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Acute Tumor Inflammation with CD4/8+ and CD11+ Prolong The Survival Effect Induced by Intratumral Injection Optimum Combination of Chemotherapy Drugs with Hydralazine as Hapten in Animal Model

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Keyword:

Intratumoral therapy; Hapten; Abscopal effect; Immune therapy

1. Abstract

1.1. Purpose

To study the survival time of mice with tumor by intratumoral injection with chemotherapy drug Adr plus hapten of DNP or Hydra and find a combination of drugs of Adr with DNP or Hydra can prolong their life, and which one of DNP or Hydra can provide a long life extension of mice with tumor.

1.2. Method

After inoculation of tumor cells into mice, tumor reach at 4~5mm size as the experiment day 0, the random groups was performed for intratumoral injection with corresponding drugs combination of Adr+DNP or Hydra and Adr or ARA-C control group, follow up to 90 days to find which combination of drugs could provide a longer their life extension of mice with tumor.

1.3. Result

Comparison the body weight between the groups, there is not significantly different but the control group maintains the stable of weight. it indicated that PYM could not alone induce a expression of immunity at all and DNP as hapten is playing a important role in stimulation of immunity reaction. There is a significant difference between the tumor volume in treatment group and control group from day1 to day 41, it showed that local injection of combination drug can control tumor growth in the difference drugs group. There is a significant difference between survival time in treatment group and the control group. The life extension rate is 31.4% for group of A1 (Adr+Hydra I) is bigger than A2 (Adr+Hydra II) which has same formulation with high dose of Hydra, and A3 (Adr+Ara-C+Hydra) which has one more drug of Ara-CA2; life extension rate for B group, B1 (Adr+DNP) is 24.4%, it is bigger than B2 (Adr+Ara-C+DNP) which has one more drug of Ara-C; Both of A1 and B1 is better than D (Adr+Ara-C) in the life extension rate, it indicated that best Hydra dose in A1 and DNP in B1 played an important role in the life extension, Hydra is a better hapten than DNP.

1.4. Conclusion

It has further confirmed the abscopal effect induced by chemotherapy drug Adr plus DNP or Hydra is optimum combination with compatibility and could prolong their life of mice, it offer a novel eclectic approach for treating cancer through the Hydra modify with TAA *in vivo* with intratumoral injection best combination with chemotherapy drug.

2. Introduction

In the present, chemotherapy is commonly used in clinic treatment for all cancer patients and always with multiple drugs for better effect which is designed based on the treatment principle and cognition of each single drug which is approved by FDA. Combination of

multiple compound chemotherapy drugs are rarely approved in various countries which is in a default state. Therefore, the commonly used combination of chemotherapy schemes are based on clinical experience and single drug pharmacology, some has certain curative effects, but all lack the basis of animal experiment approved. In order to better carry out combined chemotherapy in the treatment of cancer, multiple drug combined experiment in animal model is very necessary. Often and heavy combination of drugs is used in most some cases, patients' lives have not been prolonged but shortened by repeated use of various drugs since the combination of drugs in which the dose of each agent is pushed to the brink of unacceptable toxicity, specifically, whereas certain ratios of combined drugs can be synergistic, other ratios of the same agents may be antagonistic, implying that the most efficacious combinations may be useful to utilize certain agents at reduced doses [1]. In order to better carry out multiple drug combined chemotherapy in the treatment of advanced cancer by synergistic or more benefit like improving their immunogenicity against tumor.

