ATR1 Angiotensin II Receptor Reduces Hemoglobin S Polymerization, Phosphatidylserine Exposure, and Increases Deformability of Sickle Cell Disease Erythrocytes

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Abstract
Angiotensin II (Ang II) regulates blood volume and stimulates erythropoiesis through AT1 (ATR1) and AT2 (ATR2) receptors, found in multiple tissues, including erythrocytes. Sickle cell disease (SCD) patients present altered Ang II levels. Hemoglobin S polymerization, deformability and phosphatidylserine translocation are important features of mature erythrocytes, therefore, our hypothesis is Ang II affects these parameters and, if it does, what would be the influence of AT1R and AT2R on these effects. A polymerization assay (PA), deformability, and annexin V binding were performed in SCD erythrocytes samples adding Ang II, ATR1 antagonist (losartan or eprosartan), and ATR2 antagonist (PD123319). Through the PA test, we observed a dose-dependent polymerization inhibition effect when comparing Ang II to control. Losartan did not affect the level or the rate of Ang II inhibition, while PD123319 showed an increased level of protection against polymerization, and eprosartan brought levels back to control. Ang II was able to reduce the translocation of phosphatidylserine from the inner to the outer leaflet, a marker of eryptosis, in the presence of PD123319. Also, ATR1 showed a positive effect increasing deformability. Our data shows that ATR1 is important for maintenance of erythrocyte physiological function in SCD and for prolonging its life.

Keywords Angiotensin II · Erythrocyte · Deformability · Sickle cell disease

Introduction
Sickle cell anemia is a hereditary disorder caused by a mutation in chromosome 11 and genetically characterized by the replacement of the nitrogen base thymine (T) by adenine (A), causing the substitution of the amino acid glutamic acid for valine, in position six of the β globin chain, resulting in S hemoglobin (HbS) [1]. When HbS is deoxygenated, it leads to the formation of long polymers, assuming a rigid and elongated cell shape, known as a sickle shape. This polymerization is the primary event in the molecular pathogenesis of sickle cell disease, resulting in the distortion of the erythrocyte form and the decrease in deformability [2, 3]. These rigid cells are responsible for vaso-occlusive phenomena and hemolysis, leading to pain, hemolytic anemia, and progressive damage to multiple organs [4], which are the hallmark of the disease. In addition, this morphological modification also leads to membrane abnormalities, increasing adhesion capacity to the endothelium mediated by plasma molecules, causing obstruction and local hypoxia, with worsening of sickling, which may trigger inflammatory phenomena [2].

The increase in the hemolysis process, endothelial activation and interaction between erythrocytes, white cells,
platelets and activation of coagulation pathways are important for the pathophysiological process, and one of the factors that contribute to these processes is the exposure of phosphatidylserine from the inner leaflet to the outer layer on the erythrocyte membrane [5]. Thus, polymerization not only stimulates the adhesion process, but the opposite is also true, under prolonged conditions of hypoxia, the adhesive process being a possible biomarker of the severity of patients’ vaso-occlusive crises [6].

Angiotensin II (Ang II) is an octapeptide produced by the carboxypeptidase angiotensin converting enzyme 1 (ACE1) predominantly located on endothelial cells [7]. Ang II regulates blood volume through the hydroelectrolytic balance and it is part of the Renin – Angiotensin - Aldosterone System (RASS) that leads to vasoconstriction, among other functions, which tends to raise blood pressure [8]. Ang II can bind to AT1 (ATR1) and AT2 (ATR2) receptors, both coupled to G protein. The ATR1 is responsible for the main deleterious effects of Ang II on renal and cardiovascular physiology. Meanwhile the ATR2 has been characterized as an antagonistic response to ATR1, counterbalancing its actions, demonstrating beneficial effects [8].

