

An experimental study of Ehrlich's ascetic carcinoma in Swiss albino mice with chemotherapy

Utpal Goswami¹, Swatisikta^{2*}, Anupam Brahma³, Amit Kumar Verma⁴, Anshuman Panda⁵, Sukanta Sen⁶

¹Assistant Professor, Department of Pathology, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India

²Assistant Professor, Department of Anaesthesiology, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India

³Post Graduate Trainee, Department of Pathology, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India

⁴Associate Professor, Department of Pharmacology, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India

⁵Associate Professor, Department of General Surgery, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India

⁶Professor, Department of Pharmacology, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India

Received: 05-12-2019 / Revised: 05-01-2020 / Accepted: 12-02-2020

ABSTRACT

Background: Ehrlich-Lette ascites carcinoma (EAC) is also known as Ehrlich cell. It was originally established as an ascites tumor in mice. The current concept that cytotoxic chemotherapeutic agents are administered at a dose to the maximum a patient can tolerate before the onset of severe and even life-threatening toxicity is still in wide clinical use. This study was conducted to evaluate the response of chemotherapy in the treatment of Ehrlich's ascetic carcinoma. The various parameters to assess the response of various therapeutic schedules were regression of tumor by decrease in body weight of mice and decrease in abdominal girth; cell count of ascetic fluid and morphological changes of tumor cells after treatment with drugs and to study the percentage viability of the cells. **Materials & Methods:** A mouse bearing the tumor strain was taken from our laboratory in the Department of Pathology, IPGMER, Kolkata, where the strain was being maintained serially by inoculation of malignant cells into healthy mice every 8-10 days. In our work altogether 25 mice were taken for each set of experimental work. They were divided in four groups of 5-10 mice in each group. **Results:** All the mice in Group III revealed extensive metastasis with haemorrhagic ascetic fluid. About 60% mice of Group IV showed metastatic but there was no haemorrhagic fluid. None of the mice of Group IV survived beyond 35 days. Group III mice survived for a maximum of 25 days. Most of them died between 2-24 days. **Conclusion:** With the chemotherapy there was reduced cell count and cell viability. Actinomycin D alone produced a moderate effect on tumor cells.

Key words: Ehrlich's ascetic carcinoma, Swiss albino mice, Chemotherapy, Actinomycin D, Survival

©2020The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Cancers are caused by mutations that may be inherited, induced by environmental factors, or result from DNA replication errors.^[1]

Aging is the main risk factor for carcinogenesis in multicellular animal organisms including humans.^[2] Cancer is ranked as the first or second leading cause of death in 91 of 172 countries and is third or fourth in an additional 22 countries.^[3] Cancer is the second and fourth leading cause of adult death in urban and rural India, respectively. Cancer is now the leading cause of catastrophic health spending, distress financing, and increasing expenditure before death in India.^[4,5] In the early 1900s, the famous German chemist Paul Ehrlich set about developing drugs to treat infectious diseases.

Address for Correspondence

Dr. Swatisikta

Assistant Professor, Department of Anaesthesiology, ICARE Institute of Medical Sciences and Research, Banbishnupur, Purba Medinipur, Haldia, West Bengal 721645, India.

E-mail: drswatisikta@gmail.com

He was the one who coined the term “chemotherapy” and defined it as the use of chemicals to treat disease. He was also the first person to document the effectiveness of animal models to screen a series of chemicals for their potential activity against diseases, an accomplishment that had major ramifications for cancer drug development. In 1908, his use of the rabbit model for syphilis led to the development of arsenicals to treat this disease. Ehrlich was also interested in drugs to treat cancer, including aniline dyes and the first primitive alkylating agents, but apparently was not optimistic about the chance for success.^[6] For most primary tumors the treatment of choice is surgery and radiotherapy, which measures can be very effective for controlling localized tumors and indeed surgery and radiotherapy dominated the field of cancer therapy into the 1960s. However, at the time of diagnosis the majority of cancers have already microscopically metastasized throughout the body, leading to recurrent disease in the majority of cancer patients. In view of this, systemic chemotherapy is required to control outgrowth of metastases. Although for some invasive tumors at advanced disease stages, chemotherapy might be administered up front to allow better surgery, in essence presently the chemotherapy of cancer is the treatment of metastases, either known or assumed, except for hematological malignancies. The current concept that cytotoxic chemotherapeutic agents are administered at a dose to the maximum a patient can tolerate before the onset of severe and even life-threatening toxicity is still in wide clinical use.^[7]

