Medicina Universitaria. 2017;xxx(xx):xxx–xxx

MEDICINA UNIVERSITARIA

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REVIEW ARTICLE

Disintegrins obtained from snake venom and their pharmacological potential

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Received 15 February 2017; accepted 23 February 2017

KEYWORDS
Snake venom disintegrins;
Integrins antagonist;
Platelet aggregation;
Cell adhesion antagonist;
Metastasis;
Angiogenesis

Abstract Disintegrins are low molecular weight proteins (4–15 kDa) found in the venom of some snake species, these proteins act as integrin inhibitors. Integrins are membrane cell surface receptors formed by α–β subunits. These integrins modulate cell–cell and cell–extracellular matrix interactions. β_1 and β_3 integrins play important roles in angiogenesis and metastatic processes, suggesting that disintegrins may have utility in the development of new anticancer therapies. This review aims to show recent advances in disintegrin research and the evaluation of their biological activity in both *in vitro* and *in vivo* studies.

Introduction

Since the approval of captopril, the first protein-based medication isolated from snake venom in 1975, snake venoms have become a valuable natural pharmacopoeia from which new drugs may be developed.[1] Snakes are the vertebrae group with the greatest number of venomous species, which supposes a vast reservoir of different molecules,[2] since snake venom contains complex mixtures of hundreds of pharmacologically active molecules, mainly peptides and proteins.[3] Disintegrins are low molecular weight polypeptides (4–15 kDa) found in the venoms of some species. These selectively block integrins (family of adhesion receptors),[4] which perform important functions in processes like metastasis, diabetes, osteoporosis and inflammation.[5–9] One of the therapeutic applications of great interest for disintegrins is the development of new anti-angiogenesis, anti-metastasis, anti-proliferative and apoptosis-inductor agents for the treatment of cancer. Mortality in this pathology is frequently caused by metastasis; in this process, cancer cells interact with adjacent cells and extracellular matrix (ECM) proteins in order to proliferate and migrate to different tissue and settle there, forming a new tumor,[4] for which the interactions between cancer cells and ECM are essential. Moreover, certain types of cancer do not respond in an adequate way to

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http://dx.doi.org/10.1016/j.rmu.2017.02.004

current treatments, thus the importance of finding natural antagonist molecules which allow the modulation of interactions between cancer cells and ECM. The present revision shows the latest findings on snake venom disintegrins from different viper species (Family: Viperidae), and its biological activity *in vitro* and *in vivo*.

History

Disintegrins were discovered in 1987 by Tur-Fu Huang et al., who described a low molecular weight peptide isolated from the venom of the Asian snake *Trimeresurus gramineus*. This peptide, called tigramine, inhibits interaction between fibrinogen and glycoprotein IIb/IIIa (known as integrin $\alpha_{IIb}\beta_3$) expressed in platelets.[10]

Later in 1991, with the resolution of the echistatin structure (2ECH) isolated from the venom of *Echis carinatus* (Gariba snake)[11] structure–activity association studies of the disintegrins were initiated; those same studies led to the sequence description denominated: integrin binding domain, which is formed by three amino acids – arginine, glycine and aspartate (RGD). Despite the fact that domains different from RGD were observed, a characteristic which ought to be mentioned is that aspartate (D) is conserved in most variations found thereupon (KGD, MGD, VGD, WGD, MLD). This amino acid may be responsible for the binding of integrins with β subunit, while the N-terminal amino acid of the binding domain to integrin may determine specificity through interactions of the α subunit.[12] With the exception of the cysteines and binding domains, a variability between the disintegrins sequences was observed.[13] Like with other toxins, in addition to the binding domain, the inhibitor activity of disintegrins also depends on the location of the cysteines and their sequences, since these lead to the formation of disulphide bridges (S-S). The first report about S-S bridge repair in disintegrins (albolabrin) was published by Calvete in 1991.[14] On this basis, despite the high degree of similarities observed between disintegrins, they were able to prove that they have different patterns concerning their S-S bridges,[15] which allowed their current classification.

