CEMTIRESTAT, A NOVEL ALDOSE REDUCTASE INHIBITOR AND ANTIOXIDANT, IN MULTITARGET PHARMACOLOGY OF DIABETIC COMPLICATIONS

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Substantial evidence suggests a key role for the polyol pathway (Fig. 1) in the etiology of diabetic complications. Under diabetic conditions, in tissues that do not require insulin for glucose uptake, aldose reductase (ALR2, E.C.1.1.1.21), the first enzyme of the polyol pathway, reduces a fraction of the excessive glucose to the osmotically active sorbitol in an NADPH-dependent way. Sorbitol dehydrogenase, the second enzyme of the polyol pathway, partially consumes sorbitol, yet under hyperglycemia, sorbitol accumulates intracellularly, with ensuing cell disruption. Depletion of NADPH cell stores by aldose reductase may eventually increase the susceptibility of cells to damage by reactive oxygen species. In addition, the polyol pathway contributes to the glycation process supplying fructose, a reactive glycation agent. Through these mechanisms glucose exerts its toxicity with diabetic complications as a consequence [1,2].

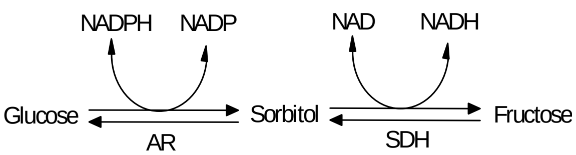

Fig. 1. Polyol pathway. AR, aldose reductase; SDH, sorbitol dehydrogenase.

Aldose reductase thus represents a promising therapeutic target in prevention of diabetic complications and a great effort is devoted to development of pharmacologically applicable inhibitors of aldose reductase (ARIs). Aldose reductase inhibitors (ARIs), such as acetic acid derivatives (epalrestat, tolrestat, zenarestat), spiro hydantoins (sorbinil), or the succinimide class of compounds (ranirestat), have been primarily investigated for their role against diabetic complications [3-5]. Yet none of them is available on the United States and Europe market owing to reported undesirable side effects. Epalrestat is approved only in Japan; it is currently the only aldose reductase inhibitor clinically used to treat subjective and objective symptoms of diabetic neuropathy. The safety and efficacy of epalrestat have not yet been established internationally [6,7].

In seeking for novel pharmacologically applicable ARIs, emphasis is given not only to the inhibitory activities but also to the physicochemical properties determining biological availability in target tissues [7,8]. A new generation of aldose reductase "differential" inhibitors was presented with ability to inhibit preferentially the reduction of either hydrophilic or hydrophobic substrates [9,10].

Elevated blood and tissue levels of markers of oxidative stress were documented under diabetic conditions [11-15]. In animal studies, antioxidant supplementation was found to attenuate hyperglycemia-related complications [15-22]. The above data provide support for the use of aldose reductase inhibitors and antioxidants to minimize consequences of glucose flux through the polyol pathway and hyperglycemia induced oxidations [22-25]. Bifunctional drugs with joint antioxidant/aldose reductase inhibitory activities would be beneficial in treatment of diabetic complications.

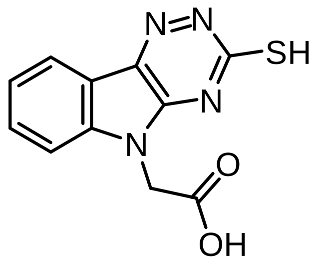

Fig. 2. Chemical structure of cemtirestat.

Recently, novel carboxymethylated mercapto-triazinoindoles have been identified as efficient inhibitors of aldose reductase [26]. Of them, 3-mercapto-5H-1,2,4-triazino[5,6-b]indole-5-acetic acid (cemtirestat, Fig. 2) was identified as the most efficient inhibitor. Currently, cemtirestat has been the subject of complex preclinical studies. By affecting both aldose reductase and oxidative stress, the compound represents an example of a promising multitarget directed agent with a therapeutic potential in prevention of diabetic complications. The present paper reviews recent results on preclinical evaluation of cemtirestat.

The inhibition of rat lens aldose reductase (ALR2) by cemtirestat was characterized by IC_{50} value in the sub-micromolar range. In addition, a closely related value was obtained for the inhibition of human recombinant AKR1B1 by cemtirestat, measured

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under identical reaction conditions as applied for the rat ALR2 assay [27].

An important feature of pharmacologically applicable ARIs is their selectivity over other members of the aldo-keto reductase family. The co-inhibition of structurally related physiologically relevant oxido-reductases may create undesired side effects. To test for selectivity, the closely related rat kidney aldehyde reductase (ALR1) was used. Approximately 400 times lower ALR1 inhibitory potency of cemtirestat points to high selectivity of cemtirestat in relation to aldose reductase. A selectivity factor above 200 was recorded for inhibition AKR1B10 by cemtirestat with respect to aldose reductase [27].

Uncompetitive inhibition was observed in relation to D,L-glyceraldehyde as a substrate with a corresponding inhibition constant Ki = 0.089 ± 0.018 μmol/L [27].

Recent studies suggest that ALR2 is important component of the detoxification system in removal of reactive aldehydes [28]. Cemtirestat was found to inhibit reduction of 4-hydroxynonenal, a toxic product of lipid peroxidation, significantly less efficiently than the reference substrate glyceraldehyde [29].

