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Research Article

MEASUREMENT OF ANTIOXIDANT POWER OF MOUTHWASHES INDICATED IN STOMATITIS

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ABSTRACT

Objective: Hospital formulations containing allopurinol and rebamipide are used in the prophylactic and therapeutic management of stomatitis, owing to their antioxidant powers. The objective of this study was to measure the antioxidant powers of Zyloric® tablets (allopurinol), Mucosta® tablets (rebamipide), different hospital formulations indicated in the management of stomatitis (allopurinol and rebamipide mouthwashes), and Azulene® 0.4% for Gargle (sodium azulene sulfonate).

Methods: We measured the antioxidant powers of Zyloric® and Mucosta® tablets, all hospital formulations indicated in the management of stomatitis (allopurinol and rebamipide mouthwashes), and the widely used Azulene® 0.4% for Gargle by employing the biological antioxidant potential test. We compared the efficacy of each of these drugs in the management of stomatitis.

Results: Azulene® 0.4% for Gargle was found to have stronger antioxidant power than Zyloric® (100 mg) and Mucosta® (100 mg) tablets dissolved in water. The antioxidant power of the solvent used in hospital formulations was similar to that of the prepared hospital formulation. Antioxidant power of the drugs themselves was not observed in both the allopurinol and rebamipide mouthwashes.

Conclusion: The antioxidant power of the drugs was not observed in both the allopurinol and rebamipide mouthwashes; therefore, hospital formulations used as antioxidants were found to be less effective in the treatment of stomatitis. However, Azulene® 0.4% for Gargle was found to be useful in the prophylactic and therapeutic management of stomatitis, owing to its antioxidant, and anti-inflammatory effects.

Keywords: Stomatitis, Bone alkaline phosphatase-test, Allopurinol mouthwash, Rebamipide mouthwash, Azulene® 0.4% for Gargle, Antioxidant power.

INTRODUCTION

Stomatitis frequently observed in cancer chemotherapy is the adverse drug reactions that affect the patient's quality of life (QOL) and treatment response rate. In particular among the anticancer drug, 5-Fluorouracil (5-FU) is known to induce stomatitis with high probability [1]. In general, the incidence of stomatitis in cancer chemotherapy of strength is less than or equal to an average of 10% in the regimen that does not include the 5-FU. In contrast, in a regimen containing 5-FU and is a Grade 3 or more stomatitis in criteria of National Cancer Institute-Common Terminology Criteria for Adverse Events is above an average of 15% [2].

In the cases of combined use of chemotherapy and radiation therapy in the treatment of head and neck cancer and implementing the high-dose chemotherapy with hematopoietic malignancies, the incidence of stomatitis is also reported that more than 50% [2]. Occurrence of stomatitis is not only physical pain of the patient, but also bring the reduction of dietary intake and reduction of the desire to continue the chemotherapy. From the fact, that prevention and mitigation of stomatitis are allowed to reduce the physical and mental distress in chemotherapy, thereby improves the QOL of patients. In order to improve the therapeutic outcome in cancer chemotherapy, efforts to avoid serious stomatitis causes the dose limiting is important.

Pathogenic mechanism of stomatitis caused by anticancer agent administration can be divided into two types [3,4]. The pathogenesis of stomatitis in chemotherapy, and that anticancer agent occurs is failure to act directly to the oral mucosa (primary stomatitis), those that cause intraoral infection by bone marrow suppression associated with such leukopenia (secondary stomatitis) has been considered. Therefore, prevention strategies are carried out by the direct and indirect administration of cytoprotective agents. Adjusting the metabolites of the cytotoxic agent are carried out for the prevention of stomatitis. Infection prevention is being carried out by the prevention of neutropenia [5]. Prevention and treatment strategies stomatitis associated with 5-FU have been reported. Those are the methods considering the pharmacological effects [6-11], and non-pharmacological method. As non-pharmacological, there are oral cavity cooling therapy [12] and helium neon laser [13-15].

