International Journal of Innovative Research in Pharmaceutical and Bio Sciences
Peer Reviewed & Refereed | Open Access | Monthly | KG Publication | ESTD: 2026
Volume 1,Issue 1,May 2026,e-ISSN: XXXX - XXXX , print-ISSN: XXXX-XXXX

Carbonic Anhydrases: Novel Targets for Anticancer Drugs

S. Dhande* and M. Gursahani
Bharati Vidyapeeth's College of Pharmacy, Sector-08, C.B.D. Belapur, Navi Mumbai-400614, India

***Abstract*— Carbonic anhydrases (CAs, EC 4.2.1.1), are a superfamily of metalloenzymes in higher vertebrates, including humans. Sixteen distinct isoenzymes of CA are known to exist in mammals and most of these isoenzymes have been extensively investigated in terms of their biochemical properties, tissue distribution, and genetic control. There are cytosolic isozymes (CA I, CA II, CA III, CA VII and CA XIII), membrane bound examples (CA IV, CA IX, CA XII and CA XIV), and mitochondrial (CA VA and CA VB) and secreted (CA VI) isoforms. It was proposed that hCA IX is a multidomain protein consisting of an N-terminal proteoglycan like (PG) domain, a CA catalytic domain, a transmembrane segment (TM), and an intracytoplasmic (IC) portion. Carbonic anhydrase IX and XII inhibitors can prove to be effective in treatment of hypoxic tumours. Moreover the side effects with these drugs will be less than those caused by other anticancer drugs as these enzymes are present constitutively in only a few tissues.**

***Index Terms*— Carbonic Anhydrase, Hypoxic Tumours, Anticancer.**

I. INTRODUCTION

Carbonic anhydrases (CAs, EC 4.2.1.1), are a superfamily of metalloenzymes in higher vertebrates, including humans. Most CAs are involved in only one physiological reaction and they act as efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate and protons ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). Sixteen distinct isoenzymes of CA are known to exist in mammals and most of these isoenzymes have been extensively investigated in terms of their biochemical properties, tissue distribution, and genetic control.

The enzyme is found in many tissues such as the gastrointestinal tract (GI), the reproductive tract, the nervous system, kidneys, lungs, skin and eyes, wherein it plays key roles in a number of physiological and pathological processes, such as pH regulation, ions and gas exchanges, calcification, photosynthesis, tonic modulation of brain excitability through modulation of amino acid receptors, and biosynthetic reactions (such as gluconeogenesis, lipogenesis, and ureagenesis).

II. CLASSIFICATION OF CARBONIC ANHYDRASES

A. Catalytic and Acatalytic Types

There are cytosolic isozymes (CA I, CA II, CA III, CA VII and CA XIII), membrane bound examples (CA IV, CA IX, CA XII and CA XIV), and mitochondrial (CA VA and CA VB) and secreted (CA VI) isoforms. Three acatalytic forms, called CA-related proteins (CARPs) (CARP VIII, CARP X and CARP XI) are also known. The expression of CA I and CA II has been most frequently investigated in a variety of tumor cells, cell lines and some carcinoma patients, but it has been difficult to find a clear-cut relationship between the expression of such CA isozymes in normal and malignant cells.

It appears that only the expression of isoforms CA IX and CA XII is strongly associated with tumorigenesis. However, no evidence of a direct relationship between malignant transformation and CA expression has been presented for CA isoforms I-VII.

III. CARBONIC ANHYDRASE IX AND XII

In 1994, carbonic anhydrase isozyme was discovered to be present in many types of tumors, and later it was denominated as CA IX. Over expression of two CA isozymes (CA IX and CA XII) are predominantly associated with many tumors and it is being postulated that they are involved in critical processes connected with cancer progression. The presence of CA IX in various tumor types has been found to correlate with poor prognosis.

CA IX is confined to few normal tissues (stomach and body cavity lining), but it is ectopically induced and highly overexpressed in many solid tumor types, through the strong transcriptional activation by hypoxia, accomplished via the hypoxia inducible factor-1 (HIF-1) transcription factor, which accumulates in tissue under the hypoxic condition that is often present in growing tumors.

CA IX has effects on cell adhesion, and it has been suggested to play a role in tumor invasion through weakening of cell-cell adhesion as E-cadherin is competing for β-catenin. In some cancer cells, the VHL (von Hippel-Lindau) gene is mutated leading to the strong upregulation of CA IX (up to 150-fold) as a consequence of constitutive HIF activation.

As CA IX is confined to few normal tissues such as the gastric mucosa (where it seems to be in a catalytically inactive state), CA IX inhibitors may show less side effects compared to other anticancer drugs which interact with their target both in the normal and cancerous tissues and hence cause an array of side effects.

