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Effect of a Thermal Spring Water on Carbohydrate-Protein Interactions in *In-Vitro* Models Implicating Normal Human Keratinocytes and Recombinant Lectins

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Abstract

Background: Sugar moiety of macromolecules is today very well known for its implications in many biological recognition mechanisms including cell-cell, extracellular matrix-cell and/or bacteria-cell interactions. In this context lectins, which are carbohydrate-binding proteins displaying a high affinity for sugar groups of other molecules, are of a great importance, notably in immune response involving bacteria, viruses and fungi. As protein-carbohydrate interactions are often mediated by ions such as calcium, zinc or magnesium, we were prompted to study the effect of a thermal spring water (which contains this type of component) on interactions existing between: 1) osidic receptors of human normal keratinocytes and 2) two lectins greatly implicated in the immune response mechanisms (*i.e.* the dectin-1 and the langerin), and their ligands. **Materials and Methods:** In a first series of experiments, we studied the effect of increasing concentrations of a thermal spring water on interactions existing between glycosylated molecules and the osidic receptors expressed at the normal human keratinocytes surface. In a second step, and in order to better understand the putative effect of our thermal spring water on the immune response, we analyzed its effect on the interactions existing between the dectin-1 (implicated in the recognition of bacteria, viruses and fungi) and the langerin (expressed by Langerhans cells, the immune cells of the cutaneous tissue), and their ligands in a model using recombinant human lectins and appropriate binding molecules. **Results:** We showed here that our thermal spring water was able to reinforce interactions between keratinocytes osidic receptors and some of their ligands, in a dose-related manner: From 8%

to 55% of increase with 10% to 30% (v/v) of thermal spring water. In the second part of our studies, we also showed that our thermal spring water was able to modulate interactions between dectin-1 and langerin and their ligands through a biphasic effect: Interactions were enhanced by more than 40% and 20% respectively with 10% of thermal spring water, and return to their basal level or lower for higher concentrations. **Conclusion:** The tested thermal spring water, probably due to its ionic composition, could significantly affect interactions of osidic receptors with their ligands. This property could be of a great interest to help immune system to maintain an appropriate "vigilance state" by using the thermal water at up to a concentration of 10%, and by avoiding any runaway reaction in case of aggression, by using concentrations higher than 10%.

Keywords

Carbohydrate-Protein Interaction, Lectin, Dectin-1, Langerin, Normal Human Keratinocytes, Immune System

1. Introduction

Carbohydrate-protein interactions are implicated into a lot of essential biological mechanisms such as cell growth and differentiation, cell adhesion, pathogen recognition, and so on [1] [2]. These interactions are also closely related to several physiopathological situations including notably microorganisms infections [3] [4], inflammatory processes [5], and immune system response [6] [7]. A good way to emphasize the key role of these interactions in such essential processes is lectins, which greatly participate to innate immunity.

Lectins are ubiquitous proteins found within numerous plant and animal tissues and organisms. They all include at least one non-catalytic domain which allows them to reversibly recognize and bind to specific mono- or oligosaccharides without altering their molecular properties [8]. By this way, they are notably implicated in the recognition of invasive microorganisms (for a review, see [9]) and greatly contribute to protective immune responses. However, lectins could also recognize damage associated molecular patterns (DAMPs) (for a review, see [10]) and be involved in immune pathology. As a result, these proteins so consist in a very valuable tool for biological investigations and research with various application fields including notably immunology.

Major classes of lectins implicated in immune response include notably calcium-dependent type lectin receptors (CLRs). As the major interactions between the carbohydrate and the protein moieties are driven by hydrogen bonds and hydrophobic and van des Vaals forces [11], calcium, but also other metallic cations such as manganese, are of a great importance for lectin binding to their ligands. In fact, these metallic cations are thought to influence the folding of lectins and stabilize them to bring their carbohydrate recognition domain closer to

the glycan epitope, which facilitate binding [12].

In order to examine if it is possible to act on carbohydrate-protein interactions and on the lectin binding capabilities, we here reasoned that a product containing metallic ions (and/or other ions) could possibly enhance and/or reduce them. We then choose to use *in vitro* models implicating recombinant lectins and human normal keratinocytes to study how a thermal spring water which has already demonstrated some significant influences on skin biology [13] [14] [15], could affect carbohydrate-protein interactions and the binding of 2 cutaneous CLRs, *i.e.* the dectin-1 and the Langerin, to their ligands.