As prevention and treatment of direct stomatitis in the treatment of cancer, there is a gargle therapy with allopurinol [8-11] and rebamipide [16,17].

Zyloric® tablets of hyperuricemia therapeutic drug have a free radical scavenging action. The action of allopurinol is it is to be effective for the prevention of stomatitis during cancer treatment.

However, about the usefulness of these drugs, while there are reports such effect was observed in stomatitis [11,18], there is a report that said the effect was not observed [19]. In 2007 of the Cochrane report, allopurinol mouthwash effect has also been described as weak and unreliable [20].

Strength of the antioxidant power of allopurinol and rebamipide has been reported so far, is estimated based on the result when it is completely dissolved agent of the drug in an organic solvent [21]. Antioxidant power when dissolved their drug in water has not been reported. In clinical, drug because it is used by dissolving in water, it is necessary that the strength of the antioxidant power when it is a drug dissolved in water are also considered.

In this study, we evaluated the strength of the antioxidant power of allopurinol and rebamipide drugs and gargling solutions when dissolved in water by using free radical analyzer (FREE [Free Radical Elective Evaluator, Italy, Diacron International s.r.l.]).

In addition, we were also measured anti-oxidizing power of sodium azulene sulfonate mouthwash which is indicated for stomatitis at the same time. And we have also examined the usefulness of two oral mouthwashes (allopurinol and rebamipide mouthwash) that has been used in stomatitis prevention and treatment of cancer treatment.

METHODS

Materials and reagents

Zyloric® (100) tablets were purchased from Kyorin Pharmaceutical Co, Ltd. (Tokyo, Japan). Mucosta® (100) tablets were purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Azulene® 0.4% for Gargle was purchased from Taisho Pharmaceutical Industries, Ltd. (Shiga, Japan).

Sodium carboxymethylcellulose (CMC-Na) was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Alkox® E-30 (Polyethylene oxide) was purchased from Meisei Kogyo Co., Ltd. Inageru® (Iota-carrageenan) was purchased from Ina Food Industry Co., Ltd. (Nagano, Japan). Pineapple taste flavor of Elental® was provided from Ajinomoto Pharmaceuticals Co., Inc. (Tokyo, Japan).

Preparation of sample solution

Samples preparation for comparing the antioxidant power among drugs was performed in the following procedure. Zyloric® (100) tablet, Mucosta® (100) tablet, and Azulene® 0.4% for Gargle were put into each of separate Erlenmeyer flask, and added to 100 ml of water to each flask. After the solutions were stirred well, each of the solutions were centrifuged at 3000 rpm for 5 minutes at 4°C. The test solutions were obtained by collecting the aqueous phase, and each of the solutions is passed through a membrane filter (0.45 μm). After that, the solution samples were measured for analyzing the antioxidant power by FREE. Allopurinol mouthwash and rebamipide mouthwash was prepared according to "hospital pharmacy formulation case studies - The compliance guidance on the preparation and use of hospital formulation" of Japanese Society of Hospital Pharmacists supervision. Process for the preparation of each mouthwash is as follows.

Procedure of preparing allopurinol method is that 100 ml of water and 1 g CMC-Na were added into the 300 ml Erlenmeyer flask. And then crushed Zyloric® tablet was added into the solvent, and stirred very well.

Procedure of preparing rebamipide mouthwash is that 1 g Alkox® E-30 and 0.4 g Inageru® were dissolved in the water, and then the grinded Mucosta® table was put into the solvent, and stirred well. Finally, for the bitterness measures of rebamipide, pineapple taste flavor of Elental® was added into the solvent.

Allopurinol mouthwash and rebamipide mouthwash were passed through a filter. Then, each the solution samples were measured for analyzing the antioxidant power by FREE.

Equipment and reagents

Measurement of antioxidant activity using a FREE (Free Radical Elective Evaluator: F.R.E.E., Diacron International s.r.l., Grosseto, Italy). It should be noted, bone alkaline phosphatase (BAP) values of healthy individuals of Japanese, has been reported to be approximately 2000 μmol/L. Reagents for analyzing were used the FREE dedicated BAP test kit.

