Review

Cancer Research

Chloroquine in Cancer Therapy: A Double-Edged Sword of Autophagy

Tomonori Kimura, Yoshitsugu Takabatake, Atsushi Takahashi, and Yoshitaka Isaka

Abstract

Autophagy is a homeostatic cellular recycling system that is responsible for degrading damaged or unnecessary cellular organelles and proteins. Cancer cells are thought to use autophagy as a source of energy in the unfavorable metastatic environment, and a number of clinical trials are now revealing the promising role of chloroquine, an autophagy inhibitor, as a novel antitumor drug. On the other hand, however, the kidneys are highly vulnerable to chemotherapeutic agents. Recent studies have shown that autophagy plays a protective role against acute kidney injury, including cisplatin-induced kidney injury, and thus, we suspect that the use of chloroquine in combination with anticancer drugs may exacerbate kidney damage. Moreover, organs in which autophagy also plays a homeostatic role, such as the neurons, liver, hematopoietic stem cells, and heart, may be sensitive to the combined use of chloroquine and anticancer drugs. Here, we summarize the functions of autophagy in cancer and kidney injury, especially focusing on the use of chloroquine to treat cancer, and address the possible side effects in the combined use of chloroquine and anticancer drugs. *Cancer Res; 73(1); 3–7. ©2012 AACR.*

Introduction

Antimalarial drugs, chloroquine and hydroxychloroquine, are promising for cancer treatment (1–3). Several clinical trials that have been conducted or are in progress have shown favorable effects of chloroquine as a novel antitumor drug (4). Although the precise mechanism remains to be determined, the anticancer effects of chloroquine may partially be because of its inhibitory action on macroautophagy (hereafter referred to as autophagy).

Autophagy

Autophagy is a major intracellular pathway for the degradation and recycling of long-lived proteins, lipid droplets, protein aggregates, mature ribosomes, glycogen, and even entire organelles such us the endoplasmic reticulum, mitochondria, and Golgi apparatus (5–7). A schematic drawing of the autophagy process is shown in Fig. 1A. Initially, parts of the cytoplasm and cellular organelles are engulfed within a double-membraned vesicle called the autophagosome. The autophagosome fuses with lysosomes to form an autolysosome, which results in the degradation of the sequestered materials by various lysosomal hydrolytic enzymes. Degradation is followed by the generation of amino acids, sugars, fatty acids, and nucleosides that are recycled for macromolecular synthesis and energy production (6, 8). This recycling system is particularly important during starvation. Autophagy occurs at low basal levels to maintain cellular homeostasis by eliminating damaged proteins and organelles. Autophagy is also strongly induced under starvation conditions to supply amino acids by degrading proteins. In addition, autophagy is upregulated when cells need to rid themselves of damaging cytoplasmic components (6).

Autophagy is currently 1 of the hottest fields in science because of recent genetic studies in mice. Because systemic autophagy-knockout mice die within a day after birth (9), tissue-specific autophagy-deficient mice have been analyzed to elucidate the role of autophagy *in vivo* (10). These studies have highlighted the importance of autophagy in several organs, including the brain, liver, heart, hematopoietic cells, and kidney (11–17).

Autophagy in the Kidney

Autophagy has an important role in proximal tubular cells of the kidney, the main target of chemotherapy-related kidney injury. Proximal tubule-specific autophagy-deficient mice exhibit proximal tubular cell degeneration and accumulation of abnormal protein with aging (16), indicating that autophagy plays a critical homeostatic role in the proximal tubular cells (18). Autophagy also plays a protective role against acute kidney injury, such as ischemia–reperfusion and cisplatin treatment-induced injury, which are more severe in proximal tubule-specific autophagy-deficient mice compared with autophagy-competent mice (16, 17). Recently, quite similar findings were also reported (19), and these data showed that autophagy plays a protective role in proximal tubular cells of the kidney against aging and acute kidney injury.

