CLINICAL

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POLYGLUCOSAMINE - ACTION ON OXIDISED LIPIDS AND DYSLIPIDEMIAS

SUMMARY

The use of chitosan) to reduce cholesterol and triglyceride levels and control body weight has been criticised as some controversial results, primarily relating to different methods and protocols, have been reported in the medical literature. The comparative inconsistency in results from clinical trials carried out in various countries (also in non-pathological individuals) should be attributed to several factors: diet, type of pathology or risk situation (if appropriate), and, above all, the type of chitosan administered.

In order to be active, chitosan has to have the appropriate molecular weight and a high water- and lipid-binding capacity. It should also be standardised in order to minimise the differences between the various production batches. Polyglucosamine (PG) is a typically standardised chitosan with the following characteristics: low molecular weight, high affinity for water and lipids and special capacity for binding oxidised lipids.

The aim of this study is to establish the activity of PG in patients suffering from type IIb hyperlipidemia, with a BMI of between 25 and 30.

A double-blind study against placebo was carried out on two groups (20 patients of both sexes, aged between 27 and 57), treated with doses of 2g/day (1g x 2) 30 minutes before main meals, for a period of three months. Their body weight, cholesterol (Total, LDL, HDL), triglyceride and oxidative stress (D-Roms test) levels were recorded before and after treatment. In the patients treated with placebo, no significant change was observed between the two periods.

In the patients treated with PG, there was a significant reduction ($p<0.05$ in the t test) in body weight (from 82.0 Kg ± 7.65 to 76.1 Kg ± 7.89), total cholesterol (from 248.3 ± 18.35 mg/dL to 214.0 ± 15.16 mg/dL) and triglycerides (from 264.3 ± 31.64 mg/dL to 224.6 ± 29.85 mg/dL). Oxidative stress had lowered from 349 ± 25.3 CARR U. to 321 ± 24.1 CARR U. ($p<0.05$ with the t test).

The results indicate that PG significantly restricts the absorption of lipids (cholesterol and triglycerides) and oxidised lipids. The product also appears to mobilize the reserve lipids in order to compensate for the energy requirements, therefore leading to weight loss.

KEY WORDS

POLYGLUCOSAMINE, DYSLIPIDEMIA, CHOLESTEROL, TRIGLYCERIDES, OXIDATIVE STRESS

INTRODUCTION

The use of **chitosan** for reducing cholesterol and triglyceride levels and for controlling body weight has been the subject of criticism, as there have been inconsistencies in the way the results have been reported in the medical literature.

Some authors reported positive results with various amounts (1-9), while some provided evidence of significant activity (10-15), and others also observed limited results with men (16).

We often refer to pathologies or risk situations such as obesity or various types of dyslipidemia. Attempts to carry out a meta-analysis on some of these studies have shown that chitosan is more active than placebo; however, this activity needs to be confirmed by more rigorous studies (17) and by using a large number of patients.

The aforementioned clinical studies fall within acceptable limits in terms of their methodological rigor, although various types of chitosan have been used (diverse formulations, doses and observation periods). Taking into account the polymeric nature of this substance and the wide range of materials from which it can be extracted (*chitin* from crayfish, crabs etc.), it would seem more appropriate to refer to chitosans themselves as being highly diverse (18-20).
The formulation is another area of disparity - it may or may not contain ascorbic acid (in varying quantities). The presence of ascorbic acid increases the capacity for forming gel and retaining lipids more effectively (21, 22).

In other words, chitosans themselves and their formulations vary greatly - therefore, each type of product (chitosan + related agents) must be standardised and evaluated for its therapeutic effect.

The aim of this study is to assess the effect of a specific chitosan, with standardised production and low molecular weight (*polyglucosamine* or *PG*), on patients suffering from Type IIb dyslipidemia who are overweight but below the limit of obesity i.e. have a Body Mass Index (BMI) of <30.

MATERIALS AND METHODS

The **polyglucosamine** (PG) used was isolated from crayfish and crabs using a grinding process, deproteinisation using NaOH and demineralisation using HCl. It then underwent hot deacetylation, followed by drying and sterilisation. The PG's molecular weight was established using the HP-SEC method (*High Performance Size Exclusion Chromatography*) combined with a TDA (*Triple Detector Array*) - the most accurate system for assessing the molecular weight of carbohydrate polymers (23).
Total cholesterol, LDL and HDL levels were determined using the standard me-

thods of digital spectrophotometry (*Free System - Diacron, Grosseto, Italy*) and dedicated reagents. Oxidative stress is assessed using the D-Roms test (24-26) - a measurement that correlates with the circulating hydroperoxides.

