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Pharmaceutical equivalence of oncology products marketed in Brazil

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ABSTRACT

The aim of this work was to assess pharmaceutical equivalence among medicinal products containing cisplatin, doxorubicin hydrochloride and paclitaxel that are marketed in Brazil by various manufacturers. We analyzed 14 lots of cisplatin injectable solution from 4 manufacturers (Labs B, C, H and I), 15 lots of doxorubicin hydrochloride injectable lyophilized powder from 5 manufacturers (Labs C, F, G, H and J) and 38 lots of paclitaxel injectable solution from 8 manufacturers (Labs A, B, C, D, E, F, G and H). All products complied with the criteria established in the Brazilian and American pharmacopoeias. The assay results for contents of cisplatin, doxorubicin hydrochloride and paclitaxel were 94.3-105.9%, 97.1-106.6% and 90.2-109.4%, respectively. Statistical analysis showed that the same products from the various manufacturers were equivalent.

Keywords: Therapeutic Equivalence. Medical oncology. Cisplatin. Doxorubicin. Paclitaxel

INTRODUCTION

The use of antineoplastic medicines has been growing worldwide, owing to the increased exposure to risk factors for cancer, such as environmental pollution, pesticides, cigarette smoke and industrial food and medicines (DeVita et al, 2005). Among the most commonly used antineoplastic agents are the platinum compounds (e.g. cisplatin, carboplatin, oxaliplatin), anthracycline antibiotics (e.g. doxorubicin, epirubicin, daunorubicin, idarubicin) and taxane agents (e.g. paclitaxel, docetaxel).

Cisplatin is a genotoxic agent widely employed in the treatment of testis, ovary and esophagus cancers and carcinomas of bladder, breast, head, neck and lung (Kartalou & Essigmann, 2001; Chaney et al, 2004; Brabec & Karsparkova, 2005; Wang & Lippard, 2005). The platinum compounds bind to DNA, blocking its duplication and transcription and inducing apoptosis or necrosis in tumor cells (Wang & Lippard, 2005; Mandic et al, 2003; Fuertes et al, 2003).

Doxorubicin hydrochloride is a mutagenic and carcinogenic agent belonging to the anthracycline antibiotic group, employed in the treatment of acute leukemia, malignant lymphomas, breast cancer, several types of sarcoma and metastatic thyroid carcinoma (DeVita et al, 2005). It binds to DNA by intercalation of the anthracycline planar ring, interfering in the synthesis of DNA and RNA (Keizer et al, 1990).

Paclitaxel is a microtubule-stabilizing agent (a taxane) extracted from fungus-infected *Taxus brevifolia* bark (Wani et al, 1971), which shows antineoplastic activity against epithelial ovary carcinoma, breast, colon, lung and head cancer, as well as Kaposi's sarcoma, associated which AIDS (Rowinsky & Donehower, 1995). It promotes the polymerization of tubulin, blocking the cell cycle in its last phase, thus preventing cell division and the consequent proliferation of neoplastic cells (Horwitz, 1992; Horwitz et al, 1993).

These antineoplastic medicines are injectable solutions (or powder for injectable solutions) and their bioequivalence is not required. In this context, verification of pharmaceutical equivalence is an important step in confirming similarity and interchangeability of those products with reference drugs (Brasil, 2010; Anvisa, 2010a; Lourenço & Pinto, 2012). The aim of this study was to assess pharmaceutical equivalence among medicinal products containing cisplatin, doxorubicin hydrochloride and paclitaxel that are marketed in Brazil by various manufacturers.

METHODS

Materials

We analyzed 14 lots of cisplatin in 10 mg and 50 mg injectable solutions from 4 manufacturers (Labs B, C, H and I), 15 lots of doxorubicin hydrochloride in 10 mg and 50 mg injectable lyophilized powders from 5 manufacturers (Labs C, F, G, H and J) and 38 lots of paclitaxel in 30 mg, 100 mg, 150 mg and 300 mg injectable solutions from 8 manufacturers (Labs A, B, C, D, E, F, G and H). Reference products of cisplatin, doxorubicin hydrochloride and paclitaxel were acquired from labs H (Fauldcispla),

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H (Fauldoxo) and D (Taxol), respectively. Each lab was organized and identified by the same code letter throughout the study (e.g., lab C is the same manufacturer of cisplatin, doxorubicin hydrochloride and paclitaxel). Chemical reference substances were obtained from the United States Pharmacopeia. Reagents and solvents were supplied by Carlo Erba, J.T.Baker and Merck. Culture media were supplied by Oxoid and Difco. LAL reagents and endotoxins were supplied by Endosafe and Cambrex.

