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Effects of Some Monoterpenes on Bovine Lens Aldose Reductase Activity

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Abstract □ Thirteen monoterpenes were examined for their effects on the bovine lens aldose reductase activity. The monoterpenes tested in this study showed mild inhibitory effects except 3-carene which did not show any significant effect. At the concentration of 10^{-3}M (+) Pulegon showed 42% inhibition on lens aldose reductase activity, which is the most potent effect among the tested substance.

Keyworks □ monoterpenes, bovine lens aldose reductase

Aldose reductase is entirely localized to the Schwann cell in peripheral nerve, to the kidney papilla, and the islets of Langerhans in pancreas. Its highest concentration is present in the lens epithelium. It is generally well known that the activity of lens aldose reductase (LAR), an allosteric enzyme of lens sorbitol pathway, is greatly enhanced in the diabetic hyperglycemic state.

Aldose reductase reduces aldoses such as glucose and galactose to their corresponding sugar alcohols. Elevated amounts of glucose in the diabetic lens would result in an intracellular accumulation of sorbitol, because polyols do not readily diffuse across cell membranes[1-5].

It is proposed that intracellular polyol accumulation causes osmotic swelling and eventual disruption of cell architecture. The strongest evidence in favor of this hypothesis is the finding that several drugs that inhibit aldose reductase significantly retard cataract formation in diabetic and galactocemic rats. A number of specific inhibitors of aldose reductase have been described in the literature[6-9].

It is still worthwhile trial to find out LAR inhibitors from the natural sources and synthetic preparations. The effects of monoterpenes on the LAR activities have not yet been reported in the literature. From this reason, the present study is motivated to investigate the effects of monoterpenes on LAR activity.

MATERIALS AND METHODS

Materials

Bovine eyes were obtained from a local abattoir soon after slaughtering, and the lenses were removed and frozen until use. Cellophane seamless tubing 18/32 inch for dialysis was purchased from Wako Chem. Co. NADPH and D,L-glyceraldehyde were purchased from Sigma Co. Monoterpenes were kindly gifted from Dr. C.K. Ryu of Ewha women's university.

Preparation of enzyme

All operations below were performed in a cold room at 4 °C. Lenses (60 g) were homogenized in 5 volumes of 5 mM phosphate buffer (pH 7.4) and centrifuged at 18,000 g for 15 minutes to remove insoluble materials. 40% ammonium sulfate was added to the supernatant. After the thick suspension had been allowed to stand with occasional stirring for 15 minutes to ensure completeness of precipitation, it was centrifuged and precipitate was discarded. Aldose reductase was then precipitated from the 40% supernatant solution by the addition of ammonium sulfate to 75% saturation and was recovered by centrifugation. The precipitated enzyme was redissolved in 5 mM phosphate buffer, and dialyzed 3×4 hours against 10 volumes of phosphate buffer. A DEAE-cellulose column (2×30 cm) was previously equilibriated with 5 mM phosphate buf-

fer. The dialyzed enzyme preparation was absorbed on the column, and the column was washed with 5 mM phosphate buffer until the absorbance at 280 nm of the eluate was less than 0.1, the elution of enzyme was accomplished with a linear gradient.

Determination of enzyme activities

rdfThe reaction mixture contained 0.1 M phosphate buffer, pH 6.2; NADPH, 2.5×10^{-4}M; D,L-glyceraldehyde, 1.5×10^{-3}M and the enzyme[10]. The total volume of this reaction mixture was adjusted to 1 m*l*. The reference blank consisted of all the above compounds except the substrate. The effect of inhibitors on the enzyme activities was determined by including in the reaction mixture the compounds being tested at the desired concentrations. The reaction was carried out at 25 °C and initiated by the addition of substrate. A unit of activity was defined as a change in absorbance of 0.001 unit per minute.

RESULTS AND DISCUSSION

Monoterpenes are widely distributed in the edible plants and many trials have been also reported for the synthesis of monoterpenes. But the effects of monoterpene derivatives on the lens aldose reductase activity have not been reported in the literature. On the basis of this consideration we started to investigate the effects of monoterpenes on the activity of the bovine LAR. Most of monoterpenes tested in this experiment showed mild inhibitory effects on the bovine LAR activity. Only 3-carene showed no effects on the enzyme activity at the concentration of 10^{-3}M and 10^{-4}M.

As shown in Table I relatively higher LAR inhibitory effects among the tested substances were found in the case of (+)pulegon, carbomethon and (−)carvon, of which inhibition percentages are 42%, 37% and 35% respectively at the concentration of 10^{-3}M. (+)Pulegon and carbomethon showed also comparable inhibitory effects, 20% and 21%, respectively at 10^{-4}M.

These mild LAR inhibitory effects of monoterpenes tested could not be construed as an applicable substances for the pharmaceutical use. But these results stimulate us to carry out the further studies on the additive and synergistic effects of monoterpene-mixtures in the edible plants and medicinal plants and to consider the applicability of the monoterpene containing herbs for the prevention and retardation of diabetic cataracts.

Further investigations on the LAR inhibitory effects of other monoterpenes and structure-activity relationships are in progress.

Table I. Effects of some Monoterpenes on Bovine Lens Aldose Reductase Activity

monoterpenes	Inhibition (%)	
	10^{-3}M	10^{-4}M
3-Carene	0	0
4-Caranol	23	17
4-Isocaranon	21	14
3-Hydroxy methyl-caran-4-on	22	17
3-Hydroxy methyl-caran-4-ol	30	16
(+) Carvon	25	14
(−) Carvon	35	14
Carbomethon	37	21
(+) Pulegon	42	20
(−) Isopulegol	25	17
(−) Menthon	23	5
4-Hydroxy methyl-menthon	31	17
Camphor	14	5

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