Antiproliferative/cytotoxic effects of molecular iodine, povidone-iodine and Lugol's solution in different human carcinoma cell lines

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Received January 22, 2016; Accepted June 16, 2016

DOI: 10.3892/ol.2016.4811

Abstract. Clinical trials have revealed that molecular iodine (I_2) has beneficial effects in fibrocystic breast disease and in cyclic mastalgia. Likewise, povidone-iodine (PVP-I), which is widely used in clinical practice as an antiseptic agent following tumour surgery, has been demonstrated to have cytotoxic effects on colon cancer and ascites tumour cells. Our previous study indicated that the growth of breast cancer and seven other human malignant cell lines was variably diminished by I_2 and iodolactones. With the intention of developing an iodine-based anticancer therapy, the present investigations extended these studies by comparing the cytotoxic capacities of I_2, potassium iodide (KJ), PVP-I and Lugol's solution on various human carcinoma cell lines. Upon staining the cell nuclei with Hoechst 33342, the cell densities were determined microscopically. While KJ alone did not affect cell proliferation, it enhanced the antiproliferative activity of I_2. In addition, PVP-I significantly inhibited the proliferation of human MCF-7 breast carcinoma, IPC melanoma, and A549 and H1299 lung carcinoma cells in a concentration corresponding to 20 μM I_2. Likewise, Lugol's solution in concentrations corresponding to 20-80 μM I_2 were observed to reduce the growth of MCF-7 cells. Experiments with fresh human blood samples revealed that the antiproliferative activity of PVP-I and I_2 is preserved in blood plasma to a high degree. These findings suggest that PVP-I, Lugol's solution, and a combination of iodide and I_2 may be potent agents for use in the development of antitumour strategies.

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Key words: iodine compounds, antiproliferative effects, human carcinoma cells

Introduction

In animal models, consumption of a molecular iodine (I_2) diet has been demonstrated to prevent N-methyl-N-nitrosourea and dimethylbenz(a)anthracene induction of breast tumours (1,2) and to reduce breast hyperplasia and perilobular/ductal fibrosis (3). Similarly, clinical trials have revealed that I_2 has beneficial effects in fibrocystic breast disease (4) and in cyclic mastalgia (5), suggesting that iodine therapy may diminish breast disease, including breast cancer progression (6,7). Povidone-iodine (PVP-I) is widely used in clinical practice as an antiseptic agent following tumour surgery. PVP-I has been demonstrated to induce the death of epithelial HeLa cells and to reduce oral mucosal tissue in rats (8). Further data supporting a potent anticancer effect of I_2 and of iodolactones has been established via cell culture investigations, which have revealed a significant decrease in cell growth in human breast cancer cell lines (9,10).

In a previous study, we showed that, in addition to breast carcinoma cells, the proliferation of seven other human malignant cell lines (neuroblastoma, glioma, melanoma, and lung, colon and pancreas carcinomas) was variably diminished by I_2 and iodolactones (11). With the intention of developing an iodine-based anticancer therapy, the present study extended this research by comparing the antiproliferative/cytotoxic capability of I_2, potassium iodide (KJ), combined KJ + I_2, PVP-I and Lugol's solution on various human carcinoma cell lines. The data suggest that PVP-I and the combination of iodide and I_2 may be potential tools to directly interfere with tumour cell growth.

Materials and methods

Materials. The following human cell lines were used in the present study: MCF-7 (breast carcinoma); HS-24, H1299 and A549 (lung carcinoma); Capan-2 and PaTu 8902 (pancreatic carcinoma), these cells lines were provided by Dr. Margarete Fischer of The Bosch Institute of Clinical Pharmacology, Stuttgart, Germany, and the IPC-298 (melanoma) cell line, provided by the Institute for Radiobiology, SanAk, Munich, Germany). The fluorescent dye Hoechst 33342 was obtained from Calbiochem (Merck Millipore, Darmstadt, Germany).