Instruments

High performance liquid chromatography (HPLC) systems were equipped with a photo-diode array detector, binary pump and auto-sampler (Agilent 1200 Series and Thermo Accela). Other equipment comprised a Karl-Fischer titrator (Mettler Toledo), analytical balance (Mettler Toledo), Steritest peristaltic pump (Millipore), water bath (Nova Ética), incubators and refrigerators (Nova Ética and Electrolab).

Pharmaceutical equivalence of cisplatin

Cisplatin was tested as specified in the Brazilian Pharmacopeia (Anvisa, 2010b). The analysis of cisplatin included identification, pH, volume, limit of transplatin, limit of trichloroplatinate, assay of cisplatin, sterility and bacterial endotoxin tests.

Cisplatin was assayed by HPLC. The mobile phase was a mixture of ethyl acetate-methanol-dimethylformamide-water (25:16:5:5). The chromatograph was equipped with a 310-nm detector and a 4.0 mm x 30 cm column containing packing L8 (essentially a monomolecular layer of aminopropylsilane chemically bonded to a totally porous silica gel support of particles 10 µm in diameter). The flow rate was about 2.0 mL per min. Solutions of the reference substance (RS), containing between 0.175 and 0.325 mg of USP cisplatin per mL, were prepared and filtered, 60 µL of each being injected. Product samples were diluted, filtered and aliquots of 60 µL were also injected. Solutions of cisplatin were inactivated with 10% sodium thiosulfate prior to disposal (Scaramel et al, 2011).

Pharmaceutical equivalence of doxorubicin hydrochloride

Doxorubicin hydrochloride was tested as specified in the United States Pharmacopeia (United States Pharmacopeia, 2012a). The analysis of doxorubicin hydrochloride included identification, pH, weight, assay of doxorubicin hydrochloride, water content, sterility and bacterial endotoxin tests.

Doxorubicin hydrochloride was assayed by HPLC. The mobile phase consisted of a mixture of water-acetonitrile-methanol-phosphoric acid (540:290:170:2). The chromatograph was equipped with a 254-nm detector and a 4.6 mm x 25 cm column containing packing L13 (trimethysilane chemically bonded to porous silica particles, 3 to 10 µm in diameter). The flow rate was approximately 1.5 mL per min. RS solutions containing between 0.07 and 0.13 mg of USP doxorubicin hydrochloride per mL were prepared and filtered, 10 µL of each being injected. Product samples were diluted, filtered and aliquots of 10 µL were also injected. Doxorubicin hydrochloride solutions were inactivated with 0.5% Asepto 75™ prior to disposal (Scaramel et al, 2011).

Pharmaceutical equivalence of paclitaxel

Paclitaxel was tested as required by the United States Pharmacopeia (United States Pharmacopeia, 2012b). The paclitaxel analysis included identification, pH, volume, assay of paclitaxel, limits of related compounds, sterility test and bacterial endotoxin test.

Paclitaxel was assayed by high performance liquid chromatography. The mobile phase used was water-acetonitrile (11:9). The chromatograph was equipped with a 227-nm detector and a 4.0 mm x 25 cm column containing packing L43 (pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 5 to 10 µm in diameter). The flow rate was about 1.5 mL per min. RS solutions containing between 0.42 and 0.78 mg of USP paclitaxel per mL were prepared and filtered, 10 µL of each being injected. Samples were diluted, filtered and aliquots of 10 µL were also injected. Solutions of paclitaxel were inactivated with 0.5% Asepto 75™ prior to disposal (Scaramel et al, 2011).

Statistical analysis

The two one-sided test (TOST) procedure was employed for equivalence testing, to compare the results of cisplatin, doxorubicin hydrochloride and paclitaxel assays of several products from different manufacturers. To test equivalence, 90% confidence intervals (90% CI) were determined, based on the residual error estimated by analysis of variance (ANOVA). We considered that an appropriate range (Δ=10%) for equivalence testing should be defined, taking the specifications for cisplatin (90-110%), doxorubicin hydrochloride (90-110%) and paclitaxel (90-110%) assays as a reference (Lourenço & Pinto, 2012).

