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Intravenous Chemotherapy with Cisplatin for Regional Lymph Node Metastases of Auricular VX2 Carcinoma

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Abstract. *Introduction: The VX2 carcinoma is well established as a useful model for studies on treatment of primary tumors of various locations including the rabbit's auricle; however, limited experience exists on the treatment modalities of lymph node (LN) metastases. In this investigation we studied the frequency and extent of lymphogenic metastatic spread of auricular VX2-carcinomas and their response to systemic chemotherapy. Materials and Methods: Induction of a right-sided auricular VX2-carcinoma in 17 healthy New Zealand white rabbits was followed by ablation of the right auricle and intravenous application of 1mg/kg KG CDDP (cisdiamminedichloroplatinum (II)) dissolved in 2ml NaCl via the left-sided auricular vein in 10 rabbits (group 1), while 7 rabbits (group 2) remained untreated. After a 24-day follow-up period, animals of both groups were sacrificed and the regional draining LN as well as the lungs were isolated and examined histopathologically. Results: Group 1. Following intravenous cisplatin therapy (ICT), 6/10 animals showed no vital tumor cells within LN metastases of the first draining LN station, while 4/10 animals had necrotic LN metastases limited to the parotideal LN. Group 2. All 7 animals showed necrotic LN metastases of the first and second draining LN station as well as pulmonary metastases. Conclusion: The auricular VX2-carcinoma, characterized by frequent lymphogenic metastatic spread and response of LN metastases to ICT, offers an excellent animal model for further studies on the optimised treatment of lymphogenic metastatic spread in HNSCC.*

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The VX2-carcinoma of the New Zealand white rabbit resembles the human head and neck squamous cell carcinoma (HNSCC) in its growth behavior since it tends to metastasize lymphatically (1). The auricular VX2-carcinoma is characterized by its easy accessibility and observation. This location has been successfully used for intra-arterial embolization (2,3) and the VX2-carcinoma is well established as a model for transcatheter arterial embolization for gynecological tumors (4). Moreover isolated perfusion of tumors transplanted into the hindlimb (5), transcatheter arterial chemoembolization with paclitaxel-lipiodol solution in rabbit VX2 liver tumors (6), intratumoral injection of agents (7), arterial and intraperitoneal administration of oily anticancer agents (8) and the combined antitumor effects of local hyperthermia and a simultaneous injection of bleomycin suspended in sesame oil (BLM-sesame oil) into the proper hepatic artery (9) have been evaluated. Furthermore, magnetic drug targeting, biodistribution of the magnetic carrier and the chemotherapeutic agent mitoxantrone after locoregional cancer treatment, has been investigated (10).

Studies on frequency, therapy and response rate of lymph node metastases of esophageal carcinomas were performed by Natsuda *et al.* (11). As complete dissection of metastatic lymph nodes in the upper mediastinum is impossible in conventional surgery for esophageal carcinoma, Natsuda *et al.* devised a form of intra-operative local adjuvant cancer chemo-therapy for metastatic lymph nodes of the mediastinum, *via* tracheal bifurcation lymph nodes located at the anatomical pivot in the upper mediastinal lymph flow. Emulsion-type bleomycin (BLM) was injected into the bifurcation lymph nodes and BLM was identified in the pulmotracheal lymph nodes within 20 minutes.

Based on the fact that the survival of patients with HNSCC depends significantly on the extent of lymphogenic metastatic spread (12), it was the aim of previous studies to evaluate the lymph node topography of New Zealand white

Table I. *Pathohistological results.*

Topography	Histology	
	Group 1 (n=10)	Group 2 (n=5)
parotideal LN	6/10 avital metastatic residual 4/10 vital, partially necrotic metastases	5/5 vital, partly necrotic metastases
caudal mandibular LN	10/10 tumor free	4/5 vital, partly necrotic metastases
pulmonary metastases	10/10 tumor free	3/5 vital, partly necrotic metastases

LN: lymph node

rabbits (13) in order to perform investigations on the extent of lymphogenic metastatic spread in untreated auricular VX2-carcinomas (14). With the background of these investigations, it was the aim of the present study to evaluate the response of rabbit cervicofacial lymph node metastases to systemic chemotherapy with cisplatin (CDDP).

