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PROFESSOR JOSÉ ROBERTO GIGLIO AND TOXINOLOGY IN BRAZIL: 48 YEARS IN RESEARCH

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ABSTRACT: This work succinctly describes the professional and scientific life of Dr. José R. Giglio, one of the most outstanding Brazilian researchers in the field of Toxinology. During his long and successful career, he has made major contributions, especially in elucidating the function, structure, and mechanisms of action of animal venom proteins (from snakes, scorpions and spiders) as well as the characterization of antibodies and several inhibitors of venoms and toxins. We present here a brief history of Dr. Giglio's personal and professional life, also reporting some of his numerous published scientific articles on venoms from snakes (*Bothrops, Crotalus*, and other genera), scorpions (*Tityus* sp), spiders (*Phoneutria* sp), their isolated toxins and natural inhibitors. Thus, this work is a tribute to Dr. Giglio in his 73rd birthday, having devoted 48 years of his life studying animal venoms, an effort that has continued even after his formal retirement from university duties.

KEY WORDS: Toxinology, animal venoms, toxins, inhibitors, homage, Giglio JR.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Dr. José Roberto Giglio (Figure 1) is one of the most outstanding Brazilian toxinologists. During his long and successful career, he has contributed a lot, especially with the elucidation of the function, structure, and mechanism of action of proteins from snake and scorpion venoms, and with the characterization of antibodies and several inhibitors of these venoms and toxins. He is an educator who was directly involved in the creation of the Department of Biochemistry and Immunology at the University of São Paulo (FMRP-USP), Brazil, and was responsible for the formation of several researchers who have made relevant contributions to scientific research. He is an outstanding citizen of the academic community, and despite his tight schedule, he never refused to take on another professional obligation that would promote the development of science. Through his loyalty and generosity, he has become a true friend to many of us.

Figure 1. Recent picture of Dr. Giglio in his laboratory.

Giglio was born in 1934 at the city of São Paulo, Brazil. In 1957, he graduated in Chemistry at the School of Philosophy, Languages and Humana Sciences, University of São Paulo [Faculdade de Filosofia Ciências e Letras, Universidade de São Paulo-USP] among a selected group of only five students, out of 30 that were enrolled in 1954. He was professor of Chemistry at the Oswaldo Cruz School [Colégio Oswaldo

Cruz] (1957-58), and worked in the Laboratory of Chemistry at São Paulo Cancer Hospital [Hospital do Câncer de São Paulo]. In 1959, Giglio started to work at the Biochemistry Department of the Ribeirão Preto School of Medicine [Faculdade de Medicina de Ribeirão Preto, (FMRP-USP)] with Dr. José Moura Gonçalves as Professor of Biochemistry where he began his first studies on snake venom and where he met his wife Ms. Albertina Exposto Giglio. They married in January 1962 and lived in the USP Campus for 40 years. Dr. Giglio created a solid family with sons and grandsons simultaneously with the development of his academic career.

Starting his PhD program in 1959 with the project entitled “Terminal amino acids of crotamine”. Giglio obtained his Doctor degree in Biochemistry in 1962 at USP under the guidance of Dr. José M. Gonçalves. In his first time abroad, he learned to analyze amino acids, becoming himself the responsible for the purification and determination of crotamine amino acids composition, being the first one to accomplish this kind of analysis in Brazil.

Dr. Giglio started his continuous and productive scientific contribution, publishing in the *Anais da Academia Brasileira de Letras*, with his supervisor Dr. José M. Gonçalves, his first complete article about spectrophotometric studies of the interaction between bovine serum albumin and pentachlorophenol (27). In 1965, Giglio had a post-doctoral training at Cornell University, USA, working on the chemistry of biomacromolecules, with studies dealing with the primary structure of thrombin.

