

Fibrosarcoma and Papillary Thyroid carcinoma in Rats administered of Platinum Analogue Daily

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Abstract: Fibrosarcoma is a malignant mesenchymal neoplasm composed predominantly of fibromatosis like stroma in which small follicles are diffusely dispersed. Papillary thyroid carcinoma (PTC), predominantly growing in a solid growth pattern with papillary projections, and composed of oncocytic cells with "Orphan annie eyes" nuclei. Cisplatin or Cis-Diamminedichloroplatinum (ii) ($Cl_2H_6N_2Pt$), it is a divalent, inorganic, water-soluble first successful platinum containing anticancer drug, used to treat various forms of cancers. However, the Carboplatin ($C_6H_{14}N_2O_4Pt$) 2nd generation and Oxaliplatin 3rd generation ($C_8H_{14}N_2O_4Pt$) analogue of Cisplatin and are the investigational chemotherapy medicine, which interferes with the genetic material or DNA inside the cancer cells and prevents them from further dividing and growing more cancer cells by forming interstrand and intrastrand cross-linking of DNA molecules. The intraperitoneal injection of 15mg/Kg BW (Group-A), 15 mg/Kg BW (Group-B), 15 mg/Kg BW (Group-C), among the three groups Group-A resulted in to fibrosarcoma or aggressive fibromatosis of the stroma, however, Group-B showed the formation of papillary thyroid carcinoma (PTC), Group-C did not show any above mentioned carcinomas. In the present study the PTC was characterized by follicles with empty colloid, cells were arranged in clusters, with nuclear crowding and overlapping, some cells still demonstrated a polygonal contour with well-defined central nucleus, finely granular, delicate, powdery chromatin presence of multinucleated giant cells or a row of palisaded tumor cells with empty lumen.

Keywords: Fibrosarcoma, Papillary Thyroid Carcinoma (PTC), Thyroid gland, Cisplatin, Carboplatin, Oxaliplatin

1. Introduction

Cisplatin, Carboplatin and Oxaliplatin are the platinum containing anticancer drugs classified as DNA alkylating agents, interferes with the genetic material or DNA inside the cancer cells and prevents them from further dividing and growing more cancer cells by forming interstrand and intrastrand cross-linking of DNA molecules (Reiter et al. 2002; Huang et al. 2003), and are used against some forms of cancer (mainly ovarian carcinoma, small cell lung cancer, non-small cell lung cancer, head and neck cancer (Timme et al. 2013).

Aggressive fibromatosis is composed of interwoven bundles of malignant spindle shape mesenchymal cells arranged in a "herringbone" and "storiform" pattern infiltrating the thyroid tissues, containing abundant collagen fibers with deposition of amyloid all over the zone. The peripheral portion of the gland showed mixed population of fibromatosis and stromal hyperplasia which was notified by interfollicular spaces filled with large number of atypical stromal cells, containing eosinophilic hyaline stroma. Sheets of follicular cells with abundant colloid led to the formation of hyperplastic nodule (Fisher et al. 2002; Rosai, 2004; Elellis and Williams, 2004; Titi et al. 2007; Yeo, 2009; Janczak et al. 2013).

Papillary thyroid carcinoma (PTC) is characterized by follicles with empty lumen or colloid, follicular cells arranged in cluster with overlapping and crowded nuclei appearing like "orphan annie eyes" containing delicate, powdery and finely granular chromatin (Rufini et al. 2007; Ito et al. 2008; Livolsi, 2011; Bellevicine et al. 2012; Yu et al. 2013; Suzuki et al. 2014; Calangui et al. 2014; Ustun et al. 2014).

Only a handful of publications have been published on fibrosarcoma and Papillary thyroid carcinoma (PTC), on human beings, therefore the present study reported the induction of fibrosarcoma after Carboplatin and PTC after Cisplatin.

2. Materials and Methods

Drugs

The anticancer drugs Carboplatin with the chemical formula ($C_6H_{14}N_2O_4Pt$) and Cisplatin ($Cl_2H_6N_2Pt$) and Oxaliplatin ($C_8H_{14}N_2O_4Pt$) manufactured by Oplex Pharma Limited, Goa, India.

Experimental Animals

Wistar albino rats (*Rattus norvegicus*) with average body weight 220-280 g were used for the experiments. Animals were maintained in the laboratory under an absolute hygienic condition as per the recommended procedures by fulfilling all the necessary ethical standards. They were housed in polypropylene box type cages, bedded with rice husk and kept at constant temperature $28 \pm 2^\circ C$ and relative humidity with 12 h light: 12h dark cycle. They were fed with pelleted diet and water *ad libitum* (Buccafusca, 2001).

