The Evolution of Chemotherapy in Tumor Remedy; The Effects of Doxorubicin and Cisplatin as Chemotherapy on Brain Tumors in Rats

Kahled Ali Ajala*

Department of Medical Laboratories, University of Elmergib, Faculty of Medical Technology-Mesallata, Libya

kaajalah@elmergib.edu.ly

Abstract

An animal model created by implantation of glioblastoma tumor in rat brains. Groups of totally count 60 rats were subjected to 2 cycles per month of 150 mg/kg body weight of 2 different chemotherapeutic agents; doxorubicin and Cisplatin for 6 months with total cycles of 12 treatments. The animals were analyzed for survival (% median increase of survival time). The antitumor efficacy was measured for the 2 chemotherapeutic agents; Doxorubicin and Cisplatin, sections for Histopathology using hematoxylin and eosin (H&E) were evaluated under light microscope. Rats treated with doxorubicin and Cisplatin had significantly higher survival times compared with control groups with 10 rats and 7 rats still alive respectively at the end of the experiment. Histology confirmed lower tumor sizes and lower values for proliferation and apoptosis in treatment groups. There was no indication of neurotoxicity. This study showed that therapy with chemotherapeutic agents especially Doxorubicin and Cisplatin offer a chemotherapeutic potential for the treatment of human brain tumor.

Keywords: Chemotherapy, rats, brain tumor, Cisplatin, doxorubicin.

1. Introduction

Tumor is considered one of the largest causes of world death Bray e. al., 2021. Especially brain tumors which are one of these types belong to the most aggressive human cancers DeAngelis e. al., 2001. Cancer has been widely spread out throughout world countries but in the last

decades, advanced success in drug treatment of tumors especially in the field of chemotherapy has been improved (Wilson et al., 2019). Chemotherapeutics have desired toxic effects in differentiating tumor cells by the means of disturbing general cellular functions, Like DNA replication and mitosis (Barker et al., 2010). Chemotherapy is commonly used in the management of patients with locally advanced cancer such as breast, ovary and brain tumors to reduce the size of the primary tumor (Malhotra et al., 2004). These chemotherapeutic agents are used to chemically stop the growth and eliminate cancer cells. However, it does not distinguish between a cancer and normal cells. The majority of people diagnosed with cancer receive chemotherapy regimen that supplied in different active ingredients to treat cancer (Bonadonna et al., 1995). Cisplatin and doxorubicin are one of the most extensively used chemotherapeutic agents for the treatment of various cancers, including that of the brain, breast and liver. Cisplatin and doxorubicin drugs are different in their chemical classes and widely applied for chemotherapy of different types of cancer (de Graaf et al.. 1996). Establishment of evidence and validation of the chemotherapy regimen for different types of tumors are one of the important issues.

2. Materials and Methods

The experimental study was conducted over a period of 6 months to evaluate the effect of two chemotherapeutic agents; Doxorubicin and Cisplatin feed additives on Rats subjected to brain tumor implantation.

2.1 Animals

Sixty adult male Wistar rats weighing 200–250 g were housed for 1 week and caged in 6 groups. They were fed optimal ration of ad libitum with standard laboratory food and water. For tumor implantation; the experimental animals were injected pentobarbital (50 mg/kg) for anesthesia. An incision of 1.5 mm was made and tumor material from the frozen stock was introduced into syringe with 21 gauge needle. The tumor material was injected deeply into the right lateral ventricle. The scalp incision was closed by glue.

2.2 Experimental design

Tumor-bearing animals were randomly divided into 6 groups and received one of the following formulations: untreated control, doxorubicin in saline (DOX), Cisplatin in saline (CIS).

2.3 Drug treatment

These preparations were injected i.v. into the tail vein using the following dose regimen: 1.5 mg/kg on weeks 1 and 3 in each month. Antitumor efficacy was estimated by increase of median survival time (IST; table) as compared to control (ISTC, %) and to DOX (ISTD, %) and CIS (ISTcis, %). Results were statistically analyzed.

- Control animals that did not receive any treatment were randomized into 2 groups (1. 2)
- Animals were randomized into 2 groups (3. 4) that were treated with DOX formulations in the dose of 1.5 mg/kg.
- Animals in groups (5, 6) are treated with 1.5 mg/kg CIS formulation.

