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Restoration of the glycoconjugates expression in the ventral prostate of androgen-deprived mice by dihydrotestosterone: A glycohistochemical study

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ABSTRACT: We investigated histochemically the expression of glycans in the mouse ventral prostate of the normal, castrated, castrated mice injected with testosterone (T) and castrated mice injected with dihydrotestosterone (DHT). The avidin-biotin-peroxidase complex technique was used with eight biotinylated lectins, on paraffin sections. Remarkably, contrarily to T, the DHT found to restore all the glycans in epithelial cells of ventral prostate. The histophysiological significance of the activation synthesis of glycoconjugates in mouse ventral prostate by DHT is discussed in the light of the other studies obtained mainly in humans.

KEYWORDS: Ventral prostate, lectins, Testosterone, Dihydrotestosterone, Glycohistochemistry.

1 INTRODUCTION

Glycoproteins play a crucial role in many biological processes namely pinocytosis, cell differentiation, cell recognition, adhesion, and in hormonal communication, they are also involved in pathological phenomena as tumorigenesis [1].

Multicellular organisms are characterized by an astonishing complexity of glycan chains. The only biochemical analysis is insufficient to grasp this complexity because it involves a tissue destruction masking the structural heterogeneity which extends down to the molecular level. Histochemistry, which is aimed at sections of organs, has the advantage of maintaining and revealing morphological relationships [2].

The use of lectins opens a new chapter in histochemistry named the glycohistochemistry. A lectin is "a protein or glycoprotein of non-immune origin, not an enzyme that binds to carbohydrates and agglutinates cells". Lectins are carbohydrate-binding proteins, which are highly variable in their amino acid sequences, widely distributed in microorganisms, viruses, animals and higher plants [3], and with different functions, structures, tissue localizations and carbohydrate-binding specificities. Lectins can be used to detect carbohydrate structures on, or in, cells and tissues in much the same way that purified antibodies can be employed to detect cell- or tissue-bound antigens using immunocytochemistry [4].

Prostate cancer develops most frequently in men over fifty years. It is the second most common type of cancer in men after lung cancer; this justifies the tireless efforts of research to improve our knowledge on this subject.

Physiology, pathology and responsiveness of the prostate against an array of hormones have been well studied [5], [6]. According to Carson and Rittmaster [7], DHT plays a beneficial role in prostate development, but it can be detrimental in adults because it causes the pathological growth of the prostate.

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The study of prostate glycoproteins is very important in order to elucidate the various aspects of this gland functioning. There are few studies which have investigated the effects of steroid hormones on the expression of glycoproteins at the prostatic epithelium cells [8]; and more, to our knowledge, no glycohistochemical study has focused on the expression of prostate glycoproteins in mouse after hormonal deprivation and injection of testosterone (T) or dihydrotestosterone (DHT). To this end we used a panel of biotinylated lectins on Bouin-fixed paraffin-embedded tissue sections [9], [10], [11], [12].

2 MATERIALS AND METHODS

2.1 ANIMALS AND TISSUE PREPARATION

Sexually mature NMRI mice (8 weeks old) were castred under sodium pentobarbital anaesthesia, and used for experiments eight weeks later. Ten castrated males are subject to hormonal administration for five days, each mice received 0.1 ml of hormone solution subcutaneously. Five males were treated with testosterone (T: 4-androsten-17β-ol-3-one) in an amount of 5µg/g, five males received 1µg/g of dihydrotestosterone (DHT: 5α-androstan-17β-ol-3-one). T and DHT are prepared by dissolving them in ether adding the appropriate volume of corn oil. Before injection, the ether-oil-hormone solution is under the hood to evaporate the ether. The controls were: both castrated and normal males injected with oil only. Mice were sacrificed 24 hours after the last injection. Control mice (sham operated) were used at the same age. Mice were maintained on a regular photoperiod of 12 hour light-12 hour dark at 23°C. Animals were anaesthesized with ether. The accessory sex glands were exposed and bathed with the fixative during dissection. The prostates were removed, fixed in Bouin's solution, embedded in paraffin and sectioned at the thickness of 5µm. After dewaxing, Lectin histochemical staining was performed using the avidin-biotin method, following optimized procedures [9], [10], [11], [2], [12].