RESULTS

A summary of the results of cisplatin, doxorubicin hydrochloride and paclitaxel contents is presented in Table 1. According to these results, all products complied with the specifications of the Brazilian or United States Pharmacopeia (Anvisa, 2010b; United States Pharmacopeia, 2012a; United States Pharmacopeia, 2012b).

According to the statistical results, cisplatin products from all 3 manufacturers (Labs B, C and I) were equivalent in content to the reference product (Lab H). These comparisons were based on two one-sided tests of the cisplatin content of the products of each manufacturer (Figure 1). All cisplatin products complied with the identification (positive), pH (between 3.5 and 6.5), volume (not less than the declared volume), sterility (sterile) and bacterial endotoxin (not more than 2.0 EU/mg of cisplatin) tests. Transplatin and trichloroplatinate contents were found to be below the specified limits (3.0% and 2.0%, respectively) for all manufacturers. (Anvisa, 2010b)

Table 1. Summary of the results for cisplatin, doxorubicin hydrochloride and paclitaxel contents in products from various labs.

Labs	Cisplatin*1	Doxorubicin hydrochloride*2	Paclitaxel*3
A	N.A.	N.A.	96.8%
B	100.5%	N.A.	98.1%
C	100.4%	102.1%	99.0%
D	N.A.	N.A.	99.7%*6
E	N.A.	N.A.	98.8%
F	N.A.	102.1%	98.6%
G	N.A.	97.1%	96.8%
H	97.1%*4	101.4%*5	100.1%
I	97.9%	N.A.	N.A.
J	N.A.	102.3%	N.A.

N.A. = Not available
*1 Products must contain ≥ 90% and ≤ 110% of the labeled amount of cisplatin, according to Brazilian Pharmacopeia (Anvisa, 2010b).
*2 Products must contain ≥ 90% and ≤ 110% of the labeled amount of doxorubicin hydrochloride, according to USP (United States Pharmacopeia, 2012a).
*3 Products must contain ≥ 90% and ≤ 110% of the labeled amount of paclitaxel, according to USP (United States Pharmacopeia, 2012b).
*4 Reference product of cisplatin (Fauldcispla).
*5 Reference product of doxorubicin hydrochloride (Fauldoxo).
*6 Reference product of paclitaxel (Taxol).

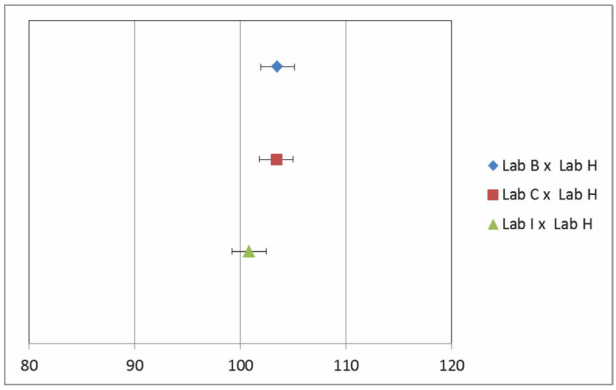

Figure 1. Equivalence tests of cisplatin injectable solutions from three manufacturers (Labs B, C and I) and the reference product (Lab H).

The two one-sided tests demonstrated equivalence between the contents of doxorubicin hydrochloride products from all 4 manufacturers (Labs C, F, G and J) and the reference product (Lab H), as shown in Figure 2. All doxorubicin hydrochloride products complied with the identification (positive), pH (between 4.5 and 6.5), weight (variation lower than 10%), water content (not more than 4.0%), sterility (sterile) and bacterial endotoxin (not more than 2.2 EU/mg of doxorubicin hydrochloride) tests. (United States Pharmacopeia, 2012a)

Injectable solutions of paclitaxel from several manufacturers were compared (Figure 3). The results showed equivalence between the contents of paclitaxel products from several manufacturers (Labs A, B, C, E, F, G and H) and the reference product (Lab D). All paclitaxel products complied with the identification (positive), pH (between 3.0 and 7.0), volume (not less than the declared volume), sterility (sterile) and bacterial endotoxin (not more than 2.0 EU/mg of paclitaxel) tests. Tests of chromatographic purity indicated that all tested products complied with the limits for baccatin III (not more than 0.8%), ethyl ester side chain (not more than 0.4%), 10-deacetylpaclitaxel (not more than 0.8%), 10-deacetyl-7-epipaclitaxel (not more than 0.5%) and 7-epipaclitaxel (not more than 0.6%). (United States Pharmacopeia, 2012b)