<Kit Contents>

Reagent 1: Thiocyanate solution (in advance 1 ml is sealed in a capped cuvette which also serves as a colorimetric cell) (Solvent: Water, isopropyl alcohol [50% or less] methanol [10% or less], ammonia [0.5% or less]).

Reagent 2: Iron oxide aqueous solution (2% or less).

Measurement principle

BAP test are using the following reaction. Trivalent iron salts ($FeCl_3$), it is red as functions by ferric ions (Fe^{3+}) when dissolved in a colorless solution containing a specific thiocyanate derivatives. Thereafter, when antioxidants are added to the solution, the trivalent iron ion (Fe^{3+}) is reduced to divalent iron ion (Fe^{2+}), then the solution is decolorized. The color change is measured with a photometer, and it is a method for evaluating antioxidant power.

1. $FeCl_3$ + AT (Colorless) → [$FeCl_3$-AT (Red)]
2. [$FeCl_3$-AT (Red)] + BP (e^-) → $FeCl_2$ + AT (Colorless) + BP

(AT: Thiocyanate, BP: Antioxidant)

In proportion to the reducing power of antioxidants, occurs fading of the solution which has been colored red. Before bleaching, the difference in absorbance after fading is shown as the reducing power (μmol/L).

Measurement method

Reagent 1 which is keeping 37°C in the cuvette was added 50 μl of reagent 2 and mixed well, then the reagent 1 in the cuvette was changed in red. After that, the reagent 1 was measured by FREE in the 505 nm photometer. Then added to the 10 μl sample in the cuvette, and after the cuvette was kept for 5 minutes to 37°C thermal insulation space of FREE. After that, the cuvette was placed into the photometer of FREE, and the antioxidant power of the sample was measured.

Statistical processing

Antioxidant power was shown as mean ± standard error. Test of the difference between the mean values of the two groups were used unpaired *t*-test. Comparison between multi-group performs one-way ANOVA, When it was significant, it was assayed using the Tukey's test as *post-hoc* test. Significance level for all tests was carried out with p=0.05. In addition, IBMSPSS statistics® 22 (IBM SPSS Japan) was used for statistical analysis.

RESULTS

Comparison of the antioxidant power of between the drugs

Zyloric® (100) tablet, Mucosta® (100) tablet, and Azulene® 0.4% for Gargle were dissolved in 100 ml of water, then the antioxidant power of each drug were determined by the BAP test (each n=5). The antioxidant power of Zyloric® tablets was 224.3±48.0 μmol/L (n=5), of Mucosta® tablets 445.6±58.8 μmol/L (n=5), and of Azulene® 0.4% for Gargle was 796.8±63.9 μmol/L (n=5). In the result of multiple comparison test (Tukey's test), a significant difference was observed among all drugs (each $p<0.05$) (Fig. 1).

Comparison of the antioxidant power between solvent

The solvents of allopurinol mouthwash and rebamipide mouthwash were measured, respectively. As a result, the solvent of allopurinol mouthwash was showed the value of the average at

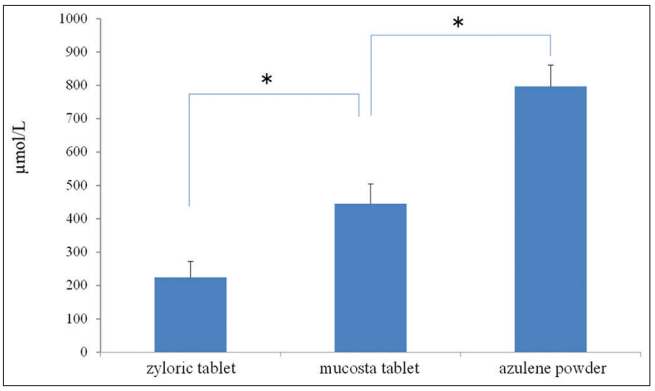

Fig. 1: Comparison of the antioxidant power of among drugs.

