OK-432 溶解液とリピオドールとの 混合液のエマルジョン化の判定

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Assessment of the emulsification of lipiodol in OK-432 suspended aqueous solution

key words : OK-432, lipiodol, emulsion, Iopamiron 300

筆者らは、後腹膜リンパ節転移に対し、OK-432 を局注し、リンパ指向性を持たせ、局注した範囲を透視下で知ると同時に徐放性を持たせることを検討している。そこで、OK-432 とリピオドールとの混合液の外観と顕微鏡下での相分離の過程と、エマルジョンの状態を経時的に観察した。

OK-432 を生理食塩水で混和、リピオドールと懸濁したが、安定したエマルジョンは得られなかった。

① そこで、非イオン性造影剤であるイオパミロン 300 を生理食塩水の代わりに用いたが、混合直後から水相の合一が観察され、3 時間後にはすでに相分離を起こしていることが外観の観察から確認された。

② また水相と油相の比重差を軽減した処方でのエマルジョンでは、合一の速度は抑制できなかったものの、沈降、浮上の要因は解消された。しかし、①と同様の経過をとり、より安定したエマルジョンは得られなかった。このように沈降と浮上による合一が避けられたものの、相分離が進行するのは水相同士との接触による合一の確率が高いというところに起因すると推測し、さらに水相と油相の混合体積比をかえた実験を試みた。

③ 比重差を軽減した水相の体積を従来の半分にまで下げ、水相：油相が 3：1 のところを 6：1 にし、エマルジョンを調製したところ、かなりの安定化傾向が得られた。これらの結果から、この系では安定したエマルジョンの状態が保持されているものと考えられた。

筆者らは後腹膜リンパ節転移に対し、OK-432 を局注する処方として、①、②、③の処方考えた。③のような、比重だけでなく体積比も考慮した処方は、筆者らの目的に適した処方であると考えた。

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OK-432 was developed with attention to the antioncotic effect of hemolytic streptococcus. It is a bacterial preparation produced by lyophilizing *Streptococcus pharyngae* (group A, type 3) strains. Clinically, OK-432 is an antioncotic agent used concomitantly with chemotherapy for prolongation of the survival period of patients with gastric carcinoma (surgery cases) or primary lung cancer and for treatment of head and neck cancer and thyroid gland cancer for which other therapies have proved ineffective. This agent is suspended in physiological saline and injected locally as an OK-432-suspended aqueous solution into a tumor or the surrounding area. At our institutions, OK-432 is injected locally into lymphonodus cancer metastasized from gastric carcinoma or the surrounding tissue. To prepare a slow-releasing OK-432 solution and to examine the uptake of the substance into the lymphonodus, an OK-432-suspended aqueous solution is mixed with Lipiodol.

In our previous report¹⁾, an assessment was made of whether or not this mixture was emulsified. The results showed that separation of the water phase from the oil phase began immediately after mixing and the phase separation three hours after preparation, as could be observed clearly with the naked eye.

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In the present study, the physiological saline was replaced by Iopamiron 300, a non-ionic aqueous contrast medium. Time-course observation of variations in the stability of the emulsions showed that this method made it possible to obtain more stable emulsions than the previous one.

The results are reported in this paper.

Methods and Materials

The experimental agents were OK-432 (5KE) : Lot No. Z1A11 (Chugai Co., Ltd.), Iopamiron 300 : Lot No. 82037 (Japan Scherring Co., Ltd.), and Lipiodol Ultrafluid (10 ml) : Lot No. 9ILU036 (Kodama Co., Ltd.). Emulsions were prepared by the three methods, (1), (2) and (3), described below and were assessed for stability.

(1) Preparation according to the method specified in this study

Iopamiron was employed in place of the physiological saline used in the previous study¹⁾. 0.5 ml of Iopamiron 300 was added to each of two vials of OK-432 (5KE) and mixed sufficiently until suspended. The two OK-432-suspended solutions were then mixed together to prepare 10 KE/ml Iopamiron 300. Two syringes were attached to a three-way cock. One syringe contained 1 ml of the suspended solution prepared as above, while the other syringe contained 3 ml of Lipiodol. Mixing and emulsification were performed for 15 minutes to ensure that the particles in the prepared emulsion reached a stationary state. After sufficient time had elapsed for emulsification, the mixed solution was placed in a test tube for observation.

The state of the emulsion was observed in two ways : observation of the process of phase separation with the naked eye and examination of the state of the emulsion under an optical microscope. Photographs were taken for both

types of observation. The time-course observations were carried out with the test tube at rest at room temperature at the following times : immediately after, 1, 2, 3, 4, 5, 10, and 20 hours after, and 1, 3 and 7 days after mixing.

(2) Preparation by reducing the difference between the specific gravities of the water and oil phases

In this preparation, the difference between the specific gravities of the water and oil phases was reduced. The diminished difference in specific gravity was expected to make the water phase, or Iopamiron phase, remain stable in the Lipiodol phase without causing sedimentation or floating.

