Preparation and Evaluation of Suppositories for RK-28 (a New Radiosensitizer) Using Rabbits

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Received August 18, 1993; accepted December 9, 1993

The pharmacokinetics of RK-28 [1-(4-hydroxy-2-butenoxy)methyl-2-nitroimidazole], a new hypoxic cell radiosensitizer, was studied following intravenous, oral, and rectal administration to rabbits. After an oral administration of RK-28 solution, the plasma concentration of RK-28 was considerably lower than that after intravenous administration through all time periods, and the absolute bioavailability was a mere 4.2%. It was presumed that a specific acid-catalyzed decomposition of RK-28 progressed in the stomach, and also, the absorbed RK-28 suffered first-pass effects in the liver. In contrast, the absolute bioavailability following the rectal administration of a solidified RK-28 suppository preparation was significantly increased in comparison with that obtained by other administration routes. Also, RK-28 emulsion suppositories were prepared by emulsifying various amounts of the drug with 1-hexadecanol and hydrogenated castor oil (HCO 60) at 80 °C, and these were administered into the rabbit rectum. The resulting absolute bioavailability was 91% for the RK-28 emulsion suppository and 86.9% for an RK-28 emulsion suppository containing small amounts of Eudispert hv. These values were better than those in rats. The rectal administration of the RK-28 emulsion suppository containing small amounts of Eudispert hv showed a preferable plasma concentration-time pattern that reflects on radiation therapy.

Keywords RK-28 emulsion suppository; 1-hexadecanol; Eudispert hv; hydrogenated castor oil (HCO 60); absolute bioavailability; radiosensitizer

The presence of radioresistant hypoxic cells is one explanation for the inability to control solid tumors using conventional radiotherapy. 1 Many types of electron-transport compounds can act as hypoxic cell radiosensitizers in experimental systems. Among these, misonidazole [1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol] (Fig. 1A) was thought to be promising, and was expected to become a useful agent for clinical radiotherapy. 2-4 Unfortunately, it has become clear that its clinical applicability at doses sufficient to produce optimum sensitization is limited due to its neurotoxicity. 5-7 Several laboratories are therefore actively looking for a compound which would be superior to misonidazole for clinical application. 8-11

RK-28 [1-(4-hydroxy-2-butenoxy)methyl-2-nitroimidazole] (Fig. 1B), a nucleoside analogue with the base component replaced by 2-nitroimidazole, was synthesized by Sakaguchi et al. 12,13 This compound showed a higher electron affinity, was more efficient in vitro, and in vivo sensitization, and was less toxic than misonidazole. 14,15

It is certain that the sensitizing effects by irradiation are maintained following intravenous infusion several times each week, but with repeated applications comes an increase in patients' complaints. Our preceding report dealt with the pharmacokinetics and formulation studies of RK-28 using rats. 16 In this report, it was suggested that the oral administration of RK-28 is inadequate because of the formation of 2-nitroimidazole followed by the specific acid-catalyzed decomposition of RK-28 in the stomach, and because of the first-pass metabolism of the drug in the liver. Furthermore, this application was limited by cumulative neurotoxicity, which may be due to the formation of 2-nitroimidazole followed by the specific acid-catalyzed decomposition of RK-28 in the stomach. As the another application, we predicted that the rectal route for RK-28 administration might be suitable. On the other hand, a time lag (approximately 0.5 h) required to nearly reach the maximum concentration of RK-28 may be favorable for producing maximum sensitizing effects by irradiation after the application of a hypoxic cell radiosensitizer to a living body, because it takes about 0.5 h to start and operate irradiation for clinical use.

In the previous report, 16 after rectal administration of a new type of suppository containing RK-28 prepared by emulsifying the drug with 1-hexadecanol and hydrogenated castor oil (HCO 60) at 80 °C (RK-28 emulsion suppository) to rats, good results were obtained compared with that for oral administration, since the drug loss caused by liver first-pass metabolism could be avoided.

