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Original Article

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ABSTRACT

Introduction: Pressurized Intra-Peritoneal Aerosol Chemotherapy (PIPAC) is a new drug delivery approach to treat peritoneal metastasis. This study evaluated two different devices, one based on aerosolization, the Capnopen ® (CAPNOMED – company) and a second, a prototype named Nebulo ® (GAMIDA - company), based on nebulization. The performance of the two devices were tested for PIPAC distribution and cell penetration of doxorubicin in a sheep model.
Methods: Doxorubicin was aerosolized for 30 min, using PIPAC into 6 ewes, 3 with Capnopen ® and 3 with Nebulo ®. The number of doxorubicin positive cells was determined using the ratio between doxorubicin fluorescence-positive cell nuclei (DOXO+) over total number of DAPI positive cell nuclei (DAPI+). Penetration depth (μm) was defined as the distance between the luminal surface and the location of the deepest DOXO+ nuclei.
Results: DOXO+ nuclei were identified in 46% of the samples. All omental samples, directly localized in front of the nebulizer head, had DOXO+ nuclei except one in the Nebulo group. There was no significant difference in penetration depth between the 2 groups. Concerning the peritoneum, 40% showed by microscope analysis a depth > 100 μm in the Capnopen group, versus 5% in the Nebulo group ($p = 0.06$).
Conclusions: Our results and limitations observed imposes a change in developing technic. A smaller size of PIPAC droplet is not an efficient option to increase significantly the tissue penetration and concentration of drugs in chemotherapy.

Pressurized Intra Peritoneal Aerosol Chemotherapy (PIPAC) is a drug delivery solution offering a new opportunity to treat patient afflicted by peritoneal metastasis. This technique has gained in popularity during the last years, as clinical results offered consistent data to support its use [1,2]. Historically only one drugs delivery system had been available for ten years, worldwide, the CapnoPen® produced by the company CAPNOMED3. At that time, this dominant situation linked to the patent had been considered as possibly problematic and had led some companies to develop other technical solutions that were different but that would allow to make PIPACs [4]. Scientific research is intense in order to offer new delivery PIPAC systems. A hopeful new opening was recently proposed by South Koreans, which offer a rotational PIPAC (RIPAC) [5]. Authors have claimed that RIPAC, with a rotative nozzle, tested in a living animal models offer a better tissue concentration and penetration depth of Doxorubicin [5].

The PIPAC drug delivery solution uses aerosolization to transform a liquid to an aerosol. The tissue drugs penetration is deeper than using intra peritoneal liquid delivery, as tested in animal model [6].

Method

Nebulization is a type of therapy that is used in some respiratory diseases like asthma, or for prolonged artificial ventilation. Nebulization transforms liquid medication into vapors that is ingested through respiration. The velocity of particle delivery is

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Fig. 1. Description and comparison of intra-peritoneal distribution pattern of doxorubicin for each peritoneal localization *O* = ovary, *E* = epiploon, C = caecum.No average or standard deviation because only one sample is analyzed per site.

very low, and nebulization is compared with a fog. Large particles (>6 μm) tend to be deposited in the upper airways, small particles (<2 μm) mainly penetrate deep in the lung and alveoli, and particles >2–<6 μm in size are typically deposited in the central and smaller airways [7].

Using this hypothesis of realizing the need for a better therapy, a private company, GAMIDA developed a new PIPAC device, based on nebulization, the Nebulo® system.

We conducted an experimental study with two customers nozzles: CapnoPen® versus Nebulo®. The evaluation of the droplet size distribution of water dispersal was not considered for comparison regarding the Nebulo® prototype proposed. We postulated that the best comparison is the evaluation of a tissue concentration and penetration depth of Doxorubicin, between the two nozzles.