Authors' Affiliation: Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan

Corresponding Author: Yoshitaka Isaka, Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Box B6, 2-2 Yamada-oka, Suita, Osaka, Japan 585-0871. Phone: 81-6-6879-3857; Fax: 81-6-6879-3857; E-mail: isaka@kid.med.osaka-u.ac.jp

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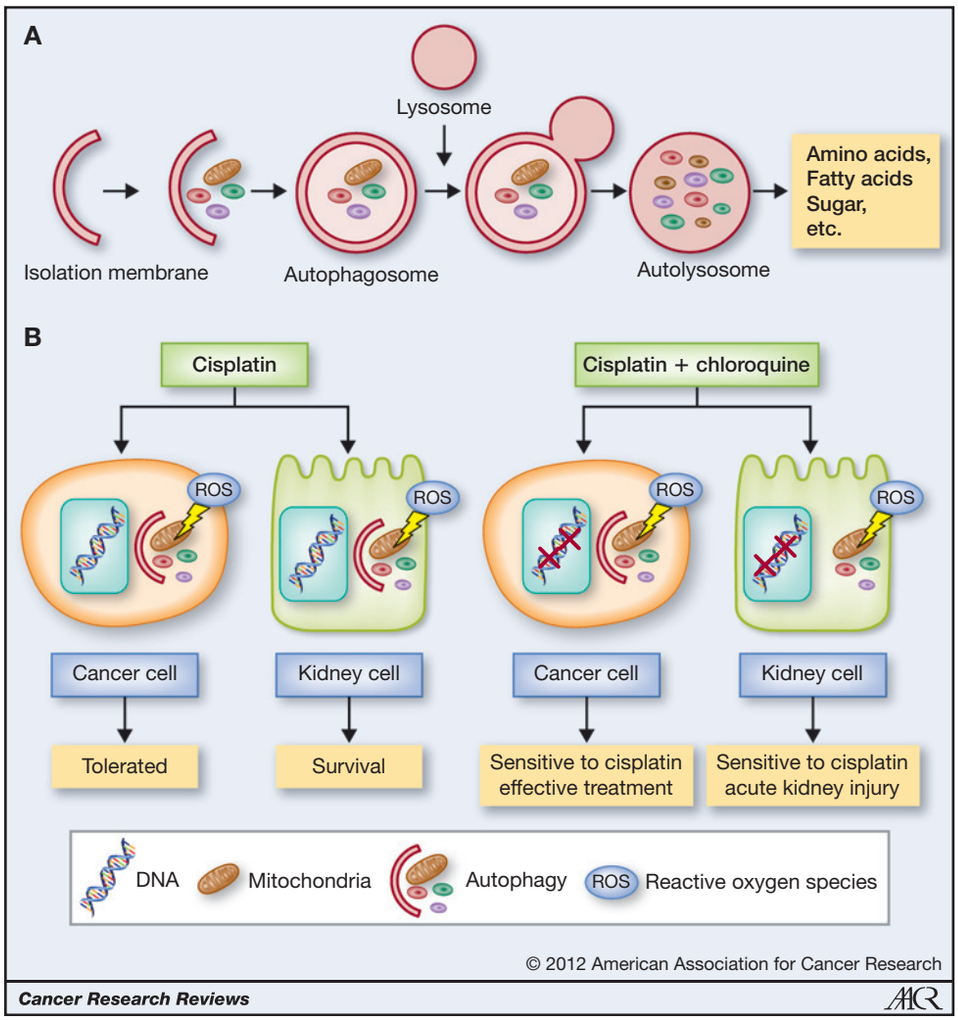

Figure 1. Combination of chloroquine and chemotherapeutic drugs (i.e., cisplatin) could exert toxic effect not only on cancer cells, but also kidney cells, which results in acute kidney injury. A, the process of autophagy. B, inhibition of autophagy by chloroquine could sensitize not only cancer cells, but also kidney cells to chemotherapy, resulting in DNA damage, production of mitochondrial reactive oxygen species, and cell death.

Autophagy and Cancer

Autophagy is considered to have 2 contrasting roles, promotion and suppression, in cancer cells. First, autophagy provides an energy source for cancer cells, which can survive in an environment that is unfavorable for normal cells. Cancer cells can survive under hypoxic and acidic conditions despite the lack of mature vessels. For energy production, cancer cells depend mainly on the glycolysis pathway (the Warburg effect), which is less efficient than the tricarboxylic acid cycle pathway; thus, cancer cells require more glucose uptake than normal cells (20). Autophagy is assumed to provide energy for cancer cells under such unfavorable conditions, and thereby, have a cancer-promoting role.

