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Enhanced cAMP/PKA pathway by seabuckthorn fatty acids in aged rats

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Abstract

Seabuckthorn fatty acids were extracted by crushing and centrifuging from china seabuckthorn fruit. We detected cyclic nucleotides concentration in serum of different stages in aged rats (from 16 to 21 months), cyclic nucleotides concentration, PKA activity and PDE activity in hepatic tissue in aged rats by seabuckthorn fatty acids. Our data showed that the serum cAMP concentration decreased, accompany with the cGMP concentration increased and the imbalance of the cAMP/cGMP ratio in aged process. This kind of change equally in the hepatic tissue, the cAMP concentration decreased, PKA activity also decreased, but no change of the cAMP particularity PDE activity. And the SBFAs raised serum cAMP level in different stages, and raised the cAMP concentration and PKA activity of hepatic tissue, but did not effect the cAMP particularity PDE activity. Our study demonstrated that it is imbalance of the cAMP/cGMP ratio in aged process. SBFAs enhanced the cAMP/PKA pathway, regulated cAMP/cGMP ratio in aged rats.

Keywords: Seabuckthorn fatty acids; Cyclic nucleotides; PKA activity; PDE activity; cAMP/PKA pathway

1. Introduction

Seabuckthorn (*Hippophae rhamnoides* L. Elaeagnaceae) is a hardy bush, which is belonged to the Elaeagnaceae family and naturally distributed over Asia and Europe. The natural habitat of seabuckthorn extends widely in China, Mongolia, Russia, and most parts of Northern Europe. It is a unique and valuable plant currently cultivated in various parts of the world. Various organs of seabuckthorn, especially berries, were used in traditional medicines, mainly in China, Mongolia, and Middle Asia. The berries of seabuckthorn have been used as a drug by traditional Tibetan and Mongolian medicine since ancient time. It has pharmacological effects on the lungs, the stomach, the spleen and the blood circulation, which was recorded in some medicinal classics, such as Sibu Yidian (Yuandanguibu et al., 1983) from the Tang Dynasty and Jing Zhu Ben Cao (Danzengpengcuo, 1986) from the Qing Dynasty. In 1977, seabuckthorn was officially for the first time listed in the Chinese Pharmacopoeia by the Ministry of Public Health (Pharmacopoeia of the PR China, 1977). Recently, the nutritional importance of seabuckthorn berries has been increased in North America, Europe and Asian (Beveridge et al., 1999). Seabuckthorn contains a series of chemical compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tanins, etc. (Guliyev et al., 2004). These compounds possess biological and therapeutic activity including antioxidant, antitumoral, hepatoprotective and immunoregulation properties (Bao et al., 1997; Geetha et al., 2003; Zeb, 2006). Oil extracts obtained from berries are used in liver diseases, absorption disorders in the gastrointestinal system, cardiovascular system, immune system, anti-cancer, anti-senility, anti-inflammation, anti-oxidant, anti-radiation and peri-menopausal period (Bao et al., 1997; Xing et al., 2002; Geetha et al., 2002; Liu, 2005; Zeb, 2006). Our previous work showed that oil extracts obtained from berries (mainly component is fatty acids, number of patent is ZL 94 1 18300.9) restrained ovarian granulose cell apoptosis, adjusted estrogen level and treated menopause syndrome (Zhao et al., 2006), and improved the postmenopausal bone metabolism, alleviated and corrected the bone loss (Liu et al., 2006). In recent years, the studies of seabuckthorn outstanding performance is anti-inflammation and immunomodulatory aspect. It is well established that cAMP modulates the response of immune

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cells to a variety of stimuli. Elevation of intracellular cAMP has been generally associated with inhibition of lymphocyte activation. The increase in intracellular cAMP can be explained through activation of PKA by dissociation of the regulatory subunits from the catalytic ones. The activated catalytic subunits phosphorylate a number of proteins, which suppress the activity of T helper 1 cells and ultimately affect gene transcription inhibiting the production of proinflammatory cytokines. In fact, agents with the ability to elevate intracellular cAMP levels have been demonstrated to posses immunosuppressive and antiinflammator properties (Castro et al., 2005). Recently research found that GPCR40 is middle, and long-chain free fatty acids (FFAs)'s receptor (Briscae et al., 2003; Kotarsky et al., 2003). And GPCR40 is a kind of G-protein-coupled receptor. Oil extracts obtained from seabuckthorn berries mainly component is middle, and long-chain free fatty acids. And it is generally agreed that elderly subjects undergo a progressive decay of their immune responsiveness which leads to an increased susceptibility to autoimmune processes, neoplasms and inflammation (Erraji-Benchekroun et al., 2005). So in the present study, we studied the age-related functional balance between cAMP, cGMP, investigated the effect of fatty acids in seabuckthorn (SBFAs) on cAMP/PKA pathway in aged rats, and presume mechanism of anti-inflammation and immunomodulatory.

