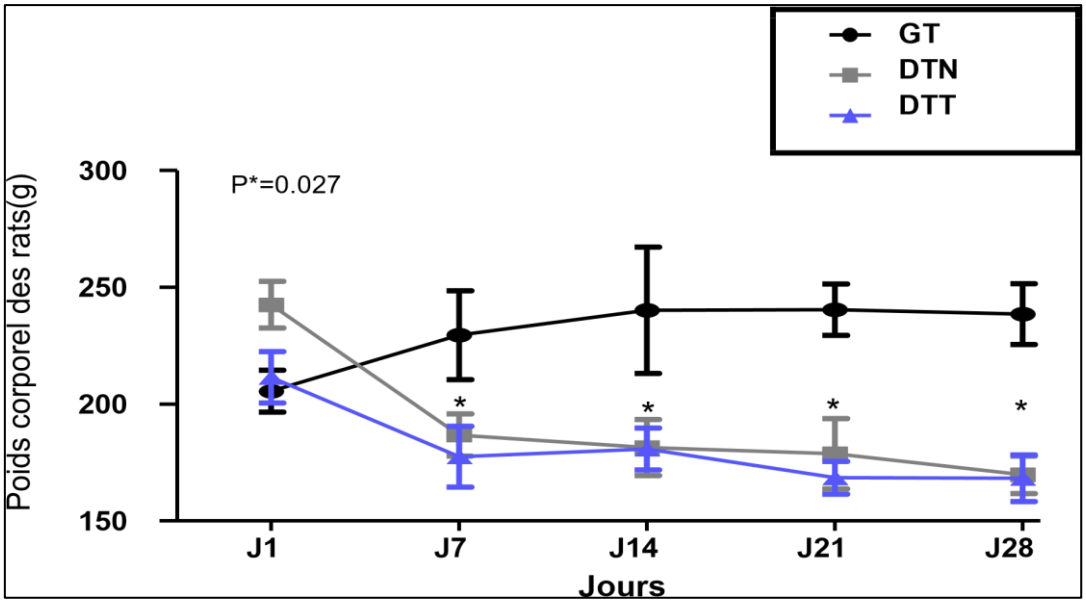

Figure 1: Body weight of three different groups of Wistar rats

3-2-Glycemic profile (Fig 2)

Statistical analysis revealed a significant distinction between groups 1 and groups 2 and 3 at D7 (P=0.0033, n=8 per group). The results show that rats in groups 2 and 3 are diabetic.

