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Research Article

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Synergistic Effect of the Combination of Polyphenols with Gemcitabineon Pancreatic Cancer Cell line AsPC-1

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Abstract

In our diet, polyphenols are micronutrients with an important role in the prevention of degenerative diseases such as cancer and cardiovascular diseases. Particularly, Pancreatic cancer is one of the most aggressive cancers, with only about 5% of patients surviving 5 years past the initial diagnosis. Despite advances with current chemotherapy combinations, overall survival outcomes are still require novel therapeutic approaches. Here, we examined the efficacy of combined treatments of polyphenols and gemcitabine the standard of treatment for patients with metastatic pancreatic cancer in human pancreatic cancer cells. For that purpose, the pro-apoptic effects of gemcitabine were studied on the human pancreatic cell line AsPC-1 in presence or absence of several polyphenols, in order to evaluate if they latter are able to potentialize gemcitabine cytotoxicity. Our study aims to investigate the implication of MDR1 (multidrug transporter)in resistance to gemcitabine and if the studied polyphenol could target this drug efflux pump in AsPC-1 cells by flow cytometric analysis. We observed that 5µg/ml gemcitabine in combination with 15 µg/ml of selected polyphenol (Catechin, Quercetin, Bergamottin, Rhamnetin) was more effective than gemcitabine alone, by increased in the percentage of dead cells up to 60%. Morever our results demonstrated that some polyphenols (Quercetin) inhibit the efflux activity of MDR1. Our study in vitro suggests therefore that chemotherapy with gemcitabine might be significantly increased upon combination with specific polyphenol. In conclusion, polyphenols may be promising agents for novel combination therapy since they potentialize the cytotoxic activity of gemcitabine to eradicate pancreatic cancer and therefore the cellular resistance.

Keywords: Pancreatic cancer, Gemcitabine, Polyphenols and Combination treatment.

Introduction

Polyphenols are natural compounds found in food such as fruits; vegetable and red win with antioxidant activity [1]. Polyphenols can help in the preventing and reducing the progression of several diseases including cardiovascular diseases, cancer and diabetes [2]. It's also known that polyphenols contribute to the body being in an anti-inflammatory state, associated with a lower risk of some chronic diseases [3]. In contrast, Many studies with polyphenols, such as flavonoids from fruits and vegetables, have shown that they are efficient a chemopreventative agents since they are able to promote apoptosis in a variety of cancer cells [4]. In addition, grapefruit juice contained dihydroxybergamotin and other furanocoumarins, which are know to inhibit the drug efflux transporters, such as MDR1 and increased the uptake of vinblastine by Caco-2 cells [5]. To enhance intracellular anticancer drug accumulation by impairing the MDR1 efflux function, the process of chemosensetization involves usually a co-administration of a MDR1 inhibitor with an anticancer [6]. Numerous compounds have been shown to inhibit the drug efflux function of MDR1 and therefore, increase the intracellular concentration of cytotoxic anti cancer agents and consequently decrease in the cellular resistance [7]. More specifically, pancreatic cancer is the eighth major form of cancer-related death worldwide, causing 227 000 deaths annually [8]. Gemcitabineis the most commonly used chemotherapeutic agent over the past decade. Current treatment modalities for advanced pancreatic cancer include gemcitabine as a single agent or in combination with multiple chemotherapeutic agents [9,10]. However, pancreatic cancer shows an important resistance against gemcitabine, whenthe major cause of chemotherapy failure with gemcitabine is the drug resistance to multiple chemotherapeutic agents [11]. Overexpression of MDR1 has been shown to induce resistance to various anticancer drugs [12]. MDR1 acts as an energy-dependent drug efflux pump, thereby decreasing the intracellular drug concentration and causing drug resistance.For example, colorectal cancer express high levels of MDR1, and this expression may contribute to the general resistance of colorectal cancer to anticancer drugs [13]. The very limited use

of chemotherapy for pancreatic cancer patients is associated with the inherent chemoresistant nature of this aggressive disease. However, MDR1 the ATP-dependentmembrane-bound drug efflux pumps,is mediators of clinically relevant chemoresistance [15]. To enhance the anticancer therapeutic efficacy and reduce the side effects, natural productswere combined with standard chemotherapy and radiotherapy [15]. In this context, the use of natural products, as a supplementary approach, to treat pancreatic cancer holds a great promise with minimal side effects [16].

