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Original article

Anticancer effects of vecuronium bromide and cisatracurium besylate on lung cancer cells (A549), *in vitro*

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ABSTRACT

Neuromuscular blocking drugs are used in anesthesia and intensive care to provide skeletal muscle relaxation. However, literature has revealed that the effects of these drugs on cancer have not been widely studied. We tested the effects of two non-depolarising neuromuscular blocking drugs, vecuronium bromide and cisatracurium besylate on lung cancer cells' (A549) proliferation, migration and viability in the presence of a cytotoxic drug, cisplatin. We demonstrated in this study that both neuromuscular blocking agents inhibit lung cancer cells' proliferation. In addition, the study revealed that vecuronium bromide and cisatracurium besylate was synergistic to cisplatin in reducing lung cancer cell proliferation. However, vecuronium inhibits lung cancer cells migration *in vitro*.

1. Introduction

Anesthesia is one of the fastest developing medical specialties. However, it is observed that various anesthetics are usually used both in patients with comorbidity of cancer and in patients for cancer resection without recourse to their effects on cancer metastatic properties and mutagenic potentials [1,2]. Neuromuscular blockade is an important component required by many anesthetists for various surgical procedures. It is often achieved by administration of neuromuscular blocking agents. Even though they do not provide anesthesia or analgesia, they abolish spontaneous respiration and enhance controlled ventilation during surgery and in intensive care. Their effects on cancer have been poorly investigated [3,4]. Neuromuscular blocking agents consist of two main types, namely the depolarizing and the non-depolarising neuromuscular blocking agents. There are many non-depolarizing neuromuscular blocking drugs which are currently used in clinical practice, however, cisatracurium besylate and vecuronium bromide are usually preferred because of their intermediary duration of action. Vecuronium bromide and cisatracurium besylate act by competitive inhibition of acetylcholine at nicotinic receptors at the postsynaptic end in the neuromuscular junction, resulting in block of neuromuscular transmission. A comprehensive literature search showed that the carcinogenic and genotoxic properties of vecuronium bromide have not been widely investigated except for data on the clinical efficacy, adverse effects, and its effect on the cellular expression of lactate dehydrogenase [5] and creatine phosphokinase [6]. Cisatracurium, a bisbenzylisoquinolinium neuromuscular blocking agent undergoes spontaneous degradation called Hofmann elimination, at physiologic pH and temperature [7,8]. This metabolic process leads to formation of two major compounds termed laudanosine [9] and monoquatenary acrylate esters [10,11]. Due to its unique metabolism, it is usually recommended in patients with renal and hepatic diseases [12] and in respiratory embarrassment demanding muscle relaxation [13]. Nigrovic earlier opined that at supra-therapeutic doses, atracurium could be injurious in isolated rat hepatocytes [14]. This finding was supported by proliferation setback induced by cisatracurium besylate and atracurium in human hepatoma (HepG2) and umbilical vein endothelial (HUVEC) cells [4]. Similarly, survival and axonal growth inhibition of neonatal and adult rat peripheral neurons

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caused by cisatracurium has been demonstrated [15]. These observations suggest that cisatracurium besylate and vecuronium bromide could be cancer suppressors in many tissues and organs in humans. Therefore, the aim of this study was to assess the effects of these non-depolarising neuromuscular blocking agents on metastatic properties of lung cancer (A549) cells *in vitro*.

2. Materials and methods

2.1. Cell culture

Lung cancer cell line (A549) was obtained from Shanghai Institute Cell Biology (Chinese Academy of Sciences, Shanghai China). Cells were cultured in RPMI 1640 (Sigma, St. Louis, USA) with high glucose containing penicillin 100 units/mL, streptomycin 100 μg/mL and 10% FBS (PAA) at 37 °C in a humidified 5% CO_2. Vecuronium bromide (Norcuron from Bedford Labs, USA) was obtained in a clinical formulation of 1 mg/mL containing 2.1 mg/mL anhydrous citric acid, 1.6 mg/mL sodium phosphate, and 9.7 mg/mL Manitol. Cisatracurium besylate (Nimbex from GlaxoSmith-Kline) was obtained in a clinical formulation of 2 mg/mL in a 35% benzene sulfonic acid solution. Dilutions were prepared in distilled water.

2.2. Cell proliferation assay

The effect of vecuronium bromide and cisatracurium besylate on cell proliferation was measured using Dojindo's CCK-8 Cell Proliferation Kit (Dojindo's Molecular Technologies, Japan).