On the other hand, autophagy also has a cancer-suppressing role. Heterozygous disruption of *Beclin 1*, an autophagy-related gene, increases the frequency of spontaneous malignancies (21, 22). Thus, *Beclin 1* has cancer-suppressing activity. Liver-specific *Atg7*-deficient mice and mice with systemic mosaic deletion of *Atg5* develop benign liver adenomas (23, 24). Although *Beclin 1* has multiple functions, including endocytosis, these observations suggest a cancer-suppressing role of autophagy. A recent study suggested that autophagy is also necessary for anti-immune responses. In response to chemotherapy, autophagy in dying cancer cells enables the release of ATP, which attracts immune cells and triggers anti-immune responses (25).

Therefore, autophagy may have different effects at different stages of cancer. Further studies are necessary to understand the precise role of autophagy in cancer cells.

Chloroquine and Cancer Treatment

Chloroquine has long been used to treat or prevent malaria. Chloroquine is also used to treat autoimmune diseases in some countries because of its immunosuppressive properties. Although the precise mechanism underlying the antimalarial effects of chloroquine remains unknown, chloroquine seems to exert its effects through the weak-base lysosome-tropic feature (26, 27). When chloroquine enters the lysosome, it becomes protonated because of the low pH within the lysosome, and accumulation of the protonated form of chloroquine within the lysosome leads to less acidic conditions and, thereby, decreased lysosomal function.

Accumulating evidences indicates that chloroquine sensitizes cancer cells to radiation and other anticancer drugs. On the basis of the data from a number of clinical trials now in process, it can be inferred that this drug may alter cancer therapeutic strategies (1).

Although the precise mechanism by which chloroquine exerts anticancer effects is unclear, 1 possible mechanism is its antiautophagic activity. Autophagy is 1 of the physiologic processes affected by chloroquine. Inhibition of lysosome activity by chloroquine arrests the latter step of autophagy, degradation of the autolysosome, which results in the failure to provide energy through the autophagy pathway. Because autophagy seems to contribute to promote cancer, chloroquine may sensitize cancer cells through inhibiting autophagy. The dosage of chloroquine usually ranges between 100 and 500 mg/day. Side effects are minimal at low doses, while many more toxic effects occur at higher doses, such as visual disturbances, gastrointestinal upset, electrocardiographic changes, headache, and pruritus.

In addition to chloroquine, other autophagy inhibitors, such as bafilomycin A1, 3-methyladenine, and pepstain A, have been studied as antitumor drugs. One notion is that these drugs, including chloroquine and its derivatives, are not specific modulators of autophagy activity, that is, these agents also have some other effects on cellular functions, such as lysosomal function and endocytosis. Targeting more specific processes of autophagy is a more preferable approach in cancer therapy, however, and is now under investigation (28).

Chloroquine in the Kidney, the Double-Edged Sword of Autophagy

The use of chloroquine in combination with other chemotherapeutic reagents may enhance cancer treatment (8, 29), but normal cells also use autophagy to maintain homeostasis, and inhibition of autophagy by chloroquine may sensitize not only cancer cells, but also normal organs to chemotherapy.

Chemotherapy causes a variety of acute and chronic organ toxicities. The kidneys are highly vulnerable to chemotherapeutic agents because many chemotherapeutic agents and their metabolites are excreted through the kidney tubular epithelial cells (30). Chemotherapy could affect the kidney, with clinical manifestations ranging from an asymptomatic rise in serum creatinine levels to acute tubular injury requiring dialysis therapy. Therefore, the use of chemotherapeutic drugs is sometimes limited by their nephrotoxic side effects. Chemotherapy-related kidney injury often leads to an insufficient treatment of cancer because kidney dysfunction requires that clinicians reduce the dose of chemotherapy to avoid further kidney injury. Kidney damage also causes other unfavorable complications, such as water and nitrogenous waste retention, electrolyte disturbances, decreased immunity, etc. Indeed, acute kidney injury is associated with poorer prognosis (31).

Chemotherapeutic drugs induce kidney damage through the injury of the proximal tubules. Cisplatin is a frequently used chemotherapeutic agent that often causes acute kidney injury. The mechanism of cisplatin-induced nephrotoxicity has not been completely elucidated, but several mechanisms have been postulated, including (i) DNA damage, (ii) mitochondrial damage, (iii) oxidative stress, and (iv) ischemic injury caused by vascular damage (32).

Recently, a protective role of autophagy against cisplatin-induced kidney injury was shown (17). Proximal tubule-specific autophagy-deficient mice showed remarkably severe kidney damage after cisplatin treatment compared with wild-type mice through several mechanisms. First, autophagy protects proximal tubular cells from mitochondrial oxidative stress (the clearance of mitochondria by autophagy is specifically called 'mitophagy'). Second, autophagy also protects proximal tubular cells from DNA damage. Third, autophagy protects proximal tubular cells from abnormal protein accumulation. In addition, autophagy protects proximal tubular cells from ischemic injury (16).