Materials and Methods

The study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for Care and Use of Laboratory Animals*, and the Animal Welfare ACT (7. U.S.C. *et seq.*); the animal use protocol was approved by the Institutional Animal Care Use Committee (IACUC) of the government of Giessen, Germany.

The study enrolled 17 healthy adult, 0.5 to 1 year-old female, specific pathogen-free, Iffa Credo New Zealand White (ICO:NZW) outbred rabbits weighing 2.5-3.5kg. They were purchased from the Behring Institute, Marburg, Germany, and were examined as described below. The animals were allowed to acclimatize for at least 3 days before use and were kept in a closed system without isolator. Housing was in accordance with IACUC of the government of Giessen guidelines in individual steel cages. Rabbits were fed with approximately 100g complete diet pellets for rabbits per day and acidified (hydrochloric acid, pH 2.7) tap water *ad libitum*, in order to reduce accidental bacterial contamination of the water and the resulting risk of infection. Environmental temperature was regulated between 18 and 20˚C and non-regulated relative air humidity was approximately 60%. Air was freshened at a rate of seven times per hour. Day/night cycle was 12/12h in artificial lighting with white lights on at 6:00 a.m.

Generation of tumor cells for carcinoma induction was performed by inoculating 0.3ml cell suspension, containing 10-20x10^6 vital tumor cells into both hind limbs of four NZW rabbits, as described in detail previously (13). For tumor implantation 0.1-0.25ml suspension, containing 10-20x10^6 vital cells, was injected concentrically between the lateral auricle edge and the central auricular artery into the cranial section of the upper third of the right auricle in all 17 animals. On the 17th day after tumor induction, all 17 animals underwent ablation of the right auricle with cold-cutting instruments under general anesthesia.

Group 1. Immediately after ablation of the right auricle, the application of intravenous chemotherapy was performed *via* the contralateral left auricular vein in 10 animals. Based on examinations of the response rate to cisplatin (CDDP) in uterine carcinomas (4), cisplatin was applied intravenously at a dose of 1mg/kg weight CDDP (cisdiammine-dichloroplatinum (II)) dissolved in 2ml NaCl per 5 sec. After a follow-up period of 24 days all animals were sacrificed.

Group 2. This group consisted of 7 animals that did not undergo further therapy after ablation of the auricle. The follow-up was equivalent to the first group.

As previously described in detail (13,14), all cervical lymph node groups as well as the lung and the liver were resected and dissected. A histological examination for detection of the response to chemotherapy with regard to lymphogenic metastatic spread and distant metastases followed.

Results

Group 1. All 10 animals survived the 24-day follow-up after systemic chemotherapy with CDDP. The size of the parotideal lymph node amounted to 24.9 mm ± 8.7 mm. Twenty-four days after ablation of the auricle (31 days after tumor induction), the histopathological examination revealed in 6/10 animals necrotic tumor tissue in the first draining lymph node station (parotid lymph node), which nearly completely filled the lymph node. However, no vital tumor cells could be depicted. The accumulation of macrophages in the marginal region in the sense of a so-called histiocytic margin indicated the already beginning phagocytosis of the avital tissue (Figure 1).

In the remaining 4/10 animals, histopathologically necrotic lymph node metastases could be found in the region of the first draining lymph node stations (parotid lymph node). Those 4 animals revealed neither metastatic spread into the secondary lymph node station nor into the lungs (Table I).

Group 2. Two out of the 7 animals were sacrificed due to weight loss of over 10% per kg on the 15th and 18th day.

