Effect of Gemcitabine on Vascular Endothelium in the Rat

J. VOKURKA

First Department of Surgery, Masaryk University, Brno, Czech Republic

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Abstract

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The aim of this work was to determine the effect of various concentrations of gemcitabine on the vascular endothelium in the rat. A total of 62 animals were used in the experiment. Gemcitabine was administered in the recommended dose (20 mg) or in the double (40 mg) or triple recommended dose (60 mg) into the abdominal aorta, splenic artery, common iliac artery, portal vein, or the jugular vein. After various intervals of survival, specimens of the vascular wall were collected for histological examination. Saline solution was administered into the vessels of the control-group of animals. The work has shown that gemcitabine at the standard, double or triple dose does not cause any serious changes to the vascular endothelium.

Gemcitabine, vascular endothelium, rat, damage

Gemcitabine is a substance with cytotoxic effect on solid tumours (Brickelmaier et al. 2002; Madajewicz et al. 2000; Vysloužil et al. 1997). It is a nucleoside derivative inhibiting the DNA synthesis. It has been approved for the therapy of defined tumour diseases or metastases in human therapy using general i.v. administration up to the prescribed dose (Kala 1999; Morgan-Meadows et al. 2002; Šefr et al. 2002; Rovný et al. 2002). The recommended dose amounts to 1000 $mg \cdot m^{-2}$ in the 30-minute i.v. infusion. The further dosage is adjusted to the foregoing toxicity. The adverse effects include myelosuppression, gastrointestinal and renal symptoms, allergy, dyspnoe, oedemas etc. The potency of the drug might be increased by its targeted administration into the appropriate supplying artery in the regional therapy of some tumour diseases (pancreatic tumours, non-small-cell lung carcinomas, mammary carcinomas, colorectal carcinomas or tumours in the maxillofacial region) (Kala 2000; Olejník 1999; Poplin et al. 1999; Šefr 1998; Vokurková 1999). The intra-arterial administration of a high concentration of the drug poses a problem owing to its possible toxicity to the vascular endothelium at the site and upwards the site in the flow direction in vascular bed (Tannock et al. 2002; Veverková 1999). The effect of gemcitabine on the vascular endothelium has not been described yet in the literature. For this reason, we prepared an experiment in order to test the effects of various gemcitabine concentrations on the arterial endothelium.

Materials and Methods

The experiment was carried on Wistar rats supplied by Top Velaz Ltd. The animals were of SPF quality, weighting between 280 g to 300 g at the start of the experiment. During the experiment, the animals were housed under standard conditions in Makrolon cages, five animals per cage. The animal housing room was air-conditioned. Relative air humidity was maintained between 50-70% and air temperature at 22 ± 2 °C. The rats were fed the DOS diet *ad libitum* and supplied with drinking water. Sterilised wood shavings were used as bedding. The procedure was performed under general anaesthesia (xylazine with ketamine hydrochloride) and aseptic conditions in the experimental room of the First Department of Surgery, St. Anna Teaching Hospital, Masaryk University in Brno. A total of 62 animals were included.

Address for correspondence:
MUDr. Jiří Vokurka, PhD.
First Department of Surgery, St. Anna Teaching Hospital
Faculty of Medicine, Masaryk University
Pekařská 53, 656 91 Brno
Czech Republic

Phone: + 420 543 182 360
Phone/Fax: + 420 543 182 373
E-mail: vokurka@med.muni.cz
http://www.vfu.cz/acta-vet/actavet.htm

Healthy rats were randomly assigned to the treatment and control groups. A pilot study was first performed in order to establish the variance of results. Statistical analysis of variance was used in the evaluation of the results.

The rats were operated on an experimental surgical table, fixed on a pad, lying on their backs with upper and lower extremities stretched out. The surgery was performed under general anaesthesia (xylazine with ketamine hydrochloride) and aseptic conditions.

The body surface of the experimental animals was assumed to amount to 200 cm^{-2}. A dose of 20 mg of gemcitabine corresponds to this body surface. The animals were assigned to five groups: the animals were given 20 mg of gemcitabine in the first treatment group, the double of this dose (40 mg) in the second group, the triple dose (60 mg) in third group, and the nine-fold quantity (180 mg) in the fourth group. In the fifth (control) group, saline was administered into the blood vessels of the rats instead of gemcitabine.