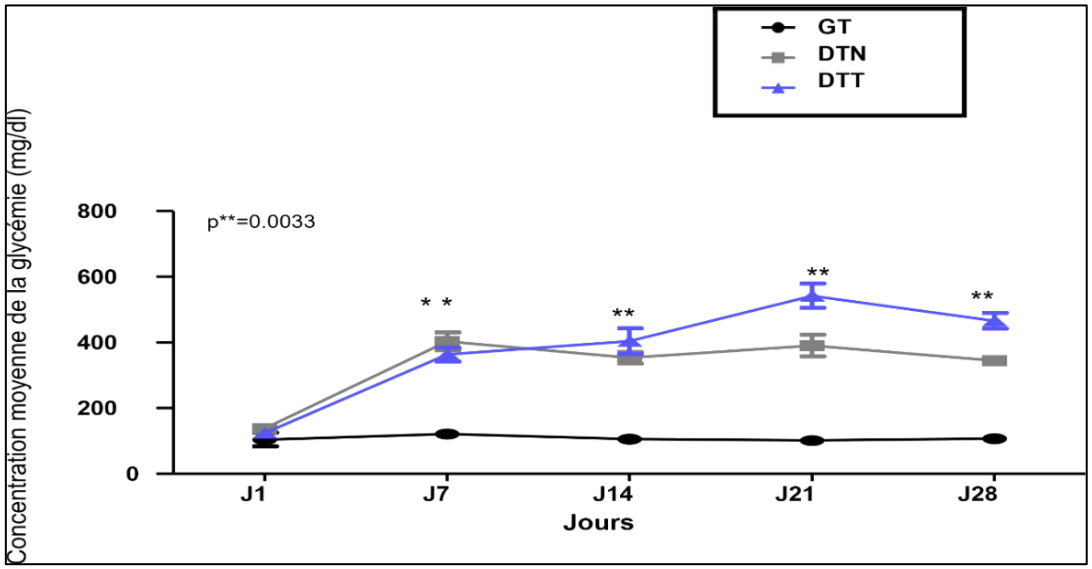

Figure 2: Average blood glucose levels in the three different groups of Wistar rats

3-3-Behavioral Analysis

Object Recognition Test (Fig 3)

We measured the time taken by rats in group 1 (GT), group 2 (DTN) and group 3 (DTT) to explore a familiar object (OF) and a new object (ON) on days 1, 7, 14, 21 and 28. In all three groups, the data showed significant differences between OF and ON time on day 1 (p**=0.0011; n=8 for group 1 normal rats; p**=0.0019; n=8 for group 2 diabetic rats and p*=0.027; n=8 for group 3 diabetic rats with SPK1 and SPK2 inhibitor). These significant differences were observed up to 21. However, we observed no significant difference between OF and ON times in group 3 rats at D 28 (p=0.7018; n=8,). These results show on the one hand that there is memory dysfunction in the object recognition test in alloxanic diabetes, and on the other that inhibition of SPK1 and 2 could be at the root of the memory dysfunction observed.