Treatments

Altogether three experiments with different regimens and with same durations were performed for studying the toxicity of Carboplatin and Cisplatin on thyroid gland (Table-1), however, the fibrosarcoma or aggressive fibromatosis were observed in Group-A and papillary thyroid carcinoma were observed in Group-B.

Histological assessment

The animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the thyroid glands were excised, fixed in Bouin's fluid for 24hrs and

preserved in 70% alcohol. The tissues were dehydrated by passing through graded series of alcohol, cleared in xylol and after embedding in paraffin blocks were prepared and cut in numerous parallel 5µm sections. For routine histological study the sections were stained with Ehrlich's haematoxylin and counter-stained with eosin.

Table 1: Experimental Design for Carboplatin, Cisplatin and Oxaliplatin (Oplex Pharma Ltd. 2mg in 1 ml) on sexually mature Wistar rats

Number of animals and sex	Treatment	Dose (mg/Kg BW/day)	Route	Duration
6male (Experimental Group-A)	Carboplatin	15mg daily	I.P.	15 days
6males (Experimental Group-B)	Cisplatin	15mg daily	I.P.	15 days
6males (Experimental Group-C)	Oxaliplatin	15mg daily	I.P.	15 days
6males (Control Group-D)	Saline	Equal volume	I.P.	Equal days

Abbreviations : E.V. = Equal volume, I.P. = Intraperitoneal, B.W. = Body weight

3. Results

Gross description

The thyroid gland morphologically demonstrated a dullness in the red colour (Group-A), rigidity in the texture (Group-B), both the lobes of thyroid gland were more or less symmetrically enlarged, surface being lobulated (Group-A) when compared with vehicle-treated control rats (Group-D).

Histopathological Changes

Vehicle-treated controls (Group-D)

Each follicle was lined by a secretory epithelium composed of a single layer of roughly cuboidal or low columnar cells (fig.1).

15 mg/Kg BW Carboplatin treatment for 15 days (Group-A)

This treatment resulted into Fibrosarcoma or Aggressive fibromatosis composed of interwoven bundles of malignant spindle shape mesenchymal cells arranged in a "herringbone" and "storiform" pattern infiltrating the thyroid tissues, containing abundant collagen fibers with deposition of amyloid all over the zone (figs. 2,3 and 4).

15 mg/Kg BW Cisplatin treatment for 15 days (Group-B)

This treatment resulted into Papillary Thyroid Carcinoma (PTC). It was characterized by papillary projections aggregation of follicular cells or psammoma bodies or clear overlapping of nuclei appearing as "orphan annie eyes", with nuclear grooves however, in some portion cytoplasm was focally elongated containing vacuoles of secretory material extruded from follicular cells (figs. 5 and 6).

15 mg/Kg BW Oxaliplatin treatment for 15 days (Group-B)

During this experiment, there were no signs of such changes as observed in the previous two experiments.