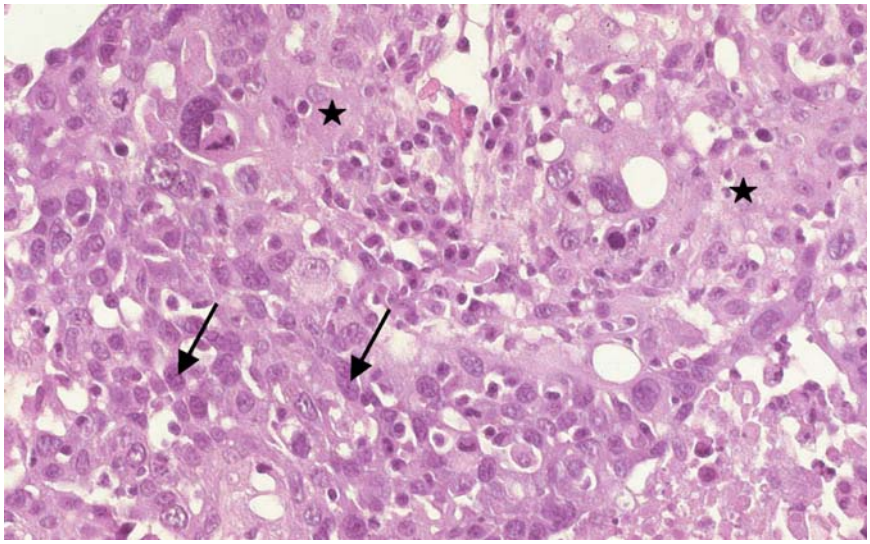

Figure 1. *Avital, necrotic residual (asterisk) of a lymph node metastasis of the parotideal lymph node with histiocytotic reaction (arrows).*

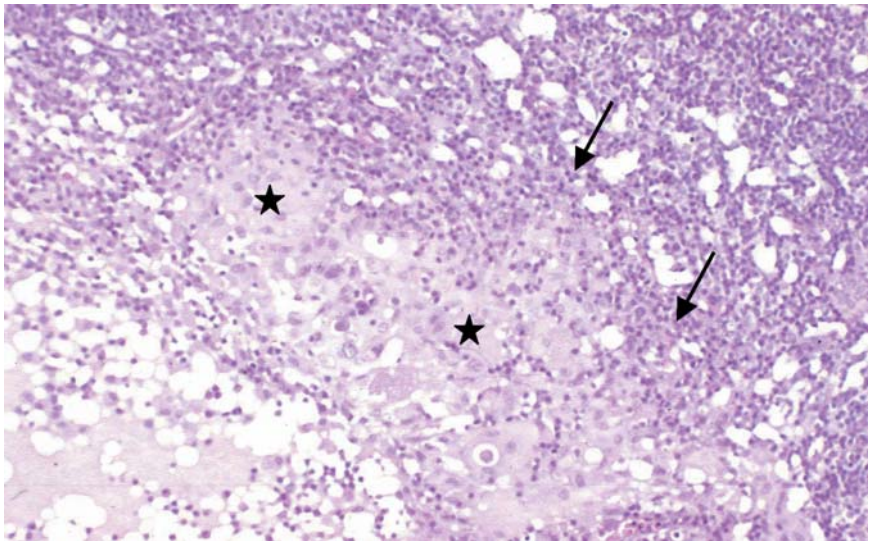

Figure 2. *Partially necrotic (asterisks), vital lymph node metastasis (arrows).*

