Incomplete copolymer degradation of in situ chemotherapy

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11 Glioblastoma; carmustine; gliadel; recurrent surgery; BCNU; in situ drug delivery.
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17 Abstract:

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20 **Introduction:** in situ carmustine wafers containing 1,3-bis(2-chloroethyl)-1-nitrosourea
21 (BCNU) are commonly used for the treatment of recurrent glioblastoma to overcome the brain-
22 blood barrier. In theory, this chemotherapy diffuses into the adjacent parenchyma and the
23 excipient degrades in maximum 8 weeks but no clinical data confirms this evolution, because
24 patients are rarely operated again.
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29 **Materials and Methods:** a 75-year-old patient was operated twice for recurrent glioblastoma,
30 and a carmustine wafer was implanted during the second surgery. Eleven months later, a third
31 surgery was performed, revealing unexpected incomplete degradation of the wafer. 1H-Nuclear
32 Magnetic Resonance was performed to compare this wafer to pure BCNU and to an unused
33 copolymer wafer.
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40 **Results:** In the used wafer, peaks corresponding to hydrophobic units of the excipient were no
41 longer noticeable, whereas peaks of the hydrophilic units and traces of BCNU were still present.
42 These surprising results could be related to the formation of a hydrophobic membrane around
43 the wafer, thus interfering with the expected diffusion and degradation processes.
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49 **Conclusions:** The clinical benefit of carmustine wafers in addition to the standard radio-
50 chemotherapy remains limited, and *in vivo* behavior of this treatment is not completely
51 elucidated yet. We found that the wafer may remain after several months. Alternative strategies
52 to deal with the blood-brain barrier, such as drug-loaded liposomes or ultrasound-opening, must
53 be explored to offer larger drug diffusion or allow repetitive delivery.
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Graphical abstract

Carmustine wafer

11 months

Unexpected non-degraded copolymer

Introduction

Tight capillary cellular junctions of the blood-brain barrier (BBB) limit drug diffusion from the systemic circulation to the brain parenchyma and prevent the use of numerous chemotherapies for central nervous system neoplasms¹. Numerous approaches aiming to breaching the BBB, including molecular engineering^{2,3} and mechanical devices⁴, have been developed to answer the challenge of bringing anti-cancer agents⁵ into the brain. In this context, *in situ* therapies, consisting in direct cell⁶ or drug⁷ infusion, have also been proposed. Carmustine wafers fall into this last category: they are biodegradable copolymers containing 3.85% of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, also known as carmustine) and are used as an *in situ* chemotherapy in glioblastoma recurrences and even, more recently, as an adjuvant first-line treatment in newly diagnosed tumors⁸. Each wafer delivers 7.7 mg of carmustine during 5 days with only 1% of the administered drug remaining in the brain tissue after 7 days⁹. Studies in non-human primates in normal brain without surgical debulking reveal a diffusion of carmustine in a 2 to 10 mm range around the wafer¹⁰. The inactive excipient consists in a degradable, random copolymer of 1,3-bis-(*p*-carboxyphenoxy)propane (CPP) and sebacic acid (SA) units in a 20:80 molar ratio connected by anhydride bonds (Figure 1a), termed poly[(1,3-bis-(*p*-carboxyphenoxy)propane)-*co*-(sebacic acid)], P(CPP-*co*-SA). P(CPP-*co*-SA) should completely degrade over a period of 6 to 8 weeks⁹. SA contains an aliphatic alkyl chain whereas CPP exhibits 2 phenyl rings per CPP unit. Therefore, as degradation occurs in an aqueous environment, it is expected that bond cleavage will first occur at the SA-SA junctions compared to SA-CPP or CPP-CPP junctions.

Although carmustine wafers have been an important subject of research and discussion, there is no report of *in vivo* local evolution in a patient, to our knowledge. Here, we report the case of a patient who underwent additional surgery months after carmustine wafers insertion, which allowed us to observe the persistence of the wafer. We provide the Proton Nuclear Magnetic Resonance (¹H-NMR) analysis of this wafer in order to investigate the possible reasons of its default of resorption.

Materials and Methods

Patient: A 75-year-old patient was admitted for glioblastoma progression, 11 months after a second resection surgery with carmustine wafer insertion. He had previously received

fractionated focal irradiation at a dose of 2 Gy per fraction given once daily 5 days per week over a period of 6 weeks, for a total dose of 59.4 Gy focal radiotherapy and 13 regimens of temozolomide at a dose of 75 mg per square meter per day, given 7 days per week from the first day of radiotherapy until the last day of radiotherapy. After a 4-week break, the patient received 7 cycles of adjuvant chemotherapy according to the standard 5-day schedule every 28 days. The dose was 150 mg per square meter for the first cycle and was increased to 200 mg per square meter beginning with the second cycle (no hematologic toxic effects were noticed). Because he presented local recurrence, his case was discussed in a multidisciplinary neuro-oncological meeting, and he underwent a third surgical resection. During the procedure, we surprisingly found remains of the partially degraded copolymer of the carmustine wafer (Figure 1b). We obtained the patient's written consent to analyze and publish anonymously his data. The wafer's spectrum by ^1H -RMN was compared to BCNU and to an unused copolymer wafer.

