SHORT COMMUNICATION

Experimental Investigations

PROTECTIVE EFFECT OF ARONIA MELANOCARP A FRUIT JUICE IN A MODEL OF CISPLATIN-INDUCED CYTOTOXICITY IN VITRO

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ABSTRACT

AIM: The aim of the present study was to investigate the protective potential of Aronia melanocarpa fruit juice in a model of cisplatin-induced cytotoxicity in the human embryonal kidney cell line HEK293T.

MATERIALS AND METHODS: The cellular viability was assessed using the MTT-dye reduction assay based on the reduction of the yellow tetrazolium dye MTT to a violet formazan product via the mitochondrial succinate dehydrogenase in viable cells. Cisplatin was applied in various concentrations either alone or after a 24-hour pretreatment of the cells with Aronia melanocarpa fruit juice at 0.1 and 0.05 mg/ml. The half maximal inhibitory concentrations (IC₅₀ values) were derived from the concentration-response curves to cisplatin.

RESULTS: Applied alone, the anticancer drug caused a prominent decrease of cellular viability with IC₅₀ 8.3 ± 1.1 µM. The juice proved to significantly ameliorate the in vitro cytotoxicity of the platinum drug, in a concentration-dependent manner. The pretreatment of the cells with Aronia melanocarpa fruit juice resulted in a significant increase (p < 0.001) of IC₅₀ for cisplatin to 25.1 ± 2.7 µM (at 0.05 mg/ml) and 34.4 ± 3.4 µM (at 0.1 mg/ml), respectively.

CONCLUSION: The protective effect of Aronia melanocarpa fruit juice observed in this study is most probably due to its well appreciated antioxidant activity as oxidative stress plays a central role in the toxic effects of cisplatin.

Key words: Aronia melanocarpa, cisplatin, cytoprotective effect, cell line, MTT assay

INTRODUCTION

Cisplatin is a highly effective antitumor agent. However, the clinical utility of the drug is limited by its nephrotoxicity. The multiple pathways which lead to cisplatin-induced renal damage and renal cell death have points of convergence and share some common modulators. The most frequent event among all the pathways is the oxidative stress that acts as both a trigger and a result.¹ The role of the oxidative stress in cisplatin-induced nephrotoxicity is indicated by the protective effect of several food-derived antioxidants such as vitamin C and α-tocopherol as well as the flavonoids quercetin and naringenin.²,³ Furthermore, in experiments in vitro, antioxidants or scavengers of reactive oxygen species (ROS) have a cytoprotective effect on cells exposed to cisplatin.²

Aronia melanocarpa fruits are one of the richest natural sources of polyphenolic antioxidants and ROS scavengers⁴ such as flavonoids (mainly from the subclass of anthocyanins), procyanidins and phenolic acids.

Aronia melanocarpa has been extensively studied as a medicinal plant in recent years. To our knowledge, no reports are available on the effect of Aronia melanocarpa fruits and juice in models of cisplatin-induced nephrotoxicity.

AIM

The aim of the present study was to investigate
the protective potential of *Aronia melanocarpa* fruit juice (AMFJ) in a model of cisplatin-induced cytotoxicity in the human embryonal kidney cell line HEK293T.

**MATERIALS AND METHODS**

*Aronia melanocarpa* fruit juice preparation and contents

AMFJ was produced from *Aronia melanocarpa* (Michx.) Elliot fruits grown in the Balkan Mountains, Bulgaria. They were handpicked in September, crushed and squeezed. The juice was filtered, pasteurized at 80°C for 10 min and stored at 0°C till the experiment. As previously described, the total phenolics, total flavonoids and total anthocyanins in AMFJ were determined spectrophotometrically and quercetin was measured by high-performance liquid chromatography.

**Cell culture and cytotoxicity assessment (MTT-dye reduction assay)**

The human embryonal kidney cell line HEK293T was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany). The cells were grown in a controlled environment – cell culture flasks at 37°C in an Heraeus BB16 Function Line incubator (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO₂. The cells were maintained in 90% RPMI-1640, supplemented with 10% FBS and 2 mM L-glutamine as monolayer cultures. Cells were kept in log phase by supplementation with fresh medium, two or three times a week.

The cellular viability was assessed using the MTT-dye reduction assay as described by Mosmann with slight modifications. The assay is based on the reduction of the yellow tetrazolium dye MTT [3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide] to a violet formazan product via the mitochondrial succinate dehydrogenase in viable cells. In brief, exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 μl/well) at a density of 1×10⁵ cells per ml and after 24-hour incubation at 37°C they were exposed to either culture medium or various concentrations of AMFJ for further 24 hours. Thereafter medium-treated or AMFJ-treated cells were exposed to various concentrations of cisplatin for 72 hours. For each concentration a set of at least 8 wells was used. After the exposure period 10 μl MTT solution (10 mg/ml in PBS) aliquots were added to each well. Thereafter the microplates were incubated for 4 hours at 37°C and the formed MTT-formazan crystals were dissolved through addition of 100 μl/ well 5% formic acid solution in 2-propanol. The MTT-formazan absorption was measured using Beckman-Coulter DTX800 multimode microplate reader at 580 nm.

Cell survival fractions were calculated as a percentage of the solvent-treated control. IC₅₀ values were derived from the concentration-response curves to cisplatin, using non-linear regression analysis (Curve fit, GraphPad Prism software).