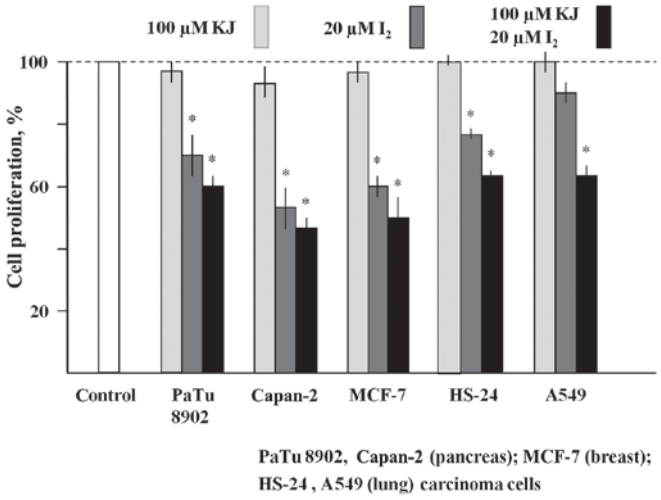

Figure 1. Effects of I_2, KJ, and the combination of the two on the proliferation of human carcinoma cells. KJ had no effect, whereas I_2 reduced cell proliferation by ≤55% in Capan-2 and 50% in MCF-7 cells. This effect was, to a certain degree, enhanced in the presence of KJ. Results are presented as the means ± standard deviations; n=4-6; *P<0.05 (*t*-test; treated samples vs. controls). I_2, molecular iodine; KJ potassium iodide.

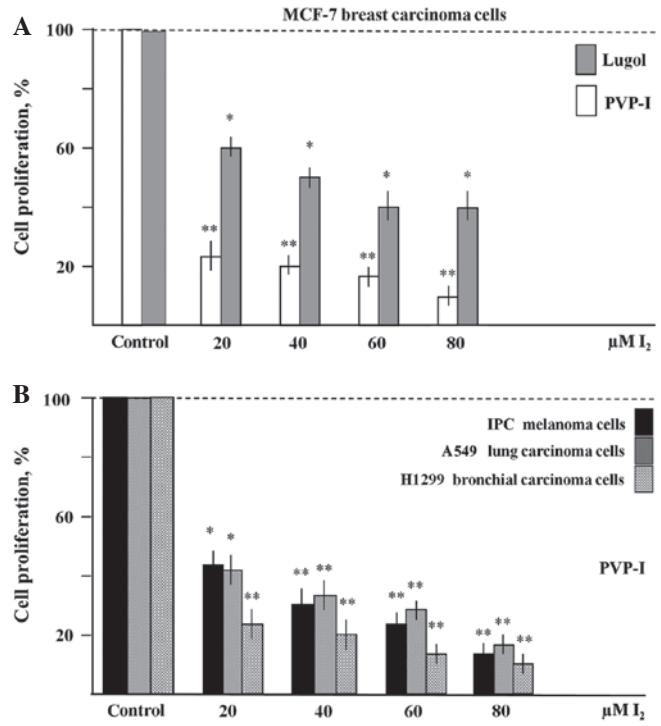

Figure 2. Antiproliferative effects of PVP-I and Lugol's solution. (A) PVP-I significantly inhibited the growth of MCF-7 breast carcinoma cells in a dose-dependent manner. Lugol's solution was markedly less effective. (B) The proliferation of IPC melanoma, A549 lung and H1299 bronchial carcinoma cells was significantly reduced, dose-dependently, in the presence of PVP-I. Results are presented as the means ± standard deviations; n=4-6; *P<0.05 and **P<0.01 (*t*-test; treated samples vs. controls). PVP-I, povidone-iodine.

Gibco Ham's F12 medium, fetal calf serum (FCS), streptomycin and penicillin were purchased from Thermo Fisher Scientific, Inc. (Carlsbad, CA, USA). I_2, KJ and Lugol's solution were obtained from Merck Millipore, and PVP-I was obtained from Mundipharma International, Ltd. (Cambridge, UK).