Our published data show that UMPIC provides an ideal intratumoral approach for the chemical de-bulking of advanced carcinoma, and hapten plays a vital role in prolonging the survival rate by immunogenicity [2-3]. Which is a good example for combination treatment in local delivery of drugs by synergistic. This immunogenicity is phenomenon of abscopal effect which is a hypothesis for treating metastatic cancer [4]. The present review briefly describes the history of radiotherapy-induced abscopal effects and local irradiation's activation of systemic antitumor immune responses [5]. Theoretically, this suggests that tumor necrosis induced by chemical drugs with hapten, it is not natural necrosis of the tumor or ionizing radiation necrosis, can also cause immune responses against tumor cells. It is reported that UMIPIC can induce an excellent anti-tumor immune response in animal models and humans [2-3,6-7]. Hapten is not a available drug in market with FDA approved, therefore, in order to find available drug as hapten induce the abscopal effect in animal model, we set up animal with tumor and treat by multiple drugs with hydralazine as hapten, then analyze and comparison the tumor weight in different group and survival time. Meanwhile, intratumoral injection of combination of drugs can induce inflammation which is acute inflammation which may help for tumor antigen presenting, in fact, tumor always has chronic inflammation which is involved the cancer development [8]. Acute inflammation induced by this treatment with drugs and hapten, it may provide a chance for interaction of tumor antigen with hapten to be strange antigens.

3. Materials and Method

1.1. Preparation of Tumor-Bearing Mouse

The 8th generation of H22 ascites tumor-bearing of mouse was maintenance for a few weeks at room temperature, mouse's abdomen was disinfected with 75% ethanol; tumor cell in ascites was extracted on the super clean bench and was diluted with normal saline according to the ratio of 1:2, next, each Babl/C mouse was inoculated with 0.2ml of H22 cells (10^7/ml) on the underarm of the left foreleg to establish the subcutaneous solid tumor model.

1.2. Treatment Method

After inoculation of tumor cells, when the longest diameter of the tumor reached about 4~5mm as the experiment day 0, the random grouping was performed for intratumoral injection with corresponding drugs combination (Table 1). Next day an experiment day 1 and mice were inoculated with 0.2ml of H22 cells (10^5/ml) on the right underarms. The second medication of same injection was performed on the experiment day 7 and 14. The body weight and tumor size was observed each others days for 48 days. Some tumors were performed pathological histochemical staining, the morphology of various tissues was prepared for observe morphological changes under the light microscope and pictures were taken as usual practice. Immunohistochemical staining of CD4, CD11, FOXp3, CD8 and CD86 for tumor of mice at day 1 and day 7 and day 30.

Table 1: Group and dosage.

Group	Drug combination	Concentration	Dose	Number of animal
		(mg/ml)	(μg/ mm^3)	
A1: Adr+Hydra	Adr+Hydra+H2O2	1.0+0.5+7.2	1.0+0.5+20.0	10
A2 Adr+Hydra	Adr+Hydra+H2O2	1.0+1.0+7.2	1.0+1.0+20.0	10
A3: Adr+Ara-C+Hydra	Adr+Ara-C+Hydra+H2O2	1.0+0.8+1.0+7.2	1.0+0.8+1.0+20.0	10
B1: Adr+DNP	Adr++ DNP +H2O2	1.0+1.0+7.2	1.0+1.0+20.0	10
B2: Adr+Ara-C+DNP	Adr+Ara-C+ DNP +H2O2	1.0+0.8+1.0+7.2	1.0+0.8+1.0+20.0	10
C: Adr	Adr	1.0	1.0	10
D: Adr+Ara-C	Adr+Ara-C	1.0+0.8	0.5+0.5	10
E:Model group	NS	9.0	9.0	10

Note: Adr:Adriamycin, Hydra:Hydralazine, Ara-C:Cytarabine.