2.2 LECTIN HISTOCHEMISTRY

Eight types of biotinylated lectins were used. Their full names, abbreviation, natural sources, saccharide specificities and binding inhibitors are listed in table 1. After deparaffination, tissue sections were incubated in methanol/0.3% H_2O_2 for 30 min at room temperature to block endogenous peroxidase activity. Sections were then washed in PBS (phosphate-buffered saline, 0.15M NaCl containing 0.01M phosphate buffer, pH 7.3±0.1) and incubated with biotinylated lectins (5µg/ml) for 10 min at room temperature. Then the sections were rinsed in PBS and incubated for 30 min with an avidin-biotin-peroxidase complex (ABC kit, Vector). After being washed with PBS, the sections were developed in 3,3'-diaminobenzidine 4HCl (DAB, Sigma)-H_2O_2 medium under microscopic control at room temperature to visualize the activity of peroxidase. The sections were rinsed with tap water, counterstained with haematoxylin, dehydrated, cleared and mounted with DPX. Controls for lectin binding included: 1- omission of the respective lectin; 2- omission of the ABC kit reagents; 3- incubation of the sections with lectin solutions to which 0.2-0.3M of the specific sugar (Table 1). In order to block nonspecific binding of biotin-avidin system reagents, sections were incubated with a blocking kit (vector lab) just prior to the addition of lectin conjugates.

The localization of lectin-specific carbohydrates in the tissues by the probes was examined with a light microscope. To estimate the intensity of the labelling in each cell site we use the following equation:

$$I = n^{-1} \sum_{i=1}^{n} S_n$$

I: Average intensity of labelling in each cell site.

n: Number of sites.

S_n: The labelling intensity in each site.

The intensity of binding was evaluated from absent (0) to very strong (3) (see table 2). To ensure the redundancy of our results, each experiment was repeated three times.

Table 1: Lectins used for histochemical characterization of glycans

Latin name	Lectin Symbol	Sugar residues or sequences recognized by lectins	Sugars found to inhibit histochemical binding
Galanthus nivalis	GNA	Manα(1-3)Man	Man
Ulex europaeus	UEA-I	Fucα(1-2)Gal	L-Fuc
Sambucus nigra	SNA	Neu5Acα(2-6)Gal	Neu5Ac
Maackia amurensis	MAA	Neu5Acα(2,3)Gal=Neu5Acα(2,3)GalNac	Neu5Ac
Arachis hypogaea	PNA	Galβ(1-3)GalNAc	Lactose
Ricinus communis	RCA-I	Galβ(1-4)GalNAc	Gal
Glycine maximus	SBA	Terminal (α,β)GalNAc	GalNAc
Succinylated Triticum vulgare	s-WGA	GlcNAc[β(1-4)GlcNAc]$_{1-2}$	GlcNAC

Abbreviations for the carbohydrates are as follows: GlcNAc: N-acetylglucosamine, GalNAc: N-acetylglactosamine, Gal: galactose, Man: mannose, Glc: glucose, L-Fuc: fucose, Neu5Ac: neuraminic acid.

3 RESULTS

The results regarding the influence of castration on the expression of glycoconjugates in the prostatic epithelial cells are included in our paper [2]. To allow easy comparison of the different experimental cases, we present our results in Table 2.

Overview of binding sites of each biotinylated lectin at the level of prostatic epithelial cells of castrated mice treated with testosterone or dihydrotestosterone.

Effects of Testosterone injection to castrated mice:

Staining with the eight used lectins remains stable at the basement membrane after injection of 5µg/g of testosterone to castrated mice (Table 2).

At the apical region, we observe an increase of an α,βGalNAc and Manα(1-3)Man expression as revealed by SBA and GNA, respectively.

The cytoplasmic staining is identical to that observed in normal males for all lectins except the staining with the RCA-I, which is more important during injection of testosterone.

The reactivity of epithelial cells with RCA-I (Fig. 1), UEA-I, SNA and MAA remains constant.

Effects of dihydrotestosterone injection to castrated mice:

If we consider the entire prostate epithelial cells after injection of DHT, we see the same expression as that observed in normal males of all glycoconjugates recognized by the used lectins. Furthermore the rate of prostatic epithelial cells expressing the synthesis of glycan chains is the same as that seen in normal males. There was a slight decrease in the synthesis of α,βGalNAc recognized by the SBA in the cytoplasm.

Table 2: Semiquantitatively-determinined intensity of lectin binding to the mouse ventral prostate epithelium.