Classification of disintegrins

Disintegrins come from the proteolytic processing of P-II metalloproteases (SVMP)[2] and conform to a family of polypeptides whose main characteristics are: their cysteine content, their low molecular weight (4–15 kDa)[4] and their actions as very selective integrins (β_1 and β_2).[11] Different criteria have been taken into account in order to classify them according to their size and the number of S-S bridges; it is possible to divide them into five groups: (1) short disintegrins, which have anywhere between 41 and 51 amino acids, as well as four S-S bridges, (2) medium disintegrins, which have around 70 amino acids and six S-S bridges, (3) long disintegrins, with about 84 amino acids and seven S-S bridges, (4) dimeric disintegrins and (5) heterodimeric disintegrins. Dimeric disintegrins contain subunits of 67 amino acids with 10 cysteines involved in the formation of four intrachain disulfides and two interchain cysteine linkages.[2]

According to the variability in their integrin-binding domains, disintegrins are classified into three main families: RGD, MLD and R/KTS.[16] Based on their inhibitor action, the classification includes RGD disintegrins, which block the $\alpha_8\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_V\beta_1$, $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ integrins.[17] MLD, which block $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$ and $\alpha_9\beta_1$ integrins, disintegrins from the VGD and MGD families which block $\alpha_5\beta_1$ integrins and KGD, which inhibit $\alpha_{IIb}\beta_3$ with a high selectivity degree; WGD has been reported as a powerful inhibitor of RGD-dependent integrins such as $\alpha_5\beta_1$, $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$. Moreover, the adhesive function of the disintegrins mentioned above is also blocked by the MVD disintegrins, while the disintegrins with KTS and RTS domains inhibit $\alpha_1\beta_1$.[17–19] Disintegrins are molecules of particular interest for many researchers due to their ability to inhibit cell interaction. The modulator properties of MLD and KTS disintegrins have been reported in researches about tumor angiogenesis and metastasis, immunosuppression of insulin-dependent diabetes mellitus (IDDM) and asthma, as well as in neurodegenerative *in vitro* studies and apoptosis.[16]

A group of emergent disintegrins is one from the recombinant chimerical (Table 1). In 2011, Minea et al., synthesized the first recombinant chimerical disintegrins, which they named vicrostatin (VCN), this was expressed in *Escherichia coli* (DE3) using recombinant technology. One of the advantages was that they were able to obtain quantities larger than 200 mg of purified and active disintegrins per liter of culture.[20]

Biological activity and potential biomedical applications of disintegrins

Snake venom is without a doubt a great source of pharmacologically active compounds, and a good example of this are disintegrins, from which two anti-platelet agents were developed and are currently in the market: Tirofiban (Aggrastat®) developed from echistatin isolated from the venom of *E. carinatus*, and Eptifibatide (Integrillin®) developed from barbourin isolated from the venom of the pygmy rattle snake (*Sistrurus miliarius barbouri*).[1] Tirofiban is a non-peptide synthetic inhibitor which acts on GpIIb/IIIa glycoproteins. A disadvantage of this antagonist is the fact that they lack specificity; thus, they inhibit functions from other RGD-dependent integrins. Nevertheless, with the substitution of lysine for arginine in the RGD domain, a molecule was produced, one with a high selectivity degree for GpIIb/IIIa,[21] which led to the development of Eptifibatide, derived from the KGD domain of the barbourin, which acts as an antagonist of the fibrogenic platelet receptor and is used to reduce the risk of acute cardiac ischemic events in patients with unstable angina or in cases of heart attacks.[22,23] During the development of phase III in the clinical trials of Eptifibatide, a significant decrease in coronary events in patients with low, medium and high-risk acute coronary syndromes was observed; the latter without a significant increase in hemorrhage. Eptifibatide also presented better pharmacokinetic characteristics, including short plasma half-life and a fast onset on their antiplatelet action.[12]

Another field of application for disintegrins is radiology, where disintegrins with radiation emission γ (^{99}mTc, ^{125}I), β particles (^{64}Cu), positrons (^{18}F) and infrared radiation are used as a tool for the visualization of tumor-dependent angiogenesis ($\alpha_V\beta3$).[24,25] As a result of the important role

Please cite this article in press as: Rivas-Mercado EA, Garza-Ocañas L. Disintegrins obtained from snake venom and their pharmacological potential. Medicina Universitaria. 2017. http://dx.doi.org/10.1016/j.rmu.2017.02.004