4. Result

Immunohistochemical stain was performed only in A1 to A3 experiment group for CD4, CD11, FOXp3, CD8 and CD86 at day 1 and day 7 to day 30 with all positive staining comparison with control group, it showed that the immunity reaction induced by the drug and DNP after injection intratumoral with drug and hapten can induce the immunity reaction, special the expression of CD11 and CD86 indicates the activation of tumor antigen presentation and both hapten of DNP and Hydra could induce the immunological response, so that both of DNP or Hydra can be used as a hapten for modify the tumor antigen in order to increase their capability of antigenicity. The following staining are the different expression of CD4, CD11, FOXp3, CD8 and CD86 (Figure 1). Control group showed inflammation as same as A group, with character of red, swollen, hot, painful, but immunohistochemical stain showed negative of CD4, CD11, FOXp3, CD8 and CD86. Comparison the body weight between the groups, there is not significantly different but the control group maintains the stable of weight. The weight of mice in A1 (Adr+HydraI), A2 (Adr+Hydra Ⅱ), and A3 (Adr+Ara-C+Hydra) group of treatment group is same as B1(Adr+DNP) and B2 (Adr+Ara-C+DNP) groups, comparison with control group C1 (adr), D (Adr+Ara-C) and E (Model group), there is not difference (Table 2 and Fig. 2). it indicated that hapten of Hydra and DNP combined with chemodrugs does not change the body weight since the toxicity is limited by local injection. There is a significant difference between the tumor volume in control group and treatment group from day to day 41 (Table 3), it showed that local injection of combination drug can control tumor growth in the difference drugs group (Figure 3). There is a significant difference between survival time in the control group and treatment group (Table 4). The life extension rate is 31.4% for group of A1(Adr+Hydra I) which is bigger than A2 (Adr+Hydra II) which has same formulation with high dose of Hydra, and A3(Adr+Ara-C+Hydra) which has one more drug of Ara-C; life extension rate for B group, B1 (Adr+DNP) is 24.4% which is bigger than B2 (Adr+Ara-C+DNP) which has one more drug of Ara-C; Both of A1 and B1 is better than D (Adr+Ara-C) in the life extension rate, it indicated that Hydra dose in A1 and DNP in B1 played a important role in the life extension, Hydra is better than DNP; A1 and B1 combination of drugs is one chemotherapy drug Adr with one hapten Hydra or DNP, D group has two chemotherapy drugs Adr and Ara-C, it showed that one drug combined one hapten is better than two drug combination produce longer life extension after intratumoral injection, it further confirmed that hapten can play a important role by modified tumor associated antigens to trig the immunological response against tumor for prolong their life extension (Figure 4). It indicated that optimum compatibility is more important than drug dosage used for control tumor growth and prolong their life.

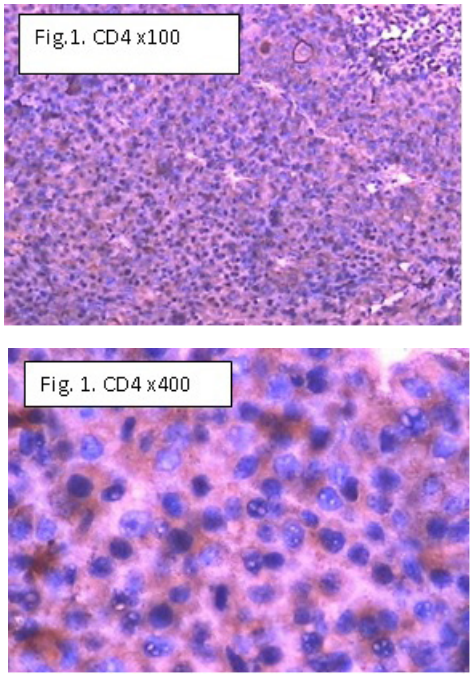
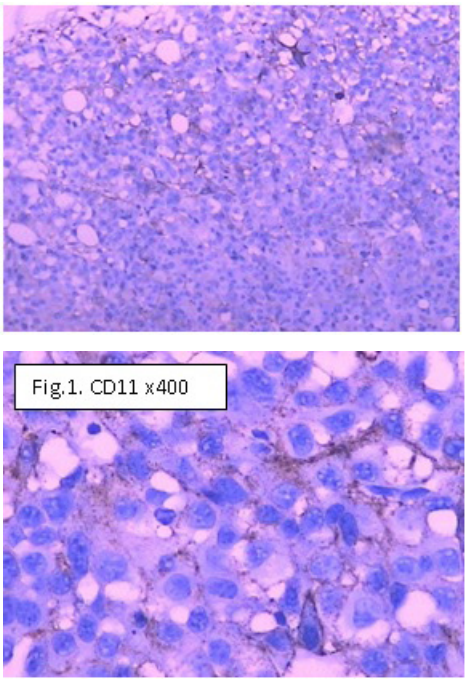
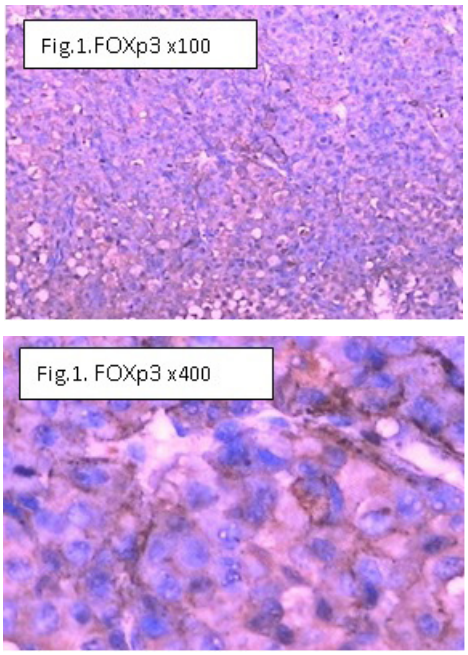


