Whey proteins as a food supplement in HIV-seropositive individuals

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Abstract

On the basis of numerous animal experiments, a pilot study was undertaken to evaluate the effect of undenatured, biologically active, dietary whey protein in 3 HIV-seropositive individuals over a period of 3 months. Whey protein concentrate was prepared so that the most thermosensitive proteins, such as serum albumin which contains 6 glutamylcysteine groups, would be in undenatured form. Whey protein powder dissolved in a drink of the patient's choice was drunk cold in quantities that were increased progressively from *8.4 to 39.2 g per day*. Patients took whey proteins without adverse side effects. In the 3 patients whose body weight had been stable in the preceding 2 months, weight gain increased progressively between 2 and 7 kg, with 2 of the patients reaching ideal body weight. Serum proteins, including albumin, remained unchanged and within normal range, indicating that protein replenishment per se was not likely the cause of increased body weight. The glutathione content of the blood mononuclear cells was, as expected, below normal values in all patients at the beginning of the study. Over the 3-month period, glutathione levels increased in all 3 cases. In conclusion, these preliminary data indicate that, in patients who maintain an adequate total caloric intake, the addition of "bioactive" whey protein concentrate as a significant portion of total protein intake increases body weight and shows elevation of glutathione (GSH) content of mononuclear cells toward normal levels. This pilot study will serve as a basis for a much larger clinical trial.

Résumé

En s'appuyant sur des données obtenues chez l'animal, nous avons entrepris une étude pilote afin d'évaluer l'effet de l'administration de protéines non-dénaturées et biologiquement actives du petit lait administrées chez 3 patients séropositifs HIV au cours d'une période de 3 mois. Le concentré protéique de petit lait a été préparé de façon à garder intact les protéines thermosensibles comme l'albumine qui contient 6 groupes glutamylcystéine. La poudre protéique a été dissoute dans un breuvage au choix du patient et absorbée froide en quantités progressivement croissantes *de 8.4 à 39.2 g par jour*. Les 3 patients n'ont présenté aucun effet secondaire. Chez les 3 patients qui ont pris ce produit de façon régulière, le poids corporel, qui était stable dans les deux mois précédents, a augmenté de façon progressive entre 2 et 7 kilos, et 2 patients ont atteint leur poids idéal. Les protéines sériques, incluant l'albumine, sont demeurées inchangées et dans les limites de la normale suggérant qu'une réplétion protéique n'était pas la cause de la prise de poids. Le contenu en glutathion des cellules mononucléées du sang était inférieur à la normale chez tous les patients au début de l'étude. Au cours des 3 mois d'administration protéique, le niveau de glutathion s'est élevé dans tous les cas. Nous concluons que ces données préliminaires indiquent

que l'addition d'un concentré protéique de petit lait non-dénaturé en quantité suffisante pour représenter une portion significative de l'apport protéique total, peut permettre un gain de poids et une normalisation du contenu en glutathion des cellules mononucléées chez des patients qui maintiennent un apport calorique total adéquat. Cette étude pilote rapporte une bonne tolérance aux protéines du petit lait dans ces conditions et ouvre la possibilité d'une étude prospective beaucoup plus large.

Introduction

Our studies have shown that the humoral immune response (number of plaque-forming cells to sheep red blood cells [SRBC] is significantly higher in mice fed a diet containing 20 g of whey protein concentrate per 100 g of diet than in mice fed formula diets of similar nutritional efficiency but where the "bioactive" whey has been replaced by other semipurified food proteins, such as casein, soy, wheat, corn, egg white, fish, beef protein, *Spirulina maxima*, *Scenedesmus* algae protein, or Purina mouse chow [1].