The biological effects observed by Ang II occur through its binding to these receptors. Losartan and eprosartan are specific antagonists of ATR1 and PD123319 is a specific antagonist of ATR2 [9–11]. Furthermore, it was found that signal transduction emitted by these receptors are regulated by Ang II as well as receptor-associated proteins such as ATRAP (angiotensin type 1 receptor-associated protein), ARAP1 (angiotensin type 1 receptor-associated protein 1), GABARAP (γ-aminobutyric acid receptor-associated protein), for ATR1 and ATIP (angiotensin type 2 receptor-interacting protein) for ATR2 [12]. Ang II receptor blockers are ATR1 antagonists causing it to bind mainly to the ATR2, stimulating renoprotective effects, as the decrease in albuminuria in patients with sickle cell anemia [13]. Thus, activation of ATR1 interferes with the expression of ATR2, suggesting a cross-relationship between them, where the blockade of ATR1 is associated with upregulation of ATR2 [9].

Roy et al., in 2018, reported the antagonism of ATR1 and ATR2 receptors in sickle cell disease, where the activation of ATR1 was related to hypostenuria, nocturia, and enuresis, compromising the patient’s quality of life. With the antagonism of ATR1 and the agonism of ATR2, it was demonstrated that one could preserve the ability to concentrate urine and prevent sickle cell glomerulopathy [14].

It is very common for patients with sickle cell disease to present kidney damage, with frequent nephropathies and an increase in blood viscosity leading to an imbalance in vascular functions, with a relative low blood pressure (compared to control population), as shown in a study carried out by [15]. This occurs since HbS polymerizes in the renal medulla causing dehydration of erythrocytes due to low oxygen partial pressure, low pH, and high osmolarity [15–17]. It has also been reported that oxidative stress resulting from sickle cell glomerulopathy leads to the formation of reactive oxygen species, which increases the conversion of oxidized angiotensinogen to Ang II, as well as increasing ATR1 levels in the kidneys of sickle cell patients [18].

Although a study conducted by Saraiva et al. [9] has shown the presence of Ang II receptors on the erythrocyte’s membrane and after our group demonstrated that Ang II, under hypo-osmotic condition, decreases hemolysis of erythrocytes from healthy erythrocytes, and that this signaling occurs via ATR2 [19], the role of Ang II and its receptors on sickle cell disease patients’ red cell membrane remains unknown. No study has demonstrated the relationship of Ang II and its receptors to the erythropoietic physiology of patients with sickle cell anemia.

Therefore, knowing that patients with sickle cell anemia have an imbalance in blood pressure and, after studies demonstrating the Ang II receptors’ expression on erythrocytes, it is important that more studies are conducted to establish the importance and role of these receptors on the erythrocyte membrane.

For this purpose, we performed four different methodologies to verify if Ang II somehow interferes in the main characteristics related to the pathophysiology of sickle cell disease; hemoglobin S polymerization, phosphatidylserine translocation and deformability capacity, and which receptor would be involved.

Methodology

Samples

The collection of sickle cell patients’ blood (exclusively SS genotype) was performed at the Institute of Hematology Arthur de Siqueira Cavalcanti (HEMORIO) and was carried out after project approval by the Ethics Committee of the Federal University of Rio de Janeiro (Protocol 1032.889.952). The project is registered in the Brazil platform under CAAE number 88140418.5.0000.5699 and was in conformity with standards set by the Declaration of Helsinki. Both men and women, over 16 years of age (for those being 16 or 17, with written consent given by the responsible person) were informed about the study and those who agreed to participate filled out the free informed consent form for collection of blood samples and subsequent use. Patients who had received transfusion in the last three months and those taking controlled medication or who had any other hemoglobinopathies (heterozygous) were excluded. We did not distinguish between patients
who used or did not use hydroxyurea, the main medicine for the treatment of sickle cell anemia in Brazil. Blood samples were collected in anticoagulant tubes with EDTA by puncture in antecubital fossa. The collected blood underwent a washing process (three times with PBS) to remove the plasma and buffy coat, leaving only the red blood cell concentrate (RBC), which were used in the experiments. All experiments were performed in triplicate and the final Ang II concentrations obtained were 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, and 10⁻⁶ M.

