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Potential benefits of quinoxaline 1, 4-dioxides in aldosterone dysmetabolism disease

—A medical hypothesis

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ABSTRACT

Quinoxaline 1, 4-dioxides (QdNOs) are quinoxaline derivatives which have been used as antimicrobial agents and growth promoters in animals widely. They are also assumed to cure human disease such as anticancer, antitubercular and inhibiting parasite. QdNOs such as carbadox and their major metabolites induced a special decline of aldosterone production from the swine adrenal *in vivo* and *in vitro*, and thus cause hypovolemia, hyponatremia and hyperkalemia. This can also be expected to be the case for human. As a mainly physiological hormone and a novel steroid with potent mineralocorticoid activity, aldosterone plays an important role in the pathophysiological process of brain, renal and heart disease progression and may be a renal and vascular risk factor. Here, we provide evidence to support the hypothesis that QdNOs may lead potential benefits in aldosterone dysmetabolism disease via the synthesis deficiency of aldosterone in adrenal and/or the cardiovascular tissues. If the hypothesis is true, it may provide a new option into the therapy for aldosterone dysmetabolism disease, especially in cardiovascular system, and thus assume a broader application of QdNOs.

Keywords: Quinoxaline 1; 4-Dioxides; Aldosterone; Adrenal Gland; Dysmetabolism; Cardiovascular Tissues

1. INTRODUCTION

Since 1940s, Quinoxaline-di-N-oxides (QdNOs) (**Figure 1**) are known as potent antibacterial agents which act on several gram-positive and -negative species [1-4]. For this reason they have been used as growth promoters in agricultural stock farming to promote growth of pigs, cattle, fish and poultry when added to the feed during rearing in dosages 25 - 100 mg/kg [5-8].

Recently, extensive study has been carried out on QdNOs which indicates that they can be also applied for treatment of human diseases. For example, QdNOs have shown a selective cytotoxicity against hypoxic cells presented in solid tumors [9-11]. It was [12] demonstrated a dose-dependent inhibition of the proliferation of T-84 human colon cancer cells by QdNOs. Other studies have put in evidence that QdNOs are endowed with antitubercular activities *in vitro*. They have inhibitor activity of clinical isolates of *Mycobacterium tuberculosis* [13] or *Mycobacterium tuberculosis* H37Rv [14-15]. Furthermore, QdNOs presented good *in vitro* inhibitor activity of *Trypanosoma cruzi,* the causative agent of Chagas' disease affects around 20 million people in Central and South America [16].

The endocrinological effects of these compounds have long been recognized. Besides their influence on metabolic hormones and epidermal growth factor in swine [17] QdNOs such as carbadox and its major metabolites induced a special decline of aldosterone production from the adrenal *in vivo* and *in vitro*, and thus cause hypovolemia, hyponatremia and hyperkalemia [18-21] Aldosterone is a steroid hormone and the most important mineralocorticoid in the human body. Clinically, excess aldosterone is associated with increased stroke risk [22]; progressive renal disease [23], higher morbidity and mortality in cardiovascular diseases such as myocardial infarction (MI) and heart failure (HF) [24-25]. Here, we provide evidence to support the hypothesis that QdNOs can lead potential benefits in cardiovascular or other diseases caused by metabolism dysfunction of aldosterone via the synthesis deficiency of aldosterone.

