STUDY ON THE RECTAL ABSORPTION OF GENTAMICIN III

HARUMASA TOYA, TOMOJI SHINMEN and YOSHIKO KATO
Research Labolatory, Sawai Pharmaceutical Co., Ltd.

MASARU YAMAZAKI and AKIRA KAMADA
Faculty of Pharmaceutical Sciences, Osaka University

(Received October 21, 1983)

The rectal administration of gentamicin was investigated using beagle dogs as experimental animals. The animals were divided into four groups : 1～2 months old(infantile), 3～4 months old (young), 1～2 years old (adult) and 8～10 years old (aged). Gentamicin was administered rectally, at the dose of 10, 30 and 50 mg/kg, without the help of absorption promoter, such as surface active agents, to each of the four groups. In adult and aged dogs, even at the dose of 50 mg/kg of gentamicin, maximum serum concentration (C_{max}) were only 2.6±0.9 μg/ml (adult) and 3.0±1.8 μg/ml (aged). No relation was found between C_{max} and dose of gentamicin. In infantile and young dogs, on the other hand, an increase of the gentamicin serum concentration was found with its increased doses. In case, when 50 mg/kg of gentamicin was administered, C_{max} was 13.6±3.8 μg/ml for infantile dogs and 8.2±3.6 μg/ml for young dogs, with both concentrations being above MIC(MIC of gentamicin against *P. aeruginosa* is 1.56～6.25 μg/ml). The relationship between the dose of gentamicin and C_{max} were shown as a linear graph.

INTRODUCTION

Rectal administration is a drug delivery system which, on account of its advantages, has been drawing a great amount of attention from researchers compared to other administration methods[1,2]. Many studies were published on the rectal administration of aminoglycoside antibiotics or some of β-lactam antibiotics[3～7]. No administration method other than injection is available for these agents. Due to the meager rectal absorption of those drugs as a single entity, pharmaceutical contrivance was required to obtain effective serum concentration. An addition of absorption promoter is, for example, one of the solutions and a lot of studies have been published on it. Mature adult animals, however, were used in most of these studies and few researchers conducted studies using infantile and aged animals. Effects on improving rectal absorption of non-ionic surface active agents in infantile, adult, and aged dogs were previously examined by us[2]. High rectal permeability, in particular, was found in infantile dogs and an addition of comparatively small amounts of surface active agent was found helpful for gaining effective serum concentrations. This finding suggests that an additional of small amounts of the surface active agents or pharmaceutical contrivance without the use of surface active agents will make clinically possible the rectal administration of antibiotics in infantile.

In this paper, we examined the rectal absorption aminoglycoside antibiotic, when no absorption promoter was given, using beagles which were divided into 4 groups : 1～2 months, 3～4 months, 1～2 years and 8～10 years old. Gentamicin in 3 dose levels : 10, 30 and 50 mg/kg were used as the test drug.

MATERIALS AND METHODS

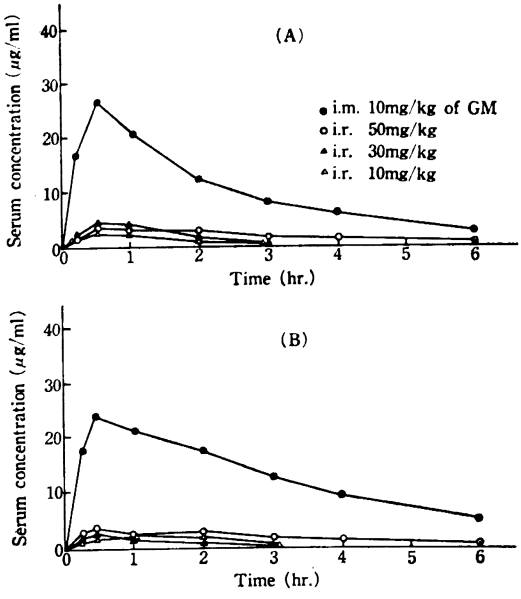
Materials Gentamicin sulfate (GM) was obtained from Shionogi Pharmaceutical Co., Ltd. Lipophilic base, Witepsol H-15(Dynamit Nobel A. G., Chemishe Werke Witten, German Fedral Republic) with a melting range of 33.5～35.5℃ and congealing range of 32.5～34.5℃ was used.

