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# The Effect of in Situ Gene Therapy of Tumor in Pregnant Mice.

KEYWORDS

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**ABSTRACT** *The aim of study to investigation the side effect of gene therapy in mice by the local injection of ( DNA with RNA of camel ) in the mice were adinoarcinoma infected, ( 30 mice were treated ,10 control and 6 healthy males ) . The method of Chloroform -phenol was utilized for extracted the nucleic acid from liver of camel . Treated mice were classified into two groups, The first group (15 mice ) subjected for local injection in the tumor while the second group(15 mice) treated by intramuscular injection. Treated females mixed with the male in order to conception. The control group and the second group were dead during 5-14 day according to the severity of case , while (13 mice ) at the first group has recovered when intra tumor injection of camel nucleic acid. Survived mice completed pregnancy subsequent by bred normal fetuses without abnormality, the birth number ranged between 5-7 born for every mice , new birth leave up to adulthood. Consequently the new generation leave with the adult male for conception and fertility. Another generation of pregnant mice has bred normal fetuses without abnormalities. In conclusion the direct injected of (DNA with RNA of camel) in the adenocarcinoma more efficient than in the intramuscular injection, in addition, no complications in the pregnant mice or fetuses , means the direct injection of genes lead to mortality of cell tumor.*

**Introduction**

Genetherapy refers to the transfer of a gene that encodes a functional protein into a cell or the transfer of an entity that will alter the expression of anendogenous gene in a cell. 1 gene therapy could be delivered by a simple injection or even a nasal spray2 . According to 3in vivo gene therapy is portable and scalable, meaning multiple doses can be administered to patients based on the disease and individual response to therapy." Higher infectious titers, or serial injections over days, may increase the number of genetically modified cells and lower the clonal dominance. Furthermore, adjusting the dose and the strength of the promoter driving corrected gene expression could optimize clinical outcomes. The observations clearly suggest that retroviral gene insertion may have been the cause of the leukaemia here. However, other factors such as genetic background and preceding viral infection may have also played a role in the development of this child's disease 4 .The most common gene therapy vectors are viruses because they can recognize certain cells and carry genetic material into the cells' genes. Researchers remove the original disease-causing genes from the viruses, replacing them with the genes needed to stop disease. This technique presents the following risks:Unwanted immune system reaction. The body's immune system may see the newly introduced viruses as intruders and attack them. This may cause inflammation and, in severe cases, organ failure. Targeting the wrong cells. Because viruses can affect more than one type of cells, it's possible that the altered viruses may infect additional cells not just the targeted cells containing mutated genes. If this happens, healthy cells may be damaged, causing other illness or diseases, including cancer.Infection caused by the virus. It's possible that once introduced into the body, the viruses may recover their original ability to cause disease. Possibility of causing a tumor. If the new genes get inserted in the wrong spot in your DNA, there is a chance that the insertion might lead to tumor formation. This has occurred occasionally in some clinical trials 5. Camel urine showed cytotoxicity against various, but not all, human cancer cell lines, with only marginal effect on non-tumorigenic epithelial and normal fibroblast cells. Treated mice were classified into two groups, The first group (15 mice ) subjected for local injection in the tumor while the second group(15 mice) treated by intramuscular injection. Gene therapy by intramuscular injection of necked DNA with RNA an able to enter the cell led to no expression and failed treatment 14.While the intranuclear disposition of exogenous DNA is highly important for the therapeutic effects of the administrated DNA 15. If the new genes get inserted in the wrong spot in your DNA, there is a chance that the insertion might lead to tumor formation, this has occurred occasionally in some clinical trials. The control group it is died at varying periods depending on the severity of the condition , the duration reached three weeks. The second group were dead during (5-14) day according to the severity of case and may be the treatment intramuscular lead to some stress of animals. Viral delivery systems are very efficient gene transfer agents, but issues associated with immunogenicity, toxicity and production have driven the clinical need for effective non-viral modes of delivery, however the expression is generally transient and decreases rapidly after 10–14 days 16. Recovered the first group with the healing of adinoarand spindle mesenchymal cells of sarcomatous component Sarcomatous component: Multinucleated giant cells with bizarre mitotic activity 17. SDC showed typical cribriform architecture, whereas anaplastic, spindled cells constituted the sarcomatoid areas. 18 Carcinomatous elements were characteristic of SDC and resembled ductal carcinoma of the breast, with a cribriform, papillary, or solid growth pattern , frequently the central portion of the cell clusters showed comedo-like necrosis, and sometimes a scirrhous 19