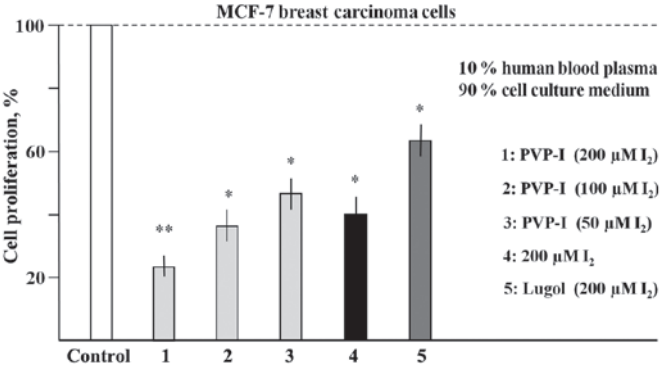

Figure 3. Antiproliferative activity of PVP-I is maintained in human blood. MCF-7 cells were cultured in medium containing 10% human blood plasma. The presence of PVP-I in the blood plasma led to a dose-dependent reduction in cell proliferation ≤70%. I_2 (molecular) was less and Lugol's solution even less effective compared with PVP-I (200μM I_2). Results are presented as the means ± standard deviations; n=4; *P<0.05 and **P<0.01 (*t*-test; treated samples vs. controls). PVP-I, povidone-iodine; I_2, molecular iodine.

Cell culture. The cells were seeded at a low density (200,000 cells per well) and cultured in Ham's F12, completed with 10% FCS and 1% penicillin/streptomycin. After 5 h, the following iodine compounds were added (final concentrations are given separately): i) KJ (100 μM), or I_2 (20 μM), or combined KJ/I_2 (100 μM/20 μM, respectively) from freshly prepared stocks dissolved in sterile water (for KJ) or pure ethanol (for I_2); ii) PVP-I corresponding to 20-80 μM I_2; iii) Lugol's solution corresponding to 20-80 μM I_2 and 60-240 μM KJ. The cells were then cultured for a further 6 days. In separate experiments, fresh sodium-heparin human blood (taken from one of the authors in MCZ Synlab, Leinfelden) was incubated with PVP-I (0.5, 1 or 2 mM I_2) or Lugol's solution (2 mM I_2) or I_2 (2 mM) for 30 min and then centrifuged (1,200 rpm, 12 min). Each supernatant (blood plasma; 200 μl) was diluted with 1,800 ml cell culture medium. MCF-7 cells were cultured with these plasma-containing medium samples for 6 days, as above.

Staining and counting of cells. In order to determine the total cell densities, cell cultures were washed twice with fresh medium in order to remove detached dead cells, stained with Hoechst 33342 (1:5,000 in phosphate-buffered saline) for 10 min, and washed again. From each cell culture well, digital images of 26 different areas of 1.5 mm^2 were captured, and the densities of stained nuclei were determined using ImageJ software (11). The results were calculated as relative values (%) compared to untreated cells (100%).

Statistical analysis. All experiments were performed in duplicate and repeated (n=4-6). Results are presented as the means ± standard deviations. In all cases, statistical significance comparing experimental values with corresponding controls was estimated by means of student t-test (for details see figure legends).

Results

Antiproliferative/cytotoxic effects of I_2, KJ and the combination of the two in different carcinoma cell lines. Various human carcinoma cell lines (PaTu 8902, Capan-2, MCF-7, HS-24 and

A549) were cultured in the presence of 100 μM KJ or 20 μM I_2, or a combination of the two (KJ + I_2) for 6 days (Fig. 1). While KJ had no antiproliferative effect, the presence of I_2 inhibited the cell growth of all cell lines tested. The antiproliferative activity was pronounced in the pancreas carcinoma line Capan-2 and the breast carcinoma line MCF-7 (reduction of cell densities by 55% and 50%, respectively, compared with untreated cells). Fig. 1 also shows that, in all cell lines, the inhibitory effect of 20 μM I_2 was enhanced in the presence of 100 μM KJ.

Antiproliferative/cytotoxic effects of PVP-I and Lugol's solution on MCF-7, IPC, A549 and H1299 cells. MCF-7 breast carcinoma, IPC melanoma, A549 lung carcinoma and H1299 bronchial carcinoma cells were cultured in the presence of the iodine compounds for 6 days and analysed as described. Fig. 2A shows that treatment with PVP-I (20-80 μM I_2) resulted in a significant reduction (by 75-85%) of total MCF-7 cell numbers when compared to untreated controls within 6 days. Similarly, Lugol's solution (20-80 μM I_2 and 60-240 μM KJ) inhibited the growth of MCF-7 cells by 40-60%. Fig. 2B shows comparable antiproliferative effects of PVP-I in IPC melanoma, A549 and H1299 cells (reduction of cell densities by 60-90%). In all experiments shown in Fig. 2, the inhibitory effects of the iodine compounds were clearly dose-dependent and significant.