Iopamiron (1 ml, $d=1.328$) was diluted in advance with 1.16 ml of purified water to obtain a similar specific gravity to that of Lipiodol ($d=1.275$ to 1.290). An emulsion was then prepared by mixing the oil phase with the water phase at a ratio of three to one according to the method of Preparation (1). Additives contained in the OK-432 were not taken into account for calculation of the specific gravity of Iopamiron, since they were believed not to significantly contribute to variations in the specific gravity. Observation was conducted in the same ways as for Preparation (1). The observation times were immediately after, 1, 3 and 15 hours after, and 1 and 7 days after mixing.

(3) Preparation by adjusting the specific gravity of the water phase to equal that of the oil phase and varying the volumetric ratio of the water phase to the oil phase

The volumetric ratio of droplets of aqueous particles to the oil phase was lowered in this preparation to reduce the frequency of collision between the two phases in expectation of further improvement in the stability of the emulsion. Prior to preparation of the emulsion, Iopamiron ($d=1.328$) was diluted with purified

Fig. 1 Immediately after the mixing

Observation with the naked eye showed a milk-white uniform emulsion. Examination under the optical microscope also revealed the formation of the emulsion. However, the water phase aggregated at a high speed.

Fig. 2 One hour after the mixing

Observation with the naked eye and under the optical microscope showed the milk-white uniform emulsion as immediately after the mixing. The aggregation of the water phase was also confirmed.

water to obtain a specific gravity similar to that of Lipiodol ($d=1.275$ to 1.290), as in the case of Preparation (2). An emulsion was then prepared by mixing the oil phase with the water phase at a ratio of six to one according to the method of Preparation (1). The same observation methods were employed as for Preparation (1). The observation times were immediately after, 1, 2, 3, 5, and 18 hours after, and 1, 2 and 5 days after mixing.

Results

(1) The time-course variations for the Lipiodol emulsions in the OK-432-suspended Iopamiron solution were as shown

Immediately after mixing of the Lipiodol and the OK-432-suspended Iopamiron solution (Fig. 1) : Observation with the naked eye showed a milk-white uniform emulsion. Examination under the optical microscope also revealed formation of an emulsion. The water phase aggregated at a high speed, however, as in the previous study¹⁾ with physiological saline. This suggested that the emulsion was relatively unstable.

One and 2 hours after mixing (Figs. 2 and 3) : Both observation with the naked eye and observation under the optical microscope showed a uniform milk-white emulsion, just as immediately after mixing. The aggregation of the

Fig. 3 Two hours after the mixing

Observation with naked eye and under the optical microscope showed the milk-white uniform emulsion as immediately after the mixing. The aggregation of the water phase was also confirmed.

Fig. 4 Three hours after the mixing

The mixture started separating into two phase—the mixture—Lipidol phase and the water phase upon observation with the naked eye. Observation under the optical microscope further revealed aggregation of the water phase.

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Three hours after mixing (**Fig. 4**) : The mixture started separating into two phases—an emulsion-maintained Lipidol phase and a water phase—upon observation with the naked eye. A process of phase aggregation and separation similar to that in the previous study¹⁾ was observed. There was the difference, however, that the separated Iopamiron water phase formed the lower layer, whereas the saline phase formed the upper layer in the previous study¹⁾. Microscopic examination further revealed aggregation of the water phase.

Four, 5, 10, and 20 hours after and 1 and 3 days after mixing (**Fig. 5**) : **Fig. 5** shows the

emulsion 3 days after mixing. Examination by the naked eye showed clearly that the separation of the water phase from the Lipidol phase had progressed further. Microscopically, the degree of aggregation of the Iopamiron emulsion was relatively reduced, partly because the concentration of the emulsion in the Lipidol phase had declined by this time. The remaining emulsion showed a tendency toward stabilization.

Seven days after mixing (**Fig. 6**) : Observation with the naked eye revealed a change was noted in the remaining emulsion, which had shown a tendency to be relatively stable. The upper part of the Lipidol phase had turned

Fig. 5 Three days after the mixing
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Fig. 6 Seven days after the mixing
Upon observation with the eye, a change was noted in the remaining emulsion which had showed a tendency to be relatively stable. The upper part of the Lipiodol phase turned light yellow and transparent, the original color of Lipiodol. This suggested that complete separation of the water and Lipiodol phase had begun.

light yellow and transparent, the original color of Lipiodol. This suggested that complete separation of the water and Lipiodol phases had begun.

(2) The time-courses variations in the preparation obtained by reducing the difference between the specific gravities of the water and oil phases were as shown

In the preparation obtained by reducing the difference between the specific gravities of the water and oil phases, the factors of aggregation, sedimentation and floating were eliminated, although aggregation was not depressed.