Type of lectin	Normal males				Castrated males				Castrated males +5µg/g testosterone				Castrated males + 1µg/g dihydrotestosterone			
	AR	C	BM	%	AR	C	BM	%	AR	C	BM	%	AR	C	BM	%
GNA	1	2*	2/3	100	3	3-3*	2/3	100	3	2-2*	2/3	100	1	2*	2/3	100
UEA-I	2	1	1	100	2	1	1	100	2	1	1	100	2	1	1	100
SNA	0	0	2	0	2	0	2	30	0	0	2	0	0	0	2	0
MAA	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
PNA	1/2	0	1	10	3	0	1	100	1	0	1	10	1/2	0	1	10
RCA-I	2	1-1*	1	100	2	2-(2)	2	100	2	2-2*	1	100	2	1-1*	1	100
SBA	2	2	1	100	2	2	1	100	3	2*	1	100	2	1	1	100
s-WGA	1	0	1	100	1	0	1	100	1	2	1	100	1	0	1	100

*AR: apical region (including microvilli); C: cytoplasm; BM: basement membrane; %: percentage of labeled cell; *: perinuclear labelling; n/n: variable reactivity in the same structure. Numbers indicate intensity on an estimated scale from 0 (unreactive) to 3 (strongly reative).*

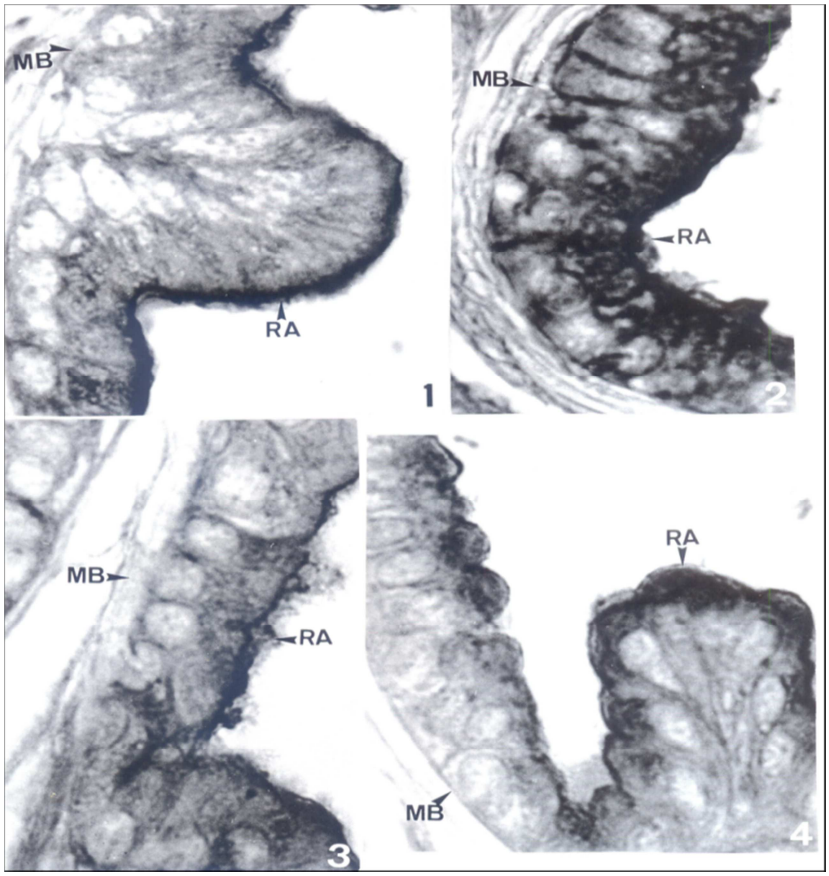

Fig 1: Binding sites of RCA-I in the ventral prostate of normal (1), castrated (2), castrated male injected with 5µg/g of testosterone (3) and castrated male injected with 1µg/g of dihydrotestosterone (4). No counterstaining X512.

4 DISCUSSION

The analysis of prostatic epithelial cells after castration shows that the nuclear and cytoplasmic volumes decrease. These cells do not change uniformly in a single glandular cell. The non-uniform regression of alveolar prostate cells after castration indicates that these cells do not exhibit the same sensitivity to androgens. Also we can imagine an unequal distribution of androgen receptors between cells. Analyzing the immunohistochemical work of Wernet et al., [13], it is easy to observe the non-uniformity of the distribution of these receptors in prostate cells. The inhibition of the activity of these receptors, may delay the progression of prostate cancer [14]. It is well known that the depletion of androgens induces apoptosis in normal prostate and early in prostate cancer, and causes a rapid regression of the gland or tumor [15], [16]. However, after some time, the prostate cancer becomes in an androgen-independent form [17].