The aim of our study is to establish whether polyphenolscould have the ability to induce apoptosis and enhance the chemotherapeutic effect of gemcitabine in vitro in an established human pancreatic cancer cell line (AsPC-1) without causing damage on the normal cells.

Material sand Methods

Chemicals and Drugs

Rhodamine 123 (RH 123) was purchased from Invitrogen. Verapamil, polyphenols and gemcitabine were purchased from Sigma-Aldrich. Final concentration of DMSO applied to cells during incubation with tested drugs was 0.5%. In the tested setup these concentrations had no adverse effects on cell viability or cell morphologyor on rhodamine-123 efflux.

Cell culture and maintenance

The human pancreatic adenocarcinoma cell line AsPC-1 cells (CRL-1682) purchased from ATCC (LGC Standards, Molsheim, France) were cultivated in the physiological nutrient-rich DMEM-based media (Sigma-Aldrich, Saint-Quentin Fallavier, France) supplemented with 10% (v/v) foetal bovine serum (Lonza, Verviers, Belgium), 2 mm glutamine, P/S (100 unit/ml and 100 lg/ml) (Sigma-Aldrich). Cells were grown in petri dishes to 70-80% confluency prior to treatment. All plates were incubated in a humidified incubator at 37°C and 5% CO_2.Then cells were incubated with gemcitabine and polyphenols for 24 hours.

Detection of apoptosis by annexin-FITC

Pancreatic cancer cells AsPC-1 cells death was assessed using AnnexinV-FITC Kit (MiltenyiBiotec) according to manufacturer's protocol. Briefly, Aspc1 cells were incubated with the gemcitabine, polyphenols and their combination for 24 hours. Cells were then washed with phosphate buffered saline (PBS) and stained with AnnexinV-FITC and PI following the manufacturer's protocol. The fluorescence intensity of AnnexinV-FITC stained cells at 530/540 nm and PI stained cells at 675/630 were analysed by Guava EasyCyte Plus capillary flow cytometer (Merck Millipore, Life Science division, Merck KgaA, Darm- stadt, Germany) and computed using the Guava ExpressPro software (Merck/Millipore/Guava Tech). The apoptotic potential of the tested drugs was compared to the apoptotic potential of celastrol, which is known to be a positif control of apoptosis.

MDR-1 function assay

MDR1-mediated efflux of rhodamine 123 was monitored on a Guava EasyCyte Plus capillary flow cytometer equipped with a 488 nm excitation laser. The accumulated intracellular fluorescence intensity of rhodamine 123 at 530/540 nm was computed on the Guava ExpressPro software (Merck/Millipore/Guava Tech) in terms of x-geometric mean arbitrary units. Dead cells were excluded based on propidium iodide staining. The inhibitory potential of tested compounds on rhodamine-123 efflux was expressed relative to maximum inhibition obtained with 100 µM verapamil in the same experiment. The experiments were repeated three times.

Statistical Analysis

Data are expressed as mean +/- standard error of mean (S.E.M.) and analysed using Graph Pad Prism5" (La Jolla, CA, USA). Statistical analysis was performed with either the one-way ANOVA test followed by Student's t-test. A P value <0.05 was considered significant. Experiments were performed at least in three separate experiments.