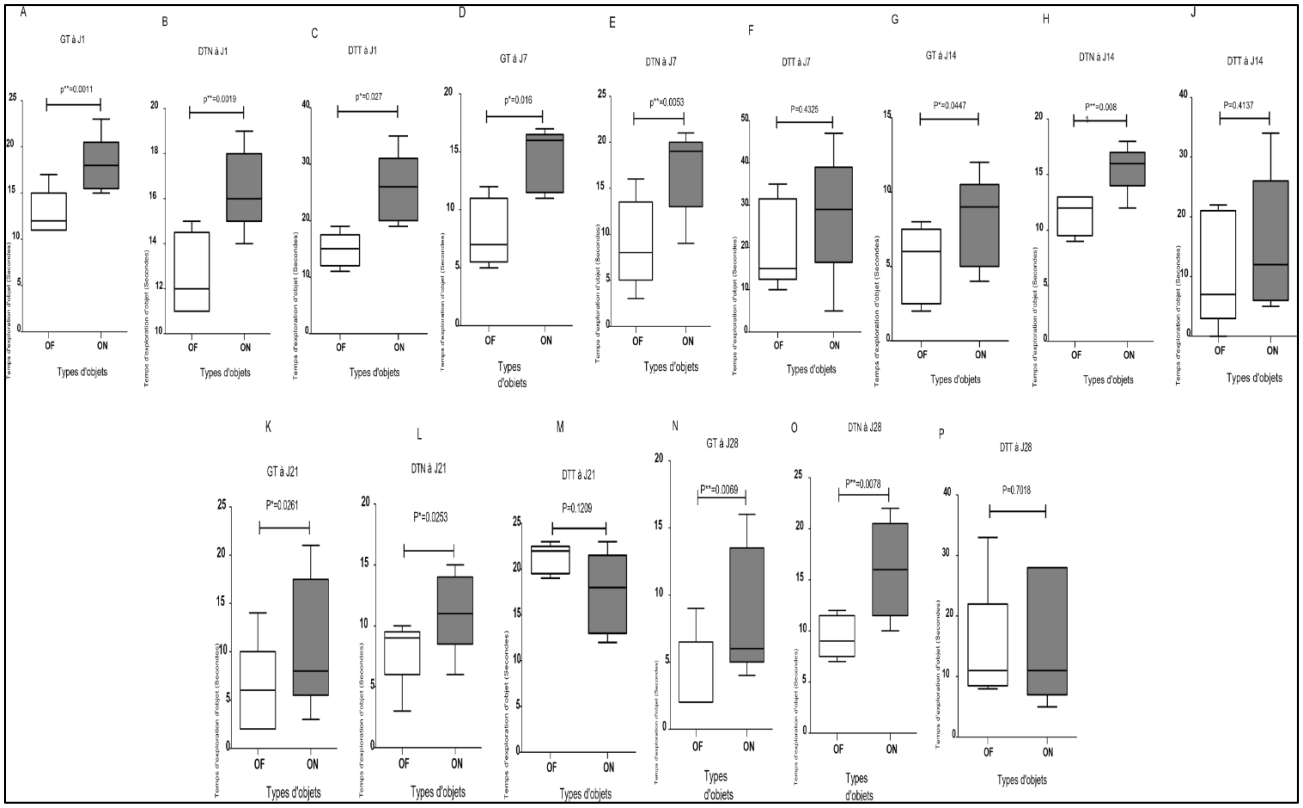

Figure 3: Object recognition test at D 1, D 7, D 14, D 21 and D 28 in Wistar rats from different groups (GT, DTN and DTT)

Radial Arm Maze (Fig 4)

Statistical analysis with the ANOVA test showed a significant disparity between the group of normal rats and the group of diabetic rats, with or without supplemental Sphingosine phosphate kinase 1 and 2 inhibitor (p=0.0029; n=8 per group). From these data, it is clear that diabetes has an impact on working memory, and that the Sphingosine kinase 1 and 2 inhibitor does not enhance working memory in diabetic rats.

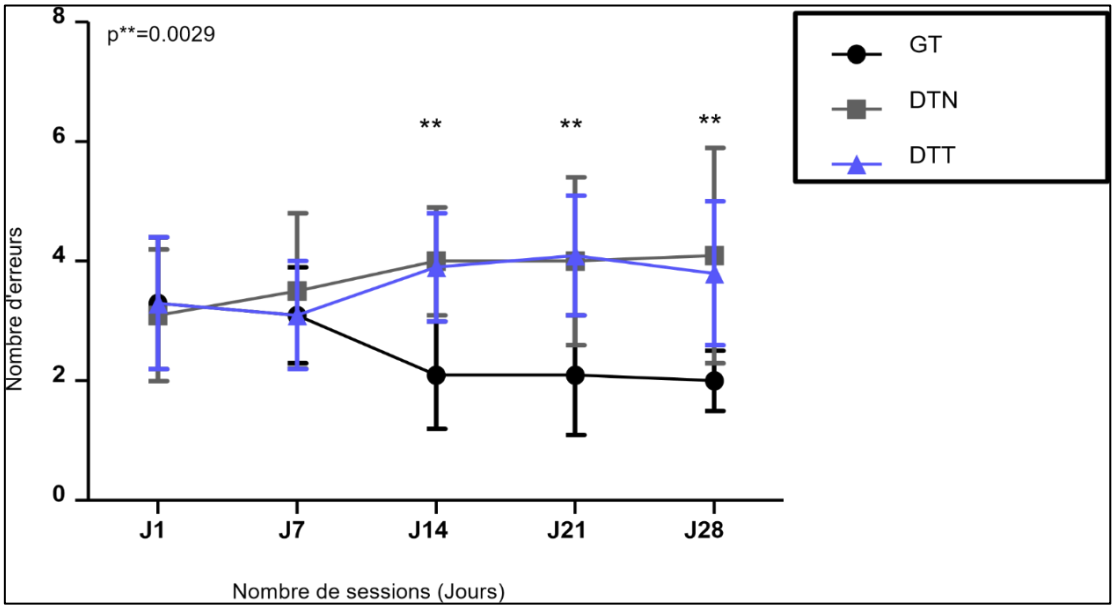

Figure 4: Spatial learning

3-4-Expression of S1P mRNA in rats

The results in Figure 5 revealed a notable disparity between rats in group 1 (GT) and those in groups 2 (DTN) and 3 (DTT) (p***= 0.001; n=8 per group). Furthermore, we found a reduction in S1P in diabetic rats with and without inhibitors, but we also