Nucleic acid, such as DNA, RNA can be delivered alone, or packaged by carrier to increase expression of a therapeutic gene or knockdown expression of specific gene 14. In this study the ( Camel gene) used in gene therapy for killed the tumor cells. It is established at the study by 6 . Camel urine showed cytotoxicity against various, but not all, human cancer cell lines, with only marginal effect on non-tumorigenic epithelial and normal fibroblast cells. Treated mice were classified into two groups, The first group (15 mice ) subjected for local injection in the tumor while the second group(15 mice) treated by intramuscular injection. Gene therapy by intramuscular injection of necked DNA with RNA an able to enter the cell led to no expression and failed treatment 14.While the intranuclear disposition of exogenous DNA is highly important for the therapeutic effects of the administrated DNA 15. If the new genes get inserted in the wrong spot in your DNA, there is a chance that the insertion might lead to tumor formation, this has occurred occasionally in some clinical trials. The control group it is died at varying periods depending on the severity of the condition , the duration reached three weeks. The second group were dead during (5-14) day according to the severity of case and may be the treatment intramuscular lead to some stress of animals. Viral delivery systems are very efficient gene transfer agents, but issues associated with immunogenicity, toxicity and production have driven the clinical need for effective non-viral modes of delivery, however the expression is generally transient and decreases rapidly after 10–14 days 16. Recovered the first group with the healing of adinoar-

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blast cells epithelial and fibroblast cells 6.

**Therefore this study included :**

1-Investigation the side effect or abnormalities in pregnancy , fetuses , and offspring's.
2-DNA with RNA extracted from liver of camel
3-Treated mice from adenocarcinoma by necked nucleic acid of camel .
4-Avoid any vectors in order to Eschews the side effect of it .

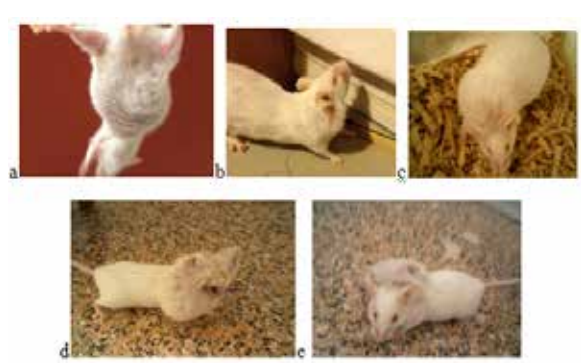
**Materials and methods**

This study conducted on ( 40 infected mice with adenocarcinoma) Figure 1, and 6 healthy males. Classified to 15 mice injected in situ tumor for gene therapy , 15 mice treated intramuscular injection , and 10 mice for control .The mice suffering from adinoarcinoma in deferent site of body like parotid gland . The total genome utilized for gene therapy extracted from the liver of camel in Iraq . Chloroform – phenol methods utilized for nucleic acid by lyses buffer solution ( 100 nM NaCl , 1 nM ETDA , 10 nM Tris-HCLpH8.0) with 5% SDS in the eppindorf tube 7 ( figure 2 ) Extraction of DNA, RNA, and protein is the basic method used in molecular biology 8. The DNA running by electrophoresis figure 3 to insure the present it. Total RNA remains in the upper aqueous phase, while most of DNA and proteins remain either in the interphase or in the lower organic phase 9. Addition of chloroform followed by centrifugation separates the solution into an aqueous phase containing the RNA and an organic phase. RNA is recovered by precipitation from the aqueous phase with isopropyl alcohol .After removal of the aqueous phase, the DNA and proteins in the sample can also be recovered by sequential precipitation with ethanol and additional isopropanol 10. Camel genome injected in to mice before and during pregnancy. Electrical current were used through the treatment of animal, it is utilized the electrical power supply provider with prop it is used pulses of 3-6 volts at the time of DNA with RNA injection. Recently, gene transfection into target cells using naked DNA, which is a simple and safe approach, has been improved by combining several physical techniques,the electroporation, gene gun, ultrasound and hydrodynamic pressure 11 Histological examination , routine stain hematoxiline –eosine used for staining and microscopical examination for tumor tissue . Electroporation enhances uptake of injected plasmid DNA into muscle and skin 12.