Figure 1. B1: experiment with BLM+DNP+H2O2.

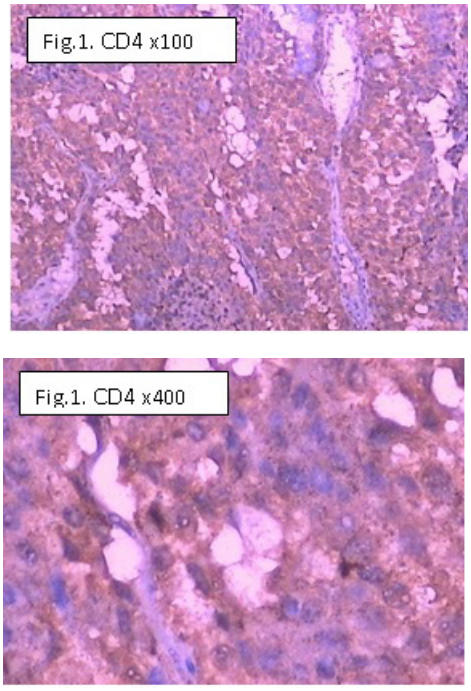
CD4 The immunohistochemical staining shows the CD4 positive at 100x and 400x in at day1, showed CD4 T Cell of special the immune reaction happened after injection intratumoral with drug and hapten day1.



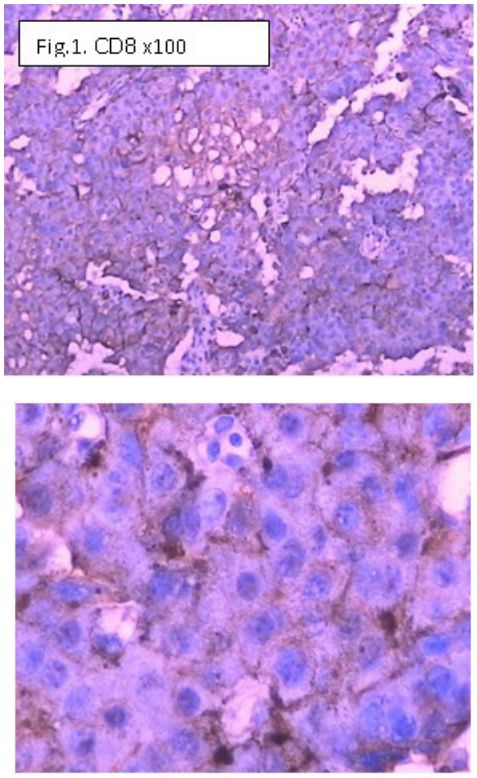
CD11. The immunohistochemical staining shows the CD11 positive at 100x and 400x at day1, showed CD11 cells activation of special the immune reaction happened after injection intratumoral with drug and hapten day 1.