4. Discussion

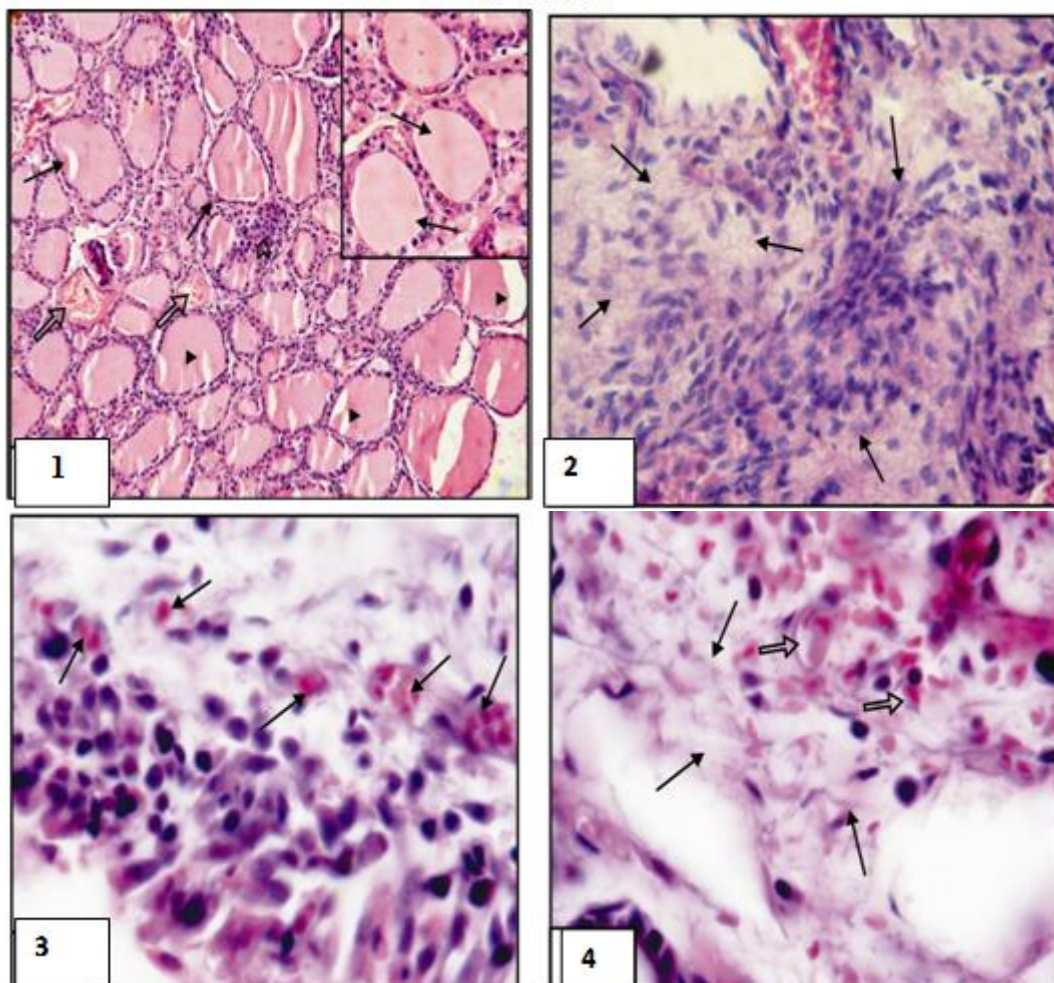
The present study revealed the formation of Fibrosarcoma / Fibromatosis of thyroid, after 15mg/KgBW for 15 days Carboplatin treatment is a malignant mesenchymal neoplasm of the fibrous soft tissue, predominantly it is consisted of fibromatosis like stroma in which small follicles appear diffusely dispersed, it is a rich cellular tumor showing more than two mitosis, containing little collagen fibers surrounded by small cells with clear cytoplasm, interwoven bundles of spindle cells admixed with plump ovoid cells and giant cells with extreme thickness of outer capsule incorporating diffusely dispersed collagen fibers and mesenchyme in the stroma infiltrating the inner portion of the gland. When old literature was perused for Comparison with our results it was noted that our findings were in conformity with the case reports on human thyroid abnormalities either reported naturally or after the induction of some exogenous factors such as Carboplatin (Fizimoto and Hidai, 1990; Samsi et al.1992; Muzukami, 1995; Sinha et al.1998; Weiss and Goldman, 2001; Fisher et al. 2002; Rosai, 2004; Elellis and Williams, 2004; Titi et al. 2007; Yeo, 2009; Janczak et al. 2013). The earlier documented reports also stated that the survival rate in fibrosarcoma is closely related to the histological grade indicated by several morphological features, such as tumor cellularity, cellular polymorphism, mitotic activity and necrosis (Scott et al. 1989; Rosai, 2004c) which is also applicable to the present study in some extent.