**Results and Discussion**

The study was aimed to investigation the side effect of gene therapy by the local injection of necked DNA with RNA in the tumor. Classical models of gene therapy rely on recombinant protein expression. In contrast, RNA interference (RNAi) therapy is a novel technology that uses short regulatory RNA sequences to modulate gene expression as its basic principle. 13 .May be the necked DNA mixed RNA disappeared by the local immunity reaction because no vector shuttle, protected against the biological environment with no entrance in the cell . The mice display macro and micro pathological features figure 4 . In the gross lesion appears redness different in the size of mass , coated with the hair or partial coated , advanced stages like cauliflower .The histological examination show mitotic figure in the cell with different stages of division, infiltrated by inflammatory cells and some areas of necrosis with some fibrosis figure 4. Microscopic findings, Carcinomatous component of carcinosarcoma: Large ductal structures with central comedo-type necrosis as salivary ductal carcinoma, intermixed remnants of duct-like forms cinoma subsequent by male mated followed pregnancy figure 5 . The number of recovered mice 13 figure 6 while 2 mice dead table 1 within 21 days , the reaction in situ injection obvious in the tumor , represented by local redness , enlarged , in the early stage of treatment subsequent by the shrink with atrophy , eventually disappeared the mass of tumor with complete covered by new white hair .

Survived mice completed pregnancy and bred normal fetuses figure 6 without abnormality. Electrophoresis of first group mice liver tissue DNA did not appeared any trace of camel DNA .We are avoid the vector in this study for transmit of nucleic acid . Create the non-viral vectors of synthetic materials for enhanced transfection efficiency of gene into mammalian cells both in vitro and in vivo, briefly over reviewing several researches about non-viral vectors, recent research trials about drug delivery system (DDS) of gene are introduced to show significance and future direction of gene delivery technology in tissue engineering 20-21.Vectors and transgenic that offer more than one mechanisms-of-action need to be explored and combined, the understanding of tumor biology, vectorlogy and immunology needs to be strengthened in order to improve efficacy and minimizing toxicity 22.Many vectors led to troubles after treatment , another reason for avoid the viral vector to avoid any reaction my stimulated the systemic immunity against therapeutic nucleic acid which used for treatment . Controlling the symptoms of infection is important because negative immune system responses may reduce the efficacy of gene therapy and make it difficult to repeat treatments ,the observations clearly suggest that retroviral gene insertion may have been the cause of the leukaemia 23 .It is still unclear exactly how this retroviral integration led to the development of neoplasia and documenting this process is an important future goal 24. Five major approaches have been tested in clinical trials: tumor suppressor gene replacement, prodrug-activating enzyme delivery, oncolytic virotherapy, antisense oligonucleotide delivery, and cytokine immuno-gene therapy 22.Although viral-mediated gene therapy has been at the forefront of the field, several non-viral gene therapy approaches have been applied to animal and other cellular models. These include plasmid-mediated gene delivery, antisense-mediated exon skipping, and oligonucleotide-mediated gene editing 25 We are conclude that the direct injection of DNA with RNA lead to toxicity and mortality of cell tumor without any complications of normal cells and without interference with the animal fertility and pregnancy.




**Fiqure 1 :** a-adenocarcinoma in the lateral side in front of right hind limbs. b- adenocarcinoma in parotid gland. c-adenocarcinoma in the two side of parotid gland extend over the head. d- before treatment the mass of tumor rotate the head at the right side . e- after treatment some

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necrotic region show with partial loss of hear.

**Figure 2 :** Shows eppindorf tube contained three phase : aqueous phase ,intermediate phase and organic phase , in the chloroform – phenol method

**Figure 3:** Camel DNA running in the gel electrophoresis . Camel chromosomal DNA bands on 2% agarose gel after 1 hour electrophoresis at 50 volt

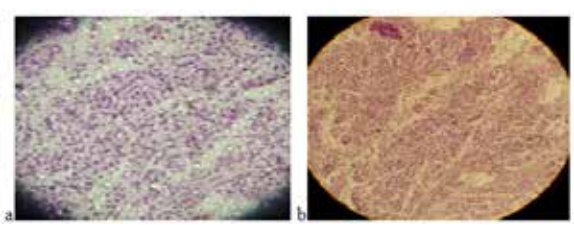

**Figure 4 :** adenocarcinoma in the parotid gland , show the mitotic figure and some necrotic defect with infiltration by inflammatory cells . (H + E×400)in a and b (H + E×200)

**Figure 5 :** pregnant mice with swelling of adenocarcinoma in the lateral side in front of right hind limbs

**Figure 6-** Healthy young fetuses with coated by hair in the age of one month .

| Mice groups | In situ tumor injection treatment | Intramuscular injection treatment | Control With out treatment |
|---|---|---|---|
| Before treatment | 15 | 15 | 10 |
| Alive | 13 | zero | zero |
| Dead | 2 | 15 | 10 |

**Table:1-**The treatment of tumor in situ of tumor and intra-muscular, with the control group

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