Thus, chloroquine, which inhibits autophagy, may promote chemotherapy-induced kidney injury through multiple pathways (Fig. 1B). Both cancer cells and kidney proximal tubular cells tolerate cisplatin through the use of autophagy because autophagy protects cells, not only from DNA damage, but also from the mitochondrial reactive oxygen species induced by the degradation of damaged mitochondria. The use of chloroquine sensitizes cancer cells to chemotherapy and leads to anticancer effects through inhibiting autophagy. On the other hand, the very effect of chloroquine to inhibit autophagy could also sensitize kidney cells to chemotherapy, leading to acute kidney injury. Consistent with this notion, chloroquine-treated mice (60 mg/kg, daily) are more vulnerable to kidney injury from ischemic–reperfusion injury than vehicle-treated mcie (33). This study may indicate possible additional effect of chloroquine in chemotherapy-induced kidney injury.

The risk factors and preventive methods for combined chemotherapy and chloroquine-induced kidney injury are likely the same as those for chemotherapy-induced kidney injury. For example, the risk factors for cisplatin-induced kidney injury (such as decreased kidney function, high-dose of cisplatin, concomitant administration of nephrotoxic drugs, previous cisplatin chemotherapy, and older age; ref. 34) could also be risk factors for combination therapy-induced kidney injury. To avoid cisplatin-induced kidney injury, the dose of cisplatin is lowered and saline is intravenously infused, and these procedures should also be applied for combination therapy-induced kidney injury. High-risk patients should be prepared for the standard approach toward prevention with close monitoring.

Even if acute kidney injury occurs in chloroquine-treated patients because of the inhibition of autophagy, it may not be possible to determine the precise cause and effect. Kidney biopsy from patients with chloroquine-associated kidney injury may not provide definitive information. Monitoring autophagy in human biopsy specimens is often difficult (35). To observe autophagy-related structures by transmission electron microscopy, perfusion fixation of tissues is recommended, but it is usually feasible in biopsy specimens and complete fixation of these specimens is difficult. Furthermore, correct identification and quantification of the autophagic compartments by electron microscopy is relatively difficult (35). Whether immunohistochemistry of autophagy-

related gene products in the tissues corresponds to autophagic activity has not been clearly shown, and this area of research requires further exploration. Methods of monitoring the activity of autophagy in human are currently unavailable and are under vigorous study.

Chloroquine in Other Organs

With regard to the autophagy-inhibiting effects, chloroquine and other autophagy inhibitors may accelerate chemotherapy-related organ injuries in other organ besides the kidney. Autophagy-deficiency exacerbates injuries in organs such as the brain, liver, heart, and hematopoietic cells (11–15); thus, the combination use of chemotherapy with chloroquine may unexpectedly worsen the function of these organs. For example, most chemotherapies often cause bone marrow depression, and the addition of chloroquine may worsen this condition. Anthracycline antibiotics, such as doxorubicin and daunorubicin, cause cardiotoxicity in a dose-dependent manner, presumably through DNA damage, and the addition of chloroquine to the therapeutic regimen could worsen the cardiotoxicity. To our knowledge, the sensitivity of organs other than the kidney has not been fully examined under the conditions of autophagy inhibition or ablation in tissue-specific autophagy-deficient mice. Therefore, the adverse effects of chloroquine in combination therapy are speculative, and further studies are required.

Conclusion

Chloroquine is a promising drug for cancer therapy and relatively safe, especially for a short period of time. Unexpected side effects on organs, such as the kidney, however, could occur, especially when combined with anticancer drugs, possibly through the inhibition of autophagy. Therefore, clinicians in the field of cancer and kidney therapy should be aware of this possibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Isaka, T. Kimura
Development of methodology: T. Kimura
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Kimura
Writing, review, and/or revision of the manuscript: Y. Isaka, T. Kimura, Y. Takabatake
Study supervision: T. Kimura
Other: A. Takahashi

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Correction: Chloroquine in Cancer Therapy: A Double-Edged Sword of Autophagy

In the printed version of this article (Cancer Res 2013;73:3–7), which was published in the January 1, 2013 issue of *Cancer Research* (1), the OnlineFirst publication date of December 27, 2012 was not included. The publisher regrets this error.

Reference

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