The protocol had been approved by ethical comity and Minister (Ministère de l'enseignement supérieur de la recherche et de l'innovation, under the number 16-42 / APAFIS 8582-201709251458172v2). The PIPAC procedure has been previously described [8]. Briefly, a PIPAC procedure was performed for adult ewes, under general anesthesia. Trocars were disposed at the same place for all animals. Doxorubicin was delivered at 3 mg with 50 cc of physiologic serum. The dosage corresponds to 1,5 mg/m2 used for human in 2018. Laparoscopic procedure was performed using a capnoperitoneum at 12 mmHg. The PIPAC procedure of Doxorubicin administration was followed by 30 min of capnoperitoneum. The animal were found not to wake up and the procedure was stopped at 30 min. A PIPAC procedure was performed for 6 sheep, 3 receiving PIPAC with CapnoPen® and 3 with Nebulo®. For each animal, 9 tissues macro- biopsies were performed in a standardized location. Dapi coloration was used for localization and confirmation that Doxorubicin was intra cellular. The number of Doxorubicin positive cells was determined using the ratio between Doxorubicin fluorescence-positive cell nuclei (DOXO+) over total number of DAPI positive cell nuclei (DAPI+). Penetration depth (μm) was defined as the distance between the luminal surface and the location of the deepest DOXO+ nuclei over the total number of cell nuclei that were stained with DAPI. Penetration depth (μm) was defined as the distance between the luminal surface and the location of the deepest DOXO+ nuclei. The procedure was uneventful and performed to completion.

Results

Tissue samples were anonymized for each ewe in order to have a blind reading regardless of the device used. Mann-Whitney tests were used to compare the impact of the intraperitoneal pressure on the peritoneal distribution of the Doxorubicin.

To assess the impact of the pneumoperitoneum pressure on the depth of penetration of doxorubicin and to take into account the correlation between the different sheep, Generalized Estimating Equations (GEE) were used for comparisons between the two groups.

A p value < 0.05 was considered statistically significant.

Statistical analyzes were performed with SPSS 15.0 et Stata 11.0 (Stata Corp., College Station, TX, USA).

Fig. 1 reports the description and comparison of intra-peritoneal distribution pattern of Doxorubicin for each peritoneal localization. The first sheep treated with Nebulo® had only one biopsy with incorporated Doxorubicin at the epiploon place. Only one sheep had biopsy with incorporated Doxorubicin at the caecum place, and is in the CapnoPen® group. The caecum is located under the rumen (part of the large stomach of the sheep), probably affecting the accessibility of that location. There was no significant difference in penetration depth between the 2 groups. Concerning the peritoneum, 40% of the microscope analysis showed a depth > 100 μm in the CapnoPen® group, versus 5% in the Nebulo® group (p = 0.06). On the ovarian tissue, the penetration depth was comparable for the 2 devices (p = 0.82). These results are summarized in Table 1.

Table 1
Comparison of the proportion of images showing a penetration depth >100 μm by histological type. N is the number of images analyzed, for all the animal of the group, n is the number of image with a positive penetration of Doxorubicin.

	Penetration depth > 100 μm				
	Nebulo® n/N (%)		CapnoPen® n/N (%)		p-value*
Peritoneum	1/22	(5)	25/62	(40)	0.06
Ovary	5/9	(56)	6/17	(35)	0,82
Caecum	2/9	(22)	4/8	(50)	*

* P value was obtained with General Estimated Equations.

Discussion

Our study present different limitations, regarding method and study design.

Our results are limited by the model used. Doxorubicin is used because of the possibility of spontaneous identification under microscopic fluorescence. However, platin drugs could offer different distributions. We can postulate that for Human, platin penetration could be different, including better. Currently the Doxorubicin drug concentration is proposed with a higher dosage, because our study was built some years ago [2]. That could explain that in our experiment, only half of the sample were positive for Doxorubicin.

That result of poor drug penetration is unsatisfactory, whatever the device was. Our results include a very small number of animals studied, that number could affect our results. We tested a new device and did not re use CapnoPen® used previously for patient in order to avoid possible bias. We use the first generation of CapnoPen®, before the company change the process [9]. We did not perform multiple biopsies in a single animal so as to increase artificially the number of statistical analyzes; but it therefore remains a limitation.

Conclusion

Our observation leads us to discourage for the development of the prototype by GAMIDA company. Considering that a smaller size of PIPAC droplet is not an acceptable option to increase dramatically the tissue penetration and concentration of drugs in chemotherapy. Because many new devices arriving for approval, our study could be considered as an important argument regarding the size of the droplet necessary to have a better clinical impact [9]

Financial and awards situation

The cost of the experiment including purchasing animal, surgical and animal facilities, laboratory evaluation of doxorubicin and a financial award for MM was given by GAMIDA company.

The Nebulo® system was provided free by the GAMIDA company.

The CapnoPen® system was provided free by the CAPNOMED company, with special thanks to Mr Stephan Gross.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.soda.2022.100078.

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