In a first part of our study, we analyzed the effect of our thermal spring water (TSW) on the ability of lectins naturally occurring at the surface of normal human keratinocytes (NHK) to bind glycans (β-chitosan, α-fucose, α-galactose, β-glucose, α-mannose 6P and α-rhamnose). We secondly focus our researches on CLRs in an acellular model using human recombinant lectins (dectin-1 and langerin) by studying the effect of increasing concentrations of our TSW on the ability of these lectins to bind their specific ligands.

2. Materials and Methods

Thermal Spring Water ionic composition (**Table 1**).

Normal human keratinocytes experiments

Interaction profile of fluoresceinylated neoglycoproteins (β-Chitobiose-BSA ref NeoCTF, α-Galactose-BSA ref NeoGaF, β-Glucose-BSA ref NeobGF, α-Mannose-6-Phosphate-BSA ref NeoMPF, α-Rhamnose-BSA ref NeoRF, α-Fucose-BSA ref NeoFF, GLYcoDiag, Orléans, France) with osidic receptors expressed at the surface of normal human keratinocytes (NHEK) were evaluated according to GLYcoDiag technology (NeoPROFILE) [16] [17]. Cells were first grown up to confluence (80% - 90%) in 96-well plates. Then, the cells were washed several times with PBS and incubated with fluorescent neoglycoproteins in presence of various concentrations of thermal spring water (30%, 20% & 10%). After 4 h of incubation at 4˚C, wells were gently rinsed with PBS, and PBS was added for the fluorescence readout (λex = 485 nm, λem = 530 nm, Fluostar OPTIMA, BMG LABTECH, France). In parallel, a calibration curve was achieved with each fluorescent neoglycoprotein solution to determine the quantity

Table 1. Mean ionic content per liter.

Sulfates	2860 mg	Potassium	45.5 mg
Chlorures	3500 mg	Silicon	42 mg
Sodium	2360 mg	Zinc	160 µg
Bicarbonates	390 mg	Manganese	154 µg
Calcium	600 mg	Copper	75 µg
Magnesium	125 mg	Iron	15 µg

Osmolarity: 275 ± 40 mOsm.

of neoglycoproteins stayed in interaction with the carbohydrate binding proteins expressed at the surface of cells.

GLYcoPROFILE® protocol

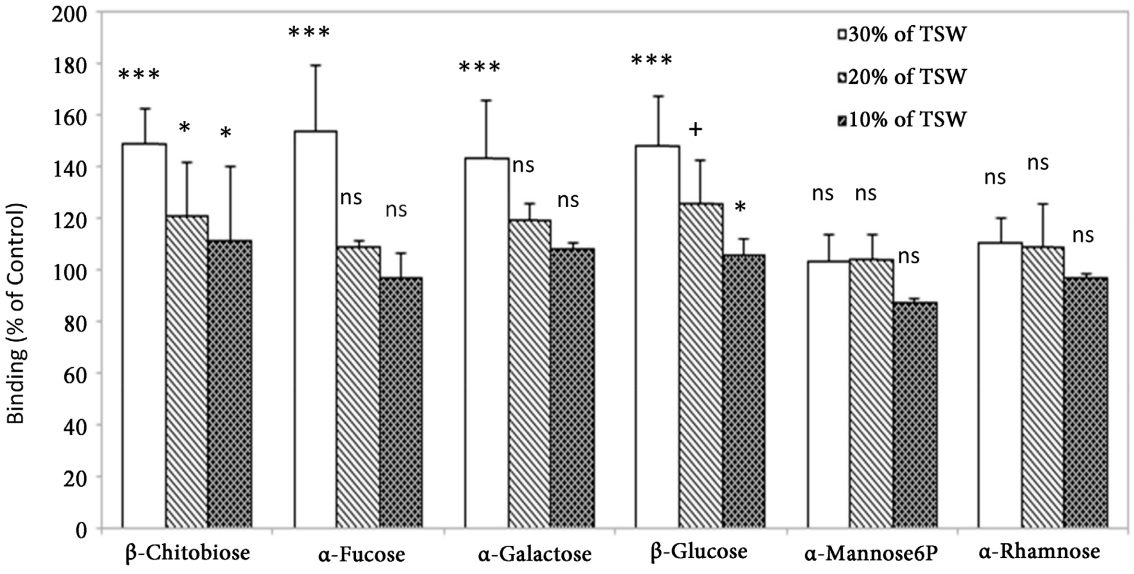
The evaluation of thermal spring water effect on the fixation of biotinylated glycosylated molecules (neoglycoproteins or glycoproteins) on lectins was determined by lectin array assays according GLYcoDiag's protocol already described [16] [17] [18]. Briefly, biotinylated glycosylated molecules (fixed concentration) were deposited in each well of LEctPROFILE. plates (GLYcoDiag, Orléans, France) under 50 µL in triplicates in presence of thermal spring water at three concentration (30%, 20% & 10%). Then, the plates and incubated two hours at room temperature. After washing with PBS buffer, the conjugate streptavidine-DTAF for fluorescence plate or extravidine-peroxydase for absorbance plate is added (50 µL) and incubated 30 min more. The plate was washed again with PBS. Finally, 100 µL of PBS was added for the readout of fluorescent plate performed with a fluorescence reader (λex = 485 nm, λem = 530 nm, Fluostar OPTIMA, BMG LABTECH, France). For the absorbance plate, the plate was washed with PBS buffer, and a solution of OPD (SIGMAFAST™ OPD (o-phenylenediamine dihydrochloride, 100 µL) for the detection of the peroxidase activity. The plate was incubated 15 min protected from light. The coloration was stopped by adding HCl (100 µL, 1 mM) and the readout performed with an absorbance reader. The signal intensity is linked with the capacity of the glycosylated molecules to be recognized by the lectin and expressed as fixation percentage. The results are compared with the absence of thermal spring water.

