ARTÍCULO

Potential use of certain lectins for the follow-up of patients operated for colorectal cancer

José Antonio Cabezas Fernández del Campo[1]*, Mario Sánchez-Martín[2], Sandra Ortega[2], Alberto Gómez-Alonso[3], Jacinto García[3], Luc Landemarre[4], Geniviève Motta[4], Michel Monsigny[4]

[1]Académico de Número de la Real Academia Nacional de Farmacia. [2]Departamento de Biología y Bioquímica Molecular. Facultad de Biología. Universidad de Salamanca. Spain. [3]Departamento de Cirugía. Hospital Universitario. Salamanca. Spain. [4]Centre de Biophysique Moleculaire. Laboratoire de Glycoconjugués. CNRS. Orleáns. France.
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e-mail: edicion@ranf.com

ABSTRACT

The reactivity of 15 lectins or their derivatives (commercially available) were determined in blood serum of a control group of 13 apparently healthy humans in comparison with: (I) a group of 28 patients of colorectal cancer, explored 1 day before surgical exeresis; (II) another group of 15 subjects analysed 4-7 days after surgery; and (III) 27 subjects investigated 7-9 months after their operation. The lectins or their derivatives were selected taking into consideration the peculiarities of their specificities for the glycoconjugate ligands in the sera. A very different reactivity was found. Results pointed to certain variability for the pathological sera analysed. In addition, differences in the lectin reactivity depending on the time of surgical exeresis (4-7 days in comparison to 7-9 months) were detected. The usefulness of the assays with certain lectins (SNA = Sambucus nigra, LEL = Licopersicon esculentum and LTL = Lotus tetragonolobus) in the follow-up of the health status of patients operated for colorectal cancer is discussed.

Key words: Colorectal cancer; Lectins; Glycoconjugates; Cancer follow-up.

RESUMEN

Uso potencial de ciertas lectinas para el seguimiento del estado de salud de pacientes operados de cáncer colorrectal

Se ha determinado la especificidad de la reacción de 15 lectinas o sus derivados (disponibles comercialmente) con glicoconjugados de sueros sanguíneos de un grupo control de 13 humanos aparentemente sanos, en comparación con: (I) un

grupo de 28 pacientes de cáncer colorrectal, explorados 1 día antes de la intervención quirúrgica; (II) otro grupo de 15 sujetos analizados 4-7 días después de dicha intervención; y (III) con 27 sujetos investigados 7-9 meses después de la operación (en estado satisfactorio de salud). Las lectinas o sus derivados fueron seleccionados tomando en consideración las peculiaridades de sus respectivas especificidades en relación con los ligandos de naturaleza glicoconjugada de los sueros analizados. Se halló reactividad diferente según los sueros. Así, se detectaron diferencias en la intensidad de la reacción, dependiendo del tiempo transcurrido desde la intervención quirúrgica (4-7 días en comparación con 7-9 meses). Por último, se discute la utilidad de las determinaciones con ciertas lectinas (las de SNA = Sambucus nigra, LEL = Licopersicon esculentum y LTL = Lotus tetragonolobus) en el seguimiento del estado de salud de personas operadas de cáncer colorrectal, como valoraciones complementarias de las habituales empleadas con esta finalidad.

Palabras clave: Cáncer colorrectal; Lectinas; Glicoconjugados; Seguimiento posoperatorio de cáncer colorrectal.

1. INTRODUCTION

The alteration of cellular glycosylation which occurs in cancer processes and the biological signification of the changes produced in certain glycoconjugate molecules have been reviewed (1-7). The increase of complex type sugar chains with a 2,6-branched outer chain might be considered (7) as the molecular basis of the so-called "Warren-Glick" phenomenon, an important discovery of the sixties observed through comparison of the glycopeptide patterns from normal and malignant cell glycoproteins. Such changes seem to involve the increased β1-6 branching of N-linked oligosaccharide truncation of the glycan chains, etc. Monosaccharides such as N-acetylneuraminic acid (Neu5Ac), N-acetylglucosamine (GlcNAc), α-L-Fucose, etc., are frequently components of these glycan moieties.

Although «strictly speaking, on a chemical basis, there are no "tumour specific" structure» (2), there are «non-specific structures» (2) (glycoconjugates) present at the tumour-cell surface which may accumulate in the sera of cancer patients and may be considered as markers for diagnosis, prognosis and metastasis.