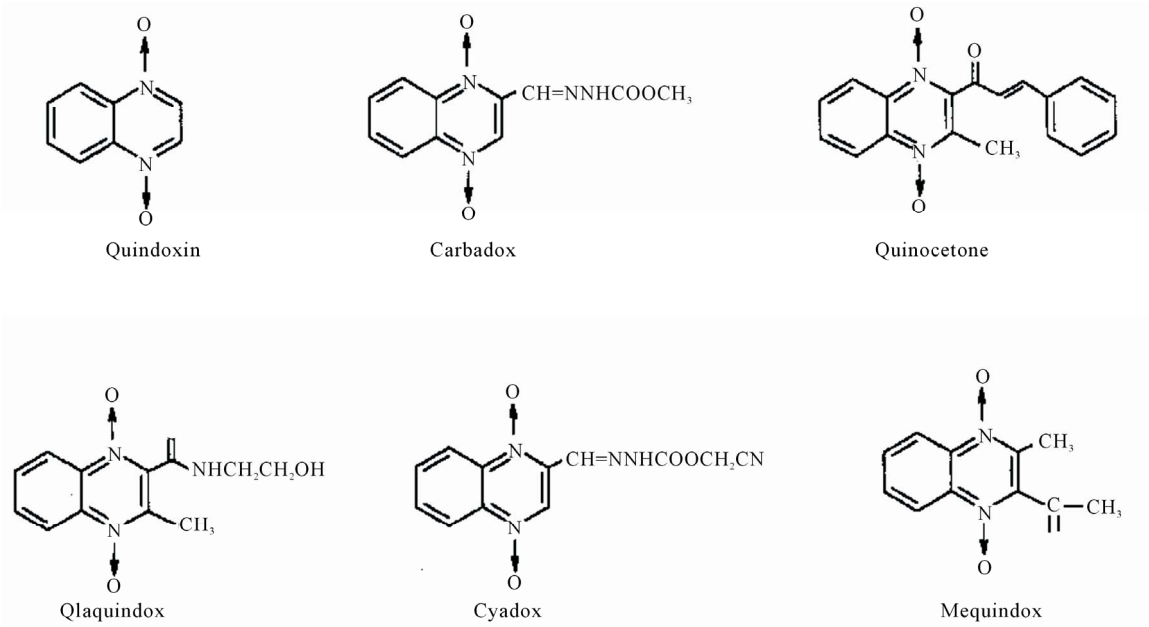

Figure 1. Chemical structures of some quinoxaline 1, 4-oxides.

2. DETAILS AND ANALYSIS

2.1. Metabolic Pathway of Aldosterone Biosynthesis

Induction of aldosterone biosynthesis can be divided into two temporal phases: an acute phase resulting from accelerated cholesterol translocation and a chronic phase involving synthesis of mRNA and enzymes [26]. There are four enzymes involved, i.e. Cholesterol desmolase (*CYP*11*A*), 21-hydroxylase (*CYP*21), 3β-Hydroxysteroid dehydrogenase (3*β-HSD*) and aldosterone synthase (*CYP*11*B*2). *CYP*11*A*, *CYP*21 and *CYP*11*B*2 are cytochromes P450 (*CYP*), membrane-bound heme-containing enzymes that accept electrons from NADPH via accessory proteins and utilize molecular oxygen to perform hydroxylations or other oxidative conversions. 3*β-HSD* is a member of the short-chain dehydrogenase family [27-28]. Biosynthetic reactions of aldosterone happened not only in adrenal but also in other tissues. *CYP*11*B*2 is expressed both in the zona glomerulosa and extra-adrenal tissues. Such synthesis has also been detected in brain [29-30], heart [31-32] and vascular smooth muscle [33-34]. Perfusion of angiotensin II increased local aldosterone production, suggesting that aldosterone is formed within the isolated tissue from a locally present substrate [35]. Even though the function of *CYP*11*B*2 in most of the tissue is not fully understood, aldosterone synthesized in these tissues can elicit important biological responses in an autocrine or paracrine fashion.

Disorder of aldosterone biosynthesis can lead to steroid hormone-related diseases such as hypoaldosteronism and hyperaldosteronism. Defective aldosterone biosynthesis may be caused by congenital adrenal hyperplasia due to *CYP*21 deficiency, in which case cortisol biosynthesis is also affected, or as an isolated defect termed *CYP*11*B*2 deficiency [27]. Primary hyperaldosteronism is a state characterized by long-standing aldosterone excess and suppressed plasma renin activity, resulting in hypertension and hypokalemia. It is the most common endocrine cause of hypertension and affects 5% - 13% of all patients and is commonly caused by aldosterone-producing adenoma (APA) or bilateral idiopathic hyperaldosteronism [36-37] (IHA).

2.2. Deleterious Effects of High Levels of Aldosterone

As a mainly physiological hormone and a novel steroid with potent mineral corticoid activity, aldosterone controls electrolyte balance, plasma volume, and vascular functions. With a low salt intake plasma aldosterone concentration may increase to high levels without any negative effects on the organism. However, growing body of evidence suggests that high plasma aldosterone levels have been shown to correlate with hypertension [38], left ventricular hypertrophy [39-41], stroke [22] and renal dysfunction [23]. Genetic and experimental models of hypertension have demonstrated that excess aldosterone induces severe injury in the heart, brain and kidneys [23]. Aldosterone synthesis in the cardiovascular system is up regulated during both acute MI and the associated remodeling of the myocardium [42], an effect

that is believed to be due to local angiotensin II production [43], suggesting that tissue-specific effects of aldosterone contribute to pathophysiological changes.