PG - average molecular weight = 145,000 D (low molecular weight) - was combined with the appropriate quantity of ascorbic acid to obtain a product with a high capacity for retaining water and emulsifying lipids.

Its capacity for retaining water is such that, in a mixing ratio between PG and water of 2:20 (mixture A), the PG suspension level should amount to at least 65% of the total volume. Its emulsifying capacity enables complete gelification of 1 ml of mixture A with 1 ml of extra-virgin olive oil (*Società Filippucci, Campello S/C - Umbria, Italy*).

PG's production process, calculation of molecular weight, and its water retention and emulsifying capacity were control elements for PG batches.

42 seemingly healthy individuals (23 men and 29 women), aged between 27 and 57, were admitted to the study. They had the following characteristics: a Body Mass Index (BMI) of between 26 and 30; total cholesterol levels >220 mg/dl; triglyceride levels of between 199 and 400 mg/dl.

Patients undergoing chronic therapy or treatment with hypolipemiants were not admitted to the study. Any illness that occurred during the study was regarded as grounds for exclusion if it led to more than four days of sickness or a stay in hospital.

No dietary precautions were requested; the only requirement was that the patients had to drink at least 1.5 litres of water per day, in addition to any water during meals.

Each patient's calorie intake was analyzed using a weekly questionnaire where the patients recorded the food quantities. This survey was carried out in the week prior to the beginning of treatment (WO), in the fourth week (W4), the eighth week (W8) and, finally, during the thirteenth (final) week of treatment (W13). These surveys allowed the differences in general calorie intake to be assessed. The food questionnaire used in the study was compiled by the Società Europea di Nutrizione Biologica (SENB - European Society of Biological Nutrition) which can be found on the website www.fia-db.com.

Based on 253 foods, the questionnaire was completed and returned by the subjects involved in the study (via email, fax, standard post or in person). If there were any inconsistencies, the individuals were contacted by telephone.

The study compared PG (*GUNA® - ARD Cholesterol*) and placebo (gum arabic, saccharose, and excipients, in identical packaging to that of the PG), both in boxes containing 48 tablets in 4 blisters.

Each tablet contained 500 mg of PG or 500 mg of placebo respectively; the administration method was 2 x 500 mg tablets, 30 minutes before the two main meals.

The tablets were taken with half a glass of water and the treatment was carried out for three consecutive months. The individuals were recruited between the first week in August and the first two weeks in September, at two gymnasiums equipped with a Medical Centre, from people who were intending to start a physical activity program in order to keep themselves in shape. The subjects selected signed an agreement to consent to delaying the start of their physical activity for three months in order to take part in the study.

After an interview to assess the admission criteria, the individuals underwent an anthropometric examination (height, body weight, abdominal circumference around the navel), followed by a blood test (after fasting for 12 hours) to assess laboratory parameters (total cholesterol, LDL, HDL, triglycerides, D-Roms test to determine oxidative stress). 103 people were assessed in order to find 42 individuals who met the admission criteria. For most of those excluded from the study, their triglyceride and/or cholesterol levels did not meet requirements. A two-week supply of the product was delivered to the individuals after a randomized allocation of treatment (PG or placebo). At a follow-up meeting/check-up, the patients were allocated the necessary quantity of the product for the remainder of the treatment period. The individuals therefore had to report to the test center for the second supply of the product and for the interview on general tolerance of the treatment. In some cases (8 PG and 2 placebo), the product was sent by post, as agreed by telephone.

Of the 42 patients, 2 (1 with PG and 1 with placebo) did not finish the trial as they could not be located when contacted for the meeting about the second supply of the product.

In the groups of individuals who were overweight with type IIb dyslipidemia and who did not undergo treatment with PG, the average differences between the values, three months apart, were equal to zero. Under the hypothesis of obtaining a clinically acceptable minimum average weight reduction equivalent to 1.5 Kg ± 1 (0.5 Kg/month of treatment), a clinically acceptable minimum cholesterol reduction equivalent to 15 mg/dL ± 10 (5 mg/dL/month) and a clinically acceptable reduction in triglycerides equivalent to 15 mg/dL ± 10 (5 mg/dL/month), the size of the sample for all three parameters, where $\beta = 1 - 0.9$ and $\alpha = 0.05$, is 15 individuals. Under the hypothesis of around a 40% *drop-out* rate, recruitment was set at **21 individuals** per group.