found strong S1P expression in the hippocampus of normal rats, but hyperglycemia could prevent S1P expression. Based on this information, it appears that diabetes impairs S1P expression, while the inhibitor of SPHK1 and SPHK2 promotes S1P expression and may be involved in the memory alterations observed in alloxan-induced diabetes.

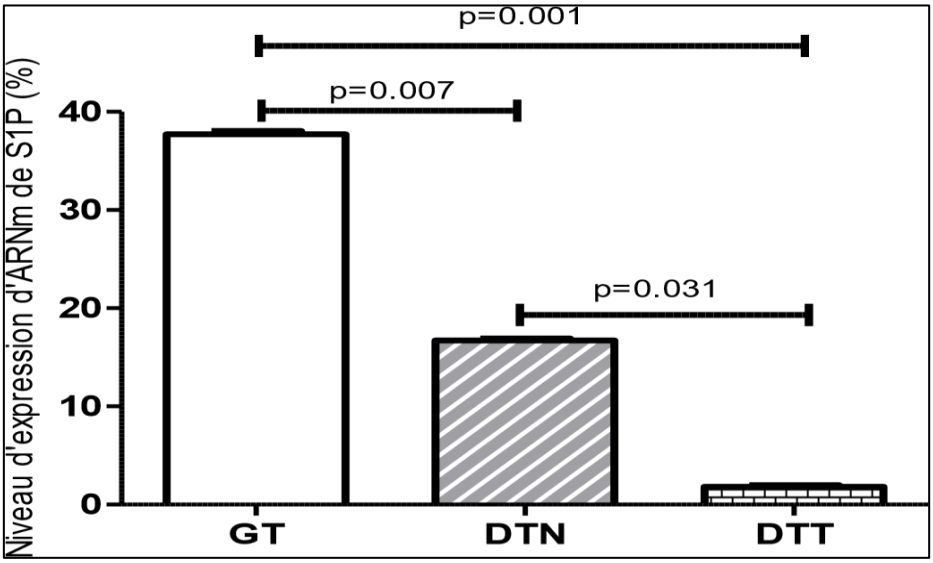

Figure 5: S1P mRNA expression in rat hippocampus

4-DISCUSSION

The aim of our study was to determine whether sphingosine-1-phosphate (S1P) can be used as a signaling pathway during alloxan diabetes-induced cognitive decline in rats. After administering alloxan monohydrate at a dose of 150 mg/kg, we observed an increase in blood glucose, followed by a decrease in body weight. In addition, we employed two behavioral tests to assess memory and learning capacity and evaluate Sp1 expression in the different groups.

Assessment of Blood Glucose and Body Weight in Rats

The weights of rats in the different groups of our study, from D 1 to D 28, were assessed progressively. The results obtained (Fig 1) are similar to those of other authors (Reyes *et al*., 2006; Auroba *et al*., 2010; Loubano-Voumbi *et al*., 2015) with regard to the period of onset of hyperglycemia. The decrease in body weight in diabetes mellitus is generally attributed to the stimulation of gluconeogenesis production. Indeed, accelerated protein and fat metabolism leads to a significant drop in body weight, resulting in increased muscle atrophy and tissue protein loss (Daisy *et al*., 2012).