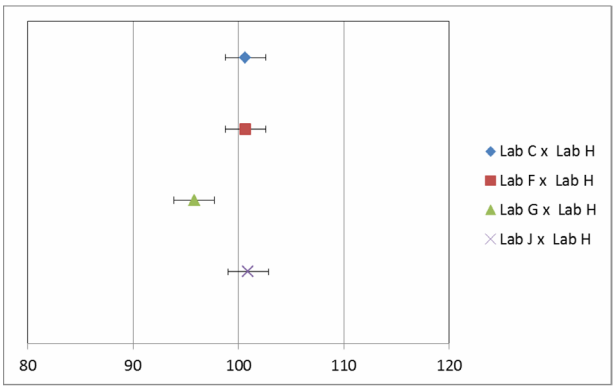

Figure 2. Equivalence tests of doxorubicin hydrochloride injectable lyophilized powder from four manufacturers (Labs C, F, G and J) and the reference product (Lab H).

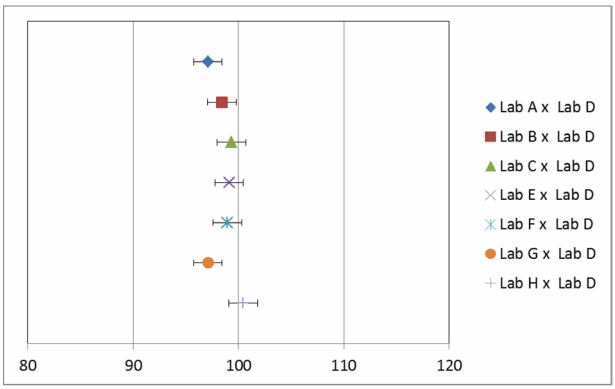

Figure 3. Equivalence tests of paclitaxel injectable solutions from six manufacturers (Labs A, B, C, E, F, G and H) and the reference product (Lab D).

DISCUSSION

Pharmaceutical equivalence testing is an important step in confirming similarity and interchangeability of pharmaceutical products, particularly regarding those that will not be tested for bioequivalence. The pharmaceutical equivalence study must consider relevant tests and assays that allow us to conclude whether the test and reference products are similar or not in efficacy (content) and safety (purity). This includes identification and content of the active pharmaceutical ingredient, pH, sterility and bacterial endotoxin tests. In some situations, pharmaceutical equivalence study should include tests for contents of impurities against limits, such as chromatographic purity, related compounds and specific organic and/or inorganic impurities (Brasil, 2010; Farmacopeia Brasileira, 2010a; Lourenço et al., 2009; Lourenço et al., 2010; Lourenço & Pinto, 2012).

Although the hypothesis test (testing for significant differences by *t*-test or analysis of variance) is usually employed to compare two or more samples in order to assess pharmaceutical equivalence, that approach is not valid and

may lead to invalid conclusions. If a test concludes there is "no statistically significant difference", it is implied that it does not supply sustainable evidence that the two products are different. However, this is not the same as saying that the two products are similar.

Equivalence testing allows us to assess and conclude whether two products may be considered pharmaceutical equivalents. Equivalence testing in this study led to results which are in accordance with the requirements defined by regulatory agencies. The number of samples tested is an important issue. Thus, the number of samples tested for paclitaxel (which included four different doses – 30 mg, 100 mg, 150 mg and 300 mg – from 8 different manufacturers) was much higher than those for cisplatin and doxorubicin hydrochloride (which included only two doses each – 10 mg and 50 mg – from four and five different manufacturers, respectively). We can therefore conclude that equivalence testing is a useful tool to assess pharmaceutical equivalence for products that will not be tested for bioequivalence or relative bioavailability.

The results allow us to conclude that all the cisplatin injectable solutions, doxorubicin injectable lyophilized powders and paclitaxel injectable solutions tested exhibit similar therapeutic efficacy and safety.

RESUMO

Equivalência Farmacêutica de produtos oncológicos comercializados no Brasil

O objetivo deste trabalho foi avaliar a equivalência farmacêutica entre medicamentos de diferentes fabricantes contendo cisplatina, doxorrubicina cloridrato e paclitaxel comercializados no Brasil. Foram analisados 14 lotes de cisplatina solução injetável de 4 fabricantes (laboratórios B, C, H e I), 15 lotes de doxorrubicina cloridrato pó liófilo injetável de 5 fabricantes (laboratórios C, F, G, H e J) e 38 lotes de paclitaxel solução injetável de 8 fabricantes (laboratórios A, B, C, D, E, F, G e H). Todos os produtos apresentaram resultados em conformidade com os critérios estabelecidos nas farmacopeias brasileira e americana. Os resultados de teores obtidos para cisplatina, doxorrubicina cloridrato e paclitaxel foram 94,3-105,9 %, 97,1-106,6 % e 90,2-109,4%, respectivamente. A análise estatística demonstrou que há equivalência entre produtos dos diferentes fabricantes avaliados.