Sample treatment: Carmustine wafer extracted from the patient and unused carmustine wafer, Gliadel[®] (mgi pharma ltd) were dissolved using 1 mL of solvable[®] solution (Perkin Elmer). The solution was stirred overnight until complete dissolution. After filtration, solvable[®] was evaporated and replaced by CDCl_3 before ^1H -NMR analysis. Carmustine, Bicnu[®] (emcure pharma UK ltd) was directly dissolved in CDCl_3 before ^1H -NMR analysis.

Analytical methods. ^1H -NMR spectroscopy was performed in 5 mm diameter tubes in CDCl_3 at 25 °C on a Bruker Avance 300 spectrometer at 300 MHz. The chemical shift scale was calibrated based on the internal solvent signals (CDCl_3 , 7.26 ppm).

Results

The ^1H -RMN analysis of the unused wafer exhibits all expected peaks of CPP (Figure 1a, b, c, and d) and SA (Figure 1e, f, and g) units belonging to the copolymer, and with the right molar ratio between CPP and SA units (Figure 1c). Peaks of BCNU were also detected in the 3.3–4.25 ppm region (Figure 1c). Interestingly, the ^1H -RMN spectrum of the partially degraded wafer showed that peaks corresponding to CPP units (peaks a–d) were no longer noticeable, whereas peaks f and g belonging to SA units were still present. Note that peak e from SA is not visible, probably because of the poor solvation of the nearby carboxylic acid functions in deuterated chloroform (the solvent used for the NMR study). Traces of BCNU were also detected demonstrating that not all BCNU was released from the wafer.

Apart from the unexpected finding of a non-degraded wafer, the incomplete clearance concerning only SA and not CPP units was rather surprising. Given SA is more hydrophilic than CPP, it was supposed to be cleared prior to CPP. Because of their greater hydrophobicity, the CPP-CPP bonds are likely more difficult to be cleaved compared to CPP-SA and SA-SA bonds in an aqueous environment. These results may be related to the potential formation of a hydrophobic membrane around the wafer, which could interfere with the normal degradation process.

Discussion

The benefit of copolymer wafers in addition to the standard radio-chemotherapy is supported by some controlled studies⁷ but remains limited (inferior to 4 months of progression-free survival). However, the higher postoperative infection rate after copolymer wafers implantation does not affect survival⁷. Although the long-term degradation of carmustine wafers has not been studied in patients, recent imaging studies prospectively investigated the MRI aspect of copolymer wafer during the years following surgery¹¹. The kinetics of changes of such images mainly helps to discriminate between a “normal” postoperative evolution and an abscess, a tumor residue or recurrence. A restricted diffusion in MRI reshaping copolymer wafer’ outline is known to be noticeable up to one year postoperatively. In light of our report and considering that it is highly uncommon to perform a recurrence glioma surgery distant from the use of copolymer wafer because of the short life expectancy of the patients, we question if such MRI findings could not be related to incomplete degradations of the P(CPP-co-SA). The local consequences of such events are unknown.

Conclusion

Despite exclusive *in situ* drug delivery during 5 days between surgery and standard radio-chemotherapy, carmustine shows limited efficiency. Our report shows that it could be related to an uncontrolled local tolerance and degradation of the copolymer. This finding underlines the importance of new studies concerning the long-term *in vivo* degradation of copolymer wafer in human. Moreover, alternative strategies to deal with the blood-brain barrier, such as drug-loaded liposomes or opening using ultrasound⁴ must be considered. They could offer larger drug diffusion and repetitive delivery compared to that observed with copolymer wafers⁹.

Disclosure: the authors declare no conflict of interest.

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Figure 1: A. Structure of polifeprosan 20: a random copolymer formed by 1,3-bis-(*p*-carboxyphenoxy)propane (CPP, green square) and sebacic acid (SA, blue square) monomers (20:80 molar ratio) connected by anhydride bonds ; B. Circled in red: carmustine wafer one year after a first surgery ; C. 1H RMN analysis of a control carmustine wafer (bottom), BCNU (middle) and the wafer extracted from patient (top). The unused wafer exhibits all expected peaks of CPP (a, b, c, and d) and SA (e, f, and g) units of the copolymer, together with peaks of BCNU in the 3.3–4.25 ppm region (c). The partially degraded wafer shows no peaks corresponding to CPP units, whereas peaks f and g belonging to SA units are still present, with traces of BCNU. Note that peak from SA is not visible, likely because of the poor solvation of the nearby carboxylic acid functions in deuterated chloroform (solvent used for the NMR study).

Carmustine wafer

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