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A Novel ADP-Ribosyl Cyclase (ARC) Regulators

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ABSTRACT

CD38, which is the main NADase enzyme with its ADP-ribose cyclase (ARC) activity, has been proposed to be involved in many human diseases. Therefore, regulating its activity is the main target of this research. This study proposed four novel regulators; nicotinamide, nicotinic acid, gallic acid, and benzamide, these regulators have a remarkable effect on the cyclase activity of ARC. The cyclase activity of ARC was measured using fluorimetric assay, the reaction was initiated with the addition of enzyme to its substrate nicotinamide guanine dinucleotide (NGD) and the production of cyclic GDP-ribose (cGDPR) was measured with different concentrations from proposed activators. The rate of ADP-ribosyl cyclase activity was increased with the addition of low concentrations of Nicotinamide and Nicotinic acid. However, Nicotinamide and Nicotinic acid are known as ARC inhibitors at high concentrations. The increase of ARC activity was also observed with Gallic acid; it is clear that Gallic acid has a strong effect on the activation of ARC and the formation of cGDPR. The last product was Benzamide, these data reflected the activation effect on ARC. Finally, the search for new ADP-ribosyl cyclase activators and the determination of their roles might provide a new therapeutic strategy for several metabolic and inflammatory conditions.

Keywords: ARC, A novel regulators, cGDPR, NGD.

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INTRODUCTION

CD38 is a member of the ADP-ribosyl cyclase family.[1,2] Intracellularly, CD38 confirmed to be a major nicotinamide adenine dinucleotide (NAD) consuming enzyme,[3] it uses NAD to produce both of NAADP and a cyclic molecule, cADPR.[4] It also hydrolyzes NAD to ADP-ribose, and nicotinamide.4 Experimental evidence accumulates to indicate that ARC can metabolize analogs of NAD, such as nicotinamide guanine dinucleotide (NGD) and nicotinamide hypoxanthine dinucleotide (NHD), releasing cyclic compounds (cGDPR and cIDPR, respectively) with fluorescent properties, but without calcium-releasing activity.[5] These fluorescent compounds are very useful as biochemical tools for studies of ADP-ribosyl cyclase activity in all eukaryotic cells. It has proved the conversion of NGD to cGDPR and nicotinamide, but no other product such as guanosine diphosphoribose (GDPR), was formed with a structure similar to cADPR except with guanine replacing adenine. The use of NGD as a substrate for assaying the cyclization reaction was found to be applicable to pure enzymes as well as crude tissue extracts, making it a useful diagnostic tool for distinguishing NAD cyclase enzymes from degradative NADases.[6] CD38 may serve as a pharmacological target for multiple conditions,[7] thus, controlling enzymatic activities of CD38 by inhibiting or by activating its cyclase activity may be used as an important tool to regulate CD38 activity, and finally, this might regulate its role in many CD38-related diseases.[8,9] Interestingly, it has been suggested an important role for both CD38 metabolites (NAADP, cADPR), in the initiation of insulin-stimulated calcium signals in human β-cells.[10] Moreover, CD38 has a negative role in chronic lymphocytic leukaemia (CLL), and patients with high CD38 expression show a shorter survival rate.[11] A more recent study demonstrated that CD38 could regulate collagen-induced arthritis (CIA) through NF-κB, and this regulatory molecule could be a novel target for the treatment of autoimmune inflammatory joint disease.[12]

Inhibiting CD38 cyclase activity has been investigated, studies have been developed from using arabiono-

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NAD, ATP. Nicotinamide derivatives, dithiothreitol, and 2,2'-dihydroxyazobenzene (DHB) as an inhibitor for CD38 cyclization reaction, to regulate its activity with intracellular localization.[13-16,7] Recently, CD38 cyclase activity was regulated by a novel a flavonoids compounds, such as kuromanin, which appears to elevate intracellular NAD levels by inhibiting CD38-cyclase activity.[3] More recently, Luteolin showed a remarkable impact on intracellular NAD^+ levels, which might be due to block NAD-cyclase activity.[17] Finally-DMSO-differentiated HL60 cells show an elevation of intracellular NAD levels,[18] which might also suggest the inhibited effect of DMSO on CD38 activities.