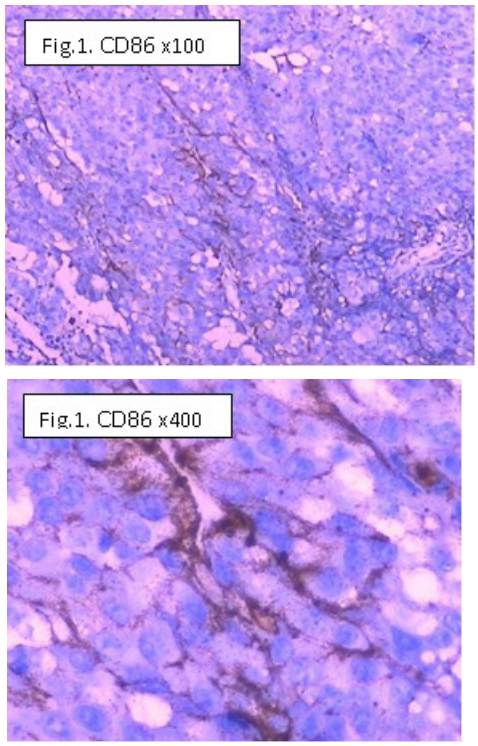
FOXp3. The immunohistochemical staining shows the FOXp3 positive at 100x and 400x in B1:BLM+DNP+H2O2 at day1, showed expression of FOXp3 for special the immune reaction happened after injection intratumoral with drug and hapten day 1.



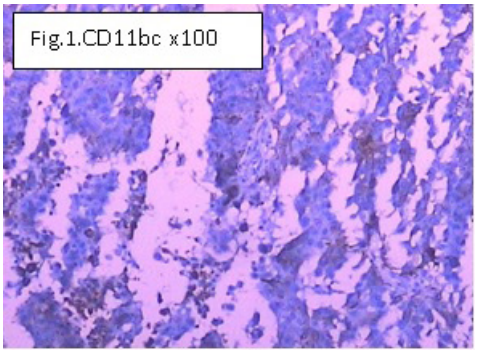
CD4. The immunohistochemical staining shows the CD4 positive at 100x and 400x in B1:BLM+DNP+H2O2 at day7, showed expression of CD4 for special the immune reaction happened after injection intratumoral with drug and hapten day 7.

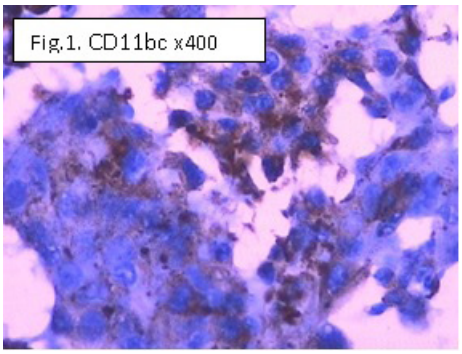


CD8. The immunohistochemical staining shows the CD8 positive at 100x and 400x in B1:BLM+DNP+H2O2 at day7, showed expression of CD8 for special the immune reaction happened after injection intratumoral with drug and hapten day 7.



CD86. The immunohistochemical staining shows the CD86 positive at 100x and 400x in B1:BLM+DNP+H2O2 at day7, showed expression of CD86 for special the immune reaction happened after injection intratumoral with drug and hapten day 7.





CD11b/c. The immunohistochemical staining shows the CD11b/c positive at 100x and 400x in B1:BLM+DNP+H2O2 at day7, showed expression of CD11bc for special the immune reaction happened after injection intratumoral with drug and hapten day 7.