In 1969, he published his first article in an international journal on the activation of bovine prothrombin by trypsin and on the identification of the residues from the N-terminal sequence of that protein, a work developed with his first doctoral student, Antonio Rossi (currently Professor at the Medical School FMRP-USP). Dr. Giglio published some articles related to bovine and porcine prothrombin and thrombin (26, 28). From 1975 to 1976, he worked at the Imperial College of London as Visiting Professor, where he got familiar with the manual sequencing of proteins, employing ribitol dehydrogenase, an important biotechnology protein obtained from *Klebsiella aerogenes* (22). From his results, Dr. Giglio and colleagues published the complete sequence of a mutant enzyme showing a higher xylitol dehydrogenase activity (31).

The first studies performed by Dr. Giglio on proteins from snake venoms were published in 1975 and dealt with the analytical investigation of crotamine, a basic neurotoxic peptide (*Mr*~ 4,880) isolated from South American rattlesnake *Crotalus d.*

terrificus venom (25), and in 1982, with studies of RAMAN spectra from the same protein which allowed him to conclude that the only Tyr residue was buried, and the Trp residues were exposed to the solvent.

Giglio became affiliated with USP as a Full Professor in 1990, devoting his life to teaching, learning and performing research, preparing post-graduate and graduate students, forming PhDs, and building a community of researchers and disciples. There are several renowned researchers who were guided by Dr. Giglio, such as Dr. Suely V. Sampaio (current Rector of USP and the first woman to occupy this position), Eliane C. Arantes, Andreimar M. Soares, Suzelei C. França, Veridiana M. Rodrigues, Maria Ines Homsi-Brandeburgo, Alessandra L. Cecchini, Silvia H. Andrião-Escarso, Adélia C. O. Cintra, Heloísa S. Selistre-de-Araújo, Antônio Rossi and others (PhDs, Full Professors of Federal or State Universities). His guidance was very important for their personal and professional maturing process. Since 1983, Dr. Giglio has been supported in his research by two excellent technicians and friends, Carlos A. Vieira and Odete A. B. Cunha, who have given him technical support of research and have played a relevant role in his several scientific publications.

Since the end of the 80's until now, Dr. Giglio has performed several studies on snake venom proteins involving their isolation and their biochemical, structural and functional characterization. Searching of new methods to study proteins, which developed concomitantly with the admission of graduate students at Dr. Giglio's Laboratory, and the contribution of other researchers from different areas created a fruitful and stimulating atmosphere for the study of these proteins following new tendencies based on the evolution in Biochemistry and resulting in the elucidation of their structure-function relationships.

SNAKE VENOMS: TOXINS AND INHIBITORS

The first protein to have its complete amino acid sequence accomplished in Brazil was BthTX-I (Figure 2A), a basic PLA_2 homologue with myotoxic activity and *Mr*~13,720, isolated from *Bothrops jararacussu* venom (20, 30); in addition, the antagonism of this myotoxic protein by polyanions was investigated (49). The possible role of the enzymatic activity of BthTX-I on neuromuscular effects induced by this myotoxin, as well as its crystallization and preliminary X-ray diffraction, was investigated in 1995 (Figure 2B). Conformational changes in BthTX-I were observed by spectroscopy studies (70), whereas mast cell degranulation induced by BthTXs

native and chemically modified with p-bromophenacyl bromide (BPB) demonstrated the dissociation between enzymatic and pharmacological activities (37). Oliveira *et al*. (52) accomplished an important study with BthTX-I and discovered that the dimer, a component of protein structure, is essential for its capability to cause membrane lesions independently of Ca^{2+}. In this same year, the position of the disulfide bridges of BthTX-I structure was determined (Figure 2A).