Substrate, cofactor and inhibitor binding to aldose reductase, as well as the protonation/deprotonation state of the enzyme are pH dependent. Moreover, under disease conditions, the cellular pH is most likely different from optimum effective values for each component. Specifically, in diabetic tissues under conditions of hyperglycemia, lower pH is expected in comparison with physiological conditions [30]. Cemtirestat was found to inhibit ALR2 very efficiently with pH optimum for inhibition activity around pH 6.2, yet very low inhibition was observed at pH 8 [31].

The crystal structure of human AKR1B1 in complex with cemtirestat was determined at pH 8.0 with a resolution of 1.26 Å. Two molecules of the inhibitor were revealed in the visualized difference electron density, one as expected in the binding pocket and a second in the vicinity of the entrance of the pocket (Fig. 3). The oxygen atom of the carboxylate group of the second inhibitor molecule and N2 of the first one formed an H-bond. The proton was most likely contributed by the nitrogen of the triazine ring of the first molecule of cemtirestat, as the carboxylate is assumed to be deprotonated under the applied conditions. The crystal structure was analyzed to rationalize the good selectivity of cemtirestat for ALR2 compared to ALR1. The conformation adopted in the binding pocket is surprising. Previously it has been proposed that addressing the protein with the specificity pocket in open state is a prerequisite for a high selectivity index however, we found in the present complex this pocket to be closed [27].

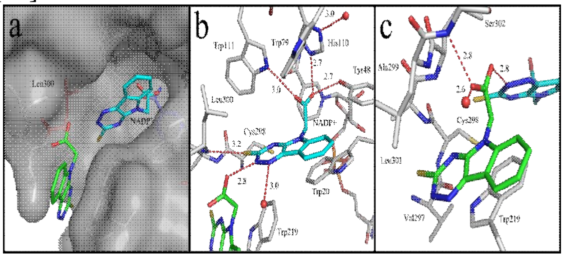

Fig. 3. High resolution X-ray assay of crystal structure of AKR1B1 complexed with cemtirestat. (a) Two molecules of cemtirestat are located in the binding site of AKR1B1; (b) Binding mode of the first molecule of cemtirestat; (c) Binding mode of the second molecule of cemtirestat [27].

The inhibitory effect of cemtirestat with respect to sorbitol accumulation in isolated rat eye lenses in ex vivo experiment was determined. Increased sorbitol levels were recorded in the isolated rat eye lenses incubated with glucose, in comparison with control incubations without glucose, reflecting increased flux of glucose through the cytosolic lens ALR2. Sorbitol accumulation was significantly and in a concentration-dependent way inhibited by cemtirestat, starting at a concentration as low as 10 μM. This finding indicates the ready uptake of cemtirestat by the eye lens tissue followed by inhibition of the cytosolic ALR2 [27].

In streptozotocin-induced diabetic rats, cemtirestat administered intragastrically (i.g., 50 mg/kg/day) for five consecutive days significantly inhibited sorbitol accumulation in the sciatic nerve. This result points to a ready uptake of cemtirestat after its i.g. administration into the central compartment, its supply to the peripheral nerves and inhibition of aldose reductase-mediated sorbitol accumulation. Yet at the dosage regimen used, the sorbitol levels in nerves were not normalized to control values. Optimization of cemtirestat dosage regimen may improve the therapeutic outcome [32].

Under diabetic conditions, enhanced flux of glucose through the polyol pathway contributes to development of oxidative stress. The studies on the antioxidant action of cemtirestat demonstrated its ability to scavenge 1,1'-diphenyl-2-picrylhydrazyl (DPPH) free radicals in a velocity exceeding that of equimolar melatonin. The total stoichiometry of DPPH scavenging by cemtirestat was determined. Under the experimental conditions used, one molecule of cemtirestat was found to quench 1.48 ± 0.06 DPPH radicals. DPPH, as a weak hydrogen atom abstractor, is considered a good kinetic model for peroxyl $ROO^{\cdot}$ radicals [33].

Taking into account charge localization in one-electron-oxidized radical of cemtirestat, spin density calculations have suggested an S-centered radical (Fig. 4). In the homogeneous system of DPPH in ethanol, the antioxidant activity of a compound tested stems from its intrinsic chemical reactivity towards radicals. In membranes, however, the apparent reactivity may be different since it is determined by the distribution ratio of the antioxidant between water and lipid compartments. In the system of isolated erythrocytes, cemtirestat was readily taken up by the cells and was found to protect intact erythrocytes against oxidative damage induced by peroxyl radicals generated intracellularly by decomposition of t-BuOOH. Osmotic fragility of the erythrocytes was not affected by cemtirestat [33].

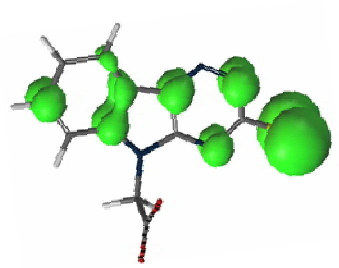

Fig. 4. Spin density of one-electron-oxidized radical of cemtirestat.

Physico-chemical indices, in addition to the structural features matching "the rule of five", along with good water solubility, point to an excellent "druglikeness" of cemtirestat.

Taken together, several advantages of cemtirestat over clinically used epalrestat are obvious, namely lower molecular weight, better water solubility, higher inhibition activity recorded both at the level of isolated enzyme and at the organ level of isolated rat eye lenses, and additional antioxidant activity. By affecting both the polyol pathway and oxidative stress, cemtirestat represents an example of a promising agent for multitarget pharmacology of diabetic complications.

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