Metformin synergistically enhances antiproliferative effects of cisplatin and etoposide in NCI-H460 human lung cancer cells*

Abstract

Objective: To test the effectiveness of combining conventional antineoplastic drugs (cisplatin and etoposide) with metformin in the treatment of non-small cell lung cancer in the NCI-H460 cell line, in order to develop new therapeutic options with high efficacy and low toxicity. Methods: We used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and calculated the combination index for the drugs studied. Results: We found that the use of metformin as monotherapy reduced the metabolic viability of the cell line studied. Combining metformin with cisplatin or etoposide produced a synergistic effect and was more effective than was the use of cisplatin or etoposide as monotherapy. Conclusions: Metformin, due to its independent effects on liver kinase B1, had antiproliferative effects on the NCI-H460 cell line. When metformin was combined with cisplatin or etoposide, the cell death rate was even higher.

Keywords: Carcinoma, non-small-cell lung; Drug therapy, combination; Metformin.

Introduction

Lung cancer (LC) is the most common and most deadly cancer worldwide. In Brazil, LC is one of the five most common cancer types, according to estimates for 2012, and it is the cancer type that took the most lives in 2010. With this data, it is evident that LC is a health challenge in Brazil and worldwide. It is known that 85% of all cases of LC are classified as non-small cell LC (NSCLC), a group composed of cancer types that are different in histology, such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, but that have similar clinical and pathological characteristics.

Chemotherapy is used in patients staged as III or IV, in order to extend survival, control the disease, and improve quality of life. In general, platinum derivatives, such as cisplatin and carboplatin, fulfill this role when combined...
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Initially, the NSCLC cell line NCI-H460, which is representative of the histologic group of large cell carcinoma, was grown to subconfluence at 37°C and 5% CO₂ in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) bovine fetal serum (Gibco/Invitrogen, Grand Island, NY, USA) and a stabilized solution of penicillin (100 IU/mL), streptomycin (100 µg/mL), and 0.5% (w/v) amphotericin B (Gibco/Invitrogen). Provided through donations from collaborators, cisplatin (Incel, 1 mg/mL injectable solution; Laboratório Darrow, Areal, Brazil), etoposide (Posidon, 20 mg/mL injectable solution; Piére, Buenos Aires, Argentina), and metformin hydrochloride (Pharma Nostra, Rio de Janeiro, Brazil) were diluted in 1× PBS.

In the in vitro cell metabolic viability test, cells were grown at a density of 7.5 x 10⁴ cells/well in 96-well plates, and, after 24 h, they were treated with each drug, in a dose-dependent manner, for 24 h. Subsequently, the medium was removed, 15 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) were added to 5 mg/mL in each well, and the plates were incubated for 4 h. Finally, 100 µL of analytical grade DMSO (Vetec, Rio de Janeiro, Brazil) were added in order to dissolve the formazan crystals. Therefore, the production of formazan by mitochondrial succinate dehydrogenase activity reflects the proportion of live cells undergoing oxidative stress caused by the drugs under investigation. In this experiment, the absorbance values obtained from the wells of untreated cells, i.e., cells exposed only to RPMI 1640 medium, represent 100% cell viability. In vitro cytotoxicity was determined by the estimate calculation of the values for inhibitory concentration of 50% for cell proliferation (IC₅₀) on the basis of the dose-response curve for each drug. After calculation of metabolic viability, evaluated with the MTT assay (absorbance at 570 nm), by using a spectrophotometer. It is of note that the experiments to obtain the dose-response curves for each drug as monotherapy and estimate the IC₅₀ values were performed in quadruplicate in three independent experiments, whereas the experiments to determine the combination index were performed in triplicate in three independent experiments. In addition, drug interaction was assessed by analysis of the combination index, the value of which was calculated by the classical isobologram equation described by Chou & Talalay, with the use of the CompuSyn software program, version 1.0 (CompuSyn, Paragon, NJ, USA). In this context, combination index values greater than 1.1 indicate antagonistic interactions, values between 0.9 and 1.1 indicate additive interactions, and values lower than 0.9 indicate synergistic interactions. Regarding the statistical analysis, the mean and standard deviation of the absorbances were calculated on the basis of the results of the MTT assay, whereas the estimated IC₅₀ values were calculated by the Prism software program, version 5 (GraphPad Inc., San Diego, CA, USA). All data on combination therapy are expressed as mean ± SD and were analyzed by one-way ANOVA followed by the Bonferroni test, with the use of the same software program.

