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Biological Essays of non-Peptide Angiotensin II AT1-Receptor Antagonists

A. Zoga[1,3], N. Giatas[1], P. Roumelioti[1], M.-E. Androutsou[1], P. Zoumpoulakis[2], T. Mavromoustakos[2], D. Vlahakos[3], E. Iliodromitis[3] and J. Matsoukas[1]

[1]Department of Chemistry, University of Patras, Patra, Greece; [2]The National Hellenic Research Foundation, Athens, Greece; [3]Onassis Cardiac Surgery Center, Athens, Greece

The octapeptide Angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH), which is the major factor of the renin-angiotensin system, plays an important role in the regulation of blood pressure and in the cause of hypertension, a recently widespread and growing disease due to the stressful and diet lifestyle of modern civilized societies. Research efforts have focused on the treatment of the disease by blocking its release and, more recently, by competing its action on AT1 Receptors. Virtually all the recognised effects of Angiotensin II are mediated by AT1 Receptors, which are blocked by AT1 Receptor antagonists.

The aim of the present work is to assess the effect of non-peptide mimetics that selectively block AT1 Angiotensin II Receptors. They belong to a new class of cardiovascular drugs that are similar to losartan. Selective AT1 Receptor blockage with losartan lowers blood pressure in Angiotensin II-dependent models of hypertension. The discovery of losartan provides an important therapy for hypertension and congestive heart failure. All the substances, which are losartan analogues, were given intravenously in anaesthetized rabbits or administered per oral in conscious rabbits (in various values of concentrations) that were made hypertensive by Angiotensin II infusion, in order to investigate their biological activity, using losartan as a reference standard.

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Synthesis of Tetrapeptides Analogs of Substance P (SP_{6-9}) and Study of Their Antiproliferative Properties *in vitro*

P. Vakalopoulou[1], A. Kortsaris[2], D. Kyriakidis[3] and *G. Stavropoulos*[1]

[1]Department of Chemistry, University of Patras, 265 00 Patra, Greece; [2]Department Research, *Theagenion* Cancer Institute, 540 07 Thessaloniki, Greece; [3]Department of Chemistry, Aristotle University of Thessaloniki, 540 07 Thessaloniki, Greece

Small peptides are currently under investigation as possible anti-tumor agents. Thus tetrapeptides, analogs of AS-I toxin, like Cys-Val-Gly-Glu-OH, showed significant antiproliferative activity on three cancer cell lines: HT-29 (human colon cancer), HeLa (human cervical cancer) and T47D (human breast cancer) (1). Analogs or C-terminal analogs of Substance P (SP) have been studied for their ability to prevent tumor growth or the proliferation of several cancer cell lines. The synthetic SP analog [D-Arg[1], D-Phe[5], D-Trp[7,9], Leu[11]]SP (antagonist D) and the C-terminal analog [Arg[6], D-Trp[7,9], MePhe[8]]SP_{6-11} (antagonist G) inhibit Small Cell Lung Cancer (SCLC) cell proliferation *in vitro* and *in* vivo (2,3).

Recently we have synthesized the C-terminal analogs [Glp[6], Glu(Bu^t)[11]]SP_{6-11} and [Glp[5], Glu(Bu^t)[11]]SP_{5-11}, which showed significant inhibition in the proliferation of the cancer cell lines HeLa and T47D at concentrations of 0.75 mM or higher (4).

In the present study a series of SP_{6-9} tetrapeptides, like Glp-Phe-Phe-Gly-OH *[1]*, Glp-D-Trp-Phe-Gly-OH *[2]*, Glp-D-Trp-MePhe-D-Trp-OH *[3]* and Arg-Phe-Phe-Gly-OH *[4]* have been synthesized, using the stepwise synthesis or the

fragment condensation method either in solution or in Solid Phase Peptide Synthesis (SPPS). The analogs were purified (HPLC) and identified (ESI-MS, ^{1}H-NMR, FT-IR). Subsequently they were tested for their antineoplastic properties in several cancer cell lines. The figure 1 exhibits the potent peptide *[3]* concentration versus cell survival fraction curve for the cancer cell lines HT-29, T47D and MCF-7 (human breast cancer) at concentrations of 10 μM or higher.

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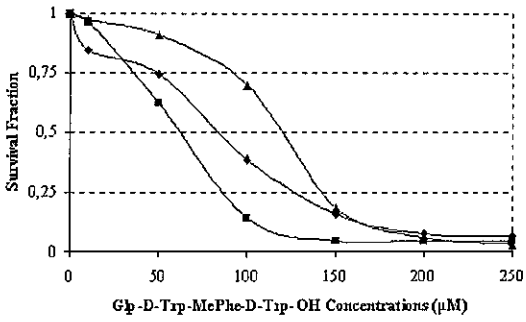

Figure 1. Antiproliferative activity of peptide Glp-D-Trp-MePhe-D-Trp-OH. -♦- MCF-7, -■- T47D, -▲- HT-29

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Biological Studies of Angiotensin II Receptor Blockers *in vivo* Experimental Models

A. Zoga[1], N. Giatas[2], P. Roumelioti[2], E. Iliodromitis[1], D. Vlahakos[1] and J. Matsoukas[2]

[1]Onassis Cardiac Surgery Center, Athens, Greece; [2]Department of Chemistry, University of Patras, Patra, Greece

The Renin-Angiotensin-Aldosterone System (RAAS) is a complex biologic mechanism that functions as an important regulator of cardiovascular and renal homeostasis. Angiotensin II (AII), which is the major factor of this system, is a potent vasoconstrictor that increases both blood pressure and systemic vascular resistance, facilitates sodium and water reabsorption and stimulates synthesis and release of aldosterone. Importantly, AII regulates blood pressure and water and electrolyte balance by the AT_1 receptor by causing vasoconstriction, stimulating aldosterone and vasopressin secretion, promoting renal tubular sodium reabsorption, augmenting peripheral noradrenergic activity and central sympathetic activity, and reducing renal flow. AII also induces vascular smooth muscle proliferation and cardiac growth through the AT_1 receptor. The identification of the physiologic roles of the RAAS and the demonstration of its potency in regulating systemic blood pressure have provided a basis for investigating blockade of this system as a potential therapeutic strategy in patients with hypertension. A recent approach involves inhibiting the effects of AII by directly blocking AII receptors. Losartan is the first orally administered, non-peptide antagonist and approved for use in treating patients with hypertension. When absorbed, it is metabolized in the liver to EXP3174, which is the active metabolite responsible for most of the drug's actions. Many other antagonists, derivatives of losartan, appeared for the treatment of hypertension in the last five years.

The aim of the present work is to assess the effect of non-peptide mimetics that selectively block AT_1 Receptors. All the substances, which are losartan analogues and were synthesized by the group of Pr. J. Matsoukas in the University of Patras, were given intravenously in anaesthetized rabbits or administered per oral in conscious rabbits (in various values of concentrations) that had AII-dependent hypertension, in order to investigate their biological activity, using losartan as a reference standard.