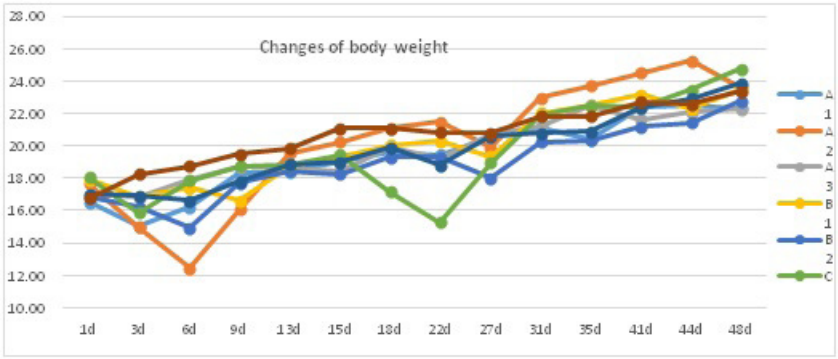


Figure 2: Changes of body weight.

P>0.05 for all group

Note:A1: Adr+Hydra Ⅰ; A2 Adr+Hydra Ⅱ; A3: Adr+Ara-C+Hydra; B1: Adr+DNP; B2: Adr+Ara-C+DNP; C: Adr; D: Adr+Ara-C; E:Model group.

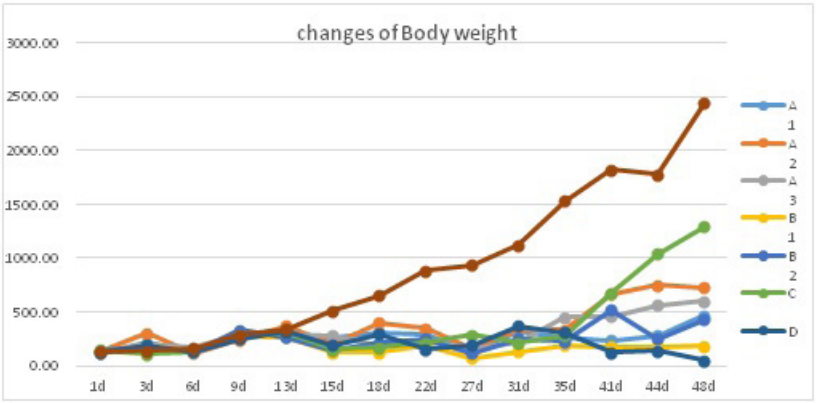


Figure 3: Change of tumor volume after treatment.

Note:A1: Adr+Hydra Ⅰ; A2 Adr+Hydra Ⅱ; A3: Adr+Ara-C+Hydra; B1: Adr+DNP; B2: Adr+Ara-C+DNP; C: Adr; D: Adr+Ara-C; E:Model group.

Table 2: Changes in body weight after administration.

Group / Weight(g)	A1: Adr+HydraI	A2 Adr+Hydra Ⅱ	A3: Adr+Ara-C+Hydra	B1: Adr+DNP	B2: Adr+Ara-C+DNP	C: Adr	D: Adr+Ara-C	E: Model group
1d	16.50±1.94	17.67±0.97	17.02±0.69	17.89±0.99	16.82±1.76	18.04±0.87	16.99±1.41	16.75±2.02
3d	15.04±0.67	14.96±1.22	16.79±1.26	16.86±0.91	16.20±1.13	15.87±1.15	16.92±0.74	18.25±1.07
6d	16.21±0.95	12.46±0.46	17.91±1.85	17.39±1.35	14.93±1.12	17.86±1.02	16.63±1.34	18.75±1.28
9d	18.35±0.48	16.06±0.82	18.68±2.65	16.59±1.43	17.72±1.74	18.72±1.37	17.82±1.45	19.51±1.03
13d	18.40±0.83	19.56±1.77	18.64±1.91	18.70±1.53	18.46±2.10	18.84±2.05	18.85±1.37	19.83±0.79
15d	19.06±0.67	20.21±1.69	18.44±2.59	19.33±1.08	18.24±2.02	19.42±1.25	19.00±1.26	21.07±0.65
18d	19.65±0.73	21.08±1.32	19.90±2.02	20.05±1.39	19.30±1.92	17.16±1.82	19.90±1.50	21.09±1.00