Our data confirm that hyperglycemia leads to a decrease in body weight. Other authors such as Luke *et al*., 2013; Saini *et al*., 2013; have also reported a significant decrease in body weight due to streptozotocin-induced diabetes compared to normal subjects. Similarly, Omari *et al*., 2011) also obtained similar results. Body weight loss may be linked to the lipolytic effects of glucocorticoids on adipose tissue. In addition to accelerating lipolysis, glucocorticoids stimulate proteolysis and gluconeogenesis. It is likely that these metabolic effects are associated with a lack of the lipogenic hormone insulin. Moreover, these results are similar to metabolic disorders observed in diabetic and non-diabetic Alzheimer's patients (Guerin *et al*., 2005).

Assessment of Cognitive Abilities in Rats

In order to survive in the wild, rodents need to learn and remember their environment. Memetic tests such as the aquatic and radial maze tests can be used to assess the learning of a spatial environment. Additional tests can detect spatial learning: object recognition, the T-maze (Crusio *et al*., 1999), the Barnes maze (Barnes *et al*., 1979), as well as non-spatial learning: fear conditioning test (Anagnostaras *et al*., 2001), avoidance experiments (Pierce *et al*., 1997).

This test involving the hippocampus leads to the conclusion that not only is there memory impairment in the hippocampus of alloxan-induced diabetic rats, but it is also possible that inhibition of SPK1 and SPK2 is at the root of the memory impairment observed in alloxan-induced diabetes. Similar to our findings, rats with hippocampal lesions have been shown to be unable to recognize objects at 10 minutes, 1 hour and 24 hours (Clark *et al*., 2000). However, rodents with deficits in this test have already demonstrated positive effects of polyphenols on object recognition. According to Yokozawa (2011), proanthocyanidins, polyphenols, have the ability to restore object recognition memory capacity in a mouse model of accelerated senescence (SAMP8) with memory problems. Curcumin consumption facilitates recognition of a new object in aged mice (Yu *et al*., 2013).

In our research, we employed the 8-branch Labyrinth test to assess short- and long-term spatial working ability in rodents. Animals were tested on their task working memory, where they had to make a chronological visit to each arm of the radial maze in each session. Each visit to an empty arm, and therefore already visited, was considered an error. In addition to the errors made, the number of correct arm visits in the first eight choices was also recorded. A significant variation (p=0.0029; n=8 per group) was found between the group of normal rats and the group of diabetic rats that received an SPK1 and SPK2 inhibitor in addition or not. These data suggest that diabetes alters working memory, and that Sphingosine kinase 1 and 2 inhibitors do not restore working memory in diabetic rats. Complementing these results, Zucker fa/fa rats (which have a mutation in the leptin receptor) have been shown to be hyperphagic, dyslipidemic, obese and have hyperinsulinism associated with glucose intolerance. Eight months after the onset of diabetes, they showed impaired memory in the Morris water maze test, long-term potentiation and neuronal loss in the CA1 hippocampal region (Biessels and Gispen, 2005). In addition, numerous studies have also validated these findings by observing age-related cognitive deficits in various tasks assessing the Rat's spatial memory (Rosenzweig and Barnes, 2003). These include tasks performed in the Morris water maze (Wang *et al.*, 2006). According to these authors, the reduced memory capacity of rats in spatial memorization and learning tasks has been shown to be caused by altered synaptic connections (Kennard and Woodruff-Pak, 2011), due to a reduction in neuronal plasticity in mice, other studies have also highlighted deficits in working memory. To illustrate, in a spontaneous alternation task, the integrity of spatial working memory, mice aged eighteen to nineteen (18-19) months performed less well than their younger counterparts aged four to five (Vandesquille *et al.*, 2011).

Hyperglycemia and Cognitive Decline

In our research, we observed a reduction in working memory in diabetic rats and problems with exploratory behavior during spatial learning (p=0.0029; n=8 per group) due to the number of errors made during open arm entry visits observed during the eight (08) branch radial maze test. It has been shown that type 1 diabetes is often linked to a reduction in reasoning speed and mental flexibility (Brands *et al*., 2005), that type 2 diabetes also has an impact on learning and memory (Awad *et al.*, 2004).