The above-mentioned changes in both the concentration and structure of many glycoconjugates might be determined by the use of lectins.

Reviews on lectins – ("carbohydrate-specific proteins that mediate cellular recognition") (8) –, depending that recognition on multiple structural features of the diversity of sialic acids, have been published (8-11). In addition, lectin assays for investigating tumour marker glycoproteins have also been reported (12, 13).

We have studied the potential use of certain lectins as indicators for the possible follow-up of the health status of human operated for colorectal cancer (who remained apparently healthy at least 7-9 months after surgery). We have employed simple assays with some selected (commercially available) lectins. To our knowledge, this aspect of the issue has received little attention. These lectins could detect changes in Neu5Ac-, GlcNAc-, α-L-Fuc, α-Gal-, α-Man- y GalNAc-containing glycoconjugates from the sera of colorectal cancer operated subjects in comparison to control sera.

2. MATERIAL AND METHODS

2.1 Patients

Serum specimens were collected from 86 adults (age 51-88 years) after informed consent had been obtained. The control group comprised 13 individual judged to be healthy on the basis of their medical history. The experimental groups comprised 72 subjects with a diagnosis of colorectal cancer as demonstrated by histological tests. Patients/formerly patients were distributed in three groups: Group I, of 28 subjects explored 1 day before surgical exeresis; group II, with 18 subjects analysed 4-7 days after surgery; and group III, with 27 subjects investigated 7-9 months after surgical exeresis.

2.2 Blood sampling

Blood was taken by venipuncture from fasting subjects between 09:00 and 10:00 under standard conditions. Samples were collected in sterile tubes (Terumo®) with coagulation activators and a silicone coated gel barrier to aid separation of the serum. After being allowed to settle for a suitable time (< 1 h) at room temperature, tubes were centrifuged at 3000 x g for 10 min. The serum thus collected was distributed in aliquots of 500 µl each and stored at -80 °C until use.

2.3 Chemicals

All reagents were of analytical grade or the highest purity available.

2.4 Protein Ddetermination

Proteins were determined by the method of Lowry et al. (14, 15).

2.5 Lecitinas

Biotinylated lectins were from Vector Laboratories (USA), except Helix pomatia (HPA) and Vicia villosa (VVA) lectins (Sigma, USA). ExtraAvidin® - Peroxidase and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were also from Sigma (USA).

2.6 Lecitin meassures

Ligands of sera from the experimental groups were analysed by their reactivity with 15 lectins/lectin derivatives in comparison to control sera.

To quantify specific carbohydrate structures carried on serum glycoconjugates, we coated ELISA plate wells with serum samples; 100 µl of serum diluted with PBS buffer/well were incubated at 4 °C overnight. After washing with PBS/Tween 20, the biotinylated-lectin solution was added and allowed to bind for 1 hour at 37 °C. The excess lectin was removed by 3 washes with PBS/Tween 20. Then, an ExtrAvidin®-Peroxidase-complex containing solution was added and allows binding for 30 min before removing the excess complex. The ExtrAvidin®-Perixodase bound, and therefore the lectin bound, was quantified by measuring the peroxidase reaction using ABTS as substrate. Other details can be found elsewhere (16).

We optimised the experimental conditions to avoid non-specific fixation of the lectins and to use convenient concentrations of both lectins and sera.

Comparison between groups was performed using analysis of variance (ANOVA). The Scheffé and Fischer testy were employed for comparison among groups using Stat View SE+GraphicsTM 1.03 software for Macintosh; P values ≤ 0.01 were considered highly significant. Correlation analysis was realized using the same software. All results were expressed as mean ± S.E.M.

3. RESULTS

The 15 lectins whose abbreviated and detailed names are indicated in Table 1 were assayed. For each lectin we used 3 different concentrations and determined their fixation on a serum dilution series (from 1/50 to 1/102400 in PBS). The results for *Lycopersicon esculentum* lectin (LEL) and for *Lotus tetragonolobus* lectin (LTL) are shown in figure 1 and figure 2, respectively. Similar results were found for the other assayed lectins (data not shown).

The optimal experimental conditions for all the lectins assayed are summarised in Table I.

In addition, we found that the optimal concentration for the ExtrAvidin®-Peroxidase complex is 2 µg/ml diluted in PBS. Thus, non-specific binding of the complex to the ELISA plate was minimal, with no decrease in fixation capacity to the biotinylated lectin. After final adaptation of the method, we measured the fixation capacity of the biotinylated lectins to ligands from pathological and normal sera. The results are shown in Table II.