Effects of iodine-containing (PVP-I, Lugol's solution, I_2) human blood samples on the proliferation of MCF-7 cells. Fresh heparinised human blood was incubated with PVP-I, Lugol's solution or with I_2 for 30 min and centrifuged to obtain blood plasma supernatants. MCF-7 cells were cultured in medium containing 10% of the plasma supernatants for 6 days. In Fig. 3, for each of these different samples a final concentration of I_2 is given, which would be expected if there were no loss of I_2 by blood cell binding or by other components removed by centrifugation.

As shown in Fig. 3, PVP-I treatment resulted in a dose-dependent inhibition of proliferation of MCF-7 cells. At calculated (maximal) final concentrations of 200, 100 and 50 μM I_2, the reductions in proliferation were 71.6, 63.1 and 56.5%, respectively. A calculated (maximal) concentration of 200 μM molecular iodine led to a growth inhibition of 69.5%. Lugol's solution, however, exhibited a markedly less effective inhibition (26.3%) in this type of experiment. These data clearly indicate that I_2 (PVP-I or molecular iodine) in human blood retains its antiproliferative/cytotoxic activity to a significant degree.

Discussion

In accordance with our previous results (11), the present data strongly confirm I_2 as a potent inhibitor of carcinoma growth. KJ was found to have no effect, but at high concentrations enhances the antiproliferative/cytotoxic effect of molecular iodine. Although the mechanisms of action were not analysed here, it is most likely that mitochondrial-mediated apoptosis pathways are involved as previously described for I_2 and iodolactones (6,9-13).

The present results also revealed a highly significant dose-dependent antiproliferative/cytotoxic effect of PVP-I in cell cultures of MCF-7 breast carcinoma, IPC melanoma, A549 lung carcinoma and H1299 bronchial carcinoma cells. At a concentration corresponding to 20 μM I_2, within 6 days the growth of MCF-7, IPC melanoma, A549 and H1299 cells was reduced by 76, 57, 59 and 77%, respectively. This is consistent with previous data demonstrating cytotoxic activity of PVP-I on CT26 colon cancer and H-22 ascites cells *in vitro* and *in vivo* (14). In the present study, Lugol's solution was also revealed to reduce the growth of MCF-7 cells (40% at a concentration corresponding to 20 μM I_2 and 60 μM KJ).

However, with respect to a therapeutic application, further knowledge concerning the resorption, systemic distribution, organ-specific uptake and metabolism of iodine compounds is necessary. A number of reports have indicated that I_2 exerts antineoplastic effects in thyroid, mammary and prostate glands, all of which were shown to take up I_2, whereas iodide supplementation had no effect (2,15,16). This is in line with the observation that I_2 treatment of patients with benign breast disease led to a bilateral reduction in breast size and a remission of disease symptoms, which was not observed when iodide was administered (17). Other authors have proposed that iodine, if ingested as I_2 at concentrations higher than 3 mg per day, acts as an antioxidant and prevents lipoperoxidation in various organs, including the brain (18). In a recent study, Delgado *et al* (19) supplemented Sprague-Dawley rats with I_2 and KJ in drinking water. They found that iodine ingestion and intestinal uptake was similar for both forms of iodine compounds. Following systemic distribution, iodide was preferentially taken up and retained by the thyroid, lactating mammary gland and breast milk, whereas pituitary, ovary and virgin mammary gland were found to take up both iodine compounds, but preferentially I_2. Taken together, it appears that I_2 is taken up, distributed systemically and preserved in blood for tissue absorption. This is in agreement with the data obtained in the present study with fresh human blood, indicating that the antiproliferative activity of PVP-I, I_2 and also, to a certain degree, Lugol's solution, is preserved in fresh human blood.

As PVP-I is already widely used in clinical practice as an antiseptic and flushing agent following tumour surgery, and PVP has even been used intravenously as a plasma expander in emergency medicine, the present data strongly suggest that PVP-I and possibly also Lugol's solution or I_2 may be potent agents for the development of potential antitumour strategies in humans, either by systemic or local stereotactic application.

Acknowledgements

The authors would like to thank Dr Heiko van der Kuip, (Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany) for providing human carcinoma cell lines, and Dr John M. Lindquist (Surgeon and General Practitioner and native speaker) for language correction and proofreading.

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