We have further shown that the immunoenhancing activity of the dietary whey protein concentrate is related, at least in part, to greater production of splenic glutathione in the "bioactive" whey protein-fed animals during the oxygen-requiring antigen-driven clonal expansion of the lymphocyte pool [2]. On the basis of available knowledge [3, 4], it was then proposed that this might reflect the ability of the lymphocytes of whey protein-fed mice to offset potential intra-cellular oxidative damage, thus responding more fully to the antigenic challenge. In fact, the capacity of a cell to recover from an oxidative insult is considered to be represented by its ability to generate intra-cellular stores of glutathione (GSH) [5]. Our studies also showed that administration of S-(n-butyl) homocystein sulfoximine, which reduces splenic glutathione by half, significantly reduced the humoral immune response of whey protein-fed mice. This was taken as further evidence for the important role of glutathione in the immunoenhancing effect of dietary whey protein [2].

Tissue glutathione concentration may be increased by the administration of gamma-glutamylcysteine so that glutathione increases in the kidney by about 50%, 40–60 min after s.c. injection of this material in mice, returning to control values 2 h later [6]. The administered gamma-glutamylcysteine is transported intact and serves as a substrate for glutathione synthetase [7].

Advances in the study of amino acid sequencing of food proteins has allowed us to investigate the occurrence of gamma-glutamylcysteine groups in whey protein and its possible relation to glutathione promotion. Indeed, whey protein concentrate from bovine milk contains substantial amounts of glutamylcysteine dipeptide groups, unlike casein, which does not increase tissue glutathione when fed to mice [1]. The glutamylcysteine groups are located primarily in the serum albumin fraction (6 groups/molecule) of the whole milk and the whey derivative. Glutamylcysteine groups are extremely rare in animal and plant edible proteins. An extensive search of all available data on amino acid sequencing of edible proteins revealed that the Glu-Cys group with disulfide link is indeed limited to whey protein, and to the ovalbumin fraction of egg white which contains 2 of these groups in a 30,000 mol. wt. molecule [8].

Our recent data [8] further indicate that the humoral immune response is highest in mice fed a dietary whey protein concentrate exhibiting the highest solubility (undenatured conformation) and, more importantly, a greater relative concentration of the thermolabile bovine serum albumin (10% 0.5) and immunoglobulins. In addition, the mice fed this type of whey protein concentrate exhibit higher levels of tissue glutathione. The presence in the serum albumin fraction of glutamylcysteine groups (rare in food protein) and the specific intramolecular bond as related to the undenatured conformation of the molecule are considered to be key factors in the glutathione-promoting activity of the protein mixture.

Our whey protein concentrate was prepared from milk treated in a proprietary fashion which is compatible with accepted standards of safety with regard to sterility. The material in question has been given the trademark name IMMUNOCAL (hereafter "Immunocal") and this was kindly provided to us for the studies reported by Immunotech Research

Corporation Ltd. of Montréal, Québec. Although the proteins contained in the concentrates from the other commercially-available sources examined were mostly in undenatured form, as indicated by relative solubility of the concentrates, the content of serum albumin and immunoglobulins in these mixtures was below the level apparently necessary to produce the significant biological activity noted above [8]. It would appear, then, that in the other whey protein concentrates examined, the very thermolabile proteins had been denatured, became insoluble, and were subsequently lost from the final preparation made available commercially. The relatively high concentrations of serum albumin and immunoglobulins of Immunocal reflect more closely the pattern of raw milk and indicate that these thermosensitive materials have been retained during the preparation of the product. These data lend support to the hypothesis that the thermolabile Glu-Cys-containing proteins, such as serum albumin in undenatured conformation, are crucial elements for the biological activity of whey protein concentrate in terms of intracellular glutathione synthesis and the other biological activities described.

Recent experiments in Japan [9] showed that spleen cells of BALB/c male mice fed a 25 g Immunocal/100 g diet for 4 weeks had an increased immune response to SRBC in vitro, and a higher content of $L3T4^+$ cells ($12.58 \times 10^6 \pm 1.36$) than mice fed on an isocaloric diet with 25 g pure casein/100 g diet ($3.69 \times 10^6 \pm 0.50$). Similarly, the spleen $L3T4^+/LYt-2^+$ ratio was 1.36 ± 0.07 in Immunocal-fed mice, and 0.55 ± 0.07 in casein-fed controls ($p < 0.001$).