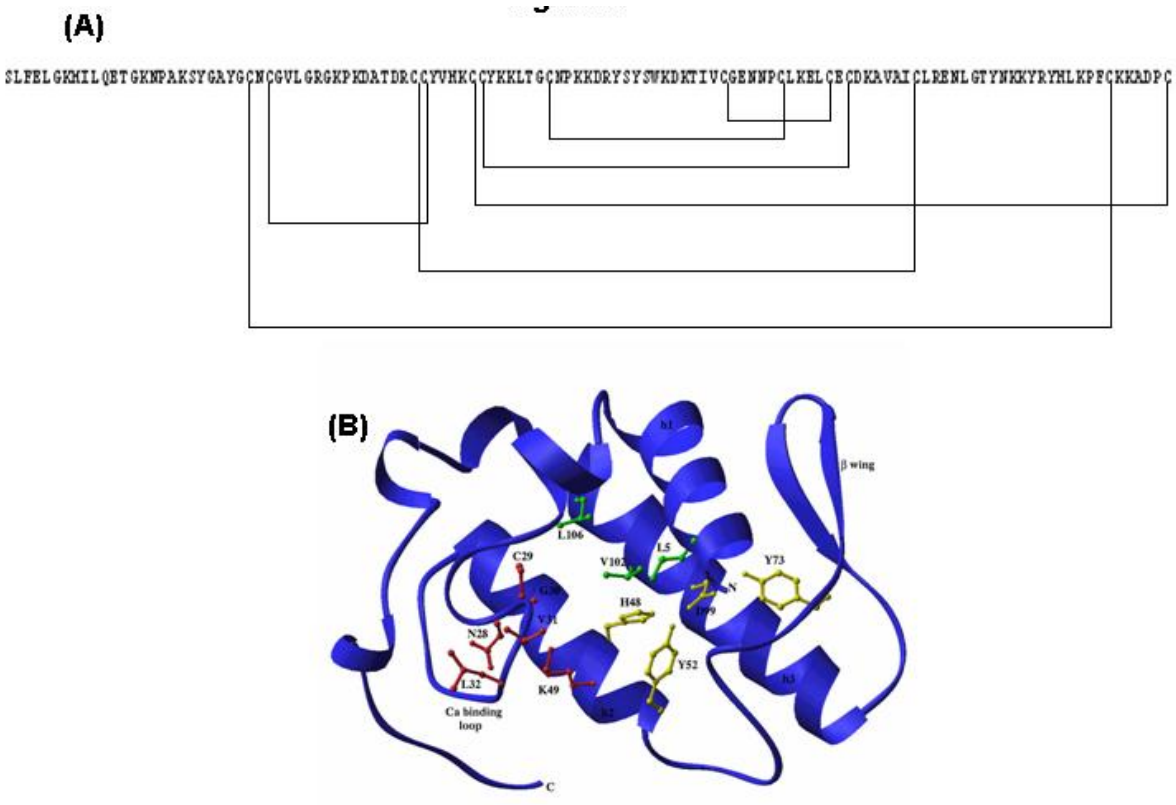

Figure 2 - Amino acid sequence of *Bothrops jararacussu* BthTX-I together with location of Cys residues in the disulfide bridges **(A)**. Ribbon diagram of monomeric BthTX-I (blue). The model has three main α-helices (h1, h2 and h3) and a pair of antiparallel β-sheet (β wing). Some residues of the three regions are shown in ball-stick representation: i) Substrate-binding domain (green), ii) Catalytic site (yellow), and iii) Ca^{++}-binding domain (red) **(B)**.

The functional and structural characterization of an acidic PLA_2 (*Mr*~13,700) composed of 122 amino acid residues exerting hypotensive and inhibitory activities of platelet aggregation, isolated from *B. jararacussu* venom, was performed (2), as well as the elucidation of the sequence of nerve growth factor (Bj-NGF) by Molecular Biology (35). In 2003, isolation and characterization of biological activities of other

acidic PLA_2 isoforms from *B. jararacussu* venom were accomplished (36). In 2004, the analysis of venom gland transcriptome of *B. jararacussu* paved the way for studies on the elucidation of the relationships between PLA_2s function and structure (34). Other studies included the evaluation of presynaptic activity of BthTX-I (55); the cloning and identification of cDNA of acidic phospholipase A_2 with bactericidal and antitumoral effects from *B. jararacussu* (60, 61); and the study of signal transduction via platelet aggregation induced by BthTX-II, applied in its native and modified form (23). This BthTX-II enzyme, a basic PLA_2 Asp49 previously isolated from the same venom (30), showing low catalytic activity, was crystallized and its primary structure completely elucidated (57).

Several other studies were developed using these isolated proteins from *Bothrops* and *Crotalus* snake venoms (3, 9, 11, 17, 18, 24, 38-43, 50, 53, 64, 73-75, 77, 78, 80, 83, 87, 88).

