

ORIGINAL ARTICLE

Arthrosipa platensis* improves macrophages arginine metabolism and prevents insulin resistance in *Psammomys obesus

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Summary. *Introduction:* Chronic low grade inflammation is generally linked to the development of obesity-related metabolic diseases and tissue-resident macrophages play a major role in the regulation of the inflammation markers in the tissues. The cyanobacterium *Arthrosipa platensis* also named spirulina is a blue green microalgae which has anti-inflammatory and anti diabetic properties. This study aimed to evaluate its potential therapeutic benefits on insulin resistance and peritoneal macrophages arginine metabolism in experimental gerbils named *Psammomys obesus*. *Methods:* A control group was fed its vegetable -based low caloric diet (halophilic plants). Two experimental groups were fed a high caloric diet for 12 weeks. Spirulina, or its vehicle (0.9% NaCl solution) were administrated daily by gastric intubation during 12 weeks. At the end of experiments, serum was collected to assess biochemical parameters and arginine metabolism was studied from isolated peritoneal macrophages. *Results:* Spirulina attenuated hyperlipidemia and prevented hyperinsulinemia, avoiding metabolic disturbances. Psammomys fed a high caloric diet displayed macrophages arginine metabolism toward pro-inflammatory phenotype while spirulina supplementation maintained peritoneal macrophages' iNOS activity similar to that of the control group. *Conclusion:* Our study suggests that *Arthrosipa platensis* is effective in improving insulin sensitivity by regulating peritoneal macrophages' arginine metabolism.

Key words: sand rat, metabolic syndrome, peritoneal macrophages, spirulina, hyperinsulinemia

Introduction

Chronic low-grade inflammation is commonly associated with the pathogenesis of several metabolic diseases, such as insulin resistance and metabolic syndrome (1). Macrophages contribute to the inflammatory process and their plasticity allows them to exchange their phenotype under certain conditions. For example, high fat diets affect macrophage polarization by switching their phenotype toward pro-inflammatory M1 macrophages, promoting the low grade inflammation and insulin resistance (2).

Based on their phenotype, macrophages differ in the way that they metabolize arginine (3): M1 macrophages metabolize arginine through the inducible

nitric oxide synthase (iNOS) pathway, producing nitric oxide (NO), while M2 macrophages metabolize arginine through the Arginase 1(Arg1) pathway, producing ornithine and urea (4).

Phytotherapy represents an alternative approach to improve insulin sensitivity by inhibiting pro-inflammatory macrophages (5). *Arthrosipa platensis*, a cyanobacteria within the Oscillatoraceae family is a rich source of proteins and essential nutrients (6). Furthermore, antioxidant and anti-inflammatory molecules such as c-phycocyanin, vitamins, β-carotene, phenolic compounds, γ-linolenic acid and minerals (7) confer to this algae many favorable effects in preventing the metabolic syndrome and its complications (8). Moreover, *A. platensis* exert anti-inflammatory actions either by inhibit-

ing the secretion of pro-inflammatory cytokines (9) or by decreasing macrophage tissue infiltration (10).

Several rodent models have been used to study the pathogenesis of metabolic syndrome. Among them, the desert gerbil *Psammomys obesus*, is an established nutritional model of insulin resistance and type 2 diabetes (11). Interestingly, this rodent remains healthy in its natural habitat when it consumes exclusively low caloric vegetation. However, it develops metabolic dysregulations when fed standard laboratory chow, which is comparatively a high energy food (12). To our knowledge, no study has reported the effect of spirulina on macrophage metabolism in the model *Psammomys obesus*.

Therefore, the present study was conducted on insulin resistant *Psammomys obesus* to investigate the effect of *Arthospira platensis* supplementation on insulin resistance and peritoneal macrophages polarization by assaying their arginine metabolism.