**HbS Polymerization assay**

The sickle cell patients’ blood was collected in EDTA tubes and centrifuged for 5 min at 750 g. After plasma and buffy coat removal, the RBC was washed with PBS (phosphate-buffered saline) three times in the same conditions mentioned above. In a 96-well plate, we added Ang II at four different concentrations (10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, final concentrations), Ang II receptor inhibitors (losartan, 100 μM; eprosartan, 20 nM and PD123319, 100 nM, final concentrations) and 2% sodium metabisulfite (HbS polymerization inductor by reacting with the water-dissolved oxygen) [20] and the final RBC volume corresponds to 1% hematocrit. After addition of all reagents, the plate was read immediately at 700 nm absorbance for 30 min in a microplate reader (Tecan GENios®), in 1-minute intervals approximately. HbS polymerization was evaluated through the turbidity resulting from the polymerization caused by the use of metabisulfite [20]. After obtaining the polymerization curve, the statistics were calculated from the area under the curve. In the experiment, a spot with DMSO (dimethylsulfoxide) was made, since losartan was dissolved in it.

**Annexin V binding**

For this experiment, an apoptosis marker, annexin V, was used according to manufacturer’s instructions. Briefly, the substances (Ang II 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M; losartan, 100 μM, final concentrations) were added together with the medium (PBS) in eppendorfs and taken to the water bath for 1 h at 37 °C. After this time, they were centrifuged for 5 min at 750 g. The supernatant was discarded. The RBC was resuspended in 50 μL of the ligation buffer. Annexin V (2.5 μL) was then added, homogenized, and left for 15 min at room temperature. Then 500 μL of PBS were added, centrifuged again for 5 min at 750 g, and the supernatant was discarded. 100 μL of the ligation buffer were added, homogenized, and transferred to a black flat bottom 96 well plate. The reading was taken with a microplate reader at 485 nm of excitation and 535 nm of emission wavelengths in order to evaluate the fluorescence emitted by annexin bound to phosphatidyl serine. All steps were taken in the dark, without direct light emission.

**Deformability**

To carry out the deformability experiment, we added Sephacryl-S 500 (a highly versatile gel filtration resin) to eppendorfs and washed the columns 3x using PBS for 5 min at 1000 g. In another eppendorf, we added PBS only, without sodium metabisulfite, so that it would perform the oxygenated control, since when we add sodium metabisulfite to the PBS, it becomes the deoxygenated control. Thus, as the experiments were performed with Ang II (10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, final concentrations) in deoxygenated medium, all points (with the exception of the oxygenated control) were performed in PBS medium containing 2% sodium metabisulfite. The content of interest was added to the sephacryl columns, then washed and centrifuged for 5 min at 3000 g in order to separate cells with deformability capacity from those that did not. Afterwards, the supernatant was removed and added to another tube with 1 mL of miliQ water, in the eppendorfs with precipitate we also added 1 mL of miliQ water (miliQ water is important to lyse erythrocytes for hemoglobin quantification at 540 nm and 700 nm). Again, the tubes were centrifuged for 5 min at 1000 g in order to separate the sephacryl resin from the samples. To perform the reading, 200 μL of the supernatant were placed in a 96-well plate and the reading was performed using optical density (OD) at 540 nm and 700 nm of deformable and non-deformable phases of RBC, using a Tecan Genius microplate reader. The deformability result for RBC, under the conditions analyzed, is obtained by the absorbance of the deformable and non-deformable hemoglobin. For this, a calculation is performed with the result of the absorbance of the deformable and non-deformable parts, as described [OD (542 nm−700 nm) of Hb in deformable RBC fraction/OD (542 nm−700 nm) of Hb in deformable RBC + non-deformable RBC fraction] * 100 [21].

**Statistical analysis**

GraphPad Prism 5 was used to plot the graphs and to perform statistical analysis, with all data expressed as Mean ± SEM. ANOVA with Tukey’s post-test was used for analysis of the significant differences (*for p < 0.05, **for p < 0.01, *** for p < 0.001 and ****p < 0.0001, when compared to control and #p < 0.05, ##p < 0.01, ###p < 0.001, and ####p < 0.0001 when compared to ANG II alone) with a confidence interval of 95%. For hemoglobin S polymerization curves, a non-linear fit line calculates with a fourth-order polynomial equation was calculated with 95% confidence intervals.
Results

Through the PA test, we observed a dose-dependent and significant polymerization inhibition effect when, with an inverted parabola curve for control and a parabola curve for Ang II, comparing different concentrations of Ang II to the control during the reading (Fig. 1A–D). Ang II inhibits ex vivo HbS polymerization (E). Reading taken at 700 nm absorbance. N = 4. Results expressed as Mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 vs control, with 95% confidence interval in One-way ANOVA and Tukey posttest.

