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Could renin-angiotensin-aldosterone system activation explain the amputations associated with canagliflozin? The nitric oxide hypothesis

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1. Commentary

One of the most important reported adverse effects events of related to sodium-glucose cotransporter-2 inhibitors (SGLT2i) is lower-limb amputations. While the reasons for the increased rate of these events are still unclear, it is unlikely that an unique explanation may explain the mechanisms by which this undesired effect occurs. In the present article we propose a hypothesis based on the observation of two patients with type 2 diabetes mellitus (DM) who, after receiving dapagliflozin, experienced erectile dysfunction (ED). In both cases the ED did not improve after adding losartan but improved markedly after starting treatment with ramipril, a type 1 angiotensin-converting enzyme inhibitor (ACEi), without the need to remove dapagliflozin. In both patients the ED was evaluated using the internationally validated test IIEF-5 (International Index of Erectile Function – 5 items) [1]. Alternative hormonal and pharmacological causes for ED were ruled out.

The function of the vascular endothelium is significantly affected with the progression of diabetes, and one its consequences can be ED, which is characterised by a reduction of the bioavailability of nitric oxide (NO) from the endothelium. The endothelium regulates the production of both vasodilator molecules, such as NO, and vasoconstrictor molecules, such as angiotensin (ANG) II. When there is endothelial dysfunction a disbalanced between both types of molecules occurs, along with a promotion of the activation and adhesion of platelets and leukocytes, which ultimately compromises the integrity of the endothelial cell barrier, one of the first steps in the development of arteriosclerosis [2]. The production of NO is dependent on the conversion of L-arginine into NO

Abbreviations: ACE, angiotensin-converting enzyme type 1; ACEi, angiotensin-converting enzyme type 1 inhibitors; ACE2, angiotensin-converting enzyme type 2; ANG, angiotensin; BK, bradykinin; DM, diabetes mellitus; ED, erectile dysfunction; eNOs, oxide nitric synthase; FAERS, Food Drug Administration event reporting system; NO, nitric oxide; RAAS, renin-angiotensin-aldosterone system; SGLT2i, sodium-glucose cotransporter-2 inhibitors.

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and citrulline by the enzyme endothelial NO synthase (eNOS), which is located in endothelial cells [2]. Through different mechanisms, DM leads to a dysfunction of the eNOS and a subsequent reduction of NO production [3]. In DM, the activation of the renin-angiotensin-aldosterone system (RAAS) occurs through the generation of ANG II, which is a potent activator of the nicotinamide adenine dinucleotide phosphate-oxidase, which, in turn, generates a superoxide anion with harmful effects. A close and balanced relationship between the RAAS, the superoxide anion and the NO has been described. If the RAAS is activated to raise blood pressure levels, then NO levels decrease, as it has been demonstrated in animal models with hypertension [4]. In patients with DM, the activation of RAAS mainly occurs at local levels through ANG II, which results, for example, in chronic renal nephropathy [5].

In addition to inhibiting the formation of ANG II, ACEi generate bradykinin (BK) and NO (Fig. 1). This occurs because angiotensin-converting enzyme type 1 (ACE) is identical to kininase II, which inactivates BK [6]. The inhibition of kininase II prevents the degradation of BK stimulating the production of NO. BK is a highly potent vasodilator peptide that is generated from endothelial prostacyclins, NO and endothelium-derived hyperpolarising factor [6].

SGLT2i causes natriuresis, decreases the circulating volume and increases the haematocrit in a sustained manner, leading to activation of the RAAS. This occurs either with or without concomitant use of RAAS inhibitor drugs [7–13]. Similarly, in renal familial glucosuria the RAAS is also activated [14]. SGLT2i causes natriuresis and activates tubuloglomerular feedback. This would lead to the inactivation of the RAAS, however the opposite occurs due to the decrease in the circulating volume. RAAS activation causes a decrease in NO as a compensatory mechanism to regulate the tone of the afferent arteriole [8]. While the clinical implications of RAAS activation induced by SGLT2i are currently unknown, in theory, they could be potentially harmful due to the resulting increase in ANG II levels. Currently, there is an ongoing clinical trial that might help clarify this relationship and answer the questions that we suggest in the present article. In this prospective