According to Allen *et al*., (2004), cognitive decline over a 7-year follow-up period is more pronounced in patients with diabetes, particularly the elderly. A relationship between diabetes and dementia is common (Duron *et al*., 2008). Hippocampal synaptic plasticity in elderly insulin-powered diabetic rats is lower than in young people. Similarly, the duration of diabetes appears to influence the impact; in insulin-privileged rats, prolonged hyperglycemia affects hippocampal neurons. The harmful consequences of hypoglycemia are also observed. A week later, diabetic rats are euthanized after receiving high doses of insulin or saline. According to Biessels and Gispen (2005), rats suffering from hypoglycemia had a greater reduction in hippocampal neuronal capacity. Based on this information, it is possible that increased glycemia has an impact on cerebral metabolism in general, as well as on the molecular mechanisms of memory and learning, which may lead to neurocognitive problems.

Expression of sphingosine-1-phosphate(S1P) mRNA in rats

Sphingolipids, found in all membranes, are lipids that metamorphose into signaling molecules that play an essential role in cellular functions such as health and disease. The concentration of S1P in cells is restricted and controlled by a subtle balance between its production and decomposition. According to Cuvillier *et al*., (1996), the balance between intracellular levels of S1P and its metabolic precursors, ceramide and sphingosine, is crucial in determining whether a cell proliferates or dies. According to Pitson *et al*., 2011), sphingosine kinases (SphKs) generate S1P, which is composed of two isoenzymes (SphK1 and SphK2), and is degraded to hexadecenal and ethanolamine phosphate by S1P lyase (SPL). Subsequently, S1P can play an intracellular signaling role or be secreted to play an autocrine or paracrine role by binding to five specific high-affinity G protein-related receptors (GPCRs), known as S1P 1-5 (Cuvillier *et al*., 2012).

In our research, we examined S1P expression in the hippocampus of rats with diabetes and investigated the role of sphingosine kinase inhibitor 1 and 2 (SPHK1 and SPHK2) by comparing S1P expression levels in three (03) groups of rats. The results of our study revealed a notable disparity between rats in group 1 and those in groups 2 and 3 (p***= 0.001; n=8 per group, Figure 5), with a reduction in S1P in diabetic rats both with and without a stimulator. In addition, there were variations between normal rats in group 1 and those in group 3 who were treated with an inhibitor of SPHK1 and SPHK2. It was also observed that S1P is present in the hippocampus of normal rats, but hyperglycemia could compromise S1P expression. This information suggests that diabetes impairs S1P expression and that the inhibitor of SPHK1 and SPHK2 promotes S1P expression, which could be responsible for the memory alterations observed in alloxan-induced diabetes. Alongside these findings, a deregulation of sphingolipid metabolism and more specifically S1P signaling has recently been identified in Alzheimer's disease (Karunakaran *et al*., 2017). Early work in neuronal cell models showed that Aβ peptide apoptosis was associated with an increase in pro-apoptotic ceramide production (Lee *et al*., 2004) or a decrease in Sphingosine kinase 1 (SPHK1) activity, leading to a decrease in pro-survival S1P content (Gomez *et al*., 2007). This deregulation of

the ceramide/S1P balance, with an increase in ceramide and a decrease in S1P, has been correlated with Aβ peptide and tau levels (He *et al.*, 2010). Inside the brain, altered sphingolipid metabolism appears to be a factor in neuronal function, as evidenced by several severe conditions other than Alzheimer's disease, such as Niemann Pick's disease, amyotrophic lateral sclerosis, Parkinson's dementia and AIDS (Mielke *et al*., 2010).

5-CONCLUSION

In sum, our research highlights significant differences in exploratory behavior and memory abilities between diabetic and normal rats, highlighting the detrimental consequences of diabetes on memory. Inhibitors of sphingosine phosphate kinase 1 and 2 appear to play a key role in reducing these dysfunctions, potentially promoting memory in diabetic rats. Furthermore, the disparity in sphingosine-1-phosphate (S1P) expression between the different groups reinforces the hypothesis that this metabolite could play an essential role as a biomarker in the context of diabetic neuropathology. These promising results pave the way for further studies into the underlying mechanisms, and could lead to new therapeutic approaches aimed at improving cognitive abilities in people with diabetes.