*Palavras-chave***:** Equivalência Terapêutica. Oncologia. Cisplatina. Doxorrubicina. Paclitaxel

REFERENCES

Agência Nacional de Vigilância Sanitária - ANVISA. Farmacopeia Brasileira. 5ª ed. Brasília: ANVISA; 2010a. p. 387-90.

Agência Nacional de Vigilância Sanitária - ANVISA. Farmacopeia Brasileira. 5ª ed. Brasília: ANVISA; 2010b. p. 787-8.

Brasil. Agência Nacional de Vigilância Sanitária. Resolução nº 31, de 11 de agosto de 2010. Dispõe sobre a realização dos Estudos de Equivalência Farmacêutica e de Perfil de Dissolução Comparativo. Diário Oficial da União, nº 154, 12 de agosto de 2010, p. 36-8.

Brabec V, Kasparkova J. Modifications of DNA by platinum complexes. Relation to resistance of tumors to platinum antitumor drugs. Drugs Resist Updat. 2005;8(3):131-46.

Chaney SG, Campbell SL, Temple B, Bassett E, Muzikansky A, Loeffler JS. Protein interactions with platinum-DNA adducts: from structure to function. J Inorg Biochem. 2004;98(10):1551-9.

DeVita VT, Hellman TSL, Rosenberg SA. Cancer: principles and practices of oncology. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.

Fuertes MA, Alonso C, Perez JM. Biochemical modulation of cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. Chem Rev. 2003;103(3):645-62.

Horwitz SB, Cohen D, Rao S, Ringel I, Shen HJ, Yang CP. Taxol: mechanisms of action and resistance. J Natl Cancer Inst Monogr. 1993;(15):55-61.

Horwitz SB. Mechanism of action of taxol. Trends Pharmacol Sci. 1992;13(4):134-6.

Kartalou M, Essigmann JM. Mechanisms of resistance to cisplatin. Mutat Res. 2001;478(1-2):23-43.

Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. Pharm Ther. 1990:47(2):219-31.

Lourenço FR, Silva ACD, Yamamoto RN, Pinto TJA. Validação de método de doseamento para aciclovir e aplicação em estudo de equivalencia farmacéutica de creme contendo aciclovir. Rev Ciênc Farm Básica Apl. 2009;30(3):273-6.

Lourenço FR, Silva ACD, Yamamoto RN, Pinto TJA. Validação de método de doseamento e aplicação em estudo de equivalência farmacêutica de solução injetável de metronidazol. Rev Ciênc Farm Básica Apl. 2010;31(1):47-51.

Lourenço FR, Pinto TJA. Assessment of pharmaceutical equivalence: difference test or equivalence test? Lat Am J Pharm. 2012;31(4):597-604.

Mandic A, Hansson J, Linder S, Shoshan MC. Cisplatin induces endoplasmic reticulum stress and nucleus-independent apoptotic signaling. J Cell Biol. 2003;278(11):9100-6.

Rowinsky EK, Donehower RC. Paclitaxel (taxol). New Engl J Med. 1995;332(15):1004-14.

Scaramel FS, Lourenço FR, Pinto TJA. Evaluation of cisplatin, doxorubicin and paclitaxel inactivation using Asepto 75™ 0.5%, sodium hypochloride 10% and sodium thiosulfate 10% by high performance liquid chromatography (HPLC) and *in vitro* cytotoxicity test. Lat Am J Pharm. 2011;30(8):1602-7.

United States Pharmacopeia. United States Pharmacopeia Convention. 35th ed. Rockville (MD): United States Pharmacopeia Convention; 2012a. p. 2981-82.

United States Pharmacopeia. United States Pharmacopeia Convention. 35th ed. Rockville (MD): United States Pharmacopeia Convention; 2012b. p. 4197-8.

Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov. 2005;4(4):307-20.

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agente from Taxus brevifolia. J Am Chem Soc. 1971;93(9):2325-7.

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