Materials and methods

Animals and diet

Psammomys obesus were captured in the area of Beni Abbes, in the Algerian Sahara (Wilaya of Béchar: 30°7' North latitude and 2°10' West longitude). They were housed in individual cages under controlled temperature and lighting conditions (25°C, 12:12 hours light:dark cycles), with free access to food and water. During a 2-week acclimatization period, the animals were fed their natural diet i.e. halophilic plants (Chenopodiaceae family) with a poor energy content (0,4 Kcal.g⁻¹). Thereafter, they were randomly divided into three groups (n=6 per group):

- Control group (C) maintained on the halophilic plants and receiving the vehicle solution (1ml of 0.9% NaCl)
- Standard group (STD) fed standard laboratory diet and receiving the vehicle solution.
- Spirulina-treated group (SP) fed standard laboratory diet plus the daily 1 ml of 10% *A. platensis* suspension by oral gavage.

The compositions of the diets are shown in Table 1. The animals were maintained under these conditions and body weight was controlled weekly. On the 12th week, diets were removed from the cages 12h before

the *P. obesus* were anesthetized, blood serum samples and peritoneal cells were collected.

All experimental procedures were authorized by the Institutional Animal Care Committee of the National Administration of Algerian Higher Education and Scientific Research, ethical approval number: 98-11, law of 22 august 1998.

Biochemical analysis

Serum glucose, triglycerides and total cholesterol were measured by a spectrophotometric method using commercial kits (Spinreact, Spain). Insulin was determined by Enzyme linked Immunosorbent Assay (DRG Insulin rat Elisa). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated as follows: (fasting glucose (mM) × fasting insulin (μ U.mL⁻¹))/22.5 (13).

Peritoneal macrophages isolation

Peritoneal macrophages were isolated as described elsewhere (14). They were removed by washing the peritoneal cavity with 3 ml of RPMI (Roswell Park Memorial Institute) 1640 culture medium containing 10% fetal calf serum (FCS) supplemented with 1% antibiotics (streptomycin 50mg.mL⁻¹, penicillin 50 IU.mL⁻¹, Sigma), 1.2% glutamine (Sigma-Aldrich) and 5% Hepes to maintain pH. Then, cells were adjusted to 10⁶ cells.mL⁻¹ medium. Macrophages were purified by adherence to the surface of non treated tissue cul-

Table 1. Composition of the diets per 100g

| Diet composition | Natural diet (%) | Standard laboratory diet (%) |
|------------------|------------------|------------------------------|
| Energy Kcal/g | 0.4 | 3.25 |
| Water | 80.8 | 9 |
| Minerals salts | 6.8 | 7.1 |
| Lipids | 0.4 | 7.5 |
| Proteins | 3.5 | 25 |
| Carbohydrates | 2.5 | 47.4 |
| Cellulose | 6 | 4 |

ture plates at 37°C for 2 h in an air/CO₂ (95%/5%) atmosphere. Non adherent cells were discarded and the remaining adherent cells (>95% macrophages (15)) were incubated in the same conditions.

After 24 h, the culture supernatant was collected to determine the production of NO, and the cells were lysed in Arginine Lysis Buffer for arginase activity assay.

NO assay

NO was determined as the amount of nitrite (16) as follows: to 50 µl of culture medium, 50 µl of 1% sulfanilamide solution and 50 µl of 0.1% naphthylethylenediamine dihydrochloride solution were added. The microplate was incubated in dark conditions at room temperature for 10 min to achieve the desired maximum absorbance and read at 540 nm using microplate reader (Biotek Instruments®, USA). The concentration of nitrite was calculated using solutions with increased concentrations of NaNO₂ as standard curve (0.001 to 0.128 mM). Results are expressed as mM.10⁻⁶ cells.

Arginase assay

The arginase activity was evaluated by a method described by Corraliza et al. (17). Briefly, 10 µl of 10 mM of MnCl₂ were added to 100 µl of cell lysate and incubated at 55°C. After 10 min, 100 µl of 0.5M L-arginine were added and the mixture was incubated for 2-3 hours at 37°C. Urea was then measured by colorimetric reagent (Spinreact, Spain.)