OBJECTIVES

This study was conducted to evaluate the response of chemotherapy in the treatment of Ehrlich’s ascetic carcinoma. The various parameters to assess the response of various therapeutic schedules were as follows:

1. To assess the regression of tumor by decrease in body weight of mice and decrease in abdominal girth
2. To study the cell count of ascetic fluid after treatment with drugs
3. To study morphological changes of tumor cells after subjecting it to antineoplastic drug
4. To study the percentage viability of the cells
5. To study the animal survival

MATERIALS & METHODS

Transplantation of Ehrlich’s ascetic carcinoma in mice (ascetic form): the proliferation of malignant cells of the Ehrlich’s ascetic carcinoma strain simulates the biology of any neoplasia. Hence this study forms a tumor model on which different antineoplastic agents have been tried to study the kind of therapy which would form the best mode of treatment. The different

antineoplastic regimen that have been undertaken in our study were effects on Ehrlich’s ascetic carcinoma (EAC) in mice when treated with actinomycin D; mitomycin C and actinomycin D and effects of EAC with radiotherapy alone.

Tumor strain

A mouse bearing the tumor strain was taken from our laboratory in the Department of Pathology, IPGMR, Kolkata, where the strain was being maintained serially by inoculation of malignant cells into healthy mice every 8-10 days. The mouse that was taken had a huge ascites from which cells were taken to inoculate mice further. Male Swiss A albino mice weighing about 16-18 grams were chosen for our study.

Transplantation

At first a preliminary diagnosis was made regarding the presence of ascites in the mice by noting the gain in weight; gain in abdominal girth and by palpating the abdomen. Then with the help of a sterile tuberculin syringe and a needle of 21 gauge, 0.5 ml sterile normal saline was taken and peritoneal cavity was punctured. This 0.5 l sterile normal saline was then pushed into the peritoneal cavity to dilute the cell suspension in the immediate vicinity of the needle and to separate any fibrin coagulum if formed, so that the cell suspension could flow out freely. Sample with whitish fluid was taken in the syringe. This was then diluted 1:10 in sterile physiological saline. A drop from this suspension was taken and changed in the Neubauer’s haemocytometer. A cell count was done under the optical microscope. The cell suspension was again diluted in sterile normal saline to obtain a desired cell suspension of about 10^7 cells/ml. Simultaneously 0.02 ml cell suspension was taken, diluted in the ratio of 1:10 in 0.15% trypan blue made up in physiological saline. A drop of cell suspension was taken and a coverslip preparation was made to study the viability of the cells. The viable cells did not take up any stain whereas the dead cells were stained blue. Percentage of viable cells was noted down. The slides made out from smears of centrifuged deposit were stained by haematoxylin and eosine which showed large malignant cells with hyperchromatic nuclei with nucleoli and scanty cytoplasm [Fig. 2]. After having confirmed the presence of malignant cells, the suspension was used to inoculate the mice. About 0.1ml of prepared suspension was then taken and diluted in 0.4 ml of sterile normal saline and this mixture (0.5 ml) was inoculated into each mouse intraperitoneally. Thus the desired cell concentration of 10^6 cells per inoculum dose was obtained. The mice were maintained on pellets prepared by Hindustan Lever Company and water ad libitum. The weight of

individual mice with their abdominal girth was noted before starting the experiment.

Animal grouping

In our work altogether 25 mice [Fig. 1] were taken for each set of experimental work. They were divided in four groups of 5-10 mice in each group. The group distribution was as follows.

Group I: Healthy mice which were maintained on feed and water only. These served as healthy controls.

Group II: Mice who were given only therapy without any preceding malignant cell inoculation.

Group III: Mice who were inoculated with the tumor cells and left as such without any treatment. These served as tumor controls [Fig. 3].

Group IV: Mice who were given therapy on the tumor they were bearing.