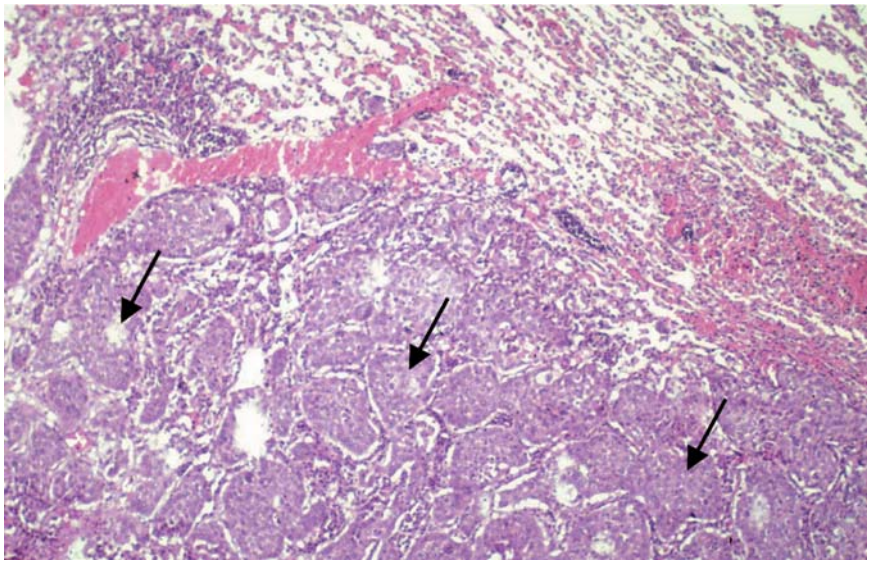

Figure 3. *Partially necrotic pulmonary metastasis (arrows).*

These animals were excluded from the study and group 2 was limited to 5 residual rabbits. In all other 5 animals, histopathologically necrotic lymph node metastases could be found in the region of the first draining lymph node station (parotid lymph node) 24 days after ablations of the auricle (31 days after tumor induction) (Figure 2). The size of the parotideal lymph node metastases amounted to 48.8 mm ± 9.1 mm. Besides tumor cell emboli within the lymphatic vessels, 4/5 animals showed a metastasis in the area of the secondary lymph node station (caudal mandibular lymph nodes). In 3/5 animals, bilateral, partly necrotic pulmonary metastases could be found (Figure 3).

Discussion

Lymph node metastases of carcinomas of the upper aerodigestive tract are of particular relevance since the 5-year survival rate is significantly reduced if two or more cervical lymph node metastases are found histologically (15,16). Prognosis additionally is reduced due to contralateral or bilateral metastases (17). Taking this into account, it is not surprising that, despite intensive efforts to optimize the treatment of the primary, the survival rate of patients who suffer from an already metastasizing HNSCC has not been significantly improved.

The examination of growth behavior and metastatic spread of HNSCC requires a representative animal model characterized by a high rate of lymphogenic metastatic spread *via* a determined lymphatic drainage. Lymphatic drainage *via* different serial lymph node stations according to the human cervicofacial topography would be ideal. Animal models to study mechanisms of lymphogenic metastatic spread often utilize tumors located in the rabbits hindlimb, which metastasize into popliteal lymph nodes (18).

The VX2-carcinoma is characterized by a very low differentiation (G3-G4) without keratinization, infiltration into surrounding structures, the formation of ulcers at a certain tumor size and the tendency to metastasize into the regional lymph nodes (19,20). Following anatomical and morphological investigations, previous studies (13) of the lymphogenic metastatic spread of auricular VX2-carcinomas showed an extremely high potential of the initial lymphogenic metastatic spread. All examined animals had parotideal lymph node metastases on the 28th day after tumor implantation. The lymphogenic metastatic spread was limited to the first draining lymph node for a period of 21 days. Four weeks after injection, a lymph node metastasis could be detected in the second lymph node station.

In this study we investigated the response rate to a systemic chemotherapy with cisplatin (CDDP). The resection of the primary tumor should exclude local complications, such as tumor bleeding or superinfection, that might lead to a premature interruption of the examination.