With this background, a pilot study was undertaken to investigate the possible beneficial effect of Immunocal in symptom-free HIV-seropositive individuals. This syndrome is characterized by immunodeficiency, low T-helper cell blood content, increased oxidative stress [10], and systemic glutathione deficiency [11].

Materials and methods

Immunocal was given orally to 3 white male HIV-seropositive individuals. These patients took the product daily in a liquid of their choice for a period of 3 months. The daily intake of pure whey protein prescribed to the patients as Immunocal was increased step-wise. During the first 4 weeks, 8.4 g were prescribed daily; in the following 4 weeks, 19.6 g; and in the final 4-week period the dose was raised to 28 g (first week) and 39.2 g (last 3 weeks). Protein intake from other sources was reduced by corresponding amounts.

Immunocal

Bovine whey protein concentrate was especially prepared for us by the "Services de recherche sur les aliments du Ministère de l'agriculture du Québec" in St-Hyacinthe, Québec, with the following characteristics: pure protein content 75% (the rest mostly lactose, some fat, and moisture), and solubility index (ph 4.6):99.5%. Protein composition as a percent of total whey protein measured by polyacrylamide gel electrophoresis [8] was: beta-lactoglobulin: $59:1 \pm 4.0$; alpha-lactalbumin: 6.6 ± 0.7; serum albumin: 9.7 ± 1.0; immunoglobulin: 24.6 ± 2.6 (mean SD).

Upon bacteriological analysis: no *staphylococci*, no *salmonella*, no *B. cereus* or *E. coli* were isolated.

Administration to patients

Glutathione content of blood mononuclear cells

Thirty ml of heparinized blood were used. Mononuclear cells concentrated by ficoll *hypaque* were resuspended in phosphate-buffered saline adjusted so that there were 10^7 cells per tube in 8 ml. After centrifugation, 900 µl of H_2O was added to the pellet to lyse all the cells. To each aliquot was added 30% sulfosalicylic acid for a final concentration of 3% in 1 ml. After 15 min incubation, the samples were centrifuged, and the clear supernatant was used for the biochemical assay according to the method of Anderson [12]. Values were expressed as nmol GSH/10^7 cells.

Lymphocyte immunophenotyping

Blood lymphocyte subsets were determined by flow-cytometry. Heparanized whole blood from patients or controls was prepared for staining for 2-color analysis using suggested techniques for cell preparation with Becton Dickenson antibodies (CD3, 4, 8). Red blood cells were lysed and the remaining cells were fixed with paraformaldehyde. Cells were analyzed within 72 h of staining to optimize dye fluorescence, and analysis was per-

formed on a Becton Dickenson FACSCAN flow cytometer. Calibration was performed with an anti-CD14/anti-CD45 control, as well as isotype IgG_2 antibodies. A control specimen was also performed in parallel to verify results.

Results

The following observations were made (Table 1):

Three patients took Immunocal daily for the 3-month period without any adverse side effects. In all these patients body weight increased progressively (from 2 to 7 kg); 2 of them (J.P., F.I.) reached ideal body weight. It is interesting to note that the body weight of patient L.S. was 101 kg 2 months and 100 kg 1 month prior to the study. Corresponding values for patient F.I. were 76.5 kg and 76.5 kg, and for patient J.P. 76.5 kg and 76 kg, respectively. Serum proteins, including albumin, remained unchanged and within normal range, indicating that protein replenishment per se was not likely the cause of increased body weight.

The glutathione content of blood mononuclear cells was as expected [11], below normal values in all patients at the onset of the study. Over the 3-month period, however, glutathione levels increased and in one case (F.I.) rose by 70% to reach normal value.

These objective changes were accompanied by a marked improvement of a subjective sense of well-being in all 3 patients.

It is noteworthy that 1 patient, over-concerned that the beneficial increase in body weight could hamper his lean appearance, drastically reduced energy and Immunocal intake during the second period of study. During this time body weight increase was reduced and glutathione failed to rise (J.P., Table 1).

