EFFECT OF A CHEMOTHERAPEUTIC DRUG OXALIPLATIN ON ACCESSORY REPRODUCTIVE GLANDS IN RAT

M. S. Sastry et al.

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Testosterone

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The toxic effect of Oxaliplatin (C₈H₁₄N₂O₄Pt), an analog of Cisplatin, the first successful platinum containing anticancer drug on the accessory glands (seminal vesicle, prostate and bulbo-urethral glands or Cowper’s gland) and evaluation of testosterone levels were studied by intraperitoneally injecting chronic low dose (4mg/KgBW/day for 15 days) and high dose (8mg/KgBW/5days) to adult male Wistar rat, Rattus rattus norvegicus. For comparing the effects the vehicle control rat was injected same amount of saline and were maintained for the same duration. Both the treatments resulted into significant suppression of body weight (p< 0.5) as well as for low dose treatment Ventral Prostate (p< 0.5), Seminal vesicle (p< 0.5), Cowper’s gland (p< 0.5) and for high dose treatment Ventral Prostate (p< 0.05), Seminal Vesicle (p< 0.5) and Cowper’s gland (p< 0.1). As compared to vehicle-treated control group treated animals revealed a number of untoward behaviour such as low appetite, withdrawn behaviour, thickness of skin, hair fall all over the body and continuous licking of testis, oral mucositis etc. Oxaliplatin treatment resulted into dose and duration dependent histopathological changes in the accessory reproductive glands as well as testosterone concentrations. Thus the results suggested antiandrogenic effect of Oxaliplatin.
INTRODUCTION

Oxaliplatin ("cis - [C1R,ZR] - 1, 2 cyclohexane diamine – N, N") [Oxalato (2-)-O-O’] platinum, Eloxiatin) is a novel platinum co-ordination complex used for the treatment of metastatic colorectal carcinoma in combination with flupiram. It interferes with the genetic material, or DNA, inside the cancer cells (Dunn et al., 1997; Cvitkovic, 1998; Huang et al., 2003). A perusal of literature on the effect of Oxaliplatin revealed that scanty and fragmentary notes are available only on the testicular atrophy or gonadal dysfunction (Dunn et al., 1997; Pectasides et al., 2004; De Giorgi et al., 2004, 2006; Corazzelli et al., 2006; Chater et al., 2007), but no detail account is available on male accessary reproductive glands (seminal vesicle, prostate and Cowper’s or bulbo-urethral glands), similarly studies on evaluation of the male gonadal hormone, testosterone are not well documented, therefore, in the present study main emphasis has been given on the evaluation of testosterone correlated to histopathological changes as these glands are androgen dependent and body weight as well as organ weight changes shows anabolic response. The targeted organs, seminal vesicle, prostate and bulbo-urethral or Cowper’s gland are essential in male physiology as their secretions contribute largely to the volume of the semen. In view of understanding the monotherapic action of this drug rat was used as a model.

MATERIALS AND METHODS

Animals and treatment
The Wistar rat, Rattus norvegicus weighing between 250 to 300gm were selected. For the present study, animals were obtained from Department of Biochemistry, RTM Nagpur University Nagpur. After a week of acclimatization to laboratory condition, Oxaliplatin was administered via disposable syringe intraperitoneally. The control animals received same amount of saline (Tables 1 and 2).

Histological assessment
The animals were sacrificed using chloroform 24 hr after the last day of each experiment. Immediately accessory reproductive glands (prostate, seminal vesicle and Cowper’s gland) were excised and were fixed in Bouin’s solution, dehydrated in ethanol and embedded in paraffin wax. The sections cut in 5 μm were stained with haematoxylin and eosin. Measurements were taken with an oculometer wherever essential.

Testosterone evaluation
For the determination of testosterone level in blood, rats were anesthetized by ether and 2mL of blood was drawn by cardiac puncture with 2mL sterile syringe. The blood was allowed to clot at room temperature for half an hr. The clotted blood was sent to NRPL Pathology laboratory, Nagpur for further processing by enzyme linked-florescent assay (Delahunt and Hirsutism, 1993).