Despite an emphasis on the study of PLA_2, other protein types were also investigated by Dr. Giglio and colleagues, such as the isolation and characterization of a new coagulant factor from *B. jararacussu* venom which is a glycoprotein with fibrinogenolytic activity (1). Recently, BjussuSP-I, another thrombin-like serine protease isolated from *B. jararacussu* venom with Mr~61,000 and pI~3.8 was also isolated and functionally characterized (69). Metalloproteases are other types of proteins that have been studied by him. Neuwiedase, a non-hemorrhagic metalloprotease isolated from *B. neuwiedi* with fibrinogenolytic activity (Mr~20,000 and pI 5.9) was characterized (62, 63). Recently, BjussuMP-II, a non-hemorrhagic metalloprotease P-I (Mr~24,000 and pI 6.0) was isolated from *B. jararacussu* venom (47).

Dr. Giglio also coauthored four review articles, being two of them on phospholipases A_2, one on PLA_2 inhibitors and another one on medicinal plants with anti-snake venom properties. The first one described the effect of chemical modifications of different amino acid residues on PLA_2s of snake venoms, especially *Bothrops*, and their effects on the enzymatic, pharmacological and toxic activities induced by these enzymes (76). The second review covered myotoxic phospholipases A_2 from *Bothrops* snake venom describing the structure-function of these enzymes, and paving the way for future perspectives in the study of these enzymes (81).

Due to the great number of venom proteins isolated and characterized, the search for new methods and compounds for an improved therapy of snakebite envenomation

has been undertaken by Dr. Giglio in his studies (15, 16, 32, 54, 56, 71, 72, 79, 80, 86, 89, 92). Dr. Giglio also participated in other two review articles describing the potential of medicinal plants against snake venoms and isolated toxins (82) and on inhibitors of snake venom PLA_2s (48).

More recently, he and others described the utilization of a library of human recombinant antibodies called Griffin.1 for fragment selection capable of inhibiting the myotoxic activity of PLA_2 from *B. jararacussu* venom (84). Now, another library is being described for recombinant antibodies capable of recognizing and inhibiting the pharmacological and enzymatic activities induced by *Crotalus d. terrificus* CB (53).

OTHER ANIMAL VENOMS

Another subject explored by Dr. Giglio, his students and collaborators, was the study of scorpion venom toxins, mainly from species of the genus *Tityus* (5-8, 10, 14, 19, 21, 29, 33, 39, 45, 46, 51, 58, 65-68, 85, 90, 91).

Fractions of *Phoneutria nigriventer* spider venom were studied by Dr. Giglio and his staff, as well as the activation of kallikrein-kininogen-kinin system in rabbit skin and the *in vivo* increase of microvascular permeability through this system (4, 13). A polypeptide from *P. nigriventer* venom was studied and showed a short-duration dose-dependent stimulation in the contraction of arterial and rabbit venous vessels (12). Marangoni *et al*. (44) described PNV1 as a protein from *P. nigriventer* venom with spasmogenic activity in the smooth muscle of rabbit vessels. Also, a polypeptide that acts relaxing cavernous bodies of rabbit *in vitro* was isolated and partially characterized (59).

CONCLUSION

In his career as professor, researcher and guide, Dr. Giglio published more than 130 articles in international journals of Biochemistry and Pharmacology and still has many works in development and/or submitted to scientific journals. He collaborated in projects developed by important research groups at USP and scientists of other national and international institutions. On May 21, 1995, *Folha de São Paulo*, one of the most distinguished Brazilian newspapers, published the names of 170 Brazilian scientists whose works caused great impact in the scientific world from 1981 to 1993 (ISI database, USA); Dr. Giglio was included in this selected group. He also had the recognition of CNPq (National Council for Scientific and Technological Development),

being honored as Emeritus Researcher. He has plenty of qualities, such as dignity, courage, sensibility and love to his profession, qualities that he maintained along his entire life, even after his formal retirement. After such a long and successful career, he should be full of satisfaction for the duty performed. However, as all great scientists, he continues performing research and helping those who look for his advice and support.

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