Table I. - Optimal biotinylated-lectin concentrations and optimal protein concentration range to detect change in several carbohydrate structures of serum glycoproteins.

Abbreviated name	Source	Lectin concentration	Protein concentration	Minimum serum dilution	Maximum serum dilution
SNA	*Sambucus nigra*	2	0.8-12.5	1/6400	1/102400
MAL II	*Maackia amurensis*	20	3-50	1/1600	1/25600
LEL	*Lycopersicon esculentum*	20	3-12.5	1/6400	1/25600
WGA	*Triticum vulgare*	20	3-50	1/1600	1/25600
LTL	*Lotus tetragonolobus*	5	3-12.5	1/6400	1/25600
ECL	*Erythrina cristagalli*	20	6-25	1/3200	1/12800
EEL	*Euonymus europaeus*	20	6-25	1/3200	1/12800
DBA	*Dolichos biflorus*	20	6-25	1/3200	1/12800
UEA I	*Ulex europaeus*	20	6-100	1/800	1/12800
UEA II	*Ulex europaeus*	40	3-50	1/1600	1/25600
HPA	*Helix pomatia*	FND	-	-	-
NPA	*Narcissus pseudonarcissus*	FND	-	-	-
SucWGA	*Triticum vulgare*	FND	-	-	-
GSL-I-B_4	*Bandeiraea simplicifolia*	FND	-	-	-
VVA	*Vicia villosa*	FND	-	-	-

Concentrations expressed as μg/ml. FND: Fixation was not detected.

Table II.- Specific fixation capacity of biotynylated lectins to ligands of pathological and control sera.

LECTIN		RESULTS		
Abbreviated name	Specificity	Reactivity	Comparison with controls	
			4-7 days	7-9 months
SNA	Neu5Ac(α2-6)Gal, Neu5Ac(α2-6Gal/*N*Ac	Very good	25% increase	20% increase
Mal II	Neu5Ac(α2-3)Gal	Good	25% increase	15% increase
LEL	(Glc*N*Ac)$_3$, (Glc*N*Ac)$_4$	Good	20% increase	6% increase
WAG	Glc*N*Ac(β1-4)$_3$>Glc*N*Ac((β1-4)$_2$>Neu5Ac	Good	12% increase	10% increase
LTL	α-L-Fuc	Good	37% decrease	No difference
ECL	Gal(β1-4)Glc*N*Ac	Good	30% decrease	24% decrease
EEL	Gal(α1-3)Gal	Good	21% decrease	16% decrease
DBA	α-Gal*N*Ac	Good	25% decrease	10% decrease
UEA I	α-L-Fuc	Weak	No difference	No difference
UEA II	L-Fuc(α1-2)Gal(β1-4)Glc	Weak	No difference	No difference
HPA	Gal*N*Ac	Very weak	No difference	No difference
NPA	α-Man	Very weak	No difference	No difference
SucWGA	Glc*N*AcNeu5Ac	Very weak	No difference	No difference
GSL-I-B_4	α-Gal	Very weak	No difference	No difference
VVA	α-Gal*N*Ac-Met > α-Gal*N*Ac-Thr	Very weak	No difference	No difference

We did not find any general correlations between sialic acids, CEA, CA 19-9 and α-fetoprotein concentration values and the affinity of the ligands for the lectins in the sera assayed (data not shown).

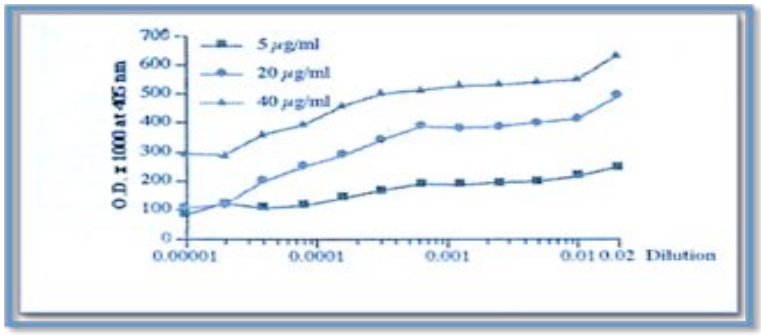

Figure 1.- Effect of lecitin concentration on LEL fixation.