RESULTS

Both the treatments resulted in the suppression of the body and organ weights (Fig. 1 and 2 respectively) and testosterone concentration (Fig. 3) and also showed regressive changes in histopathological architecture. As compared to vehicle-treated control group the low dose treated group apart from showing reduction in the body weight, revealed a number of untoward behaviour such as decrease in food intake, softness of stool, withdrawn behaviour, slow movement, but no mortality. Similarly, higher dose treatment induced diarrhoea, anemia and paleness in the extremities, tail and eyes, depression in mood, sluggishness in locomotion, extreme leaning of the body, withdrawn behaviour and so forth, even there was mortality of some animals, may be due to systemic debility and emaciation, loss of appetite, however some animals from the higher dose survived.

Seminal Vesicle

Vehicle-treated controls
The seminal vesicles in rat are a paired curved structure, about 2-3cm in height, one end draining into urethra, the gland is ensheathed into thick fibro-muscular connective tissue. The seminal vesicle of the control rat was composed of a large number of acini embedded within the fibromuscular connective tissue (Fig. 5). The acini were lined by tall columnar epithelial cells containing a prominent basal nucleus (Fig. 8). Few basal cells, almost rounded in shape and basal in position were also observed between the columnar epithelial cells. Large number of dense secretory granules were visible in the apical cytoplasm. The lumen of acini was filled with the darkly stained secretory material (Fig. 8). Lamina propria surrounding the epithelial cells was comprised of cellular connective tissue containing some smooth muscles rich in elastic fibres (Fig. 8).

**Low dose treatment (4mg/Kg BW/chronic Oxaliplatin for 15 days)**

An enormous increase in the fibro-muscular connective tissue in between the secretory tubules have resulted into remarkable reduction in their size (Fig. 4 and 6). There were either large vacuoles or streaks in the fibro-muscular tissue, suggestive of degeneration. The peripheral portion as well as some part of central tubule showed degeneration of acini due to sloughing and intermingling of secretory epithelium and emptiness due to total loss of secretory activity. However, central acini even though smaller in size showed presence...
of small amount of secretion, thus pointing to partial damage of the whole gland. The lining secretory epithelium appeared partially damaged due to irregular placement of nuclei. The ramification of secretory epithelium was highly restrictive (Fig. 6 and 9).

**High dose treatment (8mg / Kg BW/chronic Oxaloplatin for 5 days)**

After the administration of high dose of Oxaliplatin there was a remarkable alteration in the scenario, seminal vesicle was thoroughly regressed and secretory units were reduced in size (Fig. 4), peripheral connective tissue was abundant, ramification of the secretory epithelium was far too restricted, the lumen was highly delimited and contained very little colloid, if any, similarly phagocytic activity was evident in the colloid (Fig. 7 and 10).

**Prostate (Ventral Prostate)**

**Vehicle - treated control**

Out of the three lobes, dorsal, lateral and ventral, ventral prostate has been used for the present study. These lobes are connected to the urethra by fascia and a series of ducts. The prostate is a compact compound tubular gland lying in the close approximation to the bladder. The gland have a thin membranous capsule. The glandular substance was spongy. The prostate showed two clearly marked regions, a cranial peripheral unit of secreting tubules and a caudal ventral unit of collecting tubules. From the capsule thin trabeculae of fibromuscular tissue extended inward and formed the boundaries of the lobules. In the ventral prostate of control rat typical follicular pattern was evident. The lobules were formed of a closely packed network of glandular lobules or acini. Flat squamous or pseudostratified epithelium lined the large distended acini with dense secretory material. The epithelial lining was infolded and intertubular connective tissue was thin (Fig. 11 and 14).
Figure 5 to 10: (5) Seminal vesicle cross-sectioned from vehicle-treated control. The gland is encapsulated in thick connective tissue capsule (arrow). The gland is composed of a large number of acini embedded within the fibro-muscular connective tissue. Each acinus is lined by tall columnar epithelial cells. The lumen is filled with the darkly-stained secretory material (arrow head) X 100; (6) After the administration of 4mg/KgBW Oxaliplatin chronic dose for 15 days, there was an increase in the peripheral connective tissue (arrow), a restriction in the ramification of the secretory epithelium and a reduction in the amount of colloid (arrow head) in the seminal vesicle. Also please note (triangle) degeneration of peripheral secretory units due to intermingling of sloughed off epithelium and total loss of secretion X 100; (7) A single secretory acinus from the high dose treated group (8mg/KgBw/day) for 5 days. Please note far too restriction in the ramification of secretory epithelium (arrow), the lumen contain very little amount of colloid. Also note an abundant peripheral connective tissue encapsulating the acinus (arrow head) X 100; (8) A part of the secretory epithelium from vehicle-treated control. The acini are lined by tall columnar epithelial cells containing a prominent basal nucleus. Few basal cells, almost rounded in shape and basal in position in between the columnar epithelial cells. Large numbers of dense secretory granules are visible in the apical cytoplasm. The lamina propria surrounding the epithelial cells were comprised of cellular connective containing some smooth muscles rich in elastic fibres (triangle) X 400; (9) A part of the secretory epithelium from fig. 6 appear regressive, with less secretory zone and condensation, pyknosis and displacement of nuclei (arrow) X450; (10) A part of the secretory epithelium magnified from the fig. 7. The pseudostratified epithelium appears regressive and degenerated at places with little amount of colloid. Also note phagocytic activity in the colloid (arrow) X 1000