Table 1: *Division of experimental groups showing type, dose and period of treatment*

Group	Type of treatment	Dose of treatment	Period of treatment
1 and 2	Control (no ttt.)	-	Caged for 6 months
3 and 4	Doxorubicin	150 mg/kg	2 times / week for 6 months
5 and 6	Cisplatin	150 mg/kg	2 times/week for 6 months

2.4 Gross pathology

Randomly selected animals (3 of each group) were subjected to necropsy of the whole body, including the brain. Gross pathology of the brain was evaluated. Consecutively, the whole brains were fixed in phosphate-buffered formalin (4%, pH 7.4) for 4–48 hr., and then cut into 2 mm thick frontal slices, dehydrated and embedded in paraffin. Lungs, kidneys, spleens, livers and hearts were processed similarly.

2.5 Histology

Serial sections (5 μm thick) from paraffin-embedded tissues were cut and processed for staining with hematoxylin and eosin (H&E). Light microscopy visual field at a magnification of × 20 was used.

3. Statistical analysis

Means of pairwise log-rank tests were used for data evaluation. Significance levels for individual tests were adjusted according to Holm. Evaluated data are summarized descriptively by means of median survival times with their respective 95% confidence intervals. Statistical analysis used SAS/STAT procedure Life test.

4. Results

The median survival time for control groups was measured by counting the number of alive rats throughout the experiment which showed that brain tumor killed about 2 rats per month and more than 6 rats in the advanced stages of experiment (see table 2)

Table 2: *Median survival time of control groups (1 and 2) totally count 20 rats showing number of alive rats throughout 6 months of experiment*

Month	Number of alive rats
1st month	12
2nd month	10
3rd month	8
4th month	6
5th month	0
6th month	0

The excised tumors were weighed and its volume measured for the control groups which were bigger than its start volume throughout the experimental period (see table.3).

Table 3: *Tumor weight for control rats recorded in 1st and 3rd week of each month for 6 months.*

group	Treatment number (week/month)	Tumor weight
control	1	0.11±0.10
	2	0.19±0.13
	3	0.19±0.12
	4	0.22±0.16
	5	0.26±0.16
	6	0.28±0.21
	7	0.28±0.123
	8	0.35±0.22
	9	0.47±0.22
	10	0.58±0.28
	11	0.87±0.30
	12	0.95±0.33

The median survival time for groups that received Doxorubicin was measured by counting the number of alive rats throughout the experiment which showed that number of alive rats per month was improved from that of control group and 10 rats were still alive at the end of the experiment (see table 4)

Table 4: *Median survival time of Doxorubicin groups (3 and 4) totally count 20 rats showing number of alive rats throughout Doxorubicin treatment for 6 months*

Median survival time	Number of alive rats
1st month	17
2nd month	13
3rd month	13
4th month	13
5th month	10
6th month	10

The excised tumors were weighed and the volume measured was smaller than that of control group throughout the experimental period (table.5). The inhibitory rates of tumor treated by DOX were 40.0%, 42.0%, 52.0%, 57.7%, 77.0% and 88.4%, respectively for 6 months. The antitumor efficacy of DOX was illustrated to be superior to brain tumor of mice model ($P< 0.001$).

Table 5: *Tumor weight for rats received Doxorubicin recorded in 1st and 3rd week of each month for 6 months.*

group	Treatment number (week/month)	Tumor weight	P-Value
DOX TTT.	1	0.11±0.10	0.901
	2	0.19±0.12	0.622
	3	0.16±0.18	0.072
	4	0.15±0.13	0.07
	5	0.11±0.16	0.014
	6	0.10±0.10	0.08
	7	0.10±0.10	0.081
	8	0.9±0.12	0.063
	9	0.8±0.11	0.72
	10	0.8±0.11	0.068
	11	0.7±0.10	0.062
	12	0.5±0.10	0.062

The median survival time for groups that received Cisplatin was measured by counting the number of alive rats throughout the experiment which showed that number of alive rats per month was improved from that of control group and 7 rats were still alive at the end of the experiment (table 6)

Table 6: *Median survival time of Cisplatin (5 and 6) totally count 20 rats showing number of alive rats throughout Cisplatin treatment for 6 months*

Median survival time	Number of alive rats
1st month	17
2nd month	15
3rd month	13
4th month	13
5th month	8
6th month	7

The excised tumors were weighed and the volume measured was smaller than that of control group throughout the experimental period (table 7). The inhibitory rates of tumor treated by Cis were 35.0%, 42.0%, 48.0%, 52.7%, 65.0% and 77.4%, respectively for 6 months. The antitumor efficacy of CIS was illustrated to be superior brain tumor of mice model (P< 0.001).