Results

Apoptotic potential ofpolyphenols and gemcitabine

AsPC-1 cells treated for 24 hours with polyphenols do not exhibit a significant apoptotic potential in the range tested (15 µg /ml) **(figure 1)** when compared to the negative control, except Gallic acid and Rhamnetin. In contrast, AsPC-1 cells treatedfor 24 hours with gemcitabine do not exhibit a significant apoptotic potential in the range tested (0.05-25 µg /ml) **(figure 2)** when compared to the negative control.Based on the above results and literature, we choose 5 µg /ml of gemcitabine for subsequent experiments to test the effects of gemcitabine in combination with polyphenols. In the range tested, the combination of gemcitabine with polyphenolsexhibited more potent apoptotic effects than when they are administered alone **(figure 3)**. The results demonstrated therefore that, some polyphenol, like Catechin, Quercetin, Bergamottin and Rhamnetin synergizeswith gemcitabine to promote cellular apoptosis. The apoptotic potential of the investigated drugs is elucidated by an increase in the fluorescence of AnnexinV-FITC/PI stained cells.

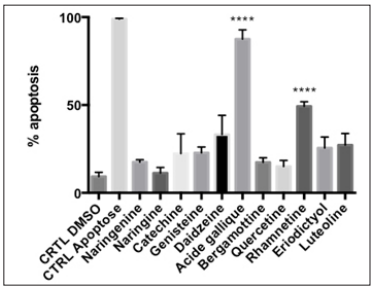

Figure 1: Apoptotic effect of polyphenols on Aspc1 cells. After incubating Aspc-1 cells 24h with polyphenols, cells death was assessed following the simultaneous staining of cells with AnnexinV-FITC and PI by capillary flow cytometry. Data are represented as Mean ± S.E.M (n=3). * Represents P < 0.5 and refers to the variation in apoptotic potential between non-treated and treated AsPC-1 cells with gemcitabine.

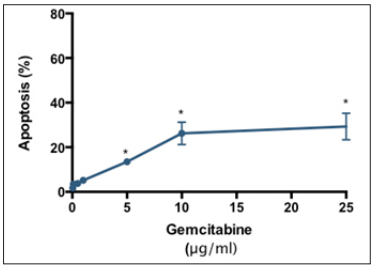

Figure 2: Apoptotic effect of gemcitabine on Aspc1 cells. After incubating Aspc1 cells 24h with gemcitabine, cells death was assessed following the simultaneous staining of cells with AnnexinV-FITC and PI by capillary flow cytometry. Figure 2 recapitulates in a dose response curves from the percentage of cells undergoing apoptosis after different treatments. Data are represented as Mean ± S.E.M (n=3). * Represents P < 0.5 and refers to the variation in apoptotic potential between non-treated and treated AsPC-1 cells with gemcitabine

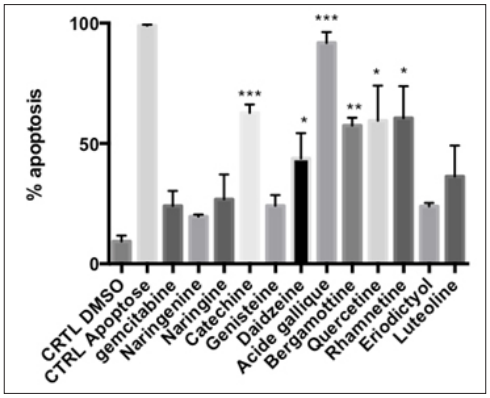

Figure 3: Apoptotic effect of polyphenols combined with gemcitabine on Aspc-1 cells. After incubating Aspc1 cells 24h with gemcitabine, cells death was assessed following the simultaneous staining of cells with AnnexinV-FITC and PI by capillary flow cytometry. Data are represented as Mean ± S.E.M (n=3). * Represents $P < 0.5$ and refers to the variation in apoptotic potential between non-treated and treated AsPC-1 cells with gemcitabine.

Quercetin effect on MDR1 Activity

Based on above results, we choose 15 µg /ml of Quercetin and 5 µg /ml of gemcitabine to evaluate their impacton MDR1. The study of the effect of Quercetin, gemcitabineor their combination on the efflux activity of MDR1 showed that in the presence of Quercetinas an inhibitory potential on the efflux activity of MDR1 when compared to the specific MDR1 inhibitor verapamil. For instant the inhibition is concluded through an increase in the intracellular fluorescence of rhodamine 123-loaded cells. Their inhibitory potential on the efflux activity of MDR1 as demonstrated by the increase in fluorescence of rhodamine 123-loaded cells (figure 4). It should be noted that even verapamil, which is considered as the standard MDR1 inhibitor.