Nevertheless, the preparation went through the same process of phase aggregation and separation as Preparation (1). Thus, the preparation obtained by this method failed to provide a more stable emulsion.

(3) The time-course variations in the preparation obtained by varying the volumetric ratio of the water phase to the oil phase were as shown

Immediately after mixing (**Fig. 7**) : In Preparations (1) and (2), the aggregation of the water proceeded at such a high speed that it could be observed microscopically. In this preparation,

Fig. 7 Immediately after the mixing
Observation with the naked eye showed a milk-white uniform emulsion, immediately after the mixing. Examination under the optical microscope also revealed the formation of the emulsion.

Fig. 8 Five days after the preparation
Observation with the naked eye did not show phase separation five days after the preparation as well. Examination under the optical microscope also revealed the formation of the emulsion.

on the other hand, although the aggregation speed was relatively slow and the aqueous particles increased in diameter, aggregation occurred extremely rarely. The diameter of the aqueous particles increased from approximately $10\ \mu\text{m}$ at the initial stage to $20\ \mu\text{m}$, at which point the particles were maintained in a nearly constant state for five days.

Observation by the naked eye also showed no phase separation five days after the preparation (Fig.8).

Discussion

Attempts have recently been made to develop new administration methods for a biological

In this preparation, aggregation speed was relatively slow and aqueous particles grew in diameter, aggregation occurred extremely rarely. The diameter of aqueous particles increased from approximately $10\ \mu\text{m}$ at the initial stage to $20\ \mu\text{m}$, at which the particles were kept in a nearly constant stage for five days.

response modifier (BRM) by introducing the concept of DDS. Studies on administration methods for OK-432 have also been conducted, including oral administration in the form of an emulsion with medium-chain triglyceride^{2,3)} and mixed administration with fibrinogen for local immunotherapy^{4,5)}.

It has also been reported that local injection of OK-432 into a tumor was effective^{6,7)}.

Because of its high viscosity, Lipiodol is used for purposes other than its original function as an oily contrast medium: ① Obstruction of blood flow, or increased thrombotic effect in more peripheral areas, and ② enhancement of the slow-releasing effect of anticancer agents, as when an anticancer agent is injected into a tumor in the form of a suspended solution with Lipiodol to make the anticancer agent slow-releasing.

Lipiodol has also been reported to be transferred to the lymphatic system⁸⁾. Lipiodol is used concomitantly, for example, to improve the efficacy of transcatheter arterial embolization for liver cancer⁹⁻¹¹⁾. Concomitant use of Lipiodol as a carrier has been studied both basically¹²⁻¹⁶⁾ and clinically^{10,17)}. Taking note of these facts, we injected Lipiodol emulsions in an OK-432-suspended aqueous solution locally into the posterior peritoneum for lymphonodus cancer metastasized from gastric carcinoma to examine the extent of uptake of OK-432 into the lymphonodus and, at the same time, to furnish the OK-432 with a slow-releasing effect^{1,18,19)}. The mixed solutions of OK-432 and Lipiodol were observed for phase separation and variations in the states of emulsion with the naked eye and under an optical microscope.

A previous study had shown that Lipiodol emulsions in OK-432-suspended physiological saline solution did not demonstrate preferable stability¹⁾. In the present study, therefore, the physiological saline was replaced by the non-ionic contrast medium Iopamiron 300. The results for Preparation (1) showed that the water(Iopamiron) phase began aggregation immediately after the mixing of the OK-432-suspended Iopamiron solution with Lipiodol. Observation with the naked eye confirmed a clear phase separation as early as three hours after preparation. In the emulsion prepared by

reducing the difference between the specific gravities of the water and oil phases (the method of Preparation (2), the aggregation factors of sedimentation and floating were eliminated, despite the failure to depress the rate of aggregation. This emulsion exhibited the same progress as Preparation (1), however. The formation of an emulsion with improved stability was not achieved.

It was assumed that this progress of phase separation, occurring despite the avoidance of water phase aggregation due to sedimentation and floating, resulted primarily from collision between the aqueous particles. An experiment was then attempted involving by varying of the volumetric ratio of the water phase to the oil phase. In Preparation (3), the difference between the specific gravities of the water and oil phases was reduced. The volume of the water phase was reduced to half that of Preparation (2): the volumetric ratio of the oil phase to the water phase was made equal to six to one, rather than three to one as in Preparation (2). The emulsion thus prepared showed a considerable improvement in stability.

These findings suggested that the stable state of the emulsion was maintained in this system. This is expected to increase the therapeutic potential of emulsion preparations with OK-432.

Assessment was made of the three methods of preparing OK-432 for local injection methods, (1), (2) and (3), for the treatment of lymphonodus cancer metastasized from gastric carcinoma. Preparation (3), which takes into account not only the specific gravity of the water and oil phases but the volumetric ratio of the former to the latter phase, is believed to be most suitable for the purpose.

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