The neurohormonal actions of aldosterone influence the prognosis of patients with HF and ischemic heart disease through two main electrolyte homeostatic actions. Firstly, overproduction of aldosterone leads to inappropriate levels of sodium and water retention, which sets up a vicious cycle whereby the kidney attempts to compensate for impaired cardiac output by releasing angiotensin II, leading to further rises in aldosterone and volume overload. Secondly, aldosterone increases the urinary excretion of magnesium and potassium leading to electrolyte imbalance, increasing the risks of ventricular arrhythmias—which in the presence of myocardial remodelling following MI or secondary to HF, increases risk of sudden death [44]. In several pathological conditions aldosterone promotes vascular damage by formation of reactive oxygen species [45]. Moreover, clinical and experimental data indicate that aldosterone and its receptor are implicated in various non-renal locations [41], in particular the heart [35], brain tissue [46], and within the vasculature [47], suggesting that aldosterone also exerts local effects in tissue levels.

Aldosterone exerts most of its biological effects on cells by occupying an intracellular receptor, termed the type I or mineralocorticoid receptor (MR), which then binds DNA and thereby influences transcription of various genes [38-49]. However, not all of the actions of aldosterone are mediated by the classic genomic pathway involving transcription and translation. There is experimental evidence that aldosterone in a number of circumstances can stimulate an increase in intracellular calcium concentration and cause vasoconstriction by a mechanism which involves protein kinase C (PKC), and which may be independent of the classical MR. Despite pharmacological antagonism of aldosterone, which is nowadays part of a commonly applied standard therapy, could markedly reduce myocardial injury, cerebral hemorrhage and renal vascular disease, most of the non-classical effects are insensitive to inhibitors of the classical cytosolic mineralocorticoid receptor [50]. Moreover, this type of chemical also has some adverse effect such as breast tenderness with or without gynecomastia. It occurs more commonly in men but not exclusively. Studies indicate that approximately 10% of men will complain of breast tenderness with use spironolactone at the 25 mg dose. Other adverse effects that are associated with spironolactone include sexual dysfunction and menstrual irregularities [51].

2.3. Evidences of Quinoxaline 1, 4-Dioxides in Aldosterone Production

Experimental studies in pigs fed with carbadox, olaquindox, and cyadox had revealed obvious decreases in aldosterone and sodium concentrations in blood, with increases in potassium levels at 150 mg/kg or above [52-53]. As these drugs are given continuously, the biogenesis of aldosterone *in vivo* is likely to be seriously impaired, even when used in the advised dosages. Pigs treated with 100 and 200 mg/kg carbadox showed a significant decline of aldosterone after five and three weeks, respectively. With olaquindox a continuous, significant decline was found from 50 mg/kg and above after five weeks. In the cyadox groups, a significant decline was measured after six weeks in the 50, 200 and 400 mg/kg groups [52]. The animals might compensate with enhanced production via the alternative pathway and/or by enhanced release of other corticosteroids with mineralocorticosteroid activity. The rapid shutdown of the mitochondrial biogenesis of corticosteroids by QdNOs will then lead to hypoaldosteronism [51,54]. Moreover, the aldosterone decline effect was also seen *in vitro*. Spierenburg *et al*. investigated that the carbadox had the ability to inhibition of aldosterone production by pig adrenal slices. A dose dependent decrease in aldosterone production was reported at the 1~40 μg/ml [55]. In a study using a suspension of porcine adrenocortical cells, QdNOs were found to reduce the output of aldosterone. The carbadox and their major metabolites induced a slowly developing but virtually irreversible inhibition of the C18 transformations from corticosterone to aldosterone. But these compounds hardly affected the alternative pathway from deoxycortisol [17-18].

Obviously, the information obtained above is rather insufficient to interpret the mechanisms of QdNOs on aldosterone production related gene expression and enzymes interactions. More detailed investigation is required to delineate the pathophysiological linkage among QdNOs exposure, aldosterone synthesis, and aldosterone dysmetabolism diseases. Recently, our previous work *in vivo* [56-57] and *in vitro* [57] have suggested that high dose of QdNOs could lead to adrnal gland impaiment and aldosterone down-regulation, combined with sodium decrease and potassium increase in rat plasma. It's demonstrated that a complex crosstalk between ROS-generating system and aldosterone secretion. The induction of oxidative stress following exposure to mequindox (MEQ) could result in a membrane damage and dysfunction of adrenal mitochondrion, and then cause the contents loss of steroid hormone including CYP11A1, CYP11B1 and CYP11B2, finally leading to the deregulation of steroid hormone secretion and unbalance of ion concentration [55]. Moreover, Higher doses of MEQ showed similar depressive responsibility for most of renin-angiotensin-aldosterone system

(RAAS) components in adrenal and kidney, indicating a down-regulation of both intra- and extra-RAAS, the upstream of aldosterone production [58].