Low dose treatment (4mg/Kg BW/chronic Oxaliplatin for 15 days)
The low dose Oxaliplatin treatment on the ventral prostate has caused partial reduction in the acinal or follicular size (Fig. 4) and secretion also, an increase in the amount of fibro muscular tissue isolating the tubules, secretory epithelium in some places showed loss of foldings indicative of reduction in the secretory activity. The epithelium exhibited vacuolation, pyknosis and condensation and displacement of nuclei, sometimes detachment from the basement membrane. Lamina propria encasing each tubule was not distinctly demarcated. The fibro-muscular tissue even appeared with scarce smooth muscle cells, may be due to their loss (Fig. 12 and 15).

High dose treatment (8mg / Kg BW/chronic Oxaliplatin for 5 days)
After the administration of high dose of Oxaliplatin (8mg / Kg BW/day) prostate gland showed some regressive changes such as reduction in the size of tubules (Fig. 4), reduction in the amount of secretory activity, irregularity in the configuration. Peculiar feature being the diminution in the number as well as height of
Figure 11 to 16: (11) Transverse section through the ventral prostate gland illustrating large acini with frequent secretory epithelial infoldings. Central lumen is filled with dense and copious amount of secretory material X 100; (12) Prostatic tubules (4 mg/kgBW/day) Oxaliplatin treated group. Note partial reduction in the acinal size and secretion (arrow), an increase in the amount of fibromuscular tissue isolating the tubules (arrow head), secretory epithelium in some places showed loss of foldings indicative of reduction in the secretory activity. Similarly there is vacuolation prevailing in the epithelium, sometimes detachment of epithelium from the basement membrane X 400; (13) Low power picture of ventral prostate (8mg/ KgBW/ day) after Oxaliplatin treatment. Note regressive changes such as reduction in the amount of secretory activity, irregularity in the configuration of acini, peculiar feature being the diminution in the number as well as height of secretory infoldings, similarly an increase in the amount of interacinal mesenchyme (arrow) X 100; (14) Few acini magnified from the prostate. Each acinus is lined by flat squamous or pseudostratified epithelium X 450; (15) Secretory prostatic epithelium at high resolution (4mg/kgBW/day). Please note vacuolation in the cells, pyknosis, condensation and displacement of nuclei, reduction in secretion pointing to degenerative changes after drug administration X 450; (16) Few prostatic tubules enlarged from high dose Oxaliplatin treatment. Note extensive vacuolation both in the nuclei and cytoplasm (arrow) as well as reduction in the amount of secretion X 1000

secretory epithelial infoldings, similarly an increase in the amount of inter-acinal mesenchyme. The epithelium showed extensive vacuolation both in the nuclei and cytoplasm as well as reduction in the amount of secretion (Figs. 13 and 16).