IV. OVERALL STRUCTURE AND ACTIVE SITE OF CAS

Structural studies on isozymes I, II, III, IV, V, XII, XIII, and XIV have revealed that all these isoforms present a considerable degree of three-dimensional similarity and a typical fold characterized by a central antiparallel β-sheet surrounding a Zn^{2+} critical for catalysis. The active site is located in a large, cone-shaped cavity that reaches the center of the protein molecule. The zinc ion is close to the bottom of the cavity and is coordinated by three conserved His residues in a tetrahedral geometry with H_2O or OH^- as the fourth ligand.

A cDNA coding for the human Carbonic anhydrase (hCA) IX protein was first cloned and investigated by Pastorek et al., and the CA IX gene was further characterized by the same group. On the basis of the sequence similarity, it was proposed that hCA IX is a multidomain protein consisting of an N-terminal proteoglycan like (PG) domain, a CA catalytic domain, a transmembrane segment (TM), and an intracytoplasmic (IC) portion. According to recent biochemical reports, the CA IX protein is dimeric, and the dimerization is mediated by the formation of an intermolecular disulfide bond between the same Cys residue located on two CA catalytic domains.

V. IN VITRO METHODS TO STUDY ACTIVITY OF CARBONIC ANHYDRASE MODULATORS

Caki-1, A-498 (human renal carcinoma cell lines) and HepG2 (human hepatocellular carcinoma cell line) can be cultured in Dubecco's modified Eagle medium (DMEM) supplemented with 10%(v/v) FBS, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. MCF-7 (human breast adenocarcinoma cell line) can be cultured in DMEM supplemented with 10% FBS, 1 mM sodium pyruvate, 1% non-essential amino acid, and 10 µg/ml insulin.

HeLa (human cervical carcinoma cell line) can be cultured in RPMI-1640 medium, 10% FBS, 2 mM L-glutamine. The cell cultures can be grown in 75-cm^2 flasks in a 37°C incubator with humidified 5% CO_2/95%

air. When the cultured cells reach 80-90% of confluence, they should be trypsinized and plated in 6-well plates at appropriate densities to obtain a sufficient quantity of cells for RNA extraction.

The levels of human CA IX and CA XII transcripts in the different cell lines can be assessed by quantitative real-time PCR using the Lightcycler detection system. Real-time PCR primers can be designed based on the complete cDNA sequences deposited in GenBank (accession numbers: NM_001216 for CA9, NM_001218 for CA12, and NM_001530 for HIF-1α). The house-keeping genes ubiquitin C (UBC) and β-2-microglobulin (B2M) can be used as internal controls.

Each PCR reaction must be performed in a total volume of 20 µl containing 1.0 µl of first-strand cDNA, 1× QuantiTect SYBR Green PCR Master Mix and 0.5 µM of each primer. The amplification and detection can be carried out as follows: after an initial 15 min activation step at 95°C, amplification should be performed in a three-step cycling procedure for 45 cycles: denaturation at 95°C for 15 sec, annealing at a temperature determined according to the Tm for each primer pair for 20 sec, and elongation at 72°C for 15 sec.

VI. CONCLUSION

Carbonic anhydrase IX and XII inhibitors can prove to be effective in treatment of hypoxic tumours. Moreover the side effects with these drugs will be less than those caused by other anticancer drugs as these enzymes are present constitutively in only a few tissues. The above mentioned in vitro methods can be used to screen potential anticancer drugs and this can prove to be a new dimension in the field of anticancer drug therapy.

ACKNOWLEDGMENT

Authors would like to thank Dr. Vilasrao Kadam, Principal, Bharati Vidyapeeth's College of Pharmacy, for providing the required facilities.

REFERENCES

[1] Supuran CT: Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008; 7:168-181.

[2] Winum JY, Rami M, Scozzafava A, Montero JL and Supuran CT: Carbonic anhydrase IX: a new druggable target for the design of antitumor agents. Med Res Rev 2008; 28:445-463.

[3] Aspatwar A, Tolvanen MEE, and Parkkila S: Phylogeny and expression of CA-related proteins. BMC molecular biology 2010; 11:25-42.

[4] Supuran CT: Carbonic anhydrase - an overview. Curr Pharma Design 2008; 14:603-614.

[5] Pastorekova S, Parkkila S, Pastorek J and Supuran CT: Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib Med Chem 2004; 19:199-229.

[6] Supuran CT, Scozzafava A and Casini A: Carbonic anhydrase inhibitors. Med Res Rev 2003; 23:146-89.

[7] Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A: Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res 2000; 60:7075-7083.

[8] Pastorek J, Pastoreková S, Callebaut I, Mornon JP, Zelník V, Opavský R et al: Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase. Oncogene 1994; 9:2877-88.

[9] Hilvo M, Baranauskiene L, Salzano AM, Scaloni A, Matulis D, Innocenti A et al: Biochemical characterization of CA IX, one of the most active carbonic anhydrase isozymes. J Biol Chem 2008; 283:27799-27809.

[10] Kallio H, Martinez A, Hilvo M, Hyrskyluoto A, Parkkila S: Cancer-Associated Carbonic Anhydrases IX and XII: Effect of Growth Factors on Gene Expression in Human Cancer Cell Lines. J Cancer Mol 2010; 5:73-78.