Methods of observations

Each mouse was weighed in gms before transplantation of tumor strain; before starting treatment and 5 days after the end of treatment. Abdominal girth of each mouse was taken in cms in the same schedule as above. The effect of therapy was recorded according to the protocol laid by Sugiura K (1961).^[8]

[+++] Indicates complete inhibition or diminution of ascites

[++] Marked inhibition indicating no abdominal distension, no gross ascites

[+] Moderate inhibition indicating slight distension; the fluid volume increased only upto $\frac{1}{4}$ of the volume increased only upto $\frac{1}{4}$ of the volume of the tumor control

[±] Slight inhibition indicating moderate abdominal distension, the fluid volume increased upto $\frac{1}{2}$ of the volume of the tumor control

[-] No effect, indicating marked abdominal distension, the fluid volume increased upto $\frac{3}{4}$ or more of the volume of the tumor control. Survival time of each mouse was noted in relation to the life of healthy and drug control mice. In case of any death of any mouse a post-mortem was carried out to find out the cause of death. Tissues from lung, liver and kidneys were taken and were fixed in 10% formalin solution. Tissues were processed as usual and paraffin sections were made and stained with haematoxylin and eosine. Each set of experiment was done twice.

RESULTS

Table 1: Effect of actinomycin D on Ehrlich's ascetic carcinoma in mice

Groups	Before therapy: after 7 days of tumor cell inoculation				After therapy: 5 days after cessation of therapy			
	Avg. Wt. Of mice (gms.)	Avg. Abdom. Girth (cm)	Avg. Cell count (per cmm)	Cell surviving after 2 hr (%)	Avg. Wt. of mice (gm)	Avg. Abdom. Girth (cm)	Avg. Cell count (per cmm)	Cell surviving after 2 hr (%)
I	19	6.3	Nil	-	20	6.5	Nil	-

Experiment

About 25 male Swiss albino mice aged around 4-6 weeks were taken and their individual weight and abdominal girth measured. Of them 15 were inoculated with the tumor cells intraperitoneally by aseptic means. The individual inoculus dose was 10^6 cells approximately. The mice were divided into 4 groups.

Group I: 5 Healthy control mice

Group II: 5 drug control mice. These healthy non-tumor bearing mice were given the drug actinomycin D in the therapeutic concentration of 2 mcg.

Group III: Tumor control mice- these 5 mice were inoculated with Ehrlich's ascetic carcinoma cells. They were kept untreated.

Group IV: Treatment group: 10 mice. These tumor bearing mice which had been inoculated with Ehrlich's ascetic carcinoma cells were subjected to treatment with actinomycin D in its therapeutic concentration.

Confirmation of Successful Transplantation

After about 5-7 days of inoculation any gain in weight and abdominal girth was noted. By careful palpation it was seen that the abdomen showed evidence of fluid collection. A peritoneal tap was done carefully in an aseptic manner and the fluid obtained from the peritoneum was simultaneously diluted in normal saline to give a 1:20 dilution. This fluid was seen under the optical microscope in a Neubauer chamber. A cell count was done and recorded. A centrifuged deposit of the cell suspension was taken and stained with Haematoxylin and eosin [Fig. 2]. The presence of large cells with hyperchromatic nuclei, nucleoli and a thin rim of basophilic cytoplasm confirmed the presence of malignant cells in the peritoneal fluid. Immediately a viable count was done and it was seen that about 90-95% cells were viable. Now a treatment schedule was adapted. Actinomycin D was obtained from Sigma chemical compound contained 5 mg in a sealed vial. It was diluted in sterile distilled water to give a concentration of 2 mcg in 0.2 ml distilled water. The period of treatment was 10 days. Actinomycin D was injected intraperitoneally carefully so that there was no spillage of actinomycin D on the peritoneum. Only after confirming that it was in the cavity which was done by first withdrawing some fluid from the cavity, only than the drug was pushed in. Thus the total dose that was given was 20 mcg per mouse.

II	18	6	Nil	-	17.5	5.8	Nil	-
III	24	7.5	4×10^5	85	32	13.3	9×10^8	75
IV	24	7.6	4×10^5	85	27	9.6	5×10^5	55

All the healthy mice gained in weight and abdominal girth. The drug control mice lost some weight. After the treatment, morphological features of aspirated cells in smear stained in haematoxylin and eosine showed the following features: degeneration of cells marked by vacuolation of cytoplasm; there is diminution in size of the nucleus and the nuclear cytoplasmic ratio had diminished [Figure. 4 & 5].

Those mice which died, a post mortem was carried out to find the cause of death. All the mice in Group III revealed extensive metastasis with haemorrhagic ascetic fluid. About 60% mice of Group IV showed metastatic but there was no haemorrhagic fluid. None of the mice of Group IV survived beyond 35 days.