On the other side, regulating NAD-cyclase activity might be a useful tool to regulate CD38-related diseases. However, limited studies were indicated an increase in CD38-cyclase activity either by using cyclic adenosine monophosphate (cAMP) or cyclic guanine monophosphate (cGMP). Moreover, CD38 expression was also elevated by oestradiol17β.[19-21]

In the recent study, the effect of some pyridine analogs (nicotinamide and nicotinic acid), and both of gallic acid and benzamide on the ADP-ribosyl cyclase activity are investigated. Pyridine analogs such as Niacin compounds (nicotinamide and nicotinic acid) are members of the vitamin B family, and they are precursors of NAD.[22] Nicotinic acid is used clinically in the treatment of hyperlipidemia, high doses of nicotinamide have also been used as clinical therapies.[23,24] Nicotinamide has been used as an inhibitor of the NAD cyclization activity of CD38. Mechanistically, nicotinamide actually forces the reverse of the cyclase reaction and produces NAD from cADPR.[25] On the other hand, nicotinic acid regulates the skeletal muscle ADP-ribosyl cyclase allosterically through regulation of NADP binding and that with high concentrations, nicotinic acid did not affect the formation of cGDPR.[26] In addition, findings referred to a possible therapeutic application of gallic acid (has the same carboxylic group of nicotinic acid) in disease.[27] Benzamide has the same amide group of nicotinamide, and used as an inhibitor for poly (ADP-ribose) polymerase (PARP),[28] where it also increased ARC activity.

The aim of the present study was to evaluate the effect of some of the pyridine analogs (Figure 1) and a similar compound on the cyclase activity of ARC in *vitro* with a range of concentrations. This study suggests that the research for CD38 activators might provide further information to regulate CD38 expression in important disease case (with high CD38 expression), such as in chronic lymphocyte leukemia in which activate CD38 expression expected to have a protective role by decreasing NAD levels and might decrease the survival rate for the cancer cells. Moreover, regulating CD38 activity might provide a protective effect in diabetic patients, and that is mediated by regulating its metabolites.

MATERIALS AND METHODS

Materials

NGD (N5131), *Aplysia* ADP-ribosyl cyclase (A9106), nicotinamide, nicotinic acid, benzamide, and gallic acid were all from Sigma (UK), with all other reagents used being of the highest quality available.

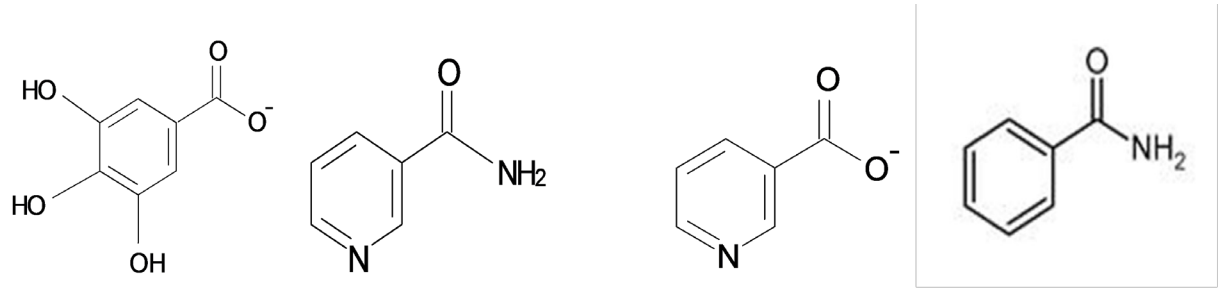
Measurement of ARC activity by fluorimetric assay

The cyclase activity of ARC was measured using the methods described by.[29,30] 1 mL of a reaction containing 50mM NaH_2PO_4 and 50 mM Na_2PO_4 (PH, 7.2) buffer, was used to dissolve the enzyme (0.04 units/µl) and NGD concentrations (25-200µM) were used in cyclase assay. The reaction was initiated with the addition of enzyme, and the production of cGDPR was measured by using a Perkin Elmer LS50-B fluorimeter, with excitation 300 nm and emission 410 nm. Different concentrations from proposed activators (nicotinamide, nicotinic acid, gallic acid and benzamide) were used in 1ml cuvette and the initial rate was monitored using the change in fluorescence per second.

RESULTS AND DISCUSSION

Kinetics of ARC

ADP-ribosyl cyclase (ARC) is known to use NAD as a substrate to produce cADPR (1), and it also uses NGD as the substrate to produce cGDPR (5, 29). To study ARC activity, we first wanted to investigate the kinetics of the reaction. Initial rate versus [substrate], a plot of the data was investigated as shown in Figure 2, which reflected that the change of fluorescence is related to NGD concentration, and the relation was linear with low NGD concentrations. Then the same data were analyzed by a representative double reciprocal plot, as shown in Figure (3) with 1/ (V) versus 1/(S). Kinetic constants of the cyclization



Gallic acid **Nicotinamide** **Nicotinic acid** **Benzamide**

Figure 1: Structures of ADP-ribosyl cyclase activators

reaction were determined and found to be; Vmax 0.34 Δ fluor. sec^{-1} , and Km 14 µM, which are slightly different from the kinetic constants previously obtained by Graeff and others (5).