In order to confirm the above preferable results in rats, we designed the study using rabbits, selected as a large mammal. The main purpose of this study is to examine whether a desirable plasma concentration-time curve can be obtained following the rectal administration of a new type of suppository containing RK-28 in rabbits. Such a study will be useful for further considerations for clinical application.

Fig. 1. Chemical Structures of Misonidazole (A) and RK-28 (B)

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MATERIALS AND METHODS

Materials  RK-28, 1-(4-hydroxy-2-butenox)methyl-2-nitroimidazole, was supplied by Kayaku Co., Ltd., Tokyo, Japan. Eudispert hv was a gift from the Higuchi Co., Tokyo, Japan. 1-Hexadecanol was obtained from Nacalai Tesque, Inc., Kyoto, Japan. Polyoxymethylene hydrog- enated castor oil (HCO 60) was generously supplied by Nikko Chemicals Co., Tokyo, Japan. All other reagents and solvents were of reagent grade.

Preparation of NK-28 Suppositories  RK-28, pale yellow crystals (mp 62—64°C), was melted at 80°C and then cooled gradually in a plastic syringe (0.9 cm i.d.). We named the resulting product solidified RK-28 suppository in this study. We also emulsified 80% (w/w) RK-28 with 15% (w/w) 1-hexadecanol as a lipophilic base and 5% (w/w) HCO 60 as an emulsifier at 80°C. By cooling it in the plastic syringe at room temperature, a new suppository named RK-28 emulsion suppository was prepared. Furthermore, an RK-28 emulsion suppository to which 2.5% (w/w) Eudispert hv was added was prepared.

Measurement of RK-28 in Rabbit Plasma  The assay method of RK-28 in plasma is basically the same as described previously.16 First, 0.4 ml of methanol was added to 0.1 ml of plasma sample. The mixture was shaken for 30 s with a vortex mixer, and was centrifuged at 12000 rpm (7740 g) for 5 min. The supernatant that was collected was filtered through a membrane filter (Tosoh H-3,2, Tosoh Co., Tokyo, Japan). The amount of RK-28 in the filtrate was determined by the HPLC (model LC-6A, Shimadzuieisakusho Co., Kyoto, Japan). The analytical conditions of HPLC were as follows: an octadecyl silica column (TSKgel ODS-80TM, 0.46×15 cm, Tosoh Co., Tokyo, Japan) was used, and measurement was made at a wavelength of 320 nm. The mobile phase used was 30% (v/v) methanol solution. The flow rate and column tem-perature were 1.0 ml/min and 40°C, respectively.

Rectal Administration in Rabbits  White male rabbits (JW, Japan SLC Co., Shizuoka, Japan) weighing 3—3.5 kg were fasted for 48 h. However, they had free access to water prior to drug administration.

A saline solution containing 15% (w/v) of RK-28 (dose is equivalent to 100 mg/kg RK-28), solidified RK-28 suppositories (doses are equivalent to 50, 100, and 200 mg/kg RK-28), and RK-28 emulsion suppositories (dose is equivalent to 100 mg/kg RK-28) were set on the position of 3 cm from the anus of the rabbits. After rectal administration, the anus was closed with an adhesive (Aronalpa, Toakaseikogyo Co., Tokyo, Japan) and a clip to prevent possible leakage. Thereafter, 0.3 ml of blood was collected at timed intervals. The collected blood was immediately centrifuged at 12000 rpm for 5 min.

Intravenous and Oral Administration of RK-28 in Rabbits  Saline solution containing 15% (w/v) of RK-28 was injected intravenously, and the same solution was administered orally with 10 ml of water via a stomach tube to the rabbits. With both methods, the assay of RK-28 in plasma was the same as described above.

Data Analysis  The area under the curve of drug concentration in plasma versus time (AUC) was calculated using the trapezoidal rule. The pharmacokinetic parameters were calculated by the program MULTI for microcomputers.17

Statistical analysis was carried out using the Student’s t test (p < 0.05).

RESULTS AND DISCUSSION

Plasma Concentration-Time Curves of RK-28 Following Administration of RK-28 Solution through Various Routes  The mean concentrations of RK-28 in plasma after intravenous, oral, and rectal administration of the drug solution are shown in Fig. 2, and the pharmacokinetic parameters obtained from plasma concentration–time curves are summarized in Table I.