The results confirm the auricular VX2-carcinoma in New Zealand white rabbits as a promising animal model for examination of the mechanisms of lymphogenic metastatic spread and the evaluation of new therapeutic concepts in the treatment of lymph node metastases (21). Following destruction of the first draining lymph node barrier, further tumor dissemination leads to a metastatic spread into the secondary lymph node station and finally hematogenous spread with development of pulmonary metastases. This behavior, corresponding to human lymphogenic metastatic spread, can be influenced therapeutically by systemic application of CDDP. In the present study, none of the treated animals showed metastases in the area of the secondary lymph node stations or distant pulmonary metastases. The necrotic tumor tissue in the area of the first draining lymph node, that could be detected histopathologically in 6/10 animals, did not contain any vital tumor cells. The marginal histocytic reaction indicated the already beginning phagocytosis of the tumor necrosis. In 4/10 animals vital necrotic lymph node metastases were found in the area of the parotid lymph node.

Barry *et al.* (22) have already documented apoptosis-related cell death induced by CDDP *in vitro*. The DNA ladder was detected within 48 h with CDDP at a low concentration (0.4 μM) or within 2 h at a high concentration (60 μM). Kuo *et al.* (23) revealed vacuolar degeneration observed 4-14 days after intra-arterial administration of VX2-tumor to rabbit hind paws. Harima *et al.* (24) investigated the effects of chemoembolisation with cisplatin on gynaecological malignancies in New Zealand white rabbits and could show that transcatheter arterial chemoembolization induced necrosis in the center and apoptosis in the periphery of the tumor as early as on the second day after treatment.

It was not the purpose of the present study to elaborate an optimal relationship between dose and effect or to find different application types with their different potential on lymph node metastases. Establishing the auricular VX2-carcinoma as an animal model for examination of the mechanisms of lymphogenic metastatic spread, it was our aim to evaluate whether cervicofacial lymph node metastases respond to a systemic CDDP chemotherapy. The auricular VX2-carcinoma offers an excellent animal model to initiate therapy studies on the optimised treatment of already detected, but also occult, lymph node metastases in HNSCC.

References

1 Mandic R, Dünne AA, Ramaswamy A, Eikelkamp N, Plehn S, Schulz S, Moll R and Werner JA: Expression of MMP-2, MMP-3 and TIMP-3 in VX-2 carcinomas of New Zealand white rabbits. Anticancer Res *22*: 3281-3284, 2002.