2.4. Nucleotide Similarity of Aldosterone Biosynthesis Enzymes in Human and Pig

Although exposure to QdNOs would lead to deregulation of aldosterone production in pigs, little information is known regarding their influence on human body. To evaluate the potential role of QdNOs in human aldosterone production, a multiple sequence alignment of the full length nucleotide sequence of *CYP*11*A* (GeneID: *Sus scrofa* 403329; *Homo sapiens* 1583), *CYP*21 (GeneID: *Sus scrofa* 403337; *Homo sapiens* 1589)and 3*β-HSD* (GeneID: *Sus scrofa* 445539; *Homo sapiens* 3284) gene were identified by performing BLASTN searches in GenBank™. *Sus scrofa* and *Homo sapiens* shared 84% identity with *CYP*11*A1*, 84% identity with *CYP*21*A* and 79% identity with 3*β-HSD*, respectively. The great similarity of aldosterone biosynthesis enzymes between human and pig confirmed the possibility that QdNOs influence aldosterone synthesis enzymes in human body. As QdNOs and their major metabolites induced a special decline of aldosterone production from the swine adrenal *in vivo* and *in vitro*, this can also be expected to be the case for human.

3. DISCUSSION

Based on above information, there is plenty of evidence to support the notion that aldosterone is an independent risk factor for brain, renal and heart disease. QdNOs may be helpful for the diseases caused by elevated levels of aldosterone. Thus we focus on the hypothesis that QdNOs could also cause aldosterone deficiency in human body and be beneficial for aldosterone dysmetabolism disease, which may suggest a newly candidate remedy for cerebrovascular system, renal and cardiovascular disease.

Although treatment with a mineralocorticoid receptor antagonist promotes blood pressure (BP) reduction and regresses target organ damage, they may be useless for non-genomic aldosterone action in clinical studies particularly in the cardiovascular system [49]. QdNOs have an effect independent of MR and perhaps act on the upstream cascade of aldosterone production, which is totally different with mineralocorticoid receptor antagonist. Further research on QdNOs may improve the understanding of their participation in the pathogenesis of aldosterone related diseases and eventually enhance the options for therapy.

There are at least three proposed mechanisms of QdNOs mediated in aldosterone deficiency and heart disease. Firstly, we postulate that the mechanism by which QdNOs inhibit the production of aldosterone, mainly by regulating some key enzymes necessary for aldosterone biosynthesis. To make known whether it is true, interaction of QdNOs with aldosterone associated physiological regulators, enzymes and cellular second messengers should be investigate to further discuss their mechanisms. Secondly, as most of enzymes involved in the biogenesis of adrenal steroids are also present in other steroid hormone producing organs, aldosterone produced within brain or heart was suggested to be similarly influenced by QdNOs. A comparable differential effect of QdNOs on the biogenesis and release of steroid hormones is to be expected. Lastly and most importantly, the chronic and continuously down regulation of aldosterone level in plasma and/or heart tissue caused by QdNOs would lead a directly shutdown of MR affinities, which may be an important direction in the therapy for heart diseases. Future pre-clinical and clinical studies associated with the precise mechanisms for QdNOs on heart diseases are anticipated. These studies should circumvent (1) appropriate pathological models *in vivo* and *in vitro*, (2) secure evaluation of QdNOs for their toxicity and adverse effects on human or other animals, (3) comparison between QdNOs and aldosterone antagonists such as eplerenone，K^+-canrenoate or spironolactone, the agents being currently used clinically [59]. All of the above hypothesis are testable in lab or in clinical.

Although QdNOs are known to their adrenal or other toxicities [19,21,60], their adverse effects can be avoided or decreased by controlling the dose. This aldosterone inhibition effect of QdNOs occurs in concentrations well below toxicity concentration found *in vivo* treatment. Moreover, some QdNOs such as cyadox is relatively less toxic and effective member of this family. Cyadox has been shown to be a very safe drug when used as a feed additive, and thus should be taken more attention than others.

4. CONCLUSIONS

It is meaningful to investigate the profitable role of QdNOs in cardiovascular or other diseases caused by metabolism dysfunction of aldosterone, which will lead to a further study in this kind of chemical. If the hypothesis is true, it may provide a new option into the therapy for aldosterone dysmetabolism disease, especially in cardiovascular system, and thus assume a broader application of QdNOs.

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