Preparation of suppository For all experi-

ments of rectal administration. A fixed dose of GM at 10, 30 and 50 mg/kg were selected. The Witepsol suppositories were prepared by the fusion method, i.e. aliquots of GM was suspended in the molten base at 40℃. The suspension was homogenized by sonication for 1 min. at 30~35℃ employing an ultrasonic cleaner (Branson 220, Branson Co., Ltd.).

***In vivo* absorption study** Male beagle dogs were used as experimental animals. The animals were divided into four groups: 1~2 months old (infantile), 3~4 months old (young), 1~2 years old (adult) and 8~10 years old (aged). Before the experiment, the animals were fasted for 12 hr. but were allowed to drink ad libitum. The total suppository weight were fixed 0.75 g for infantile and young dogs and 1.5 g for adult and aged dogs. After insertion of suppository, no expulsion or leakage was observed during the experimental period. Blood samples were taken at designated intervals. They were taken into 10-ml glass tubes and centrifuged at 3000 rpm for 5 min. The serum layer was taken into stoppered glass tubes and kept at 4℃ untill assays were carried out. For intramuscular administration, saline solution of GM was injected into the thigh muscle.

Fig.1 Serum concentration of GM after intramuscular and rectal administration in dogs. Each value is the mean of 3~8 dogs. (A) Adult dog, (B) Aged dog.



Analytical method The antimicrobial activity of GM in serum was determined by the cup method using *Bacillus subtilis* ATCC 6633 as the test organism.

RESULTS

Fig. 1 shows a graph for serum concentration-time which was obtained when GM in amounts of 10, 30 and 50 mg/kg were given to adult and

Fig.2 Serum concentration of GM after intramuscular and rectal administration in dogs. Each value is the mean of 3~5 dogs. (C) Infantile dog, (D) Young dog.

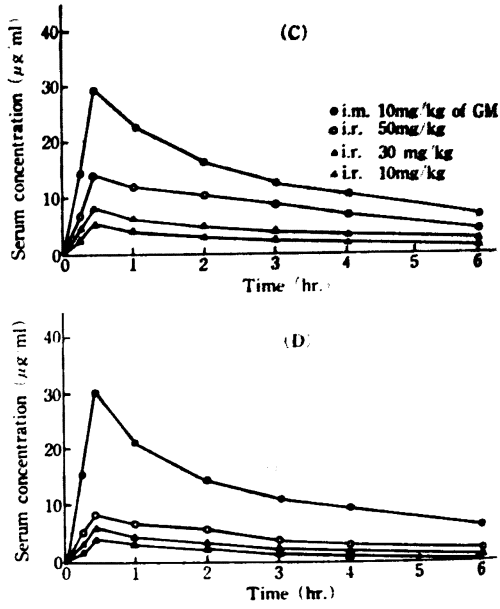
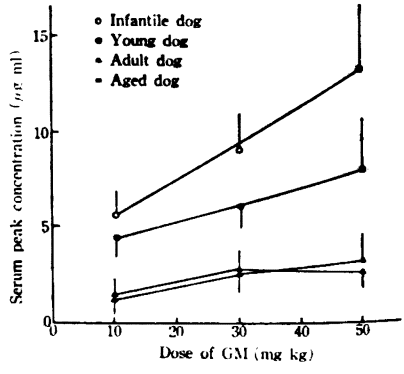


Fig.3 Correlation between dose of GM and serum peak concentration. Each value is the mean ±S.E.M. for 3~8 dogs.



aged dogs by the rectal administration and 10 mg/kg, intramusculary. Almost no absorption was found in dogs in which rectal administration was carried out, and when 50 mg/kg was given, C_{max} was only 2.6±0.9 μg/ml (adult) and 3.0±1.8 μg/kg (aged). Comparing with the intramuscular administration, the recovery of GM after rectal was extremely small (data are not shown). Fig. 2 shows serum concentration-time curves when rectal administration of GM in amount of 10, 30 and 50 mg/kg and intramuscular administration of 10 mg/kg were given to infantile and young dogs. As shown in the figure, serum concentration after rectal administration were higher than those for adult and aged dogs, and C_{max} showed increases with increased doses.