RK-28 was rapidly removed from the plasma after intravenous administration. We found 0.23 h to be the half-life for the elimination of the drug. After oral administration, the concentration of RK-28 in plasma was considerably lower than that after intravenous administration throughout all time periods. The absolute bioavailability of RK-28 was calculated as a mere 4.2%. For the rectal administration of RK-28 solution, the plasma level of RK-28 was higher than that for the oral administration, and the maximum plasma concentration (Cmax) and AUC values were improved significantly (p < 0.05) as shown in

<table>
<thead>
<tr>
<th>Route of administration and formulation (n)</th>
<th>Dose (mg/kg)</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (h)</th>
<th>MRT (h)</th>
<th>AUC∞ (µg·h/ml)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous solution of RK-28 (4)</td>
<td>100</td>
<td>122.2±8.2</td>
<td>0</td>
<td>0.62±0.04</td>
<td>61.8±3.8</td>
<td>100</td>
</tr>
<tr>
<td>Oral solution of RK-28 (4)</td>
<td>100</td>
<td>3.7±2.1</td>
<td>0.19±0.06</td>
<td>0.54±0.11</td>
<td>2.6±1.0</td>
<td>4.2±1.6</td>
</tr>
<tr>
<td>Rectal solution of RK-28 (5)</td>
<td>100</td>
<td>2.34±6.8</td>
<td>0.12±0.03</td>
<td>0.53±0.05</td>
<td>13.6±4.4</td>
<td>22.1±7.0</td>
</tr>
<tr>
<td>Solidified RK-28 suppository (3)</td>
<td>50</td>
<td>30.9±3.3</td>
<td>0.25±0.00</td>
<td>0.67±0.06</td>
<td>24.1±3.2</td>
<td>78.1±10.3</td>
</tr>
<tr>
<td>Solidified RK-28 suppository (7)</td>
<td>100</td>
<td>69.2±4.2</td>
<td>0.25±0.00</td>
<td>0.69±0.07</td>
<td>46.8±3.9</td>
<td>57.5±6.3</td>
</tr>
<tr>
<td>Solidified RK-28 suppository (4)</td>
<td>200</td>
<td>84.6±4.0</td>
<td>0.38±0.07</td>
<td>0.86±0.07</td>
<td>83.2±9.7</td>
<td>67.2±7.9</td>
</tr>
<tr>
<td>RK-28 emulsion suppository (4)</td>
<td>100</td>
<td>56.5±6.2</td>
<td>0.31±0.06</td>
<td>0.91±0.09</td>
<td>56.3±6.6</td>
<td>91.0±10.8</td>
</tr>
<tr>
<td>RK-28 emulsion suppository with 2.5% of Eudispert hv (4)</td>
<td>100</td>
<td>36.8±5.0</td>
<td>0.63±0.07</td>
<td>1.30±0.14</td>
<td>53.7±4.1</td>
<td>86.9±6.6</td>
</tr>
</tbody>
</table>

a) Each value represents the mean±S.E.M.  b) Maximum plasma concentration.  c) Time to reach Cmax.  d) Mean residence time.  e) F=[AUC∞]oral or rectal/[AUC∞]intra- venous.  f) Oral solution versus solidified RK-28 suppository (100 mg).  g) Oral solution versus RK-28 emulsion suppository.  h) Oral solution versus RK-28 emulsion suppository with Eudispert hv.  i) Rectal solution versus solidified RK-28 suppository (100 mg).  j) Rectal solution versus RK-28 emulsion suppository.  k) Solidified RK-28 suppository (100 mg) versus RK-28 emulsion suppository with Eudispert hv.  l) Significant difference was observed between them, respectively (p < 0.05).
The $C_{\text{max}}$ value and the overall plasma level of RK-28 increased with an increase in the dose of RK-28. And the linear relationship ($r=0.998$) between doses and AUCs of RK-28 was observed. The absolute bioavailabilities of RK-28 for three doses of solidified RK-28 suppositories (67.2—78.1%) increased approximately 3.04—3.53-fold compared with that following rectal administration of the drug solution. These results indicate that drug loss caused by the specific acid-catalysis decomposition in the stomach as well as by liver first-pass metabolism could be avoided by administering the above preparations into the rectum because of the increased amount of drug absorbed from that administration site, e.g., the lower part of the rectum.

