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NOTE

In vitro Radioprotective Activity of the Bryozoan *Hyalinella punctata*

BORIS PEJIN[1,*], BOJANA STANIMIROVIC[2], NEDA DJORDJEVIC[3], ALEKSANDAR HEGEDIS[1], IVO KARAMAN[4], MLADEN HORVATOVIC[4] and KSENIJA RADOTIC[1]

[1]Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia
[2]MOL d.o.o., Belgrade, Serbia
[3]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia
[4]Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

*Corresponding author: E-mail: brspjn@gmail.com; borispejin@imsi.rs

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The objective of the present study was *in vitro* evaluation of radioprotective activity of the freshwater bryozoan *Hyalinella punctata* on cultured human peripheral blood lymphocytes after irradiation with 2 Gy of ^{60}Co γ-rays. Since its water extract at concentration 0.001 mg/mL reduced the incidence of radiation-induced micronuclei for almost 30 %, it could be considered as a promising source of new natural products with the aforementioned activity. Both the content of sulphur (1.17 %), determined by gravimetric method and infrared absorption frequences (76 % similarity with those of bacitracin) of the investigated extract indicate the presence of organic sulphur compound(s) such as cyclic peptides and polypeptides which might be responsible for the observed radioprotection.

Key Words: Plumatellidae, Extract, Lymphocytes, Ionising radiation.

Bryozoa (moss animals) are a phylum which many people, both scientist and layperson alike, have little if any familiarity, despite the fact they are widely distributed throughout the world's marine and freshwater environments[1]. These invertebrates are sessile, colonial, filter-feeding organisms with about 4.000 extant species most of which are marine[2,3]. An intricate network of metabolites is present in the extracts of bryozoans including heteroatom-containing compounds[3]. However, most of the studies have focused strictly on the marine species. Freshwater bryozoans live in lotic and lentic water and feed on suspended organic particles[4]. *Hyalinella punctata* is one of the bryozoans of such origin which has been noticed in Serbia[5,6].

Radiation is an important modality in the treatment of cancer and in some instances it may be the single best solution. This kind of therapy depends on achieving a therapeutic differential (with chemical radiation sensitisers or protectors) between the cancer cell cytotoxicity and normal tissue toxicity.

In recent years, extensive studies have been conducted to evaluate the potential beneficial effects of natural products in radiorecovery and the protection of normal tissue during exposure to radiation[7]. Historically, sulfhydryl compounds were among the first radioprotectors to be identified[8]. In the course of our ongoing experiments on invertebrates[9-11], *in vitro* radioprotective activity of the bryozoan *H. punctata* was investigated for the first time.

The sample of *Hyalinella punctata* (Hancock, 1850) was collected in Belgrade (river Danube, Serbia, November 2011). Voucher specimen has been deposited in the Zoology Collection of the Department of Biology and Ecology of the University of Novi Sad, Serbia (BRY 003).

After carefully cleaning from contaminants, the bryozoan sample was lyophilised. The dried parts of *H. punctata* were ground (2 g) and extracted thrice with hot water for 1 h at room temperature. The extract was evaporated to dryness and stored at - 20 ºC until further use.

Fourier-transform infrared (FTIR) spectrum of the investigated extract was recorded in the attenuated total reflection mode (ATR) using a Nicolet 6700 FTIR Spectrometer (Thermo Scientific).

Blood samples were obtained from three healthy, non-smoking young male volunteer donors at the Medical Unit in accordance with current health and ethical regulations in Serbia[12]. Heparinised whole blood was aliquoted into sterile plastic test tubes, placed in a 15 cm × 15 cm Plexiglas container and irradiated using a ^{60}Co γ-ray source at room temperature. The radiation dose employed was 2 Gy, the dose rate was 0.45 Gy/min, the dimensions of the radiation field were 20 cm ×

20 cm and the distance from the radiation was 74 cm. After 1 h irradiation, 0.5 mL aliquots of whole blood were added to culture tubes containing 4.5 mL of PBmax karyotyping medium (Invitrogen-Gibco, Paisley, UK). Irradiated and positive control cultures were established. The optimum dose for evaluation of radioprotection was selected based on previously conducted experiments employing different concentrations of the bryozoan extract for the treatment of irradiated human lymphocytes.

For micronuclei preparation, the cytokinesis block method of Fenech *et al.*[13] was used. For each sample, at least 1000 binucleate cells were scored and micronuclei were recorded using an AxioImager A1 microscope (Carl Zeiss, Jena, Germany) with 400× or 1000× magnification. The ability of cells to proliferate *in vitro* was evaluated by counting the number of cells with one to four micronuclei on the same slides[14].

Statistical analysis was performed using the statistical software package Statistica 6.0 for Microsoft Windows. Statistical analysis was done using Student's t-test and a P value < 0.05 was considered to be significant.

The water extract of *H. punctata* has reduced the incidence of radiation-induced micronuclei (MN); a reduction of 28.17 % compared to control cells (Table-1). Both the content of sulphur (1.17 %), determined by gravimetric method[15] and infrared absorption frequences (76 % similarity with those of bacitracin; not shown here) of the investigated extract indicate the presence of organic sulphur compound(s) such as cyclic peptides and polypeptides which might be responsible for the observed biological activity[16].

TABLE-1
INCIDENCE OF MICRONUCLEI (MN) IN EXAMINED SAMPLES

Basal value of MN/1000 BN cells	Incidence of MN/1000 BN cells irradiated with 2 Gy (control)	Incidence of MN/1000 BN cells irradiated and treated with extract
7 ± 2	323 ± 21	232 ± 18

The development of radiation protectors is important not only for enhancing the effectiveness of cancer treatment but also for the studying of the underlying mechanisms of the relevant cytotoxicity[17,18]. Indeed, some radioprotectors are known for a direct effect on the cellular targets of radiation, while others act through enhancing the recovery of normal tissues[19].

Present results suggest that *H. punctata* water extract appears to confer protection against ionising radiation. Moreover, it is the first record ever of radioprotectivity of the bryozoan species as a whole. Therefore, the potential use of *H. punctata*-derived natural products in the radiation therapy is worthy for further investigation.

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