Discussion

Our preliminary data indicate that whenever patients maintain their energy intake at pre-study levels but replace a significant portion of the protein intake with Immunocal, body weight increases and mononuclear cell glutathione increases. Given that cellular glutathione is very tightly regulated [8] and that the pre-study cellular glutathione values were very similar in our 3 patients, the observed increases in cellular glutathione concentration are likely to have biological importance.

The positive effects of Immunocal observed in a very limited number of HIV-seropositive individuals acquires significance if viewed on the background of a large number of animal experiments showing elevation of cell glutathione and immune response by Immunocal [1, 2, 8, 9]. Animal studies emphasize the fact that the immunoenhancing effect of Immunocal is not related to a greater systemic nutritional efficiency when compared to several other protein sources with similar nutritional efficiency but no significant biological activity. Mice fed Immunocal did not exhibit increased body growth nor any changes in serum protein levels. Similarly, in our patients Immunocal did not produce any change in serum proteins which remained constant throughout the study. The increase in body weight observed in our patients did not correlate with increase in energy or protein intake throughout the study period but rather with improved sense of well-being. The extra protein intake through Immunocal was generally compensated by reduced intake of protein from other sources. The whey product was well tolerated in these 3 patients at different doses; no side effects were noted.

The presence of glutamylcysteine groups in the serum albumin component of the whey protein concentrate is considered to be a key factor in the glutathione-promoting and immunoenhancing activity of the protein mixture of Immunocal. Our laboratory studies indicate that whey protein concentrates, from other sources, did not produce significant biological activities while exhibiting similar nutritional efficiency. The percent serum albumin concentration in these products is (as mean ± SD, respectively): 4 ± 1 in Promod (Ross Laboratories), 4 ± 1 in Alacen 855 (New Zealand Dairy), 4.8 ± 1 in Lacprodan-80 (produced from 1989 by Danmark Protein), 4.8 ± 0.1 in Sapro (Saputo, Montreal), 4 ± 1 in Savorpro-75 (Golden Cheese, CA), 5 ± 1 in Bioisolate (Lesueur Isolates, Minneapolis) [8], and 4.3 ± 1 in Promix (Dumex, Quebec). Similarly, the content of the other thermolabile protein, immunoglobulin, was about half the value of Immunocal [8].

In conclusion, this preliminary study clearly indicates the need for further clinical investigation on the effect of Immunocal in HIV-seropositive

Table 1.

WHEY	Patient initials (age)	Weeks on whey	Energy (K cal)/ protein(g) (1)	Ideal body weight (kg)	Body weight (kg)	CD4% (2)	Helper absolute (3)	CD4/CD8 (4)	GSH n mol-10^7 cells (5)	Serum Proteins in %				
										Total	Alb	IgG	IgA	IgM
		0	2180/87	86	102.5	23	368	0.38	9.75	77	42	20.8	1.54	1.14
	L.S. (35)	6	1800/106		105	26	546	0.45	10.34	74	39	19.4	1.56	1.16
		12	2111/100		108	24	480	0.41	13.9	80	42	20.1	1.61	1.05
		0	1870/84	75.2	73.9	15	435	0.22	10.22	82	50	12.3	4.38	0.75
	F.I. (32)	6	2035/140		74.5	19	532	0.28	9.6	79	45	14.3	4.53	0.76
		12	2100/138		76	17	442	0.24	17.04	72	50	16.9	5.81	0.95
		0	2400/100	78	76	24	672	0.39	10.38	82	52	12.4	3.59	0.76
	J.P. (29)	6	2200/116		77.5	27	864	0.46	12.55	81	49	12.9	3.99	0.58
		12	1400/98		78	26	676	0.45	7.06	80	50	12.8	3.8	0.7

(1) The energy and protein intake indicated represents the mean value for the preceeding weeks.
(2) Normal range: 35–55
(3) Normal range: 580–1250
(4) Normal range: 1.42–3.56
(5) GSH: Glutathione content of mononuclear blood cells. Normal value in healthy seronegative individuals of corresponding age: 17.05 ± 2.40 (mean ± SD)

asymptomatic or symptomatic patients. Whey proteins, by providing specific substrate for glutathione replenishment in the lymphocytes, could indeed represent an adjuvant to other forms of therapy.

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