Cowper’s gland or Bulbo-urethral gland

Vehicle - treated control

The bulbo-urethral (Cowper’s) glands are small paired glands partially embedded in the skeletal muscle fibres of the urinogenital diaphragm. Each gland is divided into several lobes and opens with a single duct onto the floor of the cavernous part of the urethra. The lobes consist of secretory end - pieces or alveoli that open directly, or through small ducts, into wide cavities called ampullae. The principal ducts originates from the confluence of the ampullae of the various lobules. In the vehicle-treated rat most of the alveoli are cyst-like dilated. The alveoli and ampullae are bounded by tall glandular cells with flattened basal nuclei. The cytoplasm is full of secretion granules. These granules are bound by a unit membrane, but large irregular masses are also seen in the collecting duct which are lined by a single layer of cuboidal cells. Some granules have dense corn – like structure (Fig. 17). The secretory epithelium is so tall that it nearly fills the lumen. Small amounts of stromal and smooth muscle cells surrounds each acinus. The striking
feature of the secretory epithelium is the secretory vacuoles showing filamentous or reticular texture (Fig. 20).

**Low dose treatment (4mg/Kg BW/chronic Oxaliplatin for 15 days)**

The low dose Oxaliplatin treatment has resulted into regressive changes when compared to vehicle-treated rats. The changes include reduction in the acinal size, due to an increase in the fibro-muscular tissue causing shrinkage and compactness of tubules. (Fig. 4). Reduction in the secretory activity is evident by emptiness of some tubules. The secretory epithelium revealed reduction in height, uneven arrangements of nuclei, their pyknosis and vacuolation (Fig. 18 – 21).

Figure 17 to 22: (17) A part of Cowper’s gland from vehicle-treated control photographed to show the numerous compactly arranged secretory alveoli (arrow) which are divided by connective tissue septa surrounded by a connective tissue capsule. All the alveoli or acini appear dilated cyst-like. The ducts are lined by a single layer of cuboidal cells. Small amounts of stromal and smooth muscle cells surround each acinus. Each alveolus is lined by tall columnar secretory epithelium which nearly fill the lumen with basal nuclei and the central area occupied by secretory product, or mucosubstances which contain dark flake-like structures. Also note central duct, the ampulla (chevron) into which all alveoli drain, either directly or via a very short and narrow ductile. The cytoplasm of each cell is full of secretory product X 100; (18) A portion of bulbo-urethral gland photographed from the low Oxaliplatin regimen. Note reduction in the acinal size (arrow), regression in the height of secretory epithelium as well as reduction in the secretion. Most of the tubules lack secretion. An increase in the fibro-muscular tissue causes shrinkage and compactness of tubules X 100; (19) The bulbo-urethral gland from high dose treated group. Note reduction in the number of secretory unit due to toxic effect of the drug, their sparse distribution because of increased amount of connective tissue trabeculae (open arrow). Each unit contains regressed acini either with empty or partially filled lumen (arrow head). Correlative to this the collecting duct also shows reduction in the transporting fluid (thick arrow) X 100; (20) Few glandular lobules photographed. The acinus is lined by tall columnar cells which nearly fill the lumen with flattened basal nucleus (arrow), small amounts of stromal and smooth muscle cells surrounds each acinus (arrow head). The striking feature of the secretory epithelium is the secretory vacuoles showing filamentous or reticular texture (open arrow) X 1000; (21) Few secretory tubules magnified. Note reduction in the height of epithelium, uneven arrangements of nuclei and their pyknosis. Vacuolation prevailed in the epithelium. Secrection is partially restored X 450; (22) A part of Cowper’s gland from high dose Oxaliplatin treated group. Note remarkable reduction in the acinal size, obliteration of lumen in many tubules (arrow) random distribution of nuclei and an increase in the amount of stromal and smooth muscle cells (arrow head). The lumen lack mucin secretion (triangle) which are comparatively smaller in size than the low dose treatment X 400.
High dose treatment (8mg / Kg BW/day)
Administration of high dose Oxaliplatin (8mg / Kg BW/day) for 5 days resulted into remarkable reduction in the size of acini (Fig.3). Similarly there was reduction in the number of secretory units, their sparse distribution because of increased amount of connective tissue trabeculae. Each unit contained regressed acini either with empty or partially filled lumen, obliteration of lumen in many tubules random dispersal of nuclei and an increase in the amount of stromal and smooth muscle cells. The lumen lacked mucin secretion (Fig. 19 and 22).

Evaluation of testosterone
The low dose group showed insignificant decrease (p < 0.1) but the high dose treatment resulted into significant decrease (p < 0.001) in serum testosterone concentration compared to control values (Fig. 3).