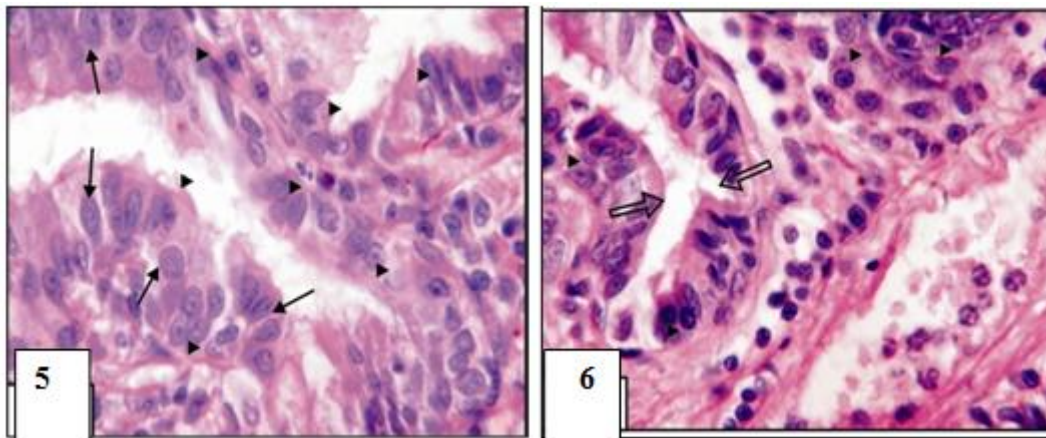
The present study also revealed the development of Papillary Thyroid Carcinoma (PTC) by 15mg/KgBW for 15 days daily Cisplatin treatment. The characteristic features described for this type of carcinoma by earlier workers are co-relative to my observations which included the formation papillae or papillary projections of the gland, containing oncocytic cells, clear overlapping of nuclei with nuclear grooves, presence of "orphan annie eyes" nuclei and "psammoma bodies". Similarly, 15mg/KgBW for 15 days daily Cisplatin treatment also demonstrated a row of palisaded tumor cells which caused development of complex papillae, follicular cells showed nuclear enlargement, intranuclear pseudoinclusions, overlapping, nuclear grooves, "orphan annie eyes" nuclei, psammoma bodies such characteristics features of PTC were co-relative with the findings of Baker and Hyland, 1985; Carcangiu et al. 1985; Green and Wilson, 1985; Chan and Saw, 1986; DeGroot et al. 1990; Sebastain et al. 2000; Akslen and LiVolsi, 2000; Ortzsebastain et al. 2000; Kukora, 2001; Baloch and Livolsi, 2002; Lam et al. 2005; Urhan et al. 2007; Rufini et al. 2007; Ito et al. 2008; Nikiforov et al. 2009; Livolsi, 2011; Bellevicine et al.2012; Yu et al. 2013; Suzuki et al. 2014; Calangiu et al. 2014; Ustun et al. 2014. The same dose and duration treatment with Cisplatin did not reveal fibromatosis of stroma as well as PTC with Carboplatin treatment. Oxaliplatin treatment did not show such type of carcinogenic changes.

PLATE-1

- Fig. 1 : Vehicle-treated control thyroid gland: Each follicle is lined by a secretory epithelium composed of a single layer of roughly cuboidal or low columnar cells (arrow). The closed cavities of the follicles contain a homogeneous, gelatinous, amber-colored colloid. This eosinophilic colloid of active gland is non-uniform, contains some non-staining vacuole-like spaces (arrow head), arising out of poor-fixation. A rich network of fenestrated blood capillaries surrounds the follicles (open arrow). A large number of stem cells are interspread between the follicles for the formation of new follicles (chevron). Inset: showing normal thyroid follicles (arrow) X 200.
- Fig. 2 : 15mg/KgBW/days of Carboplatin daily for 15 days treatment : Fibrosarcoma or aggressive fibromatosis : It is composed of interwoven bundles of malignant spindle shape mesenchymal cells arranged in a “herringbone” and “storiform” pattern which infiltrating the thyroid tissues, the background of fibrosarcoma containing abundant collagen fibers and deposition of amyloid all over the zone (arrow) X 400.
- Fig. 3 : Fibrosarcoma or fibromatosis : Peripheral portion of the gland showing the total loss of follicular structure or cellular configuration, lymphorrhages are dispersed, blood capillaries and arterioles are extensively affected (arrow), follicles appears in the form of sheets which is composed of spindle cells, arranged in a “herringbone pattern” also note the excessive increase of fibrous connective tissues and mesenchyme leads to the formation fibrosarcoma or fibromatosis X 400.
- Fig. 4 : Fibrosis :Total loss of thyrotrophes and mesenchyme with excessive growth of fibrous connective tissues with abundant collagen fibers and liquefied materials (arrow) it may be the debris of degenerating parenchymal cells with extensively affected blood capillaries (open arrow) leads to the fibrosis X 400.
- Fig. 5 : 15mg/KgBW/days of Cisplatin daily for 15 days treatment : The variant resembles papillary thyroid carcinoma (PTC) predominantly growing in a solid growth pattern and composed of oncocytic cells (arrow).The view also show clear overlapping nuclei, nuclear grooves with presence of “orphan annie eyes” nuclei and psammoma bodies (▶) X 1000.
- Fig. 6 : 15mg/KgBW/days of Cisplatin daily for 15 days treatment : Formation of papillae or papillary projections (arrow), and presence of “orphan annie eyes” nuclei (arrow) and psammoma bodies (▶) leading to papillary thyroid carcinoma X 1000.

PLATE-1





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