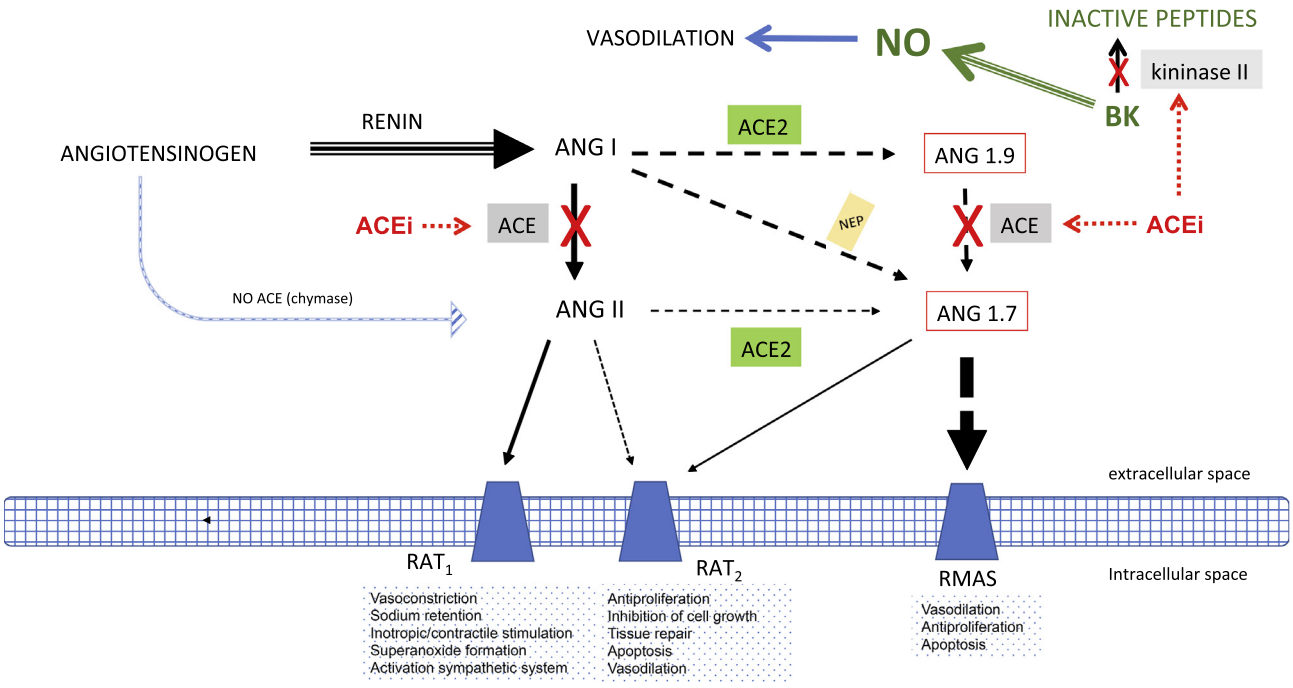

Fig. 1 – RAAS. ACEi functional scheme and generation of NO. Renin is the hormone responsible for the conversion of angiotensinogen to ANG I. ANG I would be converted to ANG II by the action of the ACE type 1. The final step of the RAAS cascade is activation of ANG II receptors by ANG II. ANG II would stimulate the receptors AT1 and AT2 and produce the different effects described. ANG 1.9 would be producing ANG 1.7 that would exert its beneficial effects through the MAS receptor. ANG 1.7 could also be formed from the NEP enzymes. ACE is also called kininase II because it participates in the breakdown of bradykinin to inactive peptides. Inhibition of ACE produces an increase in plasma bradykinin and NO. This way is represented in green. The classical cascade is represented by thick black lines. The non-classic cascade is represented by intermittent arrows. The escape route is represented in blue. RAAS: renin-angiotensin-aldosterone system; ANG I: angiotensin I; ANG II: angiotensin II; ACE: angiotensin-converting enzyme type 1; ACEi: angiotensin-converting enzyme type 1 inhibitor; ACE2: angiotensin-converting enzyme type 2; ARB: ANG II receptor type 1 blocker; RAT1: ANG II type 1 receptor; RAT2: ANG II type 2 receptor; RMAS: oncogen Mas receptor; NEP: neuro endopeptidase (neprilisine); ANG 1.9: angiotensin 1.9; ANG 1.7: angiotensin 1.7; NO: oxide nitric; BK: bradykinin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

clinical study in patients with type 2 DM and chronic kidney disease, the authors assess whether the concomitant treatment of empagliflozin and ACEi significantly increases the levels of ANG 1.7, as compared to treatment with ACEi alone (EMPagliflozin and RAs in Kidney Disease; NCT03078101).

The first trial in humans that demonstrated that SGLT2i has a hemodynamic renal effect was reported by Cherney et al in 2014 [8]. This trial included 40 patients with type 1 DM who were not taking drugs with a known effect on the RAAS. After 8 weeks of treatment with empagliflozin it was observed that ANG II and aldosterone levels were significantly elevated in the group of patients with renal hyperfiltration, together with a significant reduction of plasma NO levels. In an additional exploratory study where the intrarenal RAAS activity was measured, this was also found to increase after treatment with empagliflozin [15]. This finding has also been shown in trials using animal models with hypertension [4].