The target (main) parameters to be altered were pre-defined: body weight, total cholesterol and triglycerides. LDL and HDL cholesterol and oxidative stress were classed as secondary parameters. The averages and Standard Deviations (SD) were calculated for all the parameters.

The differences between treatment periods (basal and after three months) were assessed by applying the Student's t-test.

RESULTS

Of the 42 individuals enrolled in the study, 40 completed the trial (TABLE 1). Two patients could no longer be traced at the first check-up - patient No. 13, male, from the PG group and patient No. 1, female, from the placebo group. Therefore, the evaluation of the differential action of the two treatments is reduced to **20 patients/group**.

As we can infer from analyzing the data, the age of the subjects and the relative frequencies of the sexes, the two groups are homogeneous.

No significant differences were recorded over the various weeks between the two groups, which indicate that their diet was relatively stable. (TABLE 2)

Following treatment with PG, s**ubstantial weight loss** accompanied by **a reduction in total cholesterol and triglyceride levels** was recorded. (TABLE 3)

There was a significant reduction in LDL cholesterol levels **only** after treatment with PG. (TABLE 4)

HDL cholesterol levels did **not** alter significantly with any of the treatments studied. However, with the reduction in total cholesterol using PG, the LDL/HDL ratio improved significantly.

With regard to D-Roms test levels, normal levels are 250-300 CARR U. Therefore, in the basal survey, both groups recorded a high level of oxidative stress, which was only **reduced** significantly in the group treated with PG. (TABLE 4)

DISCUSSION

Under the test conditions used, treatment with placebo did not bring about changes in the predetermined parameters, whereas PG administered in doses of 2g/day for three months proved to significantly **reduce body weight** (an average of 13 lb, equivalent to a reduction of 1.91 in BMI, or a reduction from 27.06 to 25.15).

At the same time, a reduction in total cholesterol and triglyceride levels of 34.3 and 39.7 mg/dL respectively, equivalent to a **13.8% reduction in cholesterol and a 15% reduction in triglycerides**, was recorded.

LDL cholesterol was also reduced by treatment with PG, by 28.3 mg/dL on average. There were no significant changes in HDL cholesterol, which only increased by 1.9 mg/dL.

However, as a result of the marked reduction in LDL Cholesterol, the HDL/LDL ratio
altered significantly.

All in all, caloric intake did not alter during the trial and, therefore, the changes in the parameters studied should be traced back to the use of PG. Furthermore, from the data we can infer that the subjects generally had a ca-

AR_D Lipiban® (AR_D Cholesterol)			Placebo		
Number	Sex	Age	Number	Sex	Age
1	M	39	2	M	42
2	M	35	3	M	33
3	M	34	4	M	33
4	M	37	5	M	36
5	M	51	6	M	44
6	M	53	7	M	56
7	M	57	8	M	46
8	M	42	9	M	44
9	M	43	10	M	39
10	M	40	11	M	41
11	F	40	12	F	43
12	F	39	13	F	44
14	F	27	14	F	35
15	F	29	15	F	27
16	M	31	16	F	29
17	M	33	17	F	33
18	F	37	18	F	38
19	F	32	19	F	36
20	F	31	20	F	39
21	F	31	21	F	33
Average		38,1	Average		38,6
DS		8,09	DS		6,73

Table 1
Age and sex in the two groups. Average values ± SD (Standard Deviation) **of the individuals treated with ARD Cholesterol® (4 tablets/day) or placebo (4 tablets/day).**

Week	AR_D Lipiban® (AR_D Cholesterol)	Placebo
S 0	3315 534,5	3320 552,9
S 4	3333 501,2	3316 543,3
S 8	3339 549,5	3329 543,4
S 13	3327 513,2	3316 551,6

Table 2
Average daily values ± SD of calories consumed during the observation period in individuals treated with ARD Cholesterol or placebo.

Table 3

Average values ± SD of the main parameters (body weight, total cholesterol and triglycerides) before and after treatment with PG or placebo.