The concentration of urea was calculated by comparison to the standard curve obtained with known concentrations of urea (0.39 to 50mM). Data are expressed as mM.10⁻⁶ cells

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were achieved by Student's t test using SPSS 20.0 (SPSS Inc., Chicago, IL).

Differences were considered significant at p<0.05.

Results

Effect of Spirulina on body weight and serum parameters

At the end of the experiment, *P. obesus* fed standard diet showed significant increase in their body weight gain in comparison to that of controls (P<0.01), (Table 2), yet this weight gain was partly reduced with *A. platensis* treatment (Table 2). Moreover, in the STD group, fasting blood glucose had a tendency to be slightly higher (+34.64%) than in controls or spirulina-treated animals, albeit no statistically significant changes were observed (Table 2).

However, this hypercaloric diet induced a significantly large increase in serum insulin which rose almost 277%, as compared with control animals (p<0.001).

These changes were associated with an important increase in insulin resistance, as demonstrated by the significantly higher value of HOMA-IR in the STD animals as compared with the control animals (p<0.01). Both plasma insulin and HOMA-IR values were considerably corrected in spirulina-treated group as compared with STD group and were close to those of the control group (Table 2).

As presented in table 2, a dyslipidemia was revealed in the STD group by the increase of serum triglycerides (+180%), and total cholesterol (+101%) in

Table 2. Effect of Spirulina on body weight, glycemic homeostasis and plasma lipids in *P. obesus*

| | Control (C) | Standard (STD) | Treated (SP) |
|--|--------------|-----------------|------------------|
| Initial body weight (g) | 80 ± 4.27 | 80.3 ± 6.22 | 78 ± 3.51 |
| Final body weight (g) | 82.8 ± 5.99 | 113.6 ± 3.38** | 101.6 ± 2.60€§ |
| Glucose (mM) | 3,002 ± 0,17 | 4,042 ± 0,69 | 2,915 ± 0,48 |
| Triglycerides (g.L ⁻¹) | 0.623 ± 0.10 | 1.750 ± 0.18** | 0.643± 0.09 §§ |
| Total Cholesterol (g.L ⁻¹) | 0.633 ± 0.15 | 1.275 ± 0.12* | 0.939 ± 0.07 § |
| Insulin (µU.ml ⁻¹) | 38,23 ± 2,62 | 144,3 ± 4,98*** | 45.09 ± 4,68 §§§ |
| HOMA | 5,136 ± 0,56 | 25,652 ± 4,04** | 5,715 ± 0,94 §§ |

comparison with the control animals while oral supplementation of *A. platensis* prevented this dyslipidemia (Table 2).

Effect of spirulina on macrophages arginine metabolism

The iNOS activity was estimated from nitrates accumulation in the culture supernatant. As shown in Figure 1A, the STD group's macrophages exhibited significantly higher iNOS activity regarding the macrophages stemming from the control group ($p<0.05$). In contrast, no significant discrepancy of iNOS activity between control group and spirulina group was noted.

On the other hand, arginase activity evaluated by urea production in the lysed cells was not significantly different in the three groups (Figure 1B).

In order to estimate the effect of spirulina on macrophage M1/M2 polarization, the NO/Urea ratio was calculated. This ratio was largely elevated in the standard group as compared with the control group ($p<0.001$). Nevertheless, in macrophage cultures from spirulina-treated rats, the NO/Urea ratio decreased substantially and was comparable to that observed in control animals (Figure 2)

Discussion

Among such attractive novel therapeutic possibilities, apirulina has caught our interest because of its