Fig. 1 Ang II promotes Inhibition of HbS Polymerization. Reading performed every one minute (approximately) for 30 min. Rate of polymerization with Ang II 10⁻¹² M (A), Ang II 10⁻¹⁰ M (B), Ang II 10⁻⁸ M (C), Ang II 10⁻⁶ M (D). Ang II inhibits ex vivo HbS polymerization (E). Reading taken at 700 nm absorbance. N = 4.

and in the same way with AUC result, ranging from 46.05 a.u. of control to 31.33, 32.63, 31.57, and 32.71 a.u. for Ang II 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M all with losartan, respectively (Fig. 2E).

When performing the polymerization experiment with eprosartan, another antagonist of ATR1, it was possible to observe that the control curve with eprosartan is similar to the control curve without eprosartan. Also, when comparing Ang II curves plus eprosartan, we observed that the effect of Ang II alone is completely abrogated by eprosartan (Figs. 3A, B, C, D), being reflected in the AUC graph, with values of 73.15 a.u. for control without eprosartan, 31.14, 31.22, 32.53, and 33.6 a.u. for Ang II alone 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, respectively, 76.24 a.u. for control with eprosartan and 72.85, 69.32, 72.26 and 74.94 a.u. for Ang II 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M with eprosartan.

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respectively (Fig. 3E). Adding PD123319, ATR2 specific antagonist, the level of protection against polymerization increased in all concentrations of Ang II, showing a significant result both compared to the control and only with angiotensin, at all concentrations with Ang II plus PD123319 presenting a very low DO in the beginning of the curve and eventually matching the magnitude of the Ang II only curve (Figs. 4A–D). This was confirmed by the AUC graph with values of 46.05 a.u. for control, 30.89, 31.78, 33.01, and 34.17 a.u. for Ang II alone 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, respectively and 24.84, 26.33, 28.65 and 24.47 a.u. for Ang II 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M with PD123319, respectively, (Fig. 4E). Thus, the HbS polymerization assay demonstrated that a decrease in polymerization occurs by adding Ang II via ATR1 and not ATR2.

When analyzing the results from annexin V binding, it was possible to notice that Ang II, despite decreasing the destruction of RBCs, this decrease was not significant compared to the control, with a value of 44447.6 fluorescence arbitrary units (f.a.u.) for control, 40451.2, 43535.3, 43270.7 and 43772.8 f.a.u. for Ang II alone 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, respectively. However, in the presence of PD123319, only the two intermediate concentrations of Ang II demonstrated a decrease in annexin V binding with values of 42982, 36728, 39289.8, and 43772.8 f.a.u. respectively, (Fig. 3E). Adding PD123319, ATR2 specific antagonist, the level of protection against polymerization increased in all concentrations of Ang II, showing a significant result both compared to the control and only with angiotensin, at all concentrations with Ang II plus PD123319 presenting a very low DO in the beginning of the curve and eventually matching the magnitude of the Ang II only curve (Figs. 4A–D). This was confirmed by the AUC graph with values of 46.05 a.u. for control, 30.89, 31.78, 33.01, and 34.17 a.u. for Ang II alone 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, respectively and 24.84, 26.33, 28.65 and 24.47 a.u. for Ang II 10⁻¹² M, 10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M with PD123319, respectively, (Fig. 4E). Thus, the HbS polymerization assay demonstrated that a decrease in polymerization occurs by adding Ang II via ATR1 and not ATR2.

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44648.6 f.a.u. for Ang II 10^{-12} M, 10^{-10} M, 10^{-8} M, 10^{-6} M with PD123319, respectively, both compared to the control and compared to the experiments using only Ang II (Fig. 5A–D).