The antioxidant power of Zyloric® tablets was 224.3±48.0 μmol/L, of Mucosta® tablets was 445.6±58.8 μmol/L, and of Azulene® 0.4% for gargling was 796.8±63.9 μmol/L. Significant differences were observed among all drugs (each $p<0.05$)

3167.3±36.3 µmol/L. And the solvent of rebamipide mouthwash was showed the value of the average at 7807.1±58.8 µmol/L (Fig. 2).

Comparisons between the two groups were performed using the unpaired *t*-test, then a significant difference was observed between the two groups ($p<0.05$).

Comparison of the antioxidant power of between the formulations

The results of the strength of antioxidant power were measured between the formulations. Anti-oxidizing power of among the allopurinol mouthwash, rebamipide mouthwash, and Azulene® 0.4% for Gargle were compared. Allopurinol mouthwash was 2535.1±144.6 µmol/L, rebamipide mouthwash was 7806.0±29.0 µmol/L, and Azulene® 0.4% for Gargle was 796.8±63.9 µmol/L (Fig. 3).

In the test of the difference between the three groups of using the Tukey's test, antioxidant power is strong in the order of rebamipide mouthwash > allopurinol mouthwash > Azulene® 0.4% for Gargle was shown ($p<0.05$) (Fig. 1). In addition, as a control, the antioxidant power of the saliva of adult man was measured. And the result was about 2000 µmol/L.

DISCUSSION

The anti-oxidizing power among the Zyloric® tablets (100 mg), Mucosta® tablet (100 mg), and Azulene® 0.4% for Gargle in an aqueous solution were tested by one-way analysis of variance, and the difference significant difference was shown among them ($p<0.05$). Moreover, significant differences among all of the drugs in the multiple comparison of Tukey's test were observed ($p<0.05$). Azulene® 0.4% for Gargle is to have a stronger anti-oxidizing power than the Zyloric® tablets (100 mg) and Mucosta® tablet (100 mg) dissolved in water was shown. Traditionally, Zyloric® tablets (principal agent: Allopurinol), and Mucosta® tablets (principal agent: rebamipide) has been reported to have a very strong antioxidant power [22].

However, these reports are reported when it is completely dissolved the principal agent with an organic solvent such as dimethyl sulfoxide, and not the result of a case of dissolving the drugs in the water used in the clinic. It is reported that the solubility in water of allopurinol is 3.5×10^{-4} g/mL (25°C), and the solubility in water of rebamipide is 6.0×10^{-6} g/mL (25°C). Therefore, in the case of dissolving each one tablet of 100 mg with 100 ml water, it is considered that both of drugs are not seem to be completely dissolved in water. From an existing report, solubility and antioxidant power has been reported to be higher in allopurinol than rebamipide [22]. However, in this result, rebamipide is showed higher antioxidant power value than allopurinol. The reason for this, either of the drugs also dissolved in the state of crushed tablets in water, each of the excipients contained in the tablet can be considered that affected the solubility of the principal agent. At the same time, it is also considerable that the excipient showed antioxidant activity in solution. As a result of comparing the antioxidant powers among mouthwash formulations, rebamipide mouthwash showed the strongest antioxidant power. The reason during the preparation of the rebamipide mouthwash, citric acid contained in flavor of Elental® that is added as a flavoring agent to mask the bitter taste of rebamipide is considered that show strong antioxidant power. Allopurinol mouthwash was also showed a strong anti-oxidizing power than when it is dissolved the Zyloric® tablet in water. It was also thought to be due to the antioxidant power of the CMC-Na is used as a solvent (Fig. 2). BAP test values for the biological sample, such as healthy human blood, are commonly reported to show a value of about 2000 µmol/L.

In this study, Azulene® 0.4% for Gargle is the highest value results were showed.