Results

Initially, we performed the cell viability experiments to determine the IC₅₀ concentrations for metformin, cisplatin, and etoposide in the large cell carcinoma cell line NCI-H460, which are shown in Table 1.

Subsequently, once these data were obtained, each drug was diluted to achieve concentrations in the IC₅₀ range, as well as below and above this range, and in vitro experiments combining metformin with cisplatin and combining metformin with etoposide were performed to allow the
Discussion

Various epidemiological studies have reported the effects of metformin on the prevention and treatment of cancer.\(^{12-15}\) There have also been studies reporting that metformin can improve treatment and thus survival in patients with NSCLC and diabetes who undergo chemotherapy.\(^{16}\)

Because the safety and use of metformin has been well established since it first underwent a clinical trial in 1957, its use as a potential anticancer agent is certainly an interesting strategy.\(^{17}\)

Although in the present study the cytotoxic activity of metformin was found to be up to 200 times lower than that of etoposide or cisplatin, as determined by the IC\(_{50}\) values, the use of metformin as monotherapy reduced the metabolic viability of large cell carcinoma cells. However, in polytherapy regimens, metformin (60.58 and 30.29 mM) combined with cisplatin at 0.19 mM produced a synergistic effect and was more effective than was metformin combined with half the IC\(_{50}\) value for cisplatin (0.09 mM). Combining metformin (30.39 mM) with etoposide (0.18 mM) produced a synergistic effect and was more effective in reducing the metabolic viability of the cell line tested than was the use of etoposide (0.18 and 0.09 mM) or metformin (30.29 and 15.14 mM) as monotherapy. Furthermore, combining metformin with etoposide (30.39 mM and 0.18 mM, respectively, and 15.14 mM and 0.09 mM, respectively) produced a synergistic effect.

| Table 1 – Inhibitory concentration of viability at 50% (IC\(_{50}\)) values in the NCI-H460 cell line with the drugs tested. |
|-----------------|------------------|
| Drug            | IC\(_{50}\), mM   |
| Cisplatin       | 0.19             |
| Etoposide       | 0.37             |
| Metformin       | 60.58            |

Figure 1 – Evaluation of the effects of metformin and cisplatin (used as monotherapy or in combination therapy, at different concentrations) on the reduction in metabolic cell viability (MCV) in the NCI-H460 cell line.
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Cell carcinoma cell line used in the present study (NCI-H460) has a nonsense mutation in LKB1, which results in lack expression of its protein product. It is therefore possible to attest that the mechanisms of action of metformin in these cells are independent of LKB1 activity. Shackelford et al. showed that phenformin, a member of the biguanide class of drugs, can be used as an anticancer agent in LKB1-deficient tumors because it induces apoptosis possibly in response to metabolic stress. It has thus been suggested that a possible mechanism of action of metformin and other biguanides in LKB1-deficient tumor cells is mitochondrial complex I inhibition, which induces the production of reactive oxygen species and consequent apoptosis, because these species cannot be effectively neutralized.

Another plausible reason for the antiproliferative and synergistic effects of metformin is its preferential activity against tumor stem cells, a group of cells within the tumor mass that can be related to tumor formation, maintenance of the tumor mass, recurrence, and metastasis. Because of these characteristics, drugs that selectively target these cells are extremely promising, since, in combination with conventional chemotherapy, they could make treatment more effective and prevent disease recurrence.

Allelic loss at LKB1 occurs in various types of cancer—such as NSCLCs—especially in lung adenocarcinoma. In addition, it is believed that most mutations in LKB1 in NSCLC lead to loss of function of the encoded protein. The large cell carcinoma cell line used in the present study (NCI-H460) has a nonsense mutation in LKB1, which results in lack expression of its protein product. It is therefore possible to attest that the mechanisms of action of metformin in these cells are independent of LKB1 activity. Shackelford et al. showed that phenformin, a member of the biguanide class of drugs, can be used as an anticancer agent in LKB1-deficient tumors because it induces apoptosis possibly in response to metabolic stress. It has thus been suggested that a possible mechanism of action of metformin and other biguanides in LKB1-deficient tumor cells is mitochondrial complex I inhibition, which induces the production of reactive oxygen species and consequent apoptosis, because these species cannot be effectively neutralized.

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In summary, the present study has shown for the first time that metformin has antiproliferative effects in a large cell carcinoma cell line (NCI-H460) and that, when combined with cisplatin or etoposide, it acts synergistically, increasing the cell death rate. However, larger studies are still needed to elucidate further the mechanisms involved.

References


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