However, solidified RK-28 preparations were too hard to use and lacked the smoothness necessary for suppositories. The results of our preliminary study showed that the use of suppository bases such as polyethylene glycol 2000 or Witcostol H-12 enabled the preparation of good RK-28 suppositories for clinical use. However, rectal administration of these suppositories containing RK-28 in rats showed low bioavailabilities of RK-28, as stated in our preceding paper. This fact can be explained by our previous reports that a considerable part of the drug absorbed through wide regions of the colon and rectum may suffer first pass metabolism in the liver, because these bases ascend and spread from the anus via the upper rectum to the colon after administration.

Plasma Concentration—Time Curves of RK-28 Following Rectal Administration of Our New Emulsion Suppositories Containing RK-28 RK-28 is hydrophilic in nature (solubility; 0.2 g/ml, 20 °C, octanol/water partition coefficient; 0.5), therefore, it can not blend homogeneously with lipophilic bases. In the search for a new formula, we emulsified RK-28 using hexadecanol as a lipophilic base and HCO 60 as an emulsifier at 80 °C; then, the RK-28 emulsion suppository could be prepared by cooling the mixture to room temperature. Eudrispt hv, a copolymer of methacrylic acid and methacrylic acid methyl ester, was added further in the RK-28 emulsion suppository to control the release rate of RK-28 from the suppositories.

Figure 4 shows the mean concentration of RK-28 in plasma after the rectal administration of RK-28 emulsion suppositories. The pharmacokinetic parameters obtained from the plasma concentration–time curves are summarized in Table I.

The plasma concentration–time pattern of RK-28 given via the emulsion suppository with Eudrispt hv (2.5%, w/w) was different and showed a suitable pattern for clinical use compared with that for the emulsion suppository without Eudrispt hv. We have previously reported that methacrylic acid and methacrylic acid methyl ester copolymers formed gels with alcohols and polyhydric alcohols. Among these, the Eudragit L gel with propylene glycol was found to be useful as a sustained-release suppository base that dissolves depending on the pH in the rectum. In the present RK-28 emulsion suppositories, we recognized the gel formation between Eudrispt hv and 1-hexadecanol; the mixture of Eudrispt hv and 1-hexadecanol (mp 49—51.5 °C) formed a gel state by warming (80 °C) and agitation, and was then converted into a homogenous solid mass by cooling to...
Fig. 4. Time Course of Concentration of RK-28 in Plasma after Rectal Administration of RK-28 Emulsion Suppository (—□—) and RK-28 Emulsion Suppository with 2.5% (w/w) of Eudispert hv (—●—) to Rabbits

Dose of 100 mg/kg was administered. Each point and vertical line represents the mean and standard error for four rabbits.

room temperature. Our preliminary in vitro study showed that the release profiles of RK-28 from the emulsion suppository with Eudispert hv was suppressed in comparison to the emulsion suppository without Eudispert hv by the dissolution test with a paddle. It was assumed that the interaction between Eudispert hv and 1-hexadecanol in the RK-28 emulsion suppositories participates in the retardation of the in vivo release of drug. Further study is necessary to determine the precise mechanisms of the interaction, but the release of RK-28 from emulsion suppositories could be kept under control by adding Eudispert hv. The absolute bioavailability of RK-28 from these emulsion suppositories with or without Eudispert hv were 91 and 86.9%, respectively. On the other hand, high bioavailability is necessary for realizing a sufficient sensitizing effect by irradiation in clinical use.

Accordingly, the good plasma concentration-time profile and bioavailability of RK-28 produced by the new emulsion suppository with Eudispert hv were thought to be notable from the point of view of studying it for future clinical use.

REFERENCES