Regarding the deformability experiment, it is possible to observe that the control with deoxygenated cells, in contact with the sodium metabisulfite, presents approximately only 18.46% of deformable cells compared to 94.76% from oxygenated control. When adding Ang II, it is notable that there is a dose-dependent increase in effect in respect to concentrations, with the result being significant in all concentrations of 31.67, 34.63, 33.29, and 30.49% of deformable cells for Ang II 10^{-12} M, 10^{-10} M, 10^{-8} M, 10^{-6} M, respectively, when compared to deoxygenated control. Using Ang II plus the ATR2 antagonist, the increased deformation capacity was preserved (besides Ang II 10^{-8} M plus PD123319) with values of 31.29, 34.34, 28.65 and 30.10% of deformable cells for Ang II 10^{-12} M, 10^{-10} M, 10^{-8} M, 10^{-6} M with PD123319, respectively, when compared to deoxygenated control (Fig. 6A). The addition of losartan reverted these effects to control and Ang II plus losartan presented 19.85, 20.02, 27.87, and 22.15% of deformable cells for Ang II 10^{-12} M, 10^{-10} M, 10^{-8} M, 10^{-6} M with losartan, respectively, when compared to deoxygenated control (Fig. 6B). These results suggest that the deformability of SS RBC patients may be positively related to activation of AT1 receptors.

**Discussion**

Ang II, is the key effector of the renin-Ang II system (RAS), possessing a central role in the regulation of vascular tone, blood pressure, and electrolyte homeostasis [22].
In humans, two subtypes of Ang II receptors have been identified, the AT1 type receptor and the AT2 type receptor [23].

Ang II Receptor Blockers (ARBs) are selective antagonists of angiotensin-II receptor 1 (ATR1), while at the same time preserving potential renoprotective effects through angiotensin-II receptor 2 (ATR2) pathways [13]. Studies have shown that ATR1 and its downstream signaling pathways are often “overactivated” leading to a vicious cycle and further promoting the disease [12]. Losartan is one of the main drugs approved for the treatment of hypertension and literature has shown that in patients with sickle cell disease it can reduce albuminuria [13, 24–26]. Although losartan decreases albuminuria improving the patient’s kidney damage and is a treatment option for patients with sickle cell disease, its effects on erythrocytes have not been deeply studied. Besides, a study published by Zaidan and coworkers, 2010, with rats’ erythrocytes treated with losartan (25 mg/mL) showed a hemolysis increase in low NaCl concentrations, while our previous study, carried out in human erythrocytes, demonstrated that losartan (100 μM) was able to decrease hemolysis [19]. These results

**Fig. 4** PD123319 enhances HbS polymerization inhibition promoted by Ang II. Reading performed every one minute (approximately) for 30 min. Rate of polymerization with PD123319 plus Ang II $10^{-12}$ M (A), Ang II $10^{-10}$ M (B), Ang II $10^{-8}$ M (C), and Ang II $10^{-6}$ M (D). Ang II in the presence of PD123319 inhibits, even more, in vitro HbS polymerization (E). Reading taken at 700 nm absorbance. 4 < N < 6. Results expressed as Mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 vs control, #p < 0.05, ##p < 0.01, ###p < 0.001 and ####p < 0.0001 when compared to ANG II alone, with 95% confidence interval in One-way ANOVA and Tukey posttest.
A decrease in phosphatidylserine translocation when compared to the control and when compared to the experiment using only Ang II. 

The polymerization assay demonstrated that Ang II was able to significantly decrease the process of HbS sickling in a dose-dependent way. However, as there are two receptors capable of binding Ang II, it was necessary to perform assays individually for each receptor (ATR1 and ATR2) to unravel the possible receptor involved. Therefore, blocking ATR1 using losartan caused Ang II, present in the medium, to bind only to AT2. It was intriguing that adding losartan had a decrease in polymerization, compared to the control, with levels similar to the protection performed only by Ang II. When searching on literature, we found in silico evidence by Olubiyo and colleagues, in 2019, that demonstrated that losartan could bind itself to HbS molecules impairing its polymerization [28]. To assess this possibility, we repeated the same experiments and conditions using eprosartan, also an ATR1 antagonist, but with a molecular structure different from losartan, in the way that such interaction could not happen. In this way, eprosartan reverted completely to the polymerization levels of the control, as we expected. When adding PD123319, an ATR2 antagonist, it was possible to observe an even greater decrease in HbS polymerization. However, further studies are needed to find out exactly by which signaling pathway this protection occurs within erythrocytes.