To = Time zero

Treatment		Body weight Kg	Total cholesterol mg/dL	Trigylcerides mg/dL
AR_D Lipiban® *(AR_D Cholesterol)*	To	82,0 7,65	248,3 18,35	264,3 31,64
	Dopo 3 mesi	76,1 7,89*	214,0 15,16*	224,6 29,85*
Placebo	To	82,1 8,80	243,7 17,03	264,8 22,09
	Dopo 3 mesi	82,2 8,53	243,3 15,81	265,6 24,93
		* To vs After 3 months; Student's t-test $p < 0.05$		

Table 4

Average values ± SD of the secondary parameters (LDL and HDL cholesterol, and D-Roms test) before and after treatment with PG or placebo.

To = Time zero

Treatment		LDL cholesterol mg/dL	HDL cholesterol mg/dL	D-Roms test U. CARR.
AR_D Lipiban® *(AR_D Cholesterol)*	To	155,0 14,66	40,5 7,31	349 25,3
	Dopo 3 mesi	126,7 11,80*	42,4 7,35	321 24,1*
Placebo	To	153,7 15,06	37,1 7,06	350 26,3
	Dopo 3 mesi	151,6 13,86	38,6 7,16	355 33,2
		* To vs After 3 months; Student's t-test $p < 0.05$		

loric intake in excess of their daily activity - which was basically sedentary or moderate. This all accounts for an above-average BMI.

On average, **oxidative stress was reduced** from 349 to 321 CARR. U - a satisfactory result.

The variations in the parameters studied proved far greater than anticipated in the planning for the trial, which predicted a high number of individuals with more limited alterations. Given the relatively large number of subjects, the results are believed to be **particularly significant**. The reduction in oxidative stress requires further consideration.

From the in *vitro* experiments it is clear that PG tends to emulsify lipids and has a greater affinity for oxidised rather than unoxidised lipids. This effect indirectly affects the quality of the chilomicrons and LDLs which, after PG has been taken, form with a much lower amount of oxidised lipids and will, therefore, be less receptive to subsequent oxidation phenomena.

Furthermore, it was noted that hypercholesterolemy is very often linked with oxidative stress, due to a reduction in the reserve of antioxidants (data currently being published) and an increase in reactive-type oxidation.

Nevertheless, if within the scope of hypercholesterolemy one separates those individuals with concomitant hypertriglyceridemia, you will note that the latter, although under oxidative stress, display significantly lower D-Roms test levels and a markedly higher reserve of reduced glutathione (GSH). In subjects with lower D-Roms test levels, the phenomenon can be interpreted as an increased synthesis of triglyerides.

Unlike triglycerides of dietary origin, those produced ex novo by the body are not oxidised. This gives the lipoproteins a lower propensity to oxidative stress. In studies, it was noted that oxidised lipids enter the chilomicrons followed by the LDLs (27), making them more easily oxidisable; as a result, one notes a clear increase in D-Roms test values.

Therefore a reduction in triglyceride levels together with a reduction in oxidative stress indicates that a mobilization of lipid reserves is underway, which, as they are truly "reserves", have, by definition, a greatly reduced oxidation level.

When we consider that reducing lipids using only a diet that is low in fat is difficult to achieve and maintain, many individuals are treated over long periods of time with hypocholesterolemizing drugs. These enter into the metabolism and cause a series of enzyme changes and adjustments that subject the patient to collateral effects that are sometimes extremely significant and, in some cases, fatal. It is, therefore, useful to have a "*non-metabolic*" method of control such as PG, which does not "*enter*" into the metabolism but always remains solely within the alimentary canal.

Furthermore, the **absorbing action** of PG has an important preventive action as it tends to carry all the substances (particularly liposoluble ones), which can have an inflammatory, toxic or car-

cinogenic action on the cells of the intestinal wall. The increase in mass moving within the intestine also tends to improve various intestinal disorders, particularly those linked to low residual content in the diet.

In conclusion, the lowering of cholesterol and triglyceride levels demonstrates that PG has a *sequestering-type action* on lipids, whilst the reduction in oxidative stress indicates a *sequestering* action on oxidised lipids and mobilisation of lipid reserves to be used for energy. The latter has a beneficial effect on weight reduction in a relatively short space of time having a natural action, thus eliminating the risk of collateral effects.

The effect obtained also has an extremely favourable costs/benefits ratio (particularly in comparison with pharmacological treatments).

The results need more in-depth analysis and this will be assessed in various studies currently underway. ■

N.B. The product mentioned in this article is named **ARD LIPIBAN** in Italy and is named **ARD CHOLESTEROL** in USA.

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