Table 7: *Tumor weight for rats received Cisplatin recorded in 1st and 3rd week of each month for 6 months.*

group	**Treatment number (week/month)**	**Tumor weight**	**P-Value**
CIS TTT.	1	0.11±0.10	0.072
	2	0.19±0.12	0.07
	3	0.16±0.18	0.014
	4	0.15±0.13	0.08
	5	0.11±0.16	0.072
	6	0.10±0.10	0.081

	7	0.10±0.10	0.063
	8	0.9±0.12	0.72
	9	0.8±0.11	0.068
	10	0.8±0.11	0.062
	11	0.7±0.10	0.062
	12	0.5±0.10	0.081

- **Histologic evaluation for control groups**

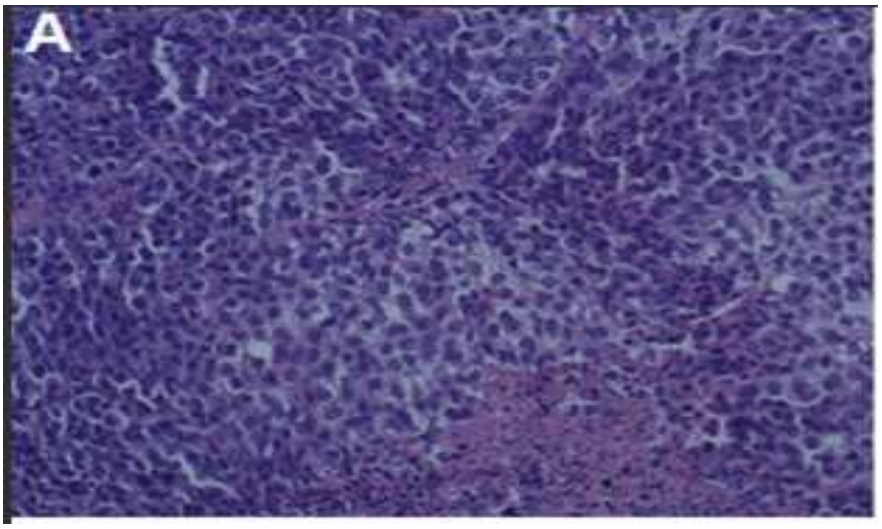

Figure 1: *Hematoxylin and eosin (H&E) stained tumor sections isolated from dead control rats.*

Histologic evaluation after Doxorubicin treatment

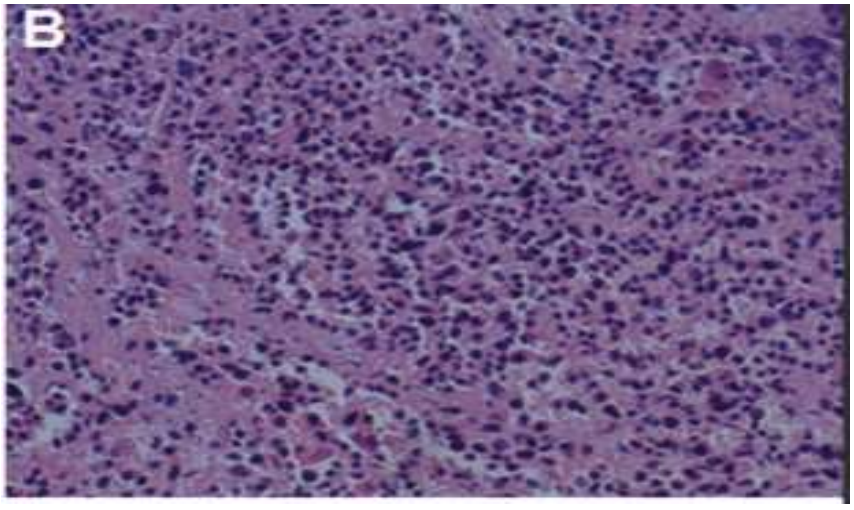

Figure 2: *Hematoxylin and eosin (H&E) stained tumor sections isolated from survival rats at the end of Doxorubicin treatment and this representative section from DOX-treated animal shows tissue necrosis.*