Annexin V binding is generally used to detect apoptotic cells as it has the ability to bind to phosphatidylserine, a marker of apoptosis, when it is increased on the outer layer of the plasma membrane. It is known that when forming HbS polymers, multiple changes occur in cells, such as an increase in calcium input and potassium output, as well as an increase in phosphatidylserine exposure [2]. Therefore, we used Ang II to check if it would be able to reduce antagonist [27] with greater affinity to binding to the receptor, to perform the experiments.

Therefore, as a prototype of ATR1 antagonist we chose losartan and eprosartan, also a nonpeptide AT1 receptor

![A](image-url)

**Fig. 5** Ang II plus PD123319 reduce annexin V binding at two intermediary concentrations. Ang II showed values similar to the control (A–D). At the concentrations of Ang II 10^{-10} M (B) and Ang II 10^{-8} M (C), in the presence of PD123319, there was a significant decrease in phosphatidylserine translocation when compared to the control

![B](image-url)

**Fig. 6** Sickle cell deformability in the presence of Angiotensin II. Ang II is able to increase the deformability capacity. Ang II in the presence of PD123319 maintained the same levels when using Ang II alone (A). However, in the presence of losartan it was possible to see a reversal of the deformation, returning to the deoxygenated control values (B). 4 < N < 7. Results expressed as Mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 vs control, *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 when compared to ANG II alone, with 95% confidence interval in One-way ANOVA and Tukey posttest.

indicate that somehow ATR1 can change the physiologic characteristics of erythrocytes.

Therefore, as a prototype of ATR1 antagonist we chose losartan and eprosartan, also a nonpeptide AT1 receptor
erythrocyte apoptosis and PD123319 to verify whether the effect was via the ATR1 receptor. The results showed that at intermediate concentrations, Ang II was able to reduce annexin V binding via ATR1, and this reduction is likely to be due to a decrease in phosphatidylserine exposure to the outer leaflet. The fact that only intermediate concentrations of Ang II were able to reduce this translocation could be due to the fact that the lowest concentration might be too low to bring a measurable effect, and, on the opposite side, a high Ang II concentration might be too high, leading to internalization of receptors. High Ang II (around 10^−6 M) was already reported as a concentration that promotes this effect on ATR1 [29, 30].

The deformation capacity of erythrocytes is a key factor for the passage of erythrocytes through blood vessels and is directly related to the pathophysiological processes of sickle cell anemia. When analyzing the results obtained after carrying out the deformability experiment, Ang II also plays an important role in increasing the deformability capacity via the ATR1 receptor, thus, increasing the viability of these erythrocytes. This set of results shows that Ang II, when binding to the ATR1 receptor, is improving its morphological and structural conditions, making it necessary to carry out more studies to demonstrate by which mechanism and pathway it occurs.

Thus, the ATR1 receptor is responsible for protecting sickle cell patient’s erythrocytes both from polymerization and from phosphatidylserine translocation, eryptosis marker, also improving deformability. However, little is known about the ATR1 signaling pathway that could provide some insight about how ATR1 exerts its effects and it would be crucial to work on this to elucidate second messengers and pathways in mature erythrocytes.

**Conclusion**

Through the evaluation of ATR1 and ATR2 involvement on some of the most basic functions of erythrocytes, it was possible to understand their role in the polymerization of HbS ex vivo, demonstrating how Ang II and its receptors act on the erythrocyte membrane of sickle cell disease patients. Thus, our data shows for the first time in literature that Ang II inhibits the polymerization of HbS ex vivo via ATR1, reducing one of the mains markers of eryptosis, probably by maintaining phosphatidylserine in the inner leaflet of the lipid bilayer instead of its translocation to the outer leaflet, which would signal apoptosis or eryptosis. Furthermore, ATR1 receptor has also been shown to positively regulate deformability of sickle cell patients’ RBC which facilitates the fluidity of these cells through the blood vessels (Fig. 7). These results point to the importance of ATR1 in maintaining the morphological function of erythrocytes in sickle cell disease [31–33].

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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