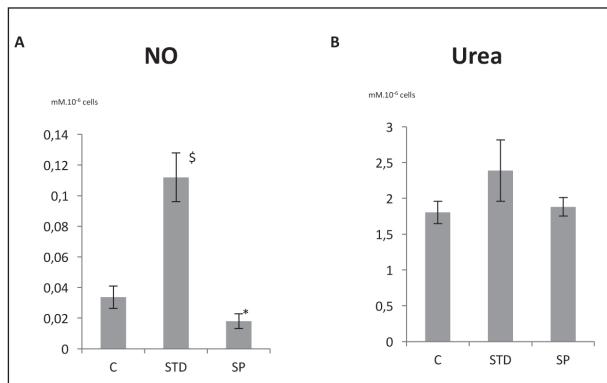


Figure 1. NO (fig A) and Urea (fig B) production by macrophages from control (C), standard (STD) and spirulina-treated (SP) *Psammomys obesus*. Data are shown as the mean \pm SEM; * $P<0.05$ (STD vs C); § $P<0.05$ (SP vs STD)

various properties. In this experiment, we studied its efficacy on metabolic syndrome and peritoneal macrophages phenotypes. Previous results from clinical and experimental studies reported that spirulina induced, at least partly, an improvement of body weight and lipid metabolism (8, 18). In our study, after feeding standard laboratory chow for 12 weeks, *P. obesus* gerbils showed metabolic disturbances in the STD group while the insulin sensitivity was restored by spirulina treatment as indicated by normalized insulinemia, HOMA-IR and plasma lipids profile. These results are in accordance with previous studies that showed beneficial effects of *spirulina* on metabolic abnormalities and insulin resistance (19, 20).

On the other hand, it has been reported that diabetic-insulin resistant rats showed alteration of peritoneal macrophages arginine metabolism (21). High caloric diet is associated, at least in the adipose tissue, with a modification in macrophage polarization, as M1 polarized macrophages with high iNOS activity become abundant while a decrease in arginase-expressing M2 macrophages is observed (2, 22).

In this work, we focus our interest on the effect of *A. platensis* on macrophages polarization and since M1 and M2 macrophages differ in the arginine metabolism pathway (3), we exploited this difference by evaluating the ratio NO/Urea in peritoneal macrophages.

Our results showed a significantly higher NO/Urea ratio in the standard group, suggesting mac-

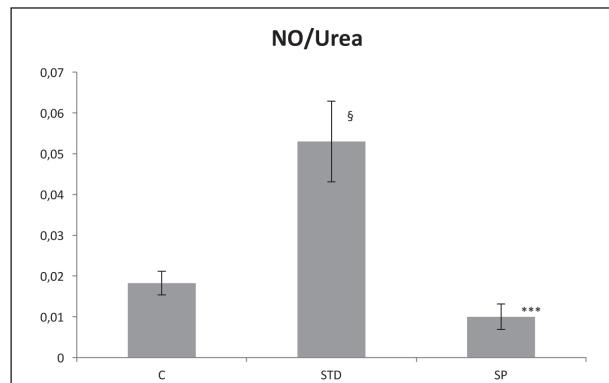


Figure 2. NO/Urea ratio calculated from NO and Urea production by macrophages from control (C), standard (STD) and spirulina-treated (SP) *Psammomys obesus*. Data are shown as the mean \pm SEM; *** $P<0.001$ (STD vs C); § $P<0.05$ (SP vs STD)

rophages polarization toward M1 macrophages, which exacerbate insulin resistance. In contrary, despite high caloric diet consumption, spirulina-treated group exhibited a lower but non significative ratio similar to that observed in the Control group. These results support similar beneficial outcomes of *A. platensis* in human medicine. A possible explanation for this may be linked to its diverse active ingredients which have antioxidant and anti-inflammatory properties (7, 23). Indeed, phycocyanin particularly has been reported to inhibit iNOS expression in macrophages stimulated by lipopolysaccharides (LPS) (24).

In summary, this study showed that macrophages's NO/Urea ratio is correlated with insulin resistance, which are both affected by spirulina intake; these findings suggest that spirulina administration at an early stage of pathogenesis prevents insulin resistance at least partly via the regulation of M1/M2 macrophages polarization.

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