Histologically, prostatic epithelium is composed of two distinct cell types. The luminal cells of the epitheliums which are highly differentiated because of being specialized in the secretory functions. These cells are very sensitive to variations of androgen. In contrast to epithelial cells, the basal cells are less differentiated and insensitive to androgens [18].

In all experimental cases, the expression of glycoconjugates at the basement membrane remains constant. Furthermore no negative reaction was observed in these structures (Table 2). From this result, we can conclude that the addition of the glycan chains in these structures is androgen-independent. Observations made by English et al., [19], are close to ours. In fact, only the epithelial cells of the ventral prostate are androgen-dependent.

The importance of basement membranes is considerable, as evidenced by the variety of infections that reach them. They contain a particular type IV collagen which is rich in glycoproteins with high levels of mannose, galactose and sialic acid [20]. In addition, the degradation of the basement membrane is an important event that characterizes prostate cancer [21].

The staining, moderate to strong, of the basement membrane by different lectins indicates its wealth into N- and O-linked oligosaccharides. GNA, SNA and RCA-I have a strong affinity against these structures, indicating the presence of significant concentrations of Manα(1-3)Man, Neu5Acα(2-6)Gal and Galβ(1-4)GalNAc respectively.

In normal males, the staining obtained with different lectins in microvilli and apical epithelial cells shows that the N- and O-linked oligosaccharides are produced in these compartments. In addition, the absence of labelling with the SNA and MAA indicates that the apical region is devoid of sialic acid; this is in agreement with the study of Saldova, et al., [22] who argue that the level of α(2-3)-linked sialic acids were significantly increased in prostate cancer patients compared with patients with benign prostate hyperplasia. Castration induces the synthesis of the glycan chains carrying the Neu5Acα(2-6)Gal residue recognized by SNA; thus injection of the T or DHT to castrated mice abolishes this synthesis at the apical epithelial cells. From these results it can be concluded that androgens have an inhibitory effect on the expression of Neu5Acα(2-6)Gal. Our results are in agreement with those of Chan and Yong [23], obtained by analyzing the glycocalyx of the epithelial cells of the ventral prostate guinea pig. Once again there is a remarkable specificity of the two lectins (*Sambucus nigra*: SNA and *Maackia amurensis*: MAA); Indeed, the absence of labelling with the MAA and moderate labelling with SNA in apical epithelial cells of the ventral prostate in castrated mice, indicate the absence of Neu5Acα(2-3)Gal disaccharide and the presence of a high rate of Neu5Acα(2-6)Gal.

In control males, the apical region of the prostate cells is rich in Fucα(1-2)Gal, Galβ(1-3)GalNAc, Galβ(1-4)GalNAc and (α,β)GalNAc as shown by the staining with UEA-I, PNA, RCA-I and SBA respectively; the injecting of one µg/g of DHT to castrated mice restores the synthesis of these glycan chains.

In this study, the labelling with RCA-I (Fig. 1) and the GNA is mainly localized in the region perinuclear epithelial cells of the ventral prostate. It has been shown that this region corresponds to the Golgi apparatus. Our observation is in agreement with the ultrastructural results of Chang and Youg [23], which have localized the labelling with Con-A and RCA-I at the Golgi cisternae. In the cytoplasm, they are the only oligosaccharides (specific GNA and RCA-I) which are activated after castration; furthermore, the expression of other glycan chains remains constant even after the hormonal changes. This is probably due to activation after castration of mannosyltransferases and galactosyltransferases. These enzymes are responsible for the transfer of galactose and mannose residues on the terminal oligosaccharide chains. The treatment of mice castrated by DHT restores the expression of these two enzymes.

5 CONCLUSION

Based on our findings of the revelation of prostate glycoprotein by the eight lectins, two major observations can be discerned. First, a neosynthesis of glycoproteins after castration, second, the DHT compared to T seems to be the most effective hormone to restore the expression of several glycoconjugates in mouse prostatic epithelial cells after hormonal deprivation**.**

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