DISCUSSION
Animals in reproductive age can be exposed to several side effects when chemotherapeutic agents are administered for cancer treatment. In the present work significant reduction in the low dose treated group (4 mg/Kg BW /chronic for 15 days) as well as in the high dose (8 mg/Kg BW /chronic for 5 days) were recorded after Oxaliplatin administration. However, with Oxaliplatin suppression of body weight have not been described previously excepting for few notes on the effect on gonads (De Giorgi et al., 2004; Charter et al., 2007). With low dose treatment in the present studies decrease in the food intake and softness of stool, but with higher dose diarrhoea, anemia, depilation, ataxia and so forth, even mortality of many animals due to systemic debility and emaciation have been observed. The animals also showed loss of appetite, sluggish locomotion and leaning of the body, piloerection and paleness in the extremities, tail and eyes, evidencing a probable anemia, depression in mood and withdrawn behaviour (Dunn et al., 1997; Pectasides et al., 2004; De Giorgi et al., 2004, 2006; Corazzelli et al., 2006; Chater et al., 2007).

It is suggested that a reduction in the total body weight may be due to decline in the circulating blood serum androgen since androgen are a potent stimulant of nitrogen retention and their administration readily leads to an increase in body weight in both men and women (Bhasin et al., 1997). Similarly androgen increases muscle mass due an increased serum concentration of potassium (Turner and Bagnara, 1976). Reduction in the testosterone levels in the present work (Fig. 3) as well as the perusal of earlier literature confirms our observations (Dunn et al., 1997; Pectasides et al., 2004; De Giorgi et al., 2004; Corazzelli et al., 2006 and Chater et al., 2007). Our results are in accordance with the results of previous workers described for Oxaliplatin. Thus with both the regimens we noted highly significant regression in the body weight correlative to mild alteration in the Leydig cell structure and function or an atrophy in the higher dose treatment suggesting a direct role of testosterone in the maintenance of body mass.

The accessory glands are morphologically and physiologically dependent on the production of the androgen and the circulating androgen which in turn are LH dependent (Lee et al., 1994; Nemeth et al., 1998) confirmed by decreased LH receptors on Leydig cells. Oxaliplatin being antiandrogenic causes involution in the weight and size of the accessory glands due to reduction in the venous blood flow from the testis (Seaman et al., 2003), similarly being a potent inhibitor of testicular 3á-hydroxysteroid oxidoreductase activity, itself bind to the catalytic binding sites of the substrates like DHT (5á-dihydroxytestosterone) thus reducing the ABP production which would have helped in the maintenance of the accessories, since it is a carrier of testosterone. At the same time loss of organ weights due to decline in protein could be due to alteration in electrolyte balance leading to loss of water in tissue (Wong et al., 1978) or through hormone target cell interaction or the expression of seminal vesicle proteins or by direct action on the metabolism of testosterone compartment of testis which are in the control of adenohypophysis. Thus it is suggestive that Oxaliplatin may not act directly on hypothalamus – hypophysis, but alters the level of serum LH and FSH through feed back regulation by testosterone.
The prostate is an equally important accessory organ as its weight and morphological appearance have served as an end point to study the biological activity of chemotherapeutic drugs. The prostatic epithelial growth and morphogenesis were found to be dependent upon an epitheliomesenchymal interaction, which in turn is regulated by androgen (Cunha et al., 1983; Blanchere et al., 2001).

The overall changes in the body weight after Oxaliplatin treatment reflects general toxicity but the gravimetric changes in the male reproductive system suggests special male reproductive toxicity. The regression of accessory glands compounding to change in the testis suggests that the androgenic compartments of the testis is also affected, as these organs are androgen dependent. Such an impact may not be direct, and only indirect through effect on the Leydig cell and steroidogenesis. In the light of the adenohypophysial control of the Leydig cell it could be hypothesized that the impact may be primarily on the adenohypophysis (gonadotrophs) and the changes in the Leydig cell and the accessory organs are only indirect manifestations.