However, even in Azulene® 0.4% for Gargle which was the highest anti-oxidizing power, BAP test values had 1000 µmol/L following numbers in the usually using regimen dosage. Therefore, the preventive effect of stomatitis by mouthwashes expected the effect of anti-oxidizing power,

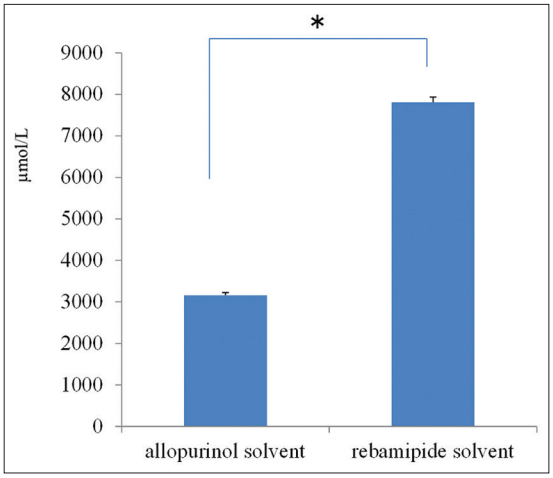

Fig. 2: Comparison of the antioxidant power between solvents.
The antioxidant power of the allopurinol mouthwash solvent was 3167.3±36.3 µmol/L, and that of the rebamipide mouthwash solvent was 7807.1±58.8 µmol/L. A significant difference was observed ($p<0.05$)

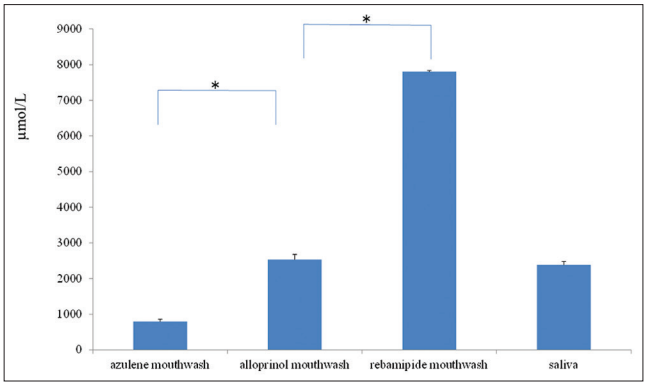

Fig. 3: Comparison of the antioxidant power among formulations.
The antioxidant power of allopurinol mouthwash was 2535.1±144.6 µmol/L, rebamipide mouthwash was 7806.0±29.0 µmol/L, and Azulene® 0.4% for gargling was 796.8±63.9 µmol/L. Significant differences were observed among all drugs (each $p<0.05$)

was considered likely too small. In fact, in previous reports about stomatitis prophylaxis by allopurinol mouthwash, both reports are present "stomatitis prophylaxis effect was observed" and "stomatitis prophylaxis effect was not observed." Then the actual effect is unknown. High antioxidant power in stomatitis formulations shown in this study results are not due to the drug is due to an anti-oxidizing power of the solvent itself. Anti-oxidizing power and anti-oxidizing power of nosocomial formulation of solvent in each hospital formulation was almost the same value. The results of this study, it is considered to be useful to use Azulene® 0.4% for Gargle than using allopurinol mouthwash and rebamipide mouthwash.

Azulene® 0.4% for Gargle for the treatment of stomatitis, as well as antioxidant activity, also have anti-inflammatory effects. Azulene® 0.4% for Gargle, which is a stomatitis of therapeutic agents, as well as anti-oxidation effect, also has anti-inflammatory effect. As Formulations products Azulene® 0.4% for Gargle, it is used azulene-xylocaine mouthwashes, which was mixed with Xylocaine in the Azulene® 0.4% for Gargle solution. Because it has also analgesic effect in addition to azulene effect, it is particularly useful for patients suffering from pain stomatitis. In conventional mouthwashes, expected antioxidant power may effect is not weak or sufficient. Needless to say, it is important to use the drugs that clinically effect is clear in the use of drugs.

CONCLUSION

In the prevention and treatment of stomatitis caused by anticancer agents, it should be used the drugs which clearly improve the QOL of patients.

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