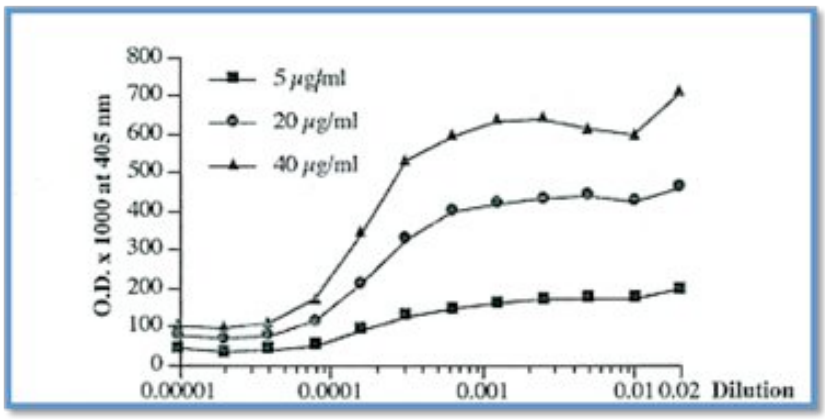

Figure 2.- Effect of lecitin concentration on LEL fixation.

4. DISCUSSION

The approximate recurrence rates following curative resection of adenocarcinoma of the colon and rectum are estimated as follows: 30% for sigmoid and rectum, 20% for right colon and 10% for transverse and left colon (17).

The follow-up the health status of operated colorectal cancer subjects with additional criteria to those now established seems to be an important aim. Furthermore, these assays would be much easier to perform on sera than in pathological tissue samples obtained after surgical operations.

In a previous paper (15), we discussed in this view the usefulness of the determination of the activities of several glycosidases and cathepsin L in the era of colorectal operated subjects 4 months after surgery. Now, we propose the use of certain lectins for the follow-up of the operated, 7-9 months after surgical exeresis.

Fucosyl or sialosyl-fucosyl, Lewisx and sialosyl Lewisx, sialosyl-dimeric Lex, Lea, monosialosyl Lea (CA 19-9), disialosyl Lea, Tn, sialosyl-Tn, galactose, and β1-6 branched oligosaccharides antigens (see their structures in reviews) (1-3,5) have been investigated/determined by monoclonal antibody, lectin or other assays in relation to invasive capacity, metastatic potential, prognosis or survival of colorectal operated patients.

We have found remarkable differences in the reactivity of the assayed lectins (see Table II). In addition, the reactivity profile shows a parallelism between SNA and MAL II, both at 4-7 days and 7-9 months after exeresis, probably due to the fact that both lectins have similarity for their specificities, as indicated in the Table. As expected, their reactivities differ from those of the other assayed lectins. Precisely, this different behaviour could suggest the possibility of choice and the selection for use as analytical tools of certain lectins such as SNA, LEL and LTL, whose profiles of reactivity are not parallel. In fact, SNA shows similarly increased values (20-25% in comparison to controls), both at 4-7 days and 7-9 months, while LEL shows a 20% increased value at 4-7 days but only a 6% increased value at 7-9 moths, and finally LTL exhibits a decrease of 37% at 4-7 days and shows not significant difference in comparison to control at 7-9 months after surgery.

A possible correlation between these results and the structure of certain antigens could be tentatively suggested as follows: SNA reactivity, with sialosyl-Tn; LEL, with sialosyl Lea; and LTL, with Lex, difucosyl Lex and sialosyl Lex.

Finally, although glycan moieties of glycoconjugates are not specific markers for serological detection of colorectal cancer, they occur in large concentrations in serum from cancer patients due to «either (a) increased biosynthesis of pre-existing “normal” molecules, which in normal tissue are present in small or even undetectable amounts» (18), or (b) the emergence of new molecules with altered glycosylation patterns (“aberrant structures”).

Monoclonal antibodies and lectins could be useful tools for detection/determinationn of both kinds of molecules: those with “normal” structure (whose concentration increases in pathological circumstances) and those with “aberrant” structure.

Since many lectins are now commercially available (at a relatively low cost) and the procedures for their measure are very simple, they may be considered as promising techniques in this view. However, the correlation between long term clinical evolution and lectin binding level will have to be ascertained for each patient in order to validate these indicators for treatment monitoring.

5. ACKNOWLEDGEMENTS

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