REFERENCES

- Allen, K. V., Frier, B. M., & Strachan, M. W. (2004). The Relationship betweentype 2 diabetes and cognitive dysfunction: longitudinal studies and their methodological limitations. *Eur J Pharmacol*, 490, 169-175.
- Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus*, 11, 8-17.
- Ibrahem, A. M. (2010). Study antidiabetic effect of Momordica charantia (bitter gourd) seeds on Alloxan induced diabetic rats. *The Iraqi Journal of Veterinary Medicine*, *34*(1), 165-170.
- Awad, N., Gagnon, M., & Messier, C. (2004). The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *Journal of clinical and experimental neuropsychology*, *26*(8), 1044-1080.
- Barnes, C. A. (1979). Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J Comp Physiol Psychol*, 93, 74-104.
- Biessels, G. J., & Gispen, W. H. (2005). The impact of diabetes on cognition: what can be learned from rodent models? *Neurobiol Aging*, *26*(Suppl.1), 36-41.
- Brands, A. M., Biessels, G. J., De Haan, E. H., Kappelle, L. J., & Kessels, R. P. (2005). The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes care*, *28*(3), 726-735.
- Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci*, 20, 8853-8860.
- Cuvillier, O., Pirianov, G., Kleuser, B., Vanek, P. G., Coso, O. A., Gutkind, S., & Spiegel, S. (1996) Suppression de la mort cellulaire programmée induite par les céramides par la sphingosine-1-phosphate. *Nature*, 381, 800-803.
- Cuvillier, O. (2012). Les récepteurs de la sphingosine 1-phosphate: de la biologie à la physiopathologie. *Med Sci (Paris)*, 28, 951-957.
- Crusio, W. E. (1999). Methodological considerations for testing learning in mice. In: Crusio, W. E., Gerlai, R. T. (Eds.). Handbook of molecular-genetic techniques for brain and behavior research. Amsterdam: Elsevier, 1999, p. 638-651
- Daisy, P., Feril, G., & Kani, J. (2012). Evaluation of antidiabetic activity of various extracts of cassia auriculatalinn. Bark on streptozotocin induced diabetic wistar rats. *Int J Pharm Pharm Sci*, *4*(4), 312-318.
- Duron, E., & Hanon, O. (2008). Vascular risk factors, cognitive decline, and dementia. *Vasc Health Risk Manag*, 4, 363-381.
- Gomez-Brouchet, A., Pchejetski, D., Brizuela, L., Garcia, V., Altié, M. F., Maddelein, M. L., Delisle, M. B., & Cuvillier, O. (2007). Rôle critique de la sphingosine kinase-1 dans la régulation de la survie de cellules de neuroblastome exposées au peptide bêta-amyloïde. *Mol Pharmacol*, 72, 341-349.
- Guérin, O., Andrieu, S., Schneider, S. M., Milano, M., Boulahssass, R., Brocker, P., & Vellas, B. (2005). Different modes of weight loss in Alzheimer disease: a prospective study of 395 patients. *The American journal of clinical nutrition*, *82*(2), 435-441.
- He, X., Huang, Y., Li, B., Gong, C. X., & Schuchman, E. H. (2010) Dérégulation du métabolisme des sphingolipides dans la maladie d'Alzheimer. *Neurobiol Vieillissement*, 31, 398-408.
- Karunakaran, I., & van Echten-Deckert, G. (2017). Sphingosine 1-phosphate - une épée à double tranchant dans le cerveau. *Biochim Biophys Acta*, 1859, 1573-1582.
- Kennard, J. A., & Woodruff-Pak, D. S. (2011). Age Sensitivity of Behavioral Tests and Brain Substrates of Normal Aging in Mice. *Front Aging Neurosci*, 3.
- Lee, J. T., Xu, J., Lee, J. M., Ku, G., Han, X., Yang, D. I., Chen, S., & Hsu, C. Y. (2004). Le peptide amyloïde bêta induit la mort des oligodendrocytes en activant la voie neutre sphingomyélinase-céramide. *J Cell Biol*, 164, 123-131.
- Loubano-Voumbi, G., Diaw, M., Ouedraogo, V., Sow, A. K., Boumba, L., Ba, A., & Samb, A. (2015). Characteristics of animal model of painfuldiabeticneuropathyinduced by alloxan in subcutaneously in rats. *Journal of Global Biosciences*, *4*(6), 2478-2485.