Fig. 3 shows the graph in which doses and C_{max} are plotted along the axis of the ordinates and the axis of the abscissa, respectively. As can be seen, there were linear relationship between C_{max} and dose of GM in infantile and young dogs. However, no relationship was found in adult and aged dogs.

DISCUSSION

Studies on the rectal absorption of aminoglycoside antibiotics have been investigated very actively by many researchers[3~7]. They showed that almost no absorption was made without the help of absorption promoter, for example, surface active agent. When surface active agent are used, careful assessment of mucosal imparement and toxicity induced by them are required[8,9], while the amount of the therapeutic agent can be reduced. This study revealed that effective GM serum concentrations can not be obtained without help of surface active agent, even at dose of 50 mg/kg, in adult and aged dogs. On the other hand, in infantile dogs, when dose of GM was over 30 mg/kg, effective serum concentration can be obtained without an addition of surface active agent. In young dogs, effective serum concentration can be obtained at dose of 50 mg/kg. This finding revealed that rectal permeability of the infantile and young dogs were appreciably higher than that of the adult and aged.

The considerable difference in the permeability of the rectal mucosa due to age has yet to be clarified. Incomplete construction of the rectal mucosa due to its immaturity and the mucosa vulnerability even to a slight stimulus were possibly responsible for the increase in the permeability. Physical stimulus at the time of insertion of suppository and a decrease in rectal pH induced by GM were major stimuli given to animals. The fact that almost no absorption of GM was found in the adult and aged dogs was probably due to the stimuli as shown above, being not so severe as to cause mucosal imparement. In spite of an extensive literature survey, information about the age difference of permeability or mucosal resistance of rectum could not be obtained. On the other hand, FEIWEL[10] and RICHARD, et al[11], in their studies on dermal permeability, reported that the permeability level of pups were far better than that of adults. The author suggested that histological difference is the most probable reason for this.

We think factors attributable to the difference of permeability by age are as follows:

1) Difference of constituent lipids and protein in the cell membrane
2) Difference of adhesion strength in the intracellular space

It is well known, as for 1), that aging causes an increase of cholesterol and lipid peroxide in the membrane[12~14], and an increase or decrease of membrane constituent substance is responsible for a big change in the permeability[15]. So, one possibility is that the difference in the composition of membrane constituent substances seemed to induce a difference in the permeability and changes in the response to stimuli in this experiment. Ca^{2+} is considered to play an important role in 2)[16]. SHAKESPEARE[17] reported on difference by age in metal ion contents in tissues. This might be influential regarding adhesion strength in a tight junction. Other factors to be involved are the qualitative difference in the microfilaments which are most important for cellular morphogenic maintenance and the difference in the thickness of muscular tunics. A histological analysis of those factors is now under way in our labolatory and will be published in the near future.

In this study, serum concentrations higher than MIC were found to be attainable without the use of absorption promoter only by increasing the doses in infantile dogs. So if the subjects are confined to pups, a more effective rectal absorption of GM is considered to be possible by improving the suppository base and its doses. Further studies will be carried out energetically to obtain more

indepth information about the above mentioned subject.

REFERENCE

1) KANAKUBO, Y.: Suppository: An overview and recent advances. Chibaigaku. 58: 7～13, 1982
2) BOER, A. G.; J. M. GUBBENS & D. D. BREIMER: Avoidance of first pass elimination of propranolol after rectal administration to rