Marine Therapy and Its Healing Properties

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Abstract: This study demonstrates the effects of Quinton’s isotonic and hypertonic solution on mononuclear cells of peripheral blood. This involved assessing cell viability, morphology, number and size of aggregated cells; possible effects on cellular proliferation; effects on cellular proliferation in different lymphocyte populations; effect on hemoglobin released into the medium.

Keywords: Aging, isotonic solution, hypertonic solution, marine therapy, sea water, immunomodulation

INTRODUCTION

The aging process is an intrinsic part of the life cycle. It is conceived as being a series of processes through which a system, in this case the human body, undergoes generally irreversible internal changes that depend more on the development of the system itself over time than on the surroundings.

Western culture connects time with the ancient god Cronus/Saturn who, with his sickle/scythe implacably demolishes everything that has been proven. To a great extent, this image of inexorability shows the fate of Western man associating the progressive deterioration of his faculties with old age.

Since the origins of man, humans have strived to find ways to postpone aging, minimize the passage of time, heal wounds, and recuperate lost youth.

Aging and longevity seem to be inseparable concepts, because apart from the impact of external aggression, the nature of the aging process seems to determine life expectancy. We now know that aging is fundamentally based on two aspects: The first is related to the genetic code itself, which seems to temporarily determine the lifespan of each species, although this does vary slightly for each individual. Since ancient times, from long-lived plant species to the fleeting existence of many invertebrates, species-to-species longevity rates have varied tremendously. The second factor involved in aging is related to our surroundings, which can either exacerbate or lessen the deterioration processes.

The human being is a multicellular organism, a double system of interdependent connections. On the one hand, the organism as a whole is interrelated with a specific type of environment, and on the other hand each of its components, namely its cells, are linked to their own “environment,” namely, the extracellular fluid — or even more accurately, the extracellular matrix. In the second half of the 20th century, Dr. A. Pischinger from Vienna University stressed how important the extracellular matrix is for correct cellular metabolism.

In current medical terms, his work proved what had already been pointed out in the 16th and 17th centuries by Paracelso and his disciples and then later by W.G. Ramon and C. Bernard (19th century) along with René Quinton, A. Nebel, and L. Vannier in the first half of the 20th century.

If aging involves the deterioration of the vital cell functions and consequently the tissue and organic functions, then looking after the cells’ vital functions would be a priori beneficial and undoubtedly delay the deterioration.

This approach was taken up by Quinton Laboratories applying over 100 years of clinical use and a solid theoretical base enhanced by the latest technology to build the third pillar of marine therapy: experimentation. In 2008 a series of studies coordinated by Dr. José Miguel Sempere at Alicante University was designed to clarify the mechanisms of action of the drinkable vials of hypertonic and isotonic solution obtained from sea water pursuant to René Quinton’s protocol. A study aimed at testing the immunomodulatory activities of Quinton’s isotonic and hypertonic solution, both in vitro and in vivo, included the possible effects of both products in vitro on human mononuclear cells of peripheral blood (PBMCs) in cultures taken from healthy individuals.

Research team studying the immunomodulatory activities of Quinton’s isotonic and hypertonic solution in healthy volunteers both in vitro and in vivo, led by Dr. D. José Miguel Sempere, Doctor of Medicine and Surgery, Immunology Specialist, and Professor at Alicante University in the Biotechnology Department, and including the following members:

- Dr. D. Francisco Navarro Doctor of Medicine and Surgery, Specialist in Rheumatology. He is Head of the Rheumatology Department and attending doctor for the Internal Medicine Service at Elche University General Hospital.
- Dr. D. Adolfo Campos, Doctor of Biology, Immunology Specialist, Head of the Immunology Section at the Alicante Transfusion Centre, and Associate Professor at Miguel Hernández University since 1985.
- Dr. D. Luz de la Sen, Doctor of Medicine, Immunology Specialist, attending doctor at the Immunology Service at Alicante University General Hospital, Associate Professor at Alicante University since 1997.
- Dr. D. Francisco Marco, doctor, Immunology Specialist, Associate Professor at Alicante University since 1997, and Head of the Allergy Department at IPI Laboratories.
individuals. This involved assessing the following general aspects during culture:

- Microscopic study of the possible effects on cell viability, morphology, number and size of aggregated cells.
- Study of the possible effects on specific and general cellular proliferation.
- Study the possible effects on cellular proliferation in different lymphocyte populations.
- Study the effect on hemoglobin released into the medium, in order to discover the possible effect of protecting or preserving the red blood cells.

In this study, 10 mL of blood was taken from 10 healthy volunteers and stored with an anticoagulant (EDTA).

**MATERIALS AND METHODS**

**Cell cultures:** The PBMNc under study were obtained by means of a Ficoll-Hypaque density-gradient centrifugation, adjusted to 1x10^6/mL and grown in Costar 96-well cell culture in the ratio of 200,000 cells/well.

The cell culture conditions were as follows:

- RPMI, supplemented with 1% antibiotic, 1% glutamine, and 10% fetal calf serum (FCS); pH = 7.3
- Quinton isotonic solution alone (ISO+); pH = 7.3
- Quinton hypertonic solution alone; pH = 7.3
- Quinton isotonic solution (ISO+) supplemented with 1% antibiotic, 1% glutamine, and 10% FCS; pH = 7.3
- Quinton hypertonic solution supplemented with 1% antibiotic, 1% glutamine, and 10% FCS; pH = 7.3
- Physiological saline solution supplemented (SS) with 1% of antibiotic, 1% of glutamine and 10% of FCS; pH = 7.3

Phytagglutinin (PHA), phorbol esters, and ionomycin (PMA+Io), anti-CD3+anti-CD28 (CD3+CD28) were used together with unstimulated cells (negative controls) as stimulants for the different conditions. The culture plates were cultivated for 4 weeks in a CO₂ incubator, at 37°C, 95% humidity and 5% CO₂.

- Inverted/optical microscope: this was used to calculate the cell viability parameters, morphological cell changes, and number and size of aggregated cells. Cell viability is analyzed using vital dyes (trypan blue).
- Flow cytometry: to analyze cell proliferation, the [5(6)]carboxyfluorescein diacetate succinimidyl ester or the CFSE (Sigma-Aldrich Co. technique was applied.

At the same time, by means of the direct immunofluorescence technique, the PBMNc were incubated with different combinations of monoclonal antibodies directed at the membrane antigens CD3, CD4, CD8, and CD25 and combined with different fluorophores (FITC, PE, PE-Cy5).

**RESULTS**

With regard to ISO+, cell viability after 4 days of cultivation was similar to that of the RPMI and that of the SS. This dropped slightly when Quinton isotonic solution was used alone. No cellular morphological changes were detected with regard to the RPMI or the SS. As for Quinton hypertonic solutions, alone or supplemented, viability was already minimum in the first 12 hours of the culture (unconfirmed data), which is why its use in later experiments was ruled out.

With regard to the number and size of the aggregated cells (Fig. 1), cell cultures with RPMI were seen to have aggregated cells in all of the stimulated wells. In the case of PMA + Io, they were small and numerous, and then bigger but fewer in the case of the PHA and CD3+CD28. In the latter case, they were considerably bigger. When ISO+ was