The effect of pyridine analogs and similar compounds on ARC activity

Nicotinamide and nicotinic acid (as pyridine analogs) are known as ARC inhibitors at high concentrations.[25,31] However, in the present study and at low concentrations of nicotinamide and nicotinic acid, the rate of ADP-ribosyl cyclase was increased compared to the control as shown in Figure4. Nicotinamide increased the rate of ARC in the concentration range 10-100 µM, the maximum effect was at 50 µM, and it decreased gradually to 100 µM, but it is still higher than the control. In the same Figure 4, the second analog, nicotinic acid, also shows a similar effect to increasing ARC activity, but with a lower effect than nicotinamide. It increased ARC activity with concentrations of 10-75 µM and with maximum effect at 50 µM, but it inhibits ARC activity in higher concentrations (100 µM).

Altogether, the results might suggest that in the first steps of the cyclase reaction, low concentration of nicotinamide (as side product) will be produced, and that will increase the cyclase activity of CD38/ARC. Still, with proceeding the reaction, the high levels of the side product will result in inhibition of the cyclase activity (CD38/ARC).

Furthermore, the effect of gallic acid on ARC activity was also investigated. The results showed an increase in the cyclase activity when gallic acid was used, as shown in Figure 5 (A), it is clear that gallic acid up to 500µM has a strong effect on the activation of ARC and formation of cGDPR as well.

The last product tested was benzamide, it has the same amide group of nicotinamide, the data reflected the activation effect on ARC with concentrations ranges from 75 µM up to 100 µM, but no ARC activity has shown in concentrations lower than 75 µM as shown in Figure 5 (B).

One of possible explanations for ARC activation is the structural features of these compounds, which may be involved in activation of ARC to format cGDPR rather than inhibition or reverse the cyclization reaction, another possible explanation for ARC activation could be due to allosteric activation.

In conclusion, the results provide evidence that increasing the rate of ADP-ribosyl cyclase activity suggests the inclusion of the pyridine analogs in the design of useful human ARC activators. Thus, regulating CD38 activity (as a member of the ARC family) by an activator expect to provide a protective effect in diabetes mediated by its metabolites. Moreover, the research for CD38 activators might provide further information to regulate CD38 expression in important disease case (with high CD38 expression), such as in chronic lymphocyte leukemia in which activate CD38 expression expected to have protective role by decreasing NAD levels and might decrease

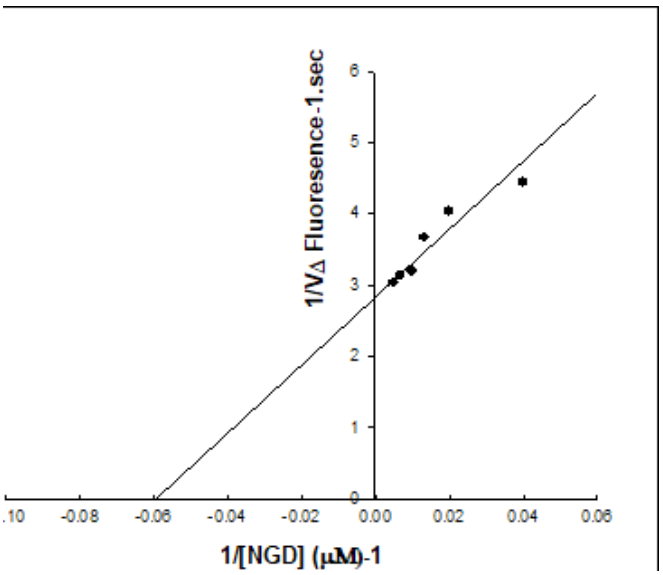


Figure 3: A double reciprocal plot of the ARC assay represents by the 1/rate(V) versus 1/[NGD](S).

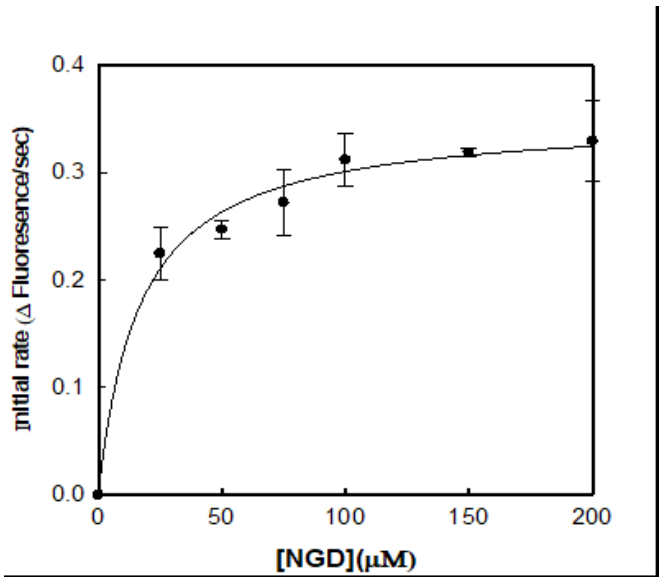


Figure 2: The graph represents the rate (ΔFluoresence/sec) versus [NGD]. The data represent (mean ± SE) (n = 6).