22d	19.44±1.04	21.46±1.28	19.18±1.82	20.30±1.02	19.27±1.77	15.25±1.68	18.78±1.38	20.81±0.79
27d	20.55±1.21	19.90±2.29	20.75±1.88	19.34±1.53	18.00±2.33	18.96±1.66	20.60±1.34	20.78±0.90
31d	21.11±1.31	22.97±0.59	21.25±1.56	22.02±1.44	20.25±2.38	21.85±0.79	20.77±1.52	21.84±1.97
35d	20.36±1.75	23.72±0.76	22.54±1.35	22.46±1.31	20.32±2.47	22.45±1.20	20.88±2.16	21.84±1.80
41d	22.35±1.26	24.52±1.26	21.61±1.58	23.16±1.12	21.18±2.87	22.44±1.57	22.38±1.29	22.70±1.83
44d	22.52±1.07	25.25±1.08	22.06±1.63	22.26±1.10	21.41±3.61	23.45±2.62	22.91±1.73	22.55±2.04
48d	22.33±1.19	23.62±1.20	22.28±2.05	23.51±1.43	22.78±2.95	24.77±0.25	23.87±1.16	23.34±1.77

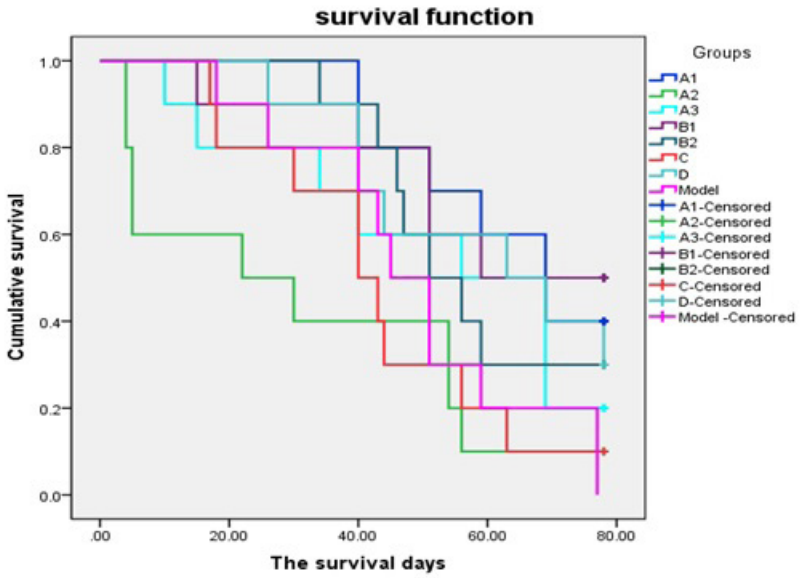


Figure 4: Survival time (days).

Table 3: Changes of tumor volume after administration.

Tumor size / Days	A1: Adr+Hydra Ⅰ	A2: Adr+Hydra Ⅱ	A3: Adr+Ara-C+Hydra	B1: Adr+DNP	B2: Adr+Ara-C+DNP	C: Adr	D: Adr+Ara-C	E: Model group
1d	113.2±48.21	132.5±32.8	132.4±44.3	131.2±24.9	140.7±35.1	147±38.6	126.4±25.8	127.8±28.5
3d	215±93.9	304.4±153.3	201.6±137.5	180.3±117.1	169.2±77.2	107.3±39.4	190.8±110.4	139.45±79.6
6d	129.15±47.3	117.1±46.9	169.5±66.2	127.95±19.3	128±49.1	128.5±55.3	131.1±106.8	158.3±77.9
9d	277.3±187.1	243.7±99.2	297.1±199.4	270.2±104.7	328.7±186.4	279.7±87.9	248.6±142.3	288.1±303.8
13d	274.9±147.1	367.0±189.5	296.6±232.0	262.3±137.1	263.9±157.5	309.8±214.1	314.1±243.0	337.1±329.7
15d	260.4±180.5	199.0±153.6	281.4±351.2	122.4±93.4	146.9±134.5	150.0±215.0	189.6±162.3	514.2±641.0
18d	307.4±277.8	400.8±404.8	193.7±145.8	124.7±149.4	216.3±173.7	166.7±111.9	292.1±272.2	648.8±625.1
22d	290.8±388.4	350±602.3	230.5±169.0	186.5±223.8	250±177.0	214.8±104.5	156.3±151.7	884.7±1000.4
27d	119.8±179.3	149.9±96.0	163.1±129.8	72±128.5	114.5±142.6	289.9±165.2	192.4±278.4	937.4±629.5
31d	321.8±479.6	337.5±402.8	217.1±202.2	130.0±208.7	251.3±336.6	214.2±183.4	372.2±652.4	1118.7±774.9
35d	258.35±356.1	332.5±387.2	452.0±645.8	190.6±289	224.7±319.4	276.1±268	311.5±527.8	1530.1±1226.1
41d	235.6±333.1	668.1±758.8	462.0±391.4	172±319.1	518.8±1080.1	675.8±671.5	121.7±270.5	1819.4±1422.2
44d	280.5±419.0	746.1±790.4	562.5±485.6	178.2±330.1	248.7±417.0	1036.8±755.5	140.3±294.1	1777.9±1311.8
48d	465.0±715.4	725±1061.8	603.1±735.9	182.1±350.6	425.0±727.4	1290.6±1176.7	48±117.5	2441.4±1763.9