Group III mice survived for a maximum of 25 days. Most of them died between 2-24 days. Therefore we can see that healthy mice gained in weight and abdominal girth whereas mice of Group II i.e. which served as drug treatment control lost weight as well as there was reduction of abdominal girth. There was a partial reduction in the malignant cell count of them mice in Group IV. None of the mice showed complete absence of malignant cells from the peritoneal fluid. All mice of Group III died within 20-25 days of inoculation. A complete cure could not be achieved by this drug schedule, which probably could have been achieved by increasing the dose of the drug.



Figure 1: Two mice, one healthy on the right and another ascetic tumor bearing mice on the left. Their age and abdominal girth were nearly similar before tumor inoculation

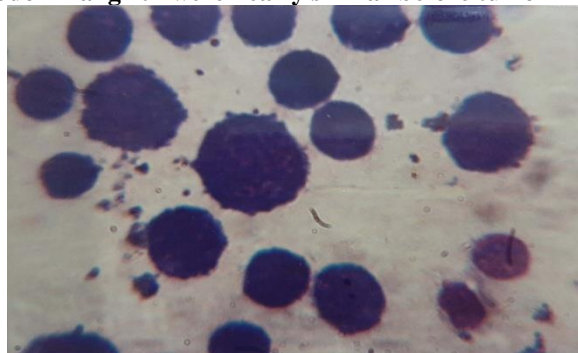


Figure 2: A haematoxylin and eosine stained smear of centrifuged deposit of peritoneal fluid. Large cells with hyperchromatic nuclei with a very thin rim of basophilic cytoplasm (x 400)

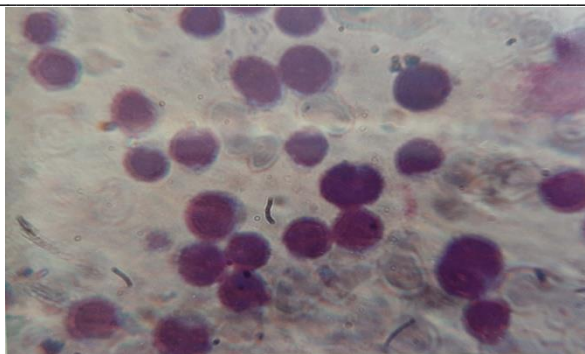


Figure 3: Confirmation of a successful tumor uptake in the peritoneum before treatment with actinomycin D (X 160)

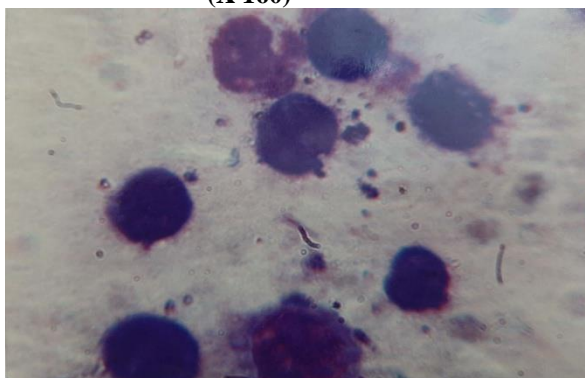


Figure 4: Morphology of cells after treatment with actinomycin D. Cells showed alterations in nuclear:cytoplasmic ratio, vacuolation of the cytoplasm, and nuclear vacuolation (x 400)

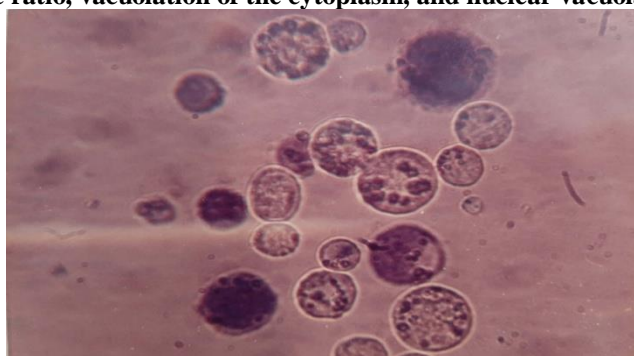


Figure 5: A viability of cells after 2 hrs of incubation showed increased number of nonviable cells as compared to control after treatment with actinomycin D (x 160)