2. Materials and methods

2.1. Plant material and extraction

China seabuckthorn fruit oils were extracted by crushing and centrifuging (yield 31%, w/w), brown and red transparent liquid. Mainly composed of palmitic acid 18.8% (g/g), palmitoleic acid 19.1% (g/g) and oleic acid 17.3% (g/g), which provided by Tiankui Biology Medical Company of Xi'an Jiaotong University NO.: 20040605, Tiangui menopause capsules (Drug certificate number of Chinese SFDA is Z20050535).

2.2. Animals and treatment

Female Wistar rats weighing 300–340 g, 10 months old, were purchased from laboratory animals research institute of Chinese Academy of Medical Sciences (Beijing, China). Rats were placed in laboratory and were reared for 5 months. Food (a standard rodent chow diet) and water were available ad libitum. The animals were kept in a room maintained at $22 \pm 2\,^{\circ}C$ and 50–60% humidity under a 12-h light/12-h dark cycle of artificial lighting (lights on at 08:00).

Animal experimental protocols were approved by the University of Hawaii Animal Care and Use Committee (IACUC) and were conducted according to the principles in the NIH Guide for the Care and Use of Laboratory Animals.

Animals were divided into 4 groups randomly, old control group, high, middle and low dose group of SBFAs. In addition, one young control group was made up of 5 months old Wistar rats. In the three administer groups, SBFAs mixed in the emulsifying agent was administered to rats with a respective dose of 4.5, 1.8, 0.72 g/kg body weight (BW). Old and young control groups administered with emulsifying agent. All groups administered 0.5 mL/100 g suspension once per day for 180 days. On the 30th day and the 100th day since administration of SBFAs, animals were anesthetized by ether (Chemistry reagent factory of Xian, Xian, China), cut tail, collected blood 1–2 mL, centrifuged at $600 \times g$, saved the serum and stored at $-20\,^{\circ}C$. Since administration for the 180th days, animals were sacrificed, anesthetized by pentobarbital sodium (Sigma, USA) and intubated common carotid artery. Collected artery blood samples, centrifuged at $600 \times g$, saved the serum and stored at $-20\,^{\circ}C$, which was used to detect cAMP concentration. Hepatic tissue was quickly removed, frozen in liquid nitrogen immediately until related analysis after rinsed with 0.9% NaCl (pre-cold in ice bath).

2.3. Methods

2.3.1. Effect of SBFAs on cyclic nucleotides in serum of different stages in aged rats

After the administration of SBFAs for 30 days (16 months) and 100 days (18.5 months), animals were anesthesiaed by ether, cut tail and collected blood 1–2 mL, centrifuged at $600 \times g$, saved the serum and store at $-20\,^{\circ}C$. After the administration 180 days (21 months), animals were anesthesiaed by pentobarbital sodium, intubated common carotid artery, collected artery blood samples, centrifuged at $600 \times g$, saved the serum and detected cAMP and cGMP concentration. cAMP and cGMP immunoassay system were purchased from R&D Systems Inc. (R&D, USA). The assay is based on the competitive binding technique in which cAMP or cGMP present in a sample competes with a fixed amount of alkaline phosphatase-labeled cAMP or cGMP for sites on a rabbit polyclonal antibody. During the incubation, the polyclonal antibody becomes bound to the goat anti-rabbit antibody coated onto the microplate. Following a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped. Determine the optical density using a high throughput multifunctional microplate test system (POLARstar OPTIMA, BMG Labtechnologies, Germany) at 405 nm. The intensity of the color is inversely proportional to the concentration of cAMP and cGMP in the sample. Operated strictly in accordance with the description.

2.3.2. Effect of SBFAs on cyclic nucleotides in hepatic tissue in aged rats

Grinded the frozen hepatic tissue to a fine powder using a mortar and pestle. Allow the liquid nitrogen to evaporate. Weigh 1 g of the frozen tissue in 10 volumes of pre-cold 5% trichloracetic acid (Chemistry reagent factory of Xian, Xian, China) and homogenize with a cold homogenizer (Xinzhi, China). Centrifuge at $600 \times g$ for 10 min. Extract supernates with 3 volumes of water-saturated ether. Dry the aqueous extracts and reconstitute with Assay Buffer ED2. The following procedure was the same with serum assay.