Table 4: Mean & median survival time and life extension rate %.

Mean and median survival time (days)			Life extension rate (%)
group	Estimate±Std.		
	mean	median	
A1	64±5	69±8	31.4
A2	31±8	22±20	-35.9
A3	52±8	56±11	6.4
B1	61±6	59±0	24.4
B2	57±5	51±7	17.0
C	43±6	40±7	-11.9
D	59±6	63±20	22.0
Model group	49±6	45±4	
Overall	52±3	51±3	

5. Discussion

The delivery into tumor by best combination of drugs with hapten produce a better efficiency by synergistic, phenomenon of abscopal is happened [2-3,4-6], which must be related with immunity of patient's body, the creation of an in situ vaccine library in tumors due to tumor-specific antigens is another attractive factor in the process of intratumoral chemotherapy [9-10]. In addition, UMIPIC induces vaccine-like effects in tumors and enhances system immunity by adding hapten [10-12]. When various autologous tumor antigens are released from tumor coagulation, cell death may trigger T cell response and induce effective immunity, such as inducing acute inflammation which is different from general inflammation. This acute inflammation could produce vaccine of tumor associate antigens. The data and our clinical data with UMIPIC and animal studies have shown that the immune response significantly improves after treatment with hapten, especially CD4+ T and B cell immunity response [11-13] and CD11b/c and CD86 expression increased by qPCR measuretheir RNA after intratumroal injection combination drug with hapten. Here, our animal studies of chemotherapy drug plus hapten has further approved the concept of abscopal effect induced by local delivery chemotherapy drug intratumoral with hapten wich alway induce a acute inflammation. Comparison of the weight and tumor volume of mice, it was found that weight is stable for control group, the tumor volume smaller of A1 and B1 group than control group. Survival time in A1 and B1 group have longer survival time to 88 days, in which A1 has same formulation of drugs comparison with A 2 has higher dose of hydra, A3 has one more drug, but A1 has great survival benefit than A2 and A3, it indicated that best combination of chemotherapy with best compatibility of drug is most important for intratumoral chemotherapy, Adr plus hapten like DNP or Hydra could work together for cancer vaccination *in vitro* by hapten modify the tumor antigens release from damage tumor cell or death tumor cells while chemotherapy drug play a role of kill tumor cell local effectively.

6. Conclusion

Chemotherapy drug of Adr plus DNP could induce the immunological effect for control tumor growth of mice and prolong their survival time, Hydra can replace DNP to make a best combination and induce an immunity reaction of mice. It has further confirmed the abscopal effect must be related with immunological reaction in body, it offer a novel eclectic approach for treating cancer through the Hydra modify with TAA *in vivo* intratumoral injection best combination with chemotherapy drug.

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