

A56**Presence of bilitranslocase in the vascular system and its role in the vasodilatation activity of anthocyanins**

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Background

Flavonoids are well known for their vasodilatation activity. Their mechanism of action is still to be elucidated. Bilitranslocase is a bilirubin-specific membrane carrier that is also responsible for the ATP-independent transport of flavonoids across the cell membrane [1]. Recently, the expression of bilitranslocase in the endothelium has been characterized [2]. The aim of the study was to examine the possible role of bilitranslocase in the vasodilatation activity of flavonoids. As a source of flavonoids we used bilberries, which are abundant in anthocyanins that show strong affinity for bilitranslocase.

Methods

A bilberry extract, of which the anthocyanin content was quantified by HPLC-DAD analysis, was used. Anthocyanins were expressed as a standard of cyanidin 3-glucoside (mg/L). Thoracic aortic rings obtained from male Wistar rats were mounted in standard organ baths filled with Krebs-Henseleit solution, maintained at 37°C with a 95% O₂ / 5% CO₂ mixture. Rings were divided into four groups: control group (intact aortic rings), endothelium denuded rings, rings with nitric oxide synthase (NOS) activity inhibited by application of L-NNA (0.1 mmol/L) and rings with inhibited bilitranslocase-mediated membrane transport. In the last group, aortic rings were pre-incubated for 30 min with mono-specific polyclonal bilitranslocase antibodies (0.24 µg/mL). After inducing submaximal contraction (60 mmol/L KCl) in all studied groups, a chemically characterized bilberry extract was applied in increasing concentrations (0.5–20 mg/L). Vascular tone was measured isometrically by a mechano-electrical transducer. Further tests were done to check the expression of bilitranslocase in the vascular system (endothelial cell line EA.hy 926 and vascular smooth muscle cell line A7r5) by Western blot analysis using bilitranslocase antibodies.

Results

Western blot analysis showed the presence of bilitranslocase on both endothelial and smooth muscle cells. Bilberry extract relaxed aortic rings in a concentration-dependent manner in the control group, but neither in endothelium-denuded aortic rings nor in rings with inhibited NOS. The maximum relaxation (19.00 ± 2.01 %, n = 5) observed at 20 mg/L in the group with inhibited bilitranslocase activity was significantly lower (p < 0.001) compared to the control group (34.95 ± 3.11%, n = 10).

Conclusions

Our results show that the vasodilatation activity of anthocyanins in bilberry extract is partly dependent on the bilitranslocase-mediated transport of flavonoids into the endothelium, followed by the activation of NOS. However, even though the bilitranslocase is also expressed on smooth muscle cells, its role in the vasodilatation activity on these cells remains negligible.

References

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