DISCUSSION

Ehrlich-Lette ascites carcinoma (EAC) is also known as Ehrlich cell. It was originally established as an ascites tumor in mice. The tumor was cultured *in vivo*, which became known as the Ehrlich cell. After 1948 Ehrlich cultures spread around research institutes all over the world. The Ehrlich cell became popular because it could be expanded by *in vivo* passage. This made it useful for biochemical studies involving large amounts of tissues. It could also be maintained *in vitro* for more carefully controlled studies. Culture techniques in large-scale, mice passage are less attractive, due to the contamination of the tumor with

multifarious host inflammatory cells. EAC is referred to as undifferentiated carcinoma, and is originally hyper-diploid. The permeability to water is highest at the initiation of the S phase and progressively decreases to its lowest value just after mitosis. Activation heats for water permeability vary during the cell cycle, ranging from 9–14 kcal/mole.^[9] Actinomycin D is a well-known antibiotic of the actinomycin group that exhibits high antibacterial and antitumor activity. Actinomycin D has been widely used in clinical practice since 1954 as an anticancer drug for treating many tumors and it is also a useful

tool in biochemistry and molecular biology. There are several mechanisms of its action that are responsible for its cytotoxic and antitumor action, these being associated with DNA functionality, leading to RNA and, consequently, protein synthesis inhibition. The two main mechanisms are intercalation to DNA and the stabilization of cleavable complexes of topoisomerases I and II with DNA, in which a phenoxazone ring localizes between GpC base pair sequence in DNA and polypeptide lactones rings occupy a position in the minor groove of the DNA helix or the drug penetrates to a place in the DNA structure where topoisomerase binds with DNA, respectively. Moreover, the slow dissociation of actinomycin D from DNA complexes, its photodynamic activity and free radical formation, as well as other biochemical effects of activity of actinomycin D may be, as suggested, important factors that influence the biological activity of this drug.^[10]

CONCLUSION

With the chemotherapy there was reduced cell count and cell viability. Actinomycin D alone produced a moderate effect on tumor cells. We can see that healthy mice gained in weight and abdominal girth whereas mice of Group II i.e. which served as drug treatment control lost weight as well as there was reduction of abdominal girth. There was a partial reduction in the malignant cell count of them mice in Group IV. None of the mice showed complete absence of malignant cells from the peritoneal fluid. All mice of Group III died within 20-25 days of inoculation. A complete cure could not be achieved by this drug schedule, which probably could have been achieved by increasing the dose of the drug. EAC has a resemblance with human

tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate.

REFERENCES

1. Tomasetti C, Li L, Vogelstein B: Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. *Science* 2017; 355:1330-1334.
2. Mallath MK, Taylor DG, Badwe RA, et al: The growing burden of cancer in India: Epidemiology and social context. *Lancet Oncol* 2014;15:e205-e212.
3. Bray F, Ferlay J, Soerjomataram I. Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; 68:394-424.
4. Rajpal S, Kumar A, Joe W. Economic burden of cancer in India: Evidence from cross-sectional nationally representative household survey, 2014. *PLoS One* 13:e0193320.
5. Smith RD, Mallath MK. History of the Growing Burden of Cancer in India: From Antiquity to the 21st Century. *Journal of Global Oncology* 2019;5, 1-15.
6. DeVita Jr VT, Chu E. A History of Cancer Chemotherapy. *Cancer Res* .2008; 68 (21):8643-8653.
7. Sparreboom A, Verweij J. Advances in cancer therapeutics. *Clin Pharmacol Ther.* 2009;85(2):113-117.
8. Sugiura K, Schmid FA, Schmid MM. Antitumor Activity of Cytosan. *Cancer Res* 1961;21:1412-1420.
9. DuPre AM, Hempling HG. Osmotic properties of ehrlich ascites tumor cells during the cell cycle. *J Cell Physiol.* 1978;97 (3): 381-96.
10. Koba M, Konopa J. Actinomycin D and its mechanisms of action. *Postepy Hig Med Dosw* 2005;59:290-8.

How to cite this Article: Goswami U, Swatisikta, Brahma A, Verma AK, Panda A, Sen S. An experimental study of Ehrlich's ascetic carcinoma in Swiss albino mice with chemotherapy. *Asian Pac. J. Health Sci.*, 2020; 7(1):91-96.

Source of Support: Nil, **Conflict of Interest:** None declared.