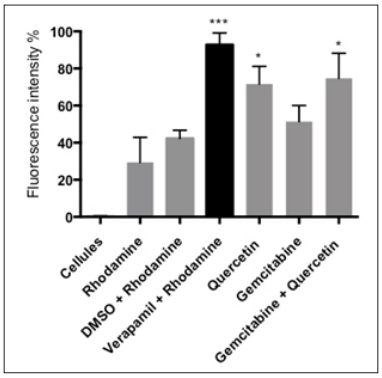

Figure 4: Effect of quercetin on the MDR1-mediated efflux of rhodamine 123. AsPC-1 cells were incubated for 24 h, in the presence of quercettin, gemcitabine or in combination. Bars represent the geometric mean values of % fluorescence intensity SD. The experiments were repeated three times. *** Represents $P < 0.001$.

Discussion

Polyphenols have an effect on human cancer cell lines, such as reduction of the number of tumors or of their growth. For example, may studies have been shown that quercetin possess anticancer property against lung carcinogenesis in mice.Specially, pancreatic cancer, due to its aggressive nature is one of the most challenging solid organs [17]. In a number of cancer types, the drug efflux pumps have been associated with chemoresistance (multiple drug resistance/MDR) [18]. However, their presence with resistance in pancreatic cancer remains to be elucidated. Gemcitabine, which has been the frontline chemotherapeutic agent against pancreatic cancer, has offered some relief over the past two decades [19]. But frequently, gemcitabine failed in the overall survival benefit [20]. In addition, recent studies indicated that natural products could provide additional strategies for monotherapy or combination treatments in pancreatic cancer due to their efficacy and low toxicity [15]. Here, we examined the efficacy of combined treatments of gemcitabine and polyphenols in human pancreatic cancer cells. Many studies demonstrated the inhibitory action of flavonoids, but also furanocoumarins of drug rejection, including MDR1. The importance of this protein inhibition is to increase the sensibilisation of cancer cells to anti-cancer drugs and the enhancing of chemosensetization process [21]. To establish whether comparable toxicity occurs in pancreatic cancer, AsPC-1 was used and results compared with those for gemcitabine and combinationwith polyphenols, to evaluate any supra-additive effect. BJ cell line was also tested, as a no transformed cell type which is similar to host components in tumours that are important for progression. BJ cells were unaffected when treated with polyphenols up to the highest concentration used 15µg /ml. These cells are not transformed, yet similar phenotypes would be represented in in vivo cancers, and tumour vasculature has proved a popular therapeutic target. No normal pancreatic cells wereavailable; such cells are not indexed in most catalogues. MDR1 inhibiting agents are pharmacologically active in vitro in concentration range from 1 to 15µg /ml [21]. A range of 15µg /ml of polyphenolswere selected for AsPC-1 cells in this study, at minimally cytotoxic doses of the micronutrient, the combination with gemcitabine showed a strong supra-additive effects at lower doses of the anti-cancer drug. Many studies indicate that the expression of drug efflux pumps MDR1 is common in pancreatic tumours and so potentially could contribute, at least in part, to the chemoresistant properties of this cancer [18]. MDR1 blockade might have a very important role in the intracellular accumulation and the cellular pharmacokinetics of many anticancer drugs. Ourresults showed that Quercetin possesses a potent inhibitory potential on MDR1 mediated efflux of rhodamine 123 when compared to verapamil and then increasing in the intracellular accumulation of gemcitabine, in the pancreatic cancer cells used, loading to more apoptosis when gemcitabine were combined with Quercetin.

In conclusion, our present data indicate that the combined treatment of polyphenols and gemcitabine induces apoptosis in the established pancreatic cell line used indicating that the effects seen are potentialize and it is strongly recommended to study and find a natural and non-toxic MDR1 blockers to potentialize the efficacyof anticancer drugs.

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