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Journal of Controlled Release 103 (2005) 7–19

journal of controlled release

www.elsevier.com/locate/jconrel

In vivo anti-tumor effect of dual release of cisplatin and adriamycin from biodegradable gelatin hydrogel

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Received 15 April 2004; accepted 1 November 2004
Available online 15 December 2004

Abstract

The objective of this paper is to investigate the in vivo anti-tumor effect by dual release of cisplatin (CDDP) and adriamycin (ADM) from a biodegradable hydrogel. Hydrogels with different water contents were prepared through the chemical crosslinking of gelatin by various concentrations of glutaraldehyde. Aqueous solution of CDDP, ADM or their mixture (CDDP+ADM) was impregnated into the freeze-dried hydrogel, followed by air-drying to obtain the dried hydrogel incorporating the corresponding drug. Irrespective of the hydrogel water content, 8–20% of CDDP incorporated and 60–80% of ADM was released from the hydrogel in the phosphate-buffered saline solution (PBS) at 37 °C within the initial 6 h and thereafter little release was observed. When intratumorally applied into mice carrying a mass of Meth-AR-1 tumor cells, the hydrogel incorporating CDDP+ADM showed significant higher anti-tumor effect on the tumor growth suppression and on survival period than other drug applications. Combination effect assay revealed that the hydrogel incorporating CDDP+ADM showed a synergistic effect between the CDDP and ADM, while the solution form showed antagonistic. The concentration of CDDP and ADM in the tumor tissue maintained at higher levels over 14 days after application. The time course of in vivo CDDP retention was in a good accordance with that of hydrogel remaining, whereas ADM was released faster, followed by the sustained release for 14 days. No practically problematic change in the mouse body and blood biochemical parameters was observed by application of the hydrogel incorporating CDDP+ADM. We conclude that dual sustained release of CDDP and ADM attached to the tumor synergistically enhanced their in vivo anti-tumor effect through the trans-tissue delivery.

Keywords: Dual release; CDDP; Adriamycin; Anti-tumor effect; Gelatin hydrogel

1. Introduction

During the last decade, many researchers have investigated combination therapy of anti-tumor drugs to augment their tumoricidal efficacy as well as to

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doi:10.1016/j.jconrel.2004.11.014

reduce the side effects caused by the high-dose injection of single drug [1–4]. Presently, combination therapy with multiple drugs is a procedure clinically common in malignant tumor treatment. Cisplatin (CDDP) and adriamycin (ADM) have been being used most widely because of their broad spectrum of cytolytic activity against solid tumors [1–4]. The combination of the two drugs is one of the most common therapeutic regimens for ovarian, uterus, prostate and bladder cancers [1,3,4]. However, it is impossible only by the intravenous administration of CDDP and ADM to achieve their sufficiently high concentration and long retention period in the tumor tissue because the molecular size is small enough to rapidly excrete from the blood circulation [5,6]. Therefore, even the combination therapy does not always contribute to any anti-tumor effects for a long time period, in spite of the clinically acceptable activity for a short time period. As one trial to tackle the drug distribution problem, localized chemotherapy, such as intraperitoneal [3], intraarterial [4] and intratumoral [7,8] administrations, has been proposed from the clinical viewpoint. However, the trials are not effective in practically improving the retention profile of drugs in the tumor tissue, although their high and retained concentration is theoretically achieved. Therefore, it is highly expected to develop a new administration form not only for higher concentration of drugs in the tumor but also for their longer retention with reduced side effects.

Recently, drug delivery system (DDS) based on the enhanced permeability and retention (EPR) effect [9] has been explored for a therapeutic approach of CDDP and ADM to experimentally demonstrate their enhanced anti-tumor effects [6,10–17]. However, such drug targeting has often augmented the anti-tumor effect because a high drug dose can be achieved reduction in drug side effects [6]. As an alternative DDS approach, local intratumoral delivery of drugs with the release carriers has been reported to enhance their tumor targetability with stability and also to achieve their sufficient retention in the tumor tissue [5,6,13,15]. However, it should be noted that the tissue localization of DDS formulation injected (i.e., the inside of the tumor tissue vs. around the normal-tumor tissue boundary) plays a critical role in the therapeutic efficacy of drugs locally released [15,16]. In addition, from the practically clinical viewpoint of gynecological or gastrointestinal cancers, there is another therapeutic big problem to be improved. Almost of all patients with such major cancers, which are usually diagnosed at advanced stage with peritoneal disseminations (such as 70–75% cases of ovarian cancers [2]), became candidates for extensive surgical operation (also called debulking surgery) as the initial primary treatment. However, the rate of complete surgery is quite low (such as only 40% of ovarian cancers [2,3]), though the residual tumor volume is one of the most important prognostic factors. These residual tumors peritoneally disseminated are the main cause of patient's death and local recurrence. However, it is practically impossible to therapeutically treat the every residual tumor by the intratumoral injection of DDS agents [5,6,13,15], because of their large numbers, small size, and wide area disseminated. Therefore, it is highly required to develop an effective new local delivery system for every residual tumor, which can be targeted directly immediately at the initial operation with the most obvious information about its location, size, and distribution. We have prepared a gelatin hydrogel sheet incorporating CDDP as a new local trans-tissue controlled delivery system [14]. This hydrogel system is so soft and flexible that can attach tightly even to the rough surface of the residual tumors. Since the CDDP release is controllable and in a direction specific manner, the hydrogel sheet enabled CDDP to increase the local concentration around the area attached as well as to retain it for a long time period, resulting in significantly enhanced anti-tumor effects with few side effects [14]. Recently, the effectiveness of the same targeting strategy was also reported from another group and the trans-tissue targeting is promising to improve the prognosis of the patients who have been treated by extensive surgical operation [18].

The objective of this study is to experimentally confirm the hypothesis that a combination local dual delivery of two anti-tumor drugs from the gelatin hydrogel results in anti-tumor effects superior to the combination therapy of drugs in the solution form. A gelatin hydrogel sheet incorporating CDDP +ADM was prepared and the in vivo anti-tumor effect and toxicity were assessed to compare with those of hydrogel sheet incorporating either CDDP or ADM