2 Van Es RJJ, Franssen O, Dullens HFJ, Bernsen MR, Bosman F, Hennik WE and Slootweg PJ: The VX carcinoma in the rabbit auricle as an experimental model for intra-arterial embolization of head and neck squamous cell carcinoma with dextran hydrogel microspheres. Lab Anim *33*: 175-184, 1999.
3 van Es RJ, Nijsen JF, Dullens HF, Kicken M, van der Bilt A, Hennink W, Koole R and Slootweg PJ: Tumour embolization of the VX2 rabbit head and neck cancer model with Dextran hydrogel and Holmium-poly(L-lactic acid) microspheres: a radionuclide and histological pilot study. J Craniomaxillofac Surg *29*: 289-297, 2001.
4 Harima Y, Harima K, Hasegawa T, Shikata N and Tanaka Y: Histopathological changes in rabbit uterus carcinoma after transcatheter arterial embolization using cisplatin. Cancer Chemother Pharmacol *38*: 317-322, 1996.
5 Davidson T, Wallace J and Carnochan P: The rabbit as an experimental model for regional chemotherapy. 1. Intra-arterial hindlimb infusion. Lab Anim *20*: 343-346, 1986.
6 Yoon CJ, Chung JW, Park JH, Yoon YH, Lee JW, Jeong SY and Chung H: Transcatheter arterial chemoembolization with paclitaxel-lipiodol solution in rabbit VX2 liver tumor. Radiology *229*: 126-131, 2003.
7 Burgener FA and Barbaric ZL: Treatment of VX2 carcinomas in rabbits by percutaneous intratumoral injection of heated 0.9% NaCl solutions. Cancer Res *39*: 3429-3434, 1979.
8 Tabaru K, Konno T, Oda T, Nagamitsu A, Ishimaru Y and Kitamura N: Treatment of VX2 carcinoma implanted in the liver with arterial and intraperitoneal administration of oily anticancer agents. Cancer Chemother Pharmacol *47*: 149-154, 2001.
9 Kitamura M, Sugimachi K, Maekawa S, Kai H, Kuwano H and Matsuda H: Destruction of VX2 tumor in rabbits by hyperthermia plus bleomycin suspended in sesame oil. Cancer Treat Rep *70*: 1263-1269, 1986.
10 Alexiou C, Jurgons R, Schmid RJ, Bergemann C, Henke J, Erhardt W, Huenges E and Parak F: Magnetic drug targeting - biodistribution of the magnetic carrier and the chemotherapeutic agent mitoxantrone after locoregional cancer treatment. J Drug Target *11*: 139-149, 2003.
11 Natsuda Y, Sugimachi K, Ueo H, Matsuzaki K, Inokuchi K and Nakamura T: Experimental study on trans-nodal cancer chemotherapy for metastatic lymph nodes. Jpn J Surg *13*: 368-372, 1983.
12 Werner JA, Dünne AA and Myers J: Functional anatomy of the lymphatic drainage system of the upper aerodigestive tract and its role in metastasis of squamous cell carcinoma. Head Neck *25*: 322-332, 2003.
13 Dünne AA, Mandic R, Ramaswamy A, Plehn S, Schulz S, Lippert BM, Moll R and Werner JA: Lymphogenic metastatic spread of auricular VX2 carcinoma in New Zealand white rabbits. Anticancer Res *22*: 3273-3279, 2002.
14 Dünne AA, Plehn S, Schulz S, Levermann A, Ramaswamy A, Lippert BM and Werner JA: Lymph node topography of the head and neck in New Zealand rabbits. Lab Anim UK *37*: 37-43, 2003.
15 Denis F, Garaud P, Manceau A, Beutter P, Garand G, le Floch O and Calais G: Prognostic value of the number of involved nodes after neck dissection in oropharyngeal and oral cavity carcinoma. Cancer Radiother *5*: 12-22, 2001.
16 Lefebvre JL, Buisset E, Van JT, Delobelle-Deroide A and Caty A: Lymph nodes as prognostic factors in pharyngolarynx SCC. *In*: Laryngeal Cancer (Smee R, Bridger GP, eds.). Amsterdam, Elsevier, 1994, pp. 589-592.
17 Ji W, Yu J and Guan C: Significance of pathological type in cervical lymph metastasis of laryngeal cancer. Lin Chuang Er Bi Yan Hou Ke Za Zhi *12*: 291-292, 1998.
18 Shigetomi A, Kotoh T, Harada T, Morikawa S and Nakamura T: Development of an experimental model for spontaneous lymph node metastasis of human esophageal carcinoma in nude mice--histopathological analysis. Hum Cell *5*: 273-281, 1992.
19 Mine H and Nakamura T: Mode of lymph node metastases in esophageal cancer induced in rabbits with VX2 carcinoma. Jpn J Surg *13*: 236-245, 1983.
20 Steward HL, Snell KC, Dunham LJ and Schlyen SM: V2 carcinome, rabbit. *In*: Atlas of Tumor Pathology, Transplantable and Transmissible Tumors of Animals, section XII-fascicle 40. Washington, AFIP, 38-42, 1959.
21 Dünne AA, Schmidt A, Kuropkat C, Ramaswamy A, Schulz S and Werner JA: The auricular VX2 carcinoma – an animal model for sentinel node concept. In Vivo *17*: 457-462, 2003.
22 Barry MA, Behnke CA and Eastman A: Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. Biochem Pharmacol *40*: 2353-2362, 1990.
23 Kuo JH: Ultrastructural study on effects of cisplatin on VX2 carcinoma cells. Bull Tokyo Med Dent Univ *37*: 35-50, 1990.
24 Harima Y, Harima K, Hasegawa T, Shikata N and Tanaka Y: Transcatheter arterial embolization with cisplatin: apoptosis in VX2 tumour uterus transplants. Anticancer Res *16*: 193-199, 1996.

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