In brief, duplicates of 3×10^3 cells/well in 96-well plates were seeded. We added varying concentrations of vecuronium bromide (0.0, 1.0, 2.0, 3.0, 5.0, 10.0 and 15.0 μM) and cisatracurium besylate (0.0, 1.0, 2.0, 3.0, 5.0, 10.0 and 15.0 μM) to the culture medium and incubated for 72 h at 37 °C in 5% CO_2. In order to measure cell proliferation, 10 μL of CCK-8 reagent was added to the culture medium and the absorbance measured one hour after addition of the reagent at 450 nm using Multiskan Go Spectrophotometer (Thermofisher Scientific, USA).

2.3. Cytotoxicity assay

Three thousand cells per well were seeded into a 96-well plate. In order to measure, cytotoxic influence of cisplatin on A549 cells in the presence of vecuronium bromide or cisatracurium besylate, two set wells were set up as control against test wells containing varying concentrations of vecuronium bromide and cisatracurium besylate. The first control wells were without drug exposure, whilst the second control wells contained only cisplatin (35 μM). The rest of the test wells contained cells treated with either vecuronium bromide with cisplatin (35 μM) or cisatracurium besylate with cisplatin (35 μM). The cytotoxic influence of cisplatin (35 μM) in the presence of vecuronium bromide or cisatracurium besylate on lung cancer cells (A549) was measured with Dojindo's CCK-8 Cell Proliferation Kit in the same manner as in cell proliferation above.

2.4. Cell migration assay

Cells were pretreated with vecuronium bromide or cisatracurium besylate at the various concentrations and grown to confluence in regular RPMI 1640 medium under optimal conditions. After 72 hours of incubation, cells were trypsinised and detached with a serum-free medium. In order to measure cell migration, 3×10^4 of A549 cells were seeded in 200 μL of serum-free medium in the upper chamber of the transwell inserts in the 24-well plate. The lower chamber of the transwell inserts was filled with 750 μL medium containing 20% FBS to serve as chemo-attractant. After 16 hours of incubation in humidified incubator with 5% CO_2, the non-migrated cells in the upper chamber were swabbed off and the plates fixed with methanol and then stained with crystal violet. The plates were observed under inverted fluorescent microscope. The number of cells were counted randomly in five field views and averaged.

2.5. Data analysis

After establishing that the absorbance was normally distributed. One-way analysis of variance with Bonferroni's correction was used for the statistical analysis. The mean absorbance of cells in the control wells was compared with that of the test wells, which were stimulated with muscle relaxants only or with muscle relaxant and cisplatin combined. Data are presented as mean ± SD for each group. The difference in mean absorbance between the control wells and the test wells was considered significant at P-value < 0.05.

3. Results

3.1. Cisatracurium besylate and vecuronium bromide inhibit A549 cells proliferation

Both cisatracurium besylate and vecuronium bromide inhibited A549 lung cancer cells proliferation in a concentration-dependent manner. The inhibition was observed in all test wells at the various concentrations compared to the control wells. For cisatracurium besylate, significant inhibition of cell proliferation occurred at concentrations of 3.0 μM to 15 μM ($P < 0.05$) whereas in the case of vecuronium bromide, significant inhibition occurred at concentrations of 5.0 μM to 15 μM ($P < 0.05$) (Fig. 1).

3.2. The combine effect of cisplatin and neuromuscular blocking agents on A549 cells

Fig. 2a and b show the cell viability of the control wells in comparison to the test wells. From Fig. 2a and b, cell viability was higher in the control wells compared with the test wells. In comparison with the control, cisplatin alone and cisplatin in combination with cisatracurium besylate significantly reduced A549 cells viability by 45% and 81% or more, respectively ($P < 0.05$). Cell viability caused by the drug combination of cisplatin with cisatracurium besylate at concentrations of 1 μM to 5 μM was fairly constant but at 10 μM and 15 μM, the viability was further reduced (Fig. 2a). Fig. 2b shows that cisplatin alone and cisplatin in combination with vecuronium bromide also caused significantly reduced cell viability by 46% and 75% or more, respectively ($P < 0.05$). Similarly, cell viability caused by the drug combination of cisplatin with vecuronium bromide at concentrations of 1 μM to 5 μM was fairly constant but at 10 μM and 15 μM, the viability was reduced (Fig. 2b).

3.3. Vecuronium bromide inhibits A549 cells migration through the transwell membrane

Fig. 3a is a photographic appearance of cells observed under inverted fluorescent microscope after migration of cells. The Fig. 3 shows that there were more cells migrating through the transwell membrane in the control compared to those of the test wells. The numbers of cells migrating through the membrane of the test wells further decrease with increasing concentration of the drug. When cells were treated with vecuronium bromide of concentration 2 μM or more, cells migration across the transwell membrane was significantly reduced (Fig. 3b) ($P < 0.05$).