- **Histologic evaluation after CIS treatment**

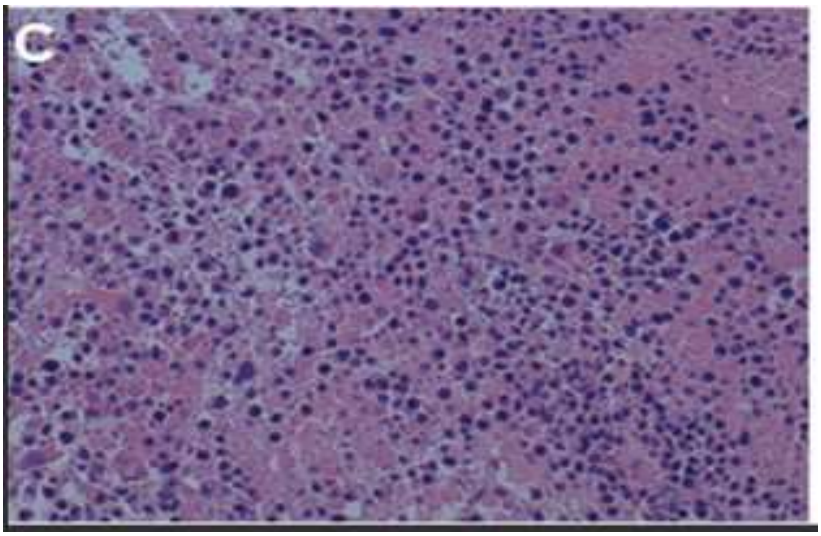

Figure 3: *Hematoxylin and eosin (H&E) stained tumor sections isolated from survival rats at the end of Cisplatin treatment and this representative section from CIS-treated animal shows tissue necrosis.*

5. Discussion

In the present study, the therapeutic potential of doxorubicin and Cisplatin for the chemotherapy of rats with implanted brain tumor was evaluated. A significant increase in survival time was found in the group of rats treated with doxorubicin and Cisplatin compared to control animals. Currently, some patients diagnosed with malignant glioblastoma receive chemotherapeutic drugs including DOX and CIS. At the time of surgery has been shown to increase 6-month survival by 50% and to prolong overall survival time. Brem et al., 1995. The results of the study of Gulyaev et al., 1999 suggested that doxorubicin was likely to be effective against brain tumors in a way that met with our present study. The results of the present experiments demonstrated the high efficacy of this formulation for therapy of rats with brain tumor. The chemotherapeutic agents provided a significant increase of the survival time in rats received DOX and CIS. compared to control groups which agreed with the study revealed by Yuan et al., 2008

In the brain sections assumed to histopathology, doxorubicin and Cisplatin -treated animals had showed a significant slower tumor growth with remarkable decrease in tumor sizes. There was no indication of short-term neurotoxicity. DOX and CIS had showed significant therapeutic levels in various studies in treating brain tumors, and successfully used to treat various types of tumor. The study by Abe et al., 1994 revealed that DOX and CIS had been used a chemotherapeutic agent and had been shown to inhibit tumor cell growth in various tumor lines and is currently used to treat a host of cancers from different malignant types of tumor in various body parts. Previous studies have shown that DOX is toxic to glioblastoma cell lines DiMeco et al., 2002. These previously mentioned studies have agreed with our point of view and showed that DOX and CIS being a potent example of chemotherapy that are highly potent inhibitor of tumors.

The results of the current study have obvious evidence that the high efficacy of these chemotherapeutic agents in rats with implanted brain tumor was obtained in the groups treated with 1.5 mg/kg of DOX and Cisplatin. These animals were sacrificed after 6 months and no signs of tumor could be observed by histologic examination. While the in vitro potency of DOX is remarkable and its current indications in treating peripheral tumors have proven efficacious, DOX has yet to be used successfully to treat malignant tumor Merker et al., 1978, In our study, we have shown that DOX and CIS being one of the most used chemotherapy have showed a remarkable tumoricidal activity against tumor cells. We found that DOX and CIS antagonized the growth of tumor cells when delivered at 1.5 mg/kg which agreed with previous studies by de Graaf et al., 1996. Cisplatin and Doxorubicin are thought to kill cells by triggering apoptosis which had been showed in the study revealed by Kishimoto et al., 2000. Chemotherapy is firmly established as a major therapeutic agent in the treatment of a wide variety of tumors when these agents had been used by 1.5 mg/kg of DOX and CIS. These animals had no signs of tumor could be observed by histologic examination by the end of the study. In addition, survival time was longer in the group treated with 1.5 mg/kg of DOX and CIS indicating a dose dependence of the treatment Success Stan et al., 1999. These results are showing a key success and promising future for chemotherapy. Our caution aimed that our experimental model has key acceptance in application to brain tumors in humans.