The RAAS consists of a classical "cascade" pathway and other pathways known as non-classical "cascades". The classical cascade ends up producing ANG II whereas the non-classical cascades would lead to the production of ANG 1.7 after the degradation of ANG II thanks to the action of the type 2 angiotensin-converting enzyme (ACE2) (Fig. 1). ANG 1.7 has vasodilatory and anti-inflammatory effects, and it favours the secretion of NO through the ANG II type 2 receptor (RAT2). ACE2 also degrades ANG I in ANG 1.9 [16]. RAAS inhibitors would be beneficial for the activation of the non-classical cascade and increases the levels of ANG 1.7 [17].

All these changes in the RAAS could potentially explain the alterations observed in the ED of patients with DM and the positive response to ACEi. Some authors have proposed that the use of ACEi might be helpful to improve ED due to its action on ANG II and BK, which are also located in the corpus cavernosum. In fact, the vascular tone of the cells in the corpus cavernosum is partially related to the balance between BK and ANG II [18].

In the patients we described before, we think that after taking dapagliflozin, the RAAS system is activated, with a compensatory reduction in the NO production, as demonstrated by Cherney et al. in patients with renal hyperfiltration [8]. This caused the patients to experience more marked symptoms of ED. It is possible that it could be certain similarities, at a physiopathological level, between Cherney et al observations and what we have clinically observed in our patients (acknowledging that there are differences between patients with type 1 and type 2 DM). In our patients there were also hyperfiltration. For example, in patient 1 creatinine was 0.69 mg/dL, and the estimated glomerular filtration rate (MDRD-4) was 130 mL/min/1.73 m^2. In other article from Cherney et al. [19], the authors point out the relationship that we have previously mentioned: *"Furthermore, if baseline renal RAS activity is highest in DM with hyperfiltration, as we have previously suggested, then, similar to observations in animals, NOS inhibition may have unopposed RAS activity leading to enhanced renal vasoconstrictive and autoregulatory effects of ANG II"*.

We raise the following question: Does the decrease in NO that occurs due to activation of the RAAS by SGLT2i also occur at the level of the arterioles of the lower limbs? As we have mentioned, the underlying mechanisms related to the endothelial dysfunction that occurs in ED can be extrapolated to the endothelial dysfunction observed in the arteries of the lower limbs of diabetic patients. Data from the United States Food and Drug Administration Adverse Event Reporting System (FAERS) in relation to ED and SGLT2i support this hypothesis [20]. Specifically, up to August 2018, 27 (52.9%) cases of ED have been described with canagliflozin, 13 (25.5%) with dapagliflozin, and 11 (21.6%) with empagliflozin [20]. The reasons why more ED and amputations occur with canagliflozin compared to the other two drugs are unknown. It would be necessary to know if patients were on treatment with RAAS inhibitors and other important additional data related to the general situation of the patients, hemodynamic state, or use of other diuretics.

Classically, it has been shown that losartan improves ED as a result of an increase of the NO available in the endothelium secondary to the stimulation of RAT2. In our patients, we initially prescribed losartan, but the ED did not respond to it. Next, we administered ramipril and after a short time we verified the positive response, which led us to suspect the importance of the BK pathway in the ED improvement.

In support of our hypothesis, this response has been previously described in the coronary arteries. Coronary endothelial function has been widely studied with the administration of acetylcholine, one of the most widely used methods to observe the endothelium function. The physiological response of the coronary tree to acetylcholine is vasodilation as a result of a stimulation of the production of NO when the endothelium is intact; However, when the endothelium is injured, acetylcholine acts directly on the muscarinic receptors of the smooth muscle leading to the opposite effect, i.e. vasoconstriction. This effect is reversed when enalapril is administered, with a vasodilatory response by the theoretical route of the BK [21]. In animal experiments, a BK-dependent coronary vasodilation by ACEi is suggested [22–24]. It has been observed that by blocking the degradation of BK, ACEi enhances the ability of BK to reduce blood pressure and stimulate the release of tissue-type plasminogen activator from the vasculature, an effect not seen with ANG II type 1 receptor blockers. These observations are concordant with the clinical improvement that we have observed in our patients.

In DM there is an endothelial dysfunction that alters the NO-dependent vasodilator response. In patients treated with SGLT2i we can observe a relative decrease in the circulating volume while the concomitant use of ACEi could compensate the deterioration of the endothelial function when acting on the BK pathway. Our clinical practice has allowed us to elaborate this hypothesis that could provide more information about the relationship between lower limb amputations and SGLT2i.

This hypothesis requires to be verified with a study assessing the levels of NO in models subjected to treatment with SGLT2i, as well as the changes resulting from co-administration of relevant medications such as ACEi. This hypothesis could explain one of the possible mechanisms behind the higher amputation risk described in diabetic patients receiving treatment with SGLT2i, acknowledging that the cause of these adverse events are likely multiple and that different precipitating factors may play a role in each particular patient.

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None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2018.12.006.

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