- Luke, U. O., Ebong, P. E., Eyong, E. U., Robert, A. E., Ufot, S. U., & Egbung, G. E. (2013). Effect of ethanolic root and twig extracts of vernoniaamygdalina (ETIDOT) on liver function parameters of streptozotocin induced hyperglycaemic and normal wistar rats. *European Scientific Journal*, *9*(30), 199-211.
- Maceyka, M., Harikumar, K. B.., Milstien, S., & Spiegel, S. (2012). Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol*, 22, 50-60.
- Mielke, M. M., & Lyketsos, C. G. (2010). Altérations de la voie sphingolipidique dans la maladie d'Alzheimer: nouveaux biomarqueurs et cibles de traitement? *Neuro Molecular Med*, 12, 331-340.
- Omari, N., Dahmani-ait akli, Y., Labrousse, F., & Hadj Bekkouche, F. (2011). Influence de la streptozotocine sur l'axe corticotrope du rat Wistar (Rattusnorvegicus). *Bulletin de la Société Royale des Sciences de Liège*, 80, 907-938.
- Pierce, J. M. (1997). Animal learning and cognition. Erlbaun (UK): Psychology Press; 1997.
- Pitson, S. M. (2011) Régulation de la sphingosine kinase et de la signalisation sphingolipidique. *Trends Biochem Sci*, 36, 97-107.
- Reyesa, B., Bautista, N. D., Tanquilut, N. C., Anunciado, R. V., Leung, A. B., & Sanchez, C. (2006). Anti-diabetic potentials of Momordicacharantia and Andrographispaniculata and their effects on estrous cyclicity of alloxan-induced diabetic rats. *Journal of Ethnopharmacology*, 105, 196-200.
- Saeedi, P., Petersohn, I., & Salpea, P. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*, 157, 107843.
- Saini, S., & Sharma, S. (2013). Antidiabetic effect of Helianthus annuus L., seeds ethanolic extract in streptozotocinnicotinamide induced type 2 diabetes mellitus. *Int J Pharm Pharm Sci*, *5*(2), 382-387.
- Sinclair, A. J., Girling, A. J., & Bayer, A. J. (2000). Cognitive dysfunction in older subjects with diabetes mellitus: impact on diabetes self-management and use of care services. *Diabetes research and clinical practice*, *50*(3), 203-212.
- Vandesquille, M., Krazem, A., Louis, C., Lestage, P., & Beracochea, D. (2011). S 18986 reverses spatial working memory impairments in aged mice: comparison with memantine. *Psychopharmacology (Berl)*, 215, 709-720.
- Yokozawa, T. (2011). Anti-aging effects of oligomeric proanthocyanidins isolated from persimmon fruits. *Drug Discoveries & Therapeutics*. doi: 10.5582/ddt.2011.v5.3.109.
- Yu, S. Y., Zhang, M., Luo, J., Zhang, L., Shao, Y., & Li, G. (2013). Curcumin ameliorates memory deficits via neuronal nitric oxide synthase in aged mice. *Prog Neuropsychopharmacol Biol Psychiatry*, 45, 47-53.