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α-Viniferin and resveratrol induced alteration in the activities of some energy metabolism related enzymes in the cestode parasite *Raillietina echinobothrida*

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ABSTRACT

α-Viniferin (AVF) and its monomer resveratrol (RESV) are natural phytostilbenes produced by several plants in response to injury or under the influence of pathogens such as bacteria or fungi. Our earlier studies have revealed that both the compounds exert anthelmintic activity through alterations of cestode tegument and its associated enzymes. The present study investigates the effects of these phytochemicals on some energy metabolism related enzymes in the fowl tapeworm, *Raillietina echinobothrida*. The phytostilbenes AVF, RESV and the reference drug praziquantel (PZQ) were tested against some selected enzymes *i.e.*, phosphoenolpyruvate carboxykinase (PEPCK), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) of *R. echinobothrida*. Exposure of the tapeworm to AVF, RESV and PZQ causes reduction in activity of PEPCK to the extent of 40.57/41.96, 24.58/23.75 and 41.11/13.47%, respectively, and LDH up to 48.95/16.25, 38.31/38.42 and 45.67/41.87%, respectively, at the time of paralysis. Whereas activity of MDH decreased by 34.22/37.7, 39.1/35.24 and 28.83/19.26%, respectively. Decrease in activities of LDH and MDH was also visible through histochemical observations. The results suggest that both the phytochemicals interfere with the energy transducing pathways by inhibiting the studied energy metabolism related enzymes of the parasite.

1. Introduction

Pathway of energy metabolism is often regarded as an important factor that determines survival of helminth parasites at low oxygen tension environment in the host (Mansour, 2002). It has been observed that many of the chemotherapeutic agents that are now in use against helminths owe their effect through selective action on certain physiological or biochemical processes in the parasite. Recent advances in the understanding of chemotherapy of helminthiasis have greatly enhanced the knowledge concerning the physiology, biochemistry and pharmacology of these worms. It is known that striking differences exist between host and parasite as per as their energy metabolism pathways are concern. These difference ranges from structure to the function of proteins and enzymes (Saz, 1981). Therefore, identification of these differences in energy metabolism pathways between the parasite and its host can lead to identification of new parasite-specific targets for drugs.

Glucose and/or glycogen are the main energy source for helminth parasites; it is degraded to phosphoenolpyruvate (PEP) by way of the Emden-Meyerhof glycolytic pathway (Mansour, 2002). Phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) is a CO_2 fixing essential regulatory enzyme of glycolysis, performs opposite role in parasitic helminth compare to its vertebrate hosts (Behm and Bryant, 1975; Colombo et al., 1978). In helminth parasites, PEPCK involves in the degradation of the glucose molecule, whereas in the vertebrate host the enzymes take part in gluconeogenesis (Mansour, 2002). Further it is established that the enzyme is linked to either guanosine 5′-diphosphate (GDP) or inosine 5′-diphosphate (IDP), but not adenosine 5′-diphosphate (ADP), whereas in mammalian hosts it is linked to guanosine 5′-triphosphate (GTP) or inosine 5′-triphosphate (ITP) to give rise ADP (Barrett, 2009). In helminthes lactate dehydrogenase (LDH; EC 1.1.1.27) reduce pyruvate to lactate. However, the prime job of LDH is to reoxidize reduced nicotinamide adenine dinucleotide (NADH) produced by the glycolytic pathway to maintain the cytoplasmic redox potential (NAD/NADH) (Marchat et al., 1996). Mansour and Bueding (1953) demonstrated that the enzyme lactate dehydrogenase of parasites is kinetically distinct from the corresponding enzymes of the host. Malate dehydrogenase (MDH, EC

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1.1.1.37) reduce oxaloacetic acid (OAA) to malate. The malate so formed in the cytosol plays a vital role in energy production (Saz, 1970). On contrary, host MDH catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH. This functional difference in the host and parasites has been studied in different parasites (Probert and Lewis, 1977; Pappas and Schroeder, 1979; Banu et al., 1992). All the three enzymes are present both in the host and the parasite; however, they are different either structurally or functionally. Therefore, these differences in the enzymes between the host and parasite can be considered as a suitable target for selective chemotherapy. Numerous commercial anthelmintic drugs like praziquantel, albendazole, levamisole, mebendazole, parbendazole and piperazine adipate found to interfere with different energy metabolism associated enzymes like LDH, MDH, hexokinase (HK), glucose-6-phospate dehydrogenase (G6PDH) and PEPCK in different helminth parasites (Sharma et al., 1987; Sharma et al., 1989; Swargiary et al., 2013). Many anthelmintics are believed to work *via* an alteration in the activity of parasite LDH. For example, benzimidazoles act primarily *via* activation of LDH, catalyzing the conversion of pyruvate to lactate (Veerakumari and Munuswamy, 2000). However, because of its limitations like high costs and resistance developed by helminth parasites against commercial drugs, people of rural India are compelled to use traditional medicinal plants as an alternative to modern treatment.