**Statistical analysis**

IC₅₀ values are presented as mean ± SEM. They were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test to identify significant difference. A level of p < 0.05 was considered significant. All analyses were performed using GraphPad Prism statistical software.

**RESULTS AND DISCUSSION**

The contents of the main biologically active substances in AMFJ are presented in Table 1. The nephroprotective potential of AMFJ was assessed in a model of cisplatin-induced cytotoxicity in the human embryonal kidney cell line HEK293T. To meet this objective the anticancer drug was applied in increasing concentrations either alone or after a 24-hour pretreatment with AMFJ at 0.1 and 0.05 mg/ml.

After 72 hours sole administration cisplatin caused a prominent decrease of cellular viability of HEK293T cells with almost total eradication

| **Table 1. Contents of main biologically active substances in *Aronia melanocarpa* fruit juice (AMFJ); the values are the mean of duplicate determinations of three samples** |
|-------------------------------|------------------|--------------------------------------|
| **Substances**               | **Amount (mg/100 ml)** | **Comment**                          |
| Total phenolics              | 709.3 ± 28.1      | Concentration based upon gallic acid as standard |
| Total flavonoids             | 189.4 ± 8.6       | Concentration based upon catechin as standard |
| Total anthocyanins           | 106.8 ± 6.2       | Concentration based upon cyanidin-3-glucoside as standard |
| Quercetin                    | 11.8 ± 0.8        |                                      |
of viable cells at the highest concentration of 40 μM (Fig. 1). The half maximal inhibitory activity (IC\textsubscript{50}) was established at 8.3 ± 1.1 μM. As evident from the modulation of concentration-response curves and from the IC\textsubscript{50} values obtained, the tested AMFJ proved to significantly ameliorate the in vitro nephrotoxicity of the platinum drug, in a concentration dependent manner (Fig. 1). The IC\textsubscript{50} values following combined treatment with cisplatin and AMFJ were 25.1 ± 2.7 μM (at 0.05 mg/ml AMFJ) and 34.4 ± 3.4 μM (at 0.1 mg/ml AMFJ), respectively. Both values were significantly higher than the IC\textsubscript{50} of cisplatin applied alone (p < 0.001).

The findings from the present study are in accordance with numerous experimental data on the protective effect of food-derived antioxidants\textsuperscript{2} including plant polyphenolics against cisplatin nephrotoxicity. Sanchez-Gonzalez et al.\textsuperscript{8} have demonstrated that quercetin, one of the flavonoids in AMFJ, prevents the nephrotoxic effect of cisplatin without affecting its anti-tumor activity.

Considering the multimodal activity of the polyphenols abundant in AMFJ, the delineation of the putative mode of nephroprotective action requires further investigations. However, as the depletion of glutathione and the generation of ROS play a central role in the toxic effects of cisplatin, we could suppose that the observed effect of AMFJ in this study is most probably due to its strong antioxidant activity, as demonstrated by many authors and reviewed by Kokotkiewicz et al.\textsuperscript{9} and Denev et al.\textsuperscript{10}

CONCLUSION

The present study revealed a protective effect of AMFJ in a model of cisplatin-induced cytotoxicity in the human embryonal kidney cell line HEK293T. As oxidative stress plays a central role in the toxic effects of cisplatin, that protective effect of AMFJ is most probably due to its well appreciated antioxidant effects.

REFERENCES

ПРОТЕКТИВНЫЙ ЭФФЕКТ ПЛОДОВОГО СОКА ИЗ ARONIA MELANOCARPA В МОДЕЛИ ЦИСПЛАТИН-ИНДУЦИРОВАННОЙ ЦИТОТОКСИЧНОСТИ IN VITRO

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РЕЗЮМЕ

ЦЕЛЬ: Исследовать протективный потенциал плодового сока из Aronia melanocarpa в модели цисплатин-индукованной цитотоксичности в человеческой эмбриональной клеточной линии НЕК297.

МАТЕРИАЛЫ И МЕТОДЫ: Клеточная жизненность оценивается с помощью МТТ теста токсичности, базирующегося на редукции желтого тетразолиевого красителя МТТ до фиолетового формазана от митохондриальной сукцинат-дегидрогеназы в жизненных клетках. Цисплатин применяется в различных концентрациях или самостоятельно, или после 24-часового „превоздействия” на клетки двумя концентрациями плодового сока из Aronia melanocarpa (0.1 and 0.05 mg/ml). Ингибитирующие концентрации 50% клеток (IC50) получены от кривых „концентрация-ответ” цислота.

РЕЗУЛЬТАТЫ: Самостоятельно примененное противопухолевое лекарство вызывает сильное снижение клеточной жизненности посредством IC50 8.3 ± 1.1 μM. В зависимости от своей концентрации сок может значимо уменьшить цитотоксичность лекарства in vitro. „Превоздействие” клеток плодовым соком из Aronia melanocarpa приводит к значимому повышению (P < 0.001) IC50 за цисплатин соответственно до 25.1 ± 2.7 μM (при 0.05 mg/ml) и 34.4 ± 3.4 μM (при 0.1 mg/ml).

ЗАКЛЮЧЕНИЕ: Наблюдаемый в этом исследовании протективный эффект плодового сока из Aronia melanocarpa по всей вероятности объясняется его хорошо документированными антиоксидантными эффектами, так как оксидативный стресс играет центральную роль в токсических эффектах цисплатина.