Statistics

Data are expressed as means ± S.E. of experiments realized at least, in triplicates (n = 3). The statistical significances were assessed by one way analysis of variance (ANOVA) followed by Holm-Sidak's tests.

3. Results and Discussion

In the selected experimental conditions, we showed that our TSW dose-dependently enhanced interactions existing between the keratinocytes' osidic receptors and some of their ligands (**Figure 1**): β-chitobiose --> +11 ($p < 0.05$); +21 ($p < 0.05$) and +49% ($p < 0.001$) vs control for 10%, 20% and 30% of TSW added within the culture medium/α-fucose --> +0 (ns); +9 (ns) and +54% ($p < 0.001$) vs control for 10%, 20% and 30% of TSW added within the culture medium/α-galactose --> +6 (ns); +19 (ns) and +44% ($p < 0.001$) vs control for 10%, 20% and 30% of TSW added within the culture medium/β-glucose --> +6 ($p < 0.05$); +26 ($p < 0.08$) and +49% ($p < 0.001$) vs control for 10%, 20%and 30% of TSW added within the culture medium. As the major interactions between carbohydrates and proteins are driven by hydrogen bonds and hydrophobic and van des Vaals forces [11], we could reasonably believe that the ionic nature of the tested thermal spring water is responsible for this effect. For α-mannose 6P and α-rhamnose, no significant effect of the TSW was observed. This is probably



ns: non-significant/*p < 0.05, Student t-test/⁺p < 0.08, Student t-test/***p < 0.001, one way ANOVA + Holm-Sidak's test.

Figure 1. Effect of increasing concentrations of a thermal spring water on interactions between different *β*-glycans and normal human keratinocytes osidic receptors.

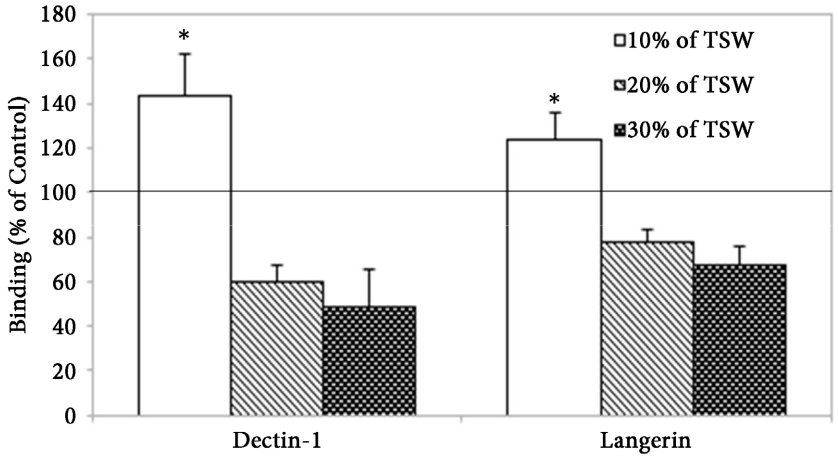
due to high interactions already existing at the basal level as if receptors were close to a "saturation state".