Our previous study revealed that the phytostilbenes α-viniferin (AVF) and resveratrol (RESV) of the anthelmintic medicinal plant *Carex baccans* induce alterations in the tegumental architecture and activities of some vital tegumental enzymes, namely acid phosphatase, alkaline phosphatase and adenosine triphosphatase in the cestode *Raillietina echinobothrida* (Giri and Roy, 2014; Roy and Giri, 2015). In addition, an *in vivo* cestocidal activity of resveratrol has also been proved against the zoonotic helminth *Hymenolepis diminuta* (Giri et al., 2015). However, hardly any information is available regarding the influence of these phytochemicals on the energy metabolism related key enzymes of helminth parasites. Therefore, the present study aimed to investigate the effects of AVF and RESV, two phytostelbenes, on some key energy metabolism related enzymes of the parasite *R. echinobothrida*.

2. Materials and methods

2.1. Chemicals and reagents

α-Viniferin was obtained from BioBioPha Co., Ltd., Yunnan Province, P.R. China. Praziquantel (P4668) and Resveratrol (R5010) were obtained from Sigma Chemicals (St. Louis, USA). The required enzymes and co-enzymes were purchased either from Himedia or Sisco Research Laboratories (Mumbai, India). For all chemical preparations Millipore water was used.

2.2. In vitro *treatment*

Live mature *R. echinobothrida* were collected from the intestine of freshly slaughtered domestic fowl (*Gallus gallus domesticus*), kept in 0.9% phosphate-buffered saline (PBS, pH 7.2) and immediately exposed to phytochemicals and the reference drug PZQ. Parasites were incubated separately at 37 ± 1 °C with 0.135 mg AVF, 0.136 mg RESV and 0.135 mg PZQ per mL of PBS, dissolved in 0.01% dimethylsulfoxide (DMSO). Control worms were maintained in PBS having 0.01% DMSO only. Five replicates for each set of incubation medium were used and the time taken for attaining paralytic state, as well as death of the parasites was recorded. Control, AVF, RESV and PZQ exposed parasites were retrieved from the incubation media at paralysis and were processed for histochemical and biochemical studies to evaluate activities of PEPCK, MDH and LDH.

2.3. Histochemical localization of LDH and MDH

Adult control and treated parasites were directly subjected for cryosectioning and cut into sections at a thickness of 14–16 μm in a Leica CM 1850 cryostat (Leica Biosystems, Germany) at −23 °C. The histochemical localization of LDH and MDH of *R. echinobothrida* was carried out following the method of Pearse (1972). The difference in stain intensity of the control and treated parasites were analyzed by ImageJ (1.481v) software.

2.4. Biochemical assay

2.4.1. Tissue processing

A 10% tissue homogenate (w/v) was prepared in an ice cold 0.25 M sucrose solution containing 0.15 M Tris–HCl (pH 7.5) in an ice bath. The homogenate was centrifuged at 10,000 rpm for 20 min (Hermle Z 233MK-2) and the resultant supernatant was used for different enzyme activity. All steps were carried out at 4 °C.

2.4.2. Preparation of subcellular fractions

Mitochondrial and cytosolic fractions were prepared by differential centrifugation of a 10% tissue homogenate in fractionating buffer containing 50 mM Tris–HCl (pH 7.5), 250 mM sucrose and 2 mM EDTA following the method of Zenka and Prokopic (1987). The homogenate was centrifuged at 500 rpm for 10 min. The pellet was removed and the supernatant used as crude tissue homogenate. Half of the tissue supernatant was re-centrifuged at 10,000 rpm for 30 min. The supernatant thus formed was collected and used as cytosolic fraction. The precipitated sediment was re-suspended in the same buffer and centrifuged at 12,000 rpm for another 30 min. The supernatant was discarded and the pellet was suspended in the buffer and sonicated for 20 s and used as the mitochondrial fraction. All the steps were carried out at 4 °C.

2.4.3. Enzyme assay

PEPCK assay was carried out following McManus and Smyth (1982). An assay mixture of PEPCK consists of 23.3 mM Tris–HCl (pH 7.4), 4 mM PEP, 40 mM $MgSO_4$, 7 mM $NaHCO_3$, 40 mM KCl, 0.1 mM GDP, 0.2 mM NADH, MDH 8 units and tissue extract 50 μL. Similarly, LDH and MDH activities were measured following Bergmeyer (1974), and Bergmeyer and Bernt (1981) with little modification in the concentrations of the chemicals. For LDH, the assay composition consisted of 47 mM phosphate buffer (pH 7.4), 0.4 mM pyruvic acid, 0.2 mM NADH, and 50 μL of tissue supernatant. Similarly, for MDH the assay mixture composed of 26.63 mM phosphate buffer (pH 7.4), 0.5 mM oxaloacetic acid, 0.2 mM NADH and 50 μL tissue supernatant. All the enzymes were measured spectrophotometrically by observing the oxidation of NADH (Beckman Model-26) at 340 nm wavelength. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the oxidation of 1 μM of NADH per minute. The protein content of all the tissues was estimated using bovine serum albumin (BSA) as a standard protein (Lowry et al., 1951).

Statistical analysis

Data collected and presented as mean ± SEM ($n = 5$). Statistical analysis was carried out by comparing the respective control values and the experimental values using Student's *t*-test. The differences at $P \leq 0.05$ were regarded as statistically significant.