Gemcitabine was administered by slow infusion with an especially finished needle of the smallest diameter (0.33×12 mm) so that the traumatism to the vascular wall be minimised. After opening the abdominal cavity by median laparotomy, the drug was administered into either the abdominal aorta or the splenic artery or the common iliac artery or the portal vein. The site of the drug administration was then followed up for possible bleeding and it was marked with a clip. Then the abdominal cavity was carefully stitched up and the animal was wakened. Most often, gemcitabine was administered into the jugular vein (i.e. without laparotomy). At pre-set time intervals, the experimental animals were euthanised with a Thiopental injection into the thoracic cavity. Specimens of the relevant marked vessel were taken starting at the site of injection and further in the blood flow direction for the histological examination of the possible damage to the vascular wall.

The results were analysed statistically with Fisher's exact test, using the software CSS: STATISTICA, version 3.1 company manual of StatSoft Tulsa, OK 74104, USA.

The histologically established damage to the vascular wall was categorized into four degrees: Degree 0 represented vessels without demonstrable signs of damage. Degree 1 included cases with stagnating erythrocytes or mildly changed properties of the endothelium. Animals with mild swelling of the endothelium represented the degree 2. Degree 3 included cases of possibly serious damage to the vascular endothelium.

The experimental work was approved by the Ethics Committee of the St. Anna Teaching Hospital, and also by the Expert Committee for the Work with Experimental Animals of the Masaryk University Medical Faculty in Brno (No 26/97).

Results and Discussion

The degree of vascular wall damage in dependence on the administered dose of gemcitabine and the days elapsed from the drug administration to the collection of histological specimens is shown in Table 1.

Table 1
Degree of the vascular wall damage versus the dose of the administered solution

Dose of gemcitabine (mg)	Days	Damage			
		degree 0	degree 1	degree 2	degree 3
0	0-4	2	2	1	0
20	0-4	6	0	0	0
40	0-4	10	0	1	0
40	9-10	3	0	0	0
40	30	8	2	0	0
40	61	4	0	0	0
40	>90	4	0	1	0
60	9-10	2	0	0	0
60	30	1	2	0	0

When a dose of gemcitabine exceeding the triple of the recommended dose was used in the pilot experiment, the experimental animals died due to toxicity of the drug. In all such cases necrosis of the liver and spleen was found in the histological examination. Therefore we only used the 60 mg dose of gemcitabine as the maximum dose in the main experiments (i.e. the triple of the standard dose).

In no case the degree 3 damage to the vascular endothelium was found.

The 0 dose of gemcitabine means that 1 ml of saline was injected into the vessel instead of gemcitabine.

In the first evaluation of the effect of gemcitabine, the number of control animals with and

without endothelium damage was compared with the total number of treated animals again with and without damaged vascular endothelium. Fisher's exact test was used for the evaluation of the inter-group differences with the resulting values of $p = 0.037$. Because this result was unexpected (the control group showed greater damage than the treatment group), the two-sided variant of the test was used, which yielded the value of $p > 0.05$. Thus the effect of gemcitabine on the vascular wall cannot be considered significant.

Using a similar technique, the numbers of animals with and without vascular wall damage were compared with respect to the dose of gemcitabine. Even here the results were not statistically significant.

There was no statistically significant difference due to the dose of gemcitabine applied into the blood vessel and the degree of the cell wall damage (Table 2).

Table 2

Degree of the vascular wall damage versus the dose of gemcitabine

Dose of gemcitabine (mg)	damage 0	damage 1 + 2
20	6	0
40	29	4
60	3	2

Undoubtedly the changed haemodynamics caused by the administration of the drug can cause changes to the vascular endothelium. In spite of this, in most cases the vascular endothelium being examined showed no signs of "breaking" and the changes observed rather corresponded to normal variants. No signs giving evidence for the necrosis or destruction of the vascular endothelium or for its separation were observed. In no case serious changes to the cell wall were observed (degree 3 damage) after the administration of gemcitabine into the vascular lumina.

Our work has demonstrated that even the double or triple standard dose of gemcitabine administered into the blood vessel with the purpose of potential chemotherapy resulted in no serious changes to the vascular endothelium and the cell wall.

Vliv gemcitabinu na cévní endotel u potkana

Cílem práce bylo ověřit účinek různých koncentrací gemcitabinu na cévní endotel u potkana.V experimentu bylo použito celkem 62 zvířat. Do abdominální aorty, a. lienalis, a. iliaca communis, v. portae, nebo v. jugularis byl aplikován gemcitabine v doporučené dávce, nebo dvojnásobku doporučené dávky, nebo trojnásobku doporučené dávky. Po různě dlouhých intervalech přežívání byly provedeny odběry cévní stěny k histologickému vyšetření. Kontrolní skupině zvířat byl aplikován do cév fyziologický roztok. V práci bylo prokázáno, že gemcitabin v dvojnásobné, ani trojnásobné dávce nezpůsobil žádné závažné změny na cévním endotelu.

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