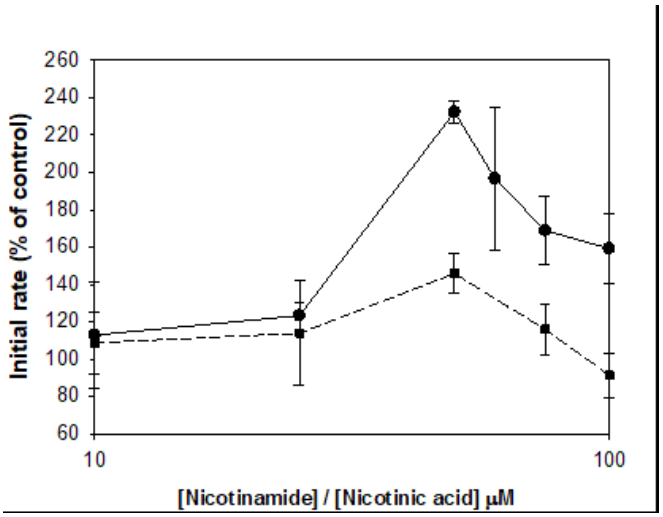


Figure 4: The rate of ADP-ribosyl cyclase in the presence of nicotinamide (solid line) and nicotinic acid (dashed line), the assays were conducted at 25°C, 100µM NGD, and pH 7.2 (n = 3-4).

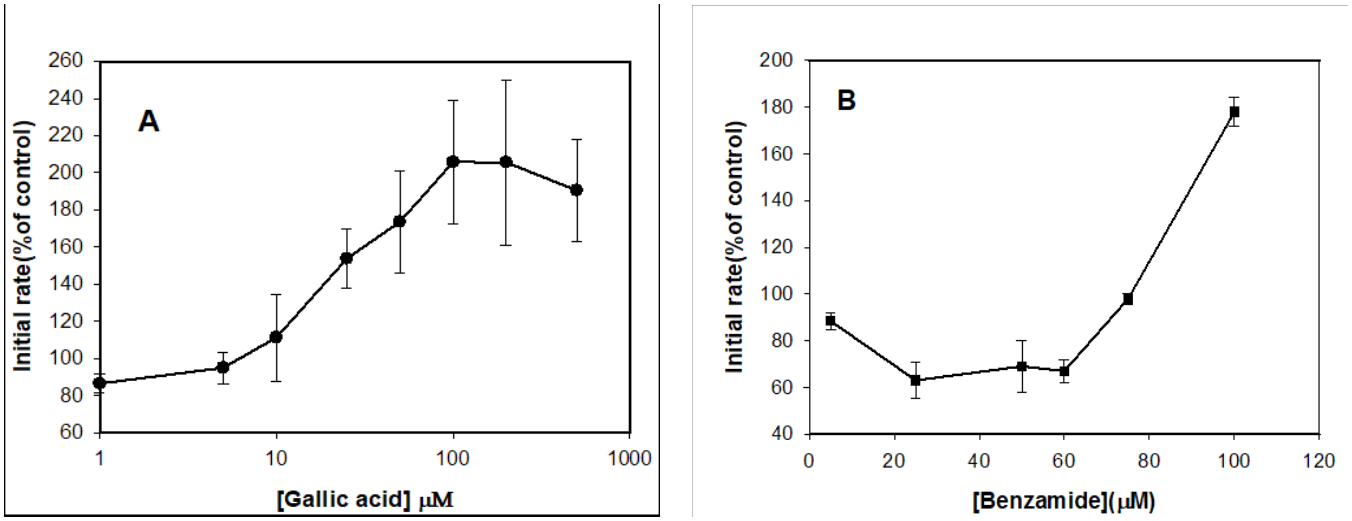


Figure 5: The rate of ADP-ribosyl cyclase represents by an initial rate in the presence of (A) Gallic acid, (B) benzamide, the assays were conducted at 25°C, 100μM NGD, and pH 7.2 (n=3-9). Each data represents (Mean ± SE).

the survival rate for the cancer cells, that because in CD38 knockout mice, NAD levels were increased as previously reported by Barbosa and others.[9]

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