In order to focus our research on the possible helping activity of our TSW in the immune system response, we decided in a second part of this study, to analyze its effects on the binding capabilities of CLRs 1) implicated in this biological response, and 2) able to recognize the glycans identified in the first part of our study as "responsive" elements (*β*-chitobiose, a-galactose, *α*-fucose, *β*-glucose). Then, we choose to study the Dectin-1, which bind to *β*(1,3)-glucans (=glucose polymers mainly linked by *β*-1,3-glycosidic bonds) [19], and the Langerin, which recognize a series of carbohydrates including notably fucose and glucose residues [20].

Relations between these lectins and their ligands were analyzed by using a validated model calling human recombinant dectin-1 and langerin.

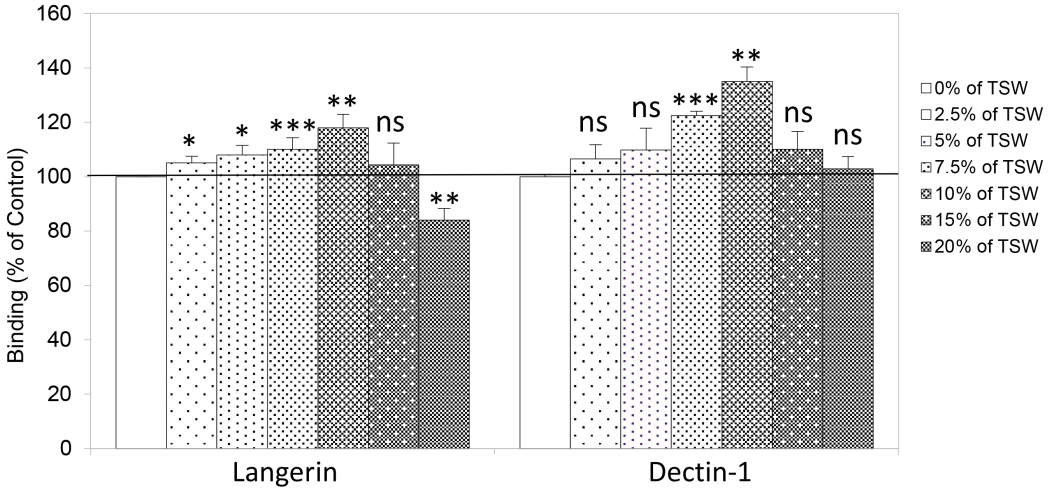
In the selected experimental conditions, the tested TSW was able to modulate interactions between dectin-1 and langerin and their ligands through a biphasic effect: Interactions were enhanced by 43.3% ($p < 0.05$) for dectin-1 and by 23.8% ($p < 0.05$) for langerin when TSW was introduced at 10% in the incubation medium and for higher concentrations of TSW, signals returned to the basal level or lower (**Figure 2**).

At last, we realized an additional experiment in order to precise the dose-related effect of our TSW on dectin-1 and langerin interactions with their ligands. As showed in **Figure 3**, the tested TSW significantly increased the interactions between the two studied CLRs and their ligands when it was added in the reactional medium at 7.5% and 10%. As observed in the precedent experiment, the tested TSW has no effect on the studied interactions or reduced them



*p < 0.05; one way ANOVA + Holm-Sidak's test.

Figure 2. Effect of increasing concentrations of a thermal spring water on interactions betweendectin-1 and langerin, and their ligands (β-glucan and α-fucose, respectively).



ns: non-significant/*p < 0.05/**p < 0.01/***p < 0.001; one way ANOVA + Holm-Sidak's test.

Figure 3. Effect of increasing concentrations of a thermal spring water on interactions betweendectin-1 and langerin, and their ligands (β-glucan and α-fucose, respectively): A sharper focus.

for concentrations superior to 10%.

As our TSW contains various ions, notably Ca^{2+}, we can suggest that these activities could be linked to the ability of these metallic cations to activate CLRs [12]. Some works are currently running in our laboratory in order to precise the nature of the TSW elements which are implicated in this effect.

4. Conclusion

We can so conclude that the tested TSW, probably due to its content of calcium, manganese and/or other metallic cations, is significantly able to affect interactions between osidic receptors and their ligands. This water could significantly act on particular carbohydrate-protein interactions and notably those concerning CLRs particularly important in the immune system response to skin aggres-

sion, such as dectin-1 and langerin. In a very interesting way, the biphasic effect of this TSW depending on its concentration of use could be of a great interest to efficiently support immune system activity: An appropriate "vigilance state" could be reached by using the TSW at concentrations up to 10% whereas higher concentrations could avoid any runaway reaction in case of skin aggression for example.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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