The treatment has resulted into gross reduction of seminal vesicle being significant with the higher dose, similarly an enormous increase in the fibro-muscular connective tissue enveloping the acini as well as in between them have caused noticeable reduction being significant with the higher dose, which also showed necrotic changes (Blanchere et al., 2001). Some peripheral as well as some central acini showed more degenerative changes in the tubule due to sloughing off of secretory epithelium and emptiness due to loss of secretory activity, however, central acini despite of reduction in the size showed presence of colloid indicative of partial damage. The high dose resulted in remarkable alteration in the scenereo due to thorough regression of gland, enhanced restriction in ramification of secretory epithelium, abundancy of peripheral connective tissue, high delamination of lumen with very little colloid and if any, with prevalence of phagocytic activity.

In control rats the prostate revealed columnar epithelium which was reduced to cuboidal and squamous because of great distention of the tubules and copious amount of secretion in the lumen. However, in the low dose treated group (4 mg / Kg BW/ chronic for 15 days) there was partial reduction in the size of acinus and secretion, an increase in the amount of fibromuscular tissue isolating the tubules, reduction in the folding of secretory epithelium indicative of reduction in the secretory activity assisted by prevalence of vacuolation in the epithelium, degenerative changes included detachment of epithelium from the basement membrane, pyknosis, condensation and displacement of nuclei. The degenerative or apoptotic changes were further enhanced in the chronic high dose (8 mg/Kg BW). The peculiar feature being the diminution in the number as well as height of secretory infoldings indicative of great reduction in the secretory function of the gland, and further increase in the interacinal mesenchyme. Thus the main cytological alterations were decrease in cell size (simple or cellular atrophy), cell loss (numeric atrophy). On comparison it was found that the prostate shows more histopathological changes than the seminal vesicle since it is more androgen dependent (Surfin and Coffey, 1974).

Cowper’s glands are small appendages of the male genital tract. They are involved in the immune defence of the genitourinary tract (Riva et al., 1990), play a role in fertility and secrete many glycoproteins, including Prostate specific antigen PSA (Beil and Hart, 1973). Hafez and Spring-Mills, 1980 described mucous as well as serous types of cells in the alveoli. These gland secrete mucosubstance, but also some neutral mucosubstnaces. The acid mucosubstances consist mainly of sulfated acid mucosubstances and a smaller amount of carboxylated acid mucosubstances (Sialo-mucin) (Holm-Nielson, 1976; Riva et al., 1988). The secretions of the bulbo-urethral glands are emitted at the beginning of the ejaculatory phase and it is conceivable that, besides secretions from the prostate, some components of the first seminal fraction could be produced by these glands (Mann and Lutwak-Mann, 1981). These glands can be affected by neoplastic, infectious, stone and especially cystic disease syringocele (Pedron et al., 1997). Our results are in consonance with their observations, thus in the low dose neoplastic drug Oxaliplatin treatment (4 mg / KgBW /day for 15 days), we observed reduction in the size of acini, regression in the height of the columnar epithelium which nearly filled the complete acinus in the vehicle-treated group, reduction in the amount of secretion or even total loss from most of the tubules. The reduction or the shrinkage of the acini is in equivalence with
the increase of fibro-muscular tissue defining stromal-epithelial interactions (Blanchere et al., 2001) as well as Dunker and Kreiglstein, 2002 have shown expression of transforming growth factor beta (TGF-β) which is important in areas undergoing morphogenetic events, for instance those involving epithelium-mesenchymal interactions or differentiation. Similarly the flattened basal nuclei showed pyknosis and displacement. The above mentioned changes also points to androgen dependency of the gland, castration leads to apoptotic changes in the epithelium (reduction in DNA and protein content) but administration of exogeneous testosterone restores the original condition proving the dependency upon testosterone (Boronikhina and Iatskovskii, 2006). The high dose treatment (8 mg/kgBW/day for 5 days) further enhanced the regressive changes.

The aforementioned histopathological changes may due to androgen deprivation. 5α- dehydrotestosterone and its preferential retention within the nucleus where it is bound to androgen receptor via the action of 5α-reductase or due to an increased 17β-hydroxydsteroid dehydrogenase activity causing an increased conversion of testosterone to androstenedione or of dihydrotestosterone to 5-androstenedione, thus lowering the intracellular concentration of DHT derived from testosterone. It was also noted that prostate shows more histopathological changes than the seminal vesicle since it is more androgen dependent.

In conclusion, the Oxaliplatin manifested a strong antiandrogenic effects, thereby causing reduction of most of the androgenic parameters due to androgen deprivation.

REFERENCES