6. Conclusion

In conclusion, this study showed that Chemotherapy provides therapeutically effective treatment of doxorubicin and Cisplatin against tumor and also provides clear evidence that this approach offers new opportunities for chemotherapy of brain tumors. At the present stage, doxorubicin and Cisplatin which are types of chemotherapies appear to be one of the most effective chemotherapy in the treatment of in the CNS tumor. There are no indications of short-term neurotoxicity.

References

1. Abe T, Hasegawa S, Taniguchi K, Yokomizo A, Kuwano T, Ono M, Mori T, Hori S, Kohno K and Kuwano M: Possible involvement of multidrug-resistance-associated protein (MRP) gene expression in spontaneous drug resistance to vincristine, etoposide and doxorubicin in human glioma cells. Int J Cancer 58(6): 860-864, 1994.

2. Barker N, Bartfeld S, Clevers H. Tissue-resident adult stem cell populations of rapidly self-renewing organs. Cell Stem Cell. 2010;7(6):656-670. doi:10.1016/j.stem.2010.11.016.

3. Bonadonna G, Valagussa P, Moliterni A, Zambetti M, Brambilla C. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer: the results of 20 years of follow-up. N Engl J Med. 1995; 332:901-906.

4. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer. 2021;127(16):3029-3030. doi:10.1002/cncr.33587

5. Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black K, Sisti M, Brem S, Mohr G, Muller P, Morawetz R and Schold SC: Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery of biodegradable polymers of chemotherapy for recurrent gliomas. Lancet 345(8956): 1008- 1012, 1995.

6. de Graaf H, Willemse PH, Bong SB, Piersma H, Tjabbes T, van Veelen H, Coenen JL, de Vries EG. Dose intensity of standard adjuvant CSF with granulocyte colony-stimulating factor for premenopausal patients with node-positive breast cancer. Oncology. 1996; 53:289-294.

7. DeAngelis LM. Brain tumors. N Engl J Med 2001; 344: 114– 23. CrossrefCASPubMedWeb of Science®Google Scholar

8. DiMeco F, Li KW, Tyler BM, Wolf AS, Brem H and Olivi A: Local delivery of mitoxantrone for the treatment of malignant brain tumors in rats. J Neurosurg 97: 1173-1178, 2002.

9. Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. Pharm Res 1999;16:1564 –9.

10. Kishimoto S, Miyazawa K, Terakawa Y, Ashikari H, Ohtani A, Fukushima S, Takeuchi Y. Cytotoxicity of cis-[((1R,2R)-1,2-cyclohexanediamine-N,N')bis(myristato)]-plati num (II) suspended in Lipiodol in a newly established cisplatin- resistant rat hepatoma cell line. Jpn J Cancer Res 2000; 91:1326-32.

11. Malhotra V., V. J. Dorr, A. P. Lyss, C. M. Anderson, S. Westgate, M. Reynolds, B. Barrett, M. C. Perry, Clin Breast Cancer 2004, 5, 337..

12. Merker PC, Lewis MR, Walker MD and Richardson EP Jr: Neurotoxicity of doxorubicin (doxorubicin) perfused through the cerebrospinal fluid spaces of the rhesus monkey. Toxicol Appl Pharmacol 44(1): 191-205, 1978.

13. Stan AC, Casares S, Radu D, Walter GF and Brumeanu TD: Doxorubicin-induced cell death in highly invasive human gliomas. Anticancer Res 19(2A): 941-950, 1999.

14. Wilson BE, Jacob S, Yap ML, Ferlay J, Bray F, Barton MB. Estimates of global chemotherapy demands and corresponding physician workforce requirements for 2018 and 2040: a population-based study. Lancet Oncol. 2019;20(6):769-780. doi:10.1016/S1470-2045(19)30163-9.

15. Yuan JN, Chao Y, Lee WP, Li CP, Lee RC, Chang FY, Yen SH, Lee SD, Whang-Peng J. Chemotherapy with etoposide, doxorubicin, cisplatin, 5-fluorouracil, and leucovorin for patients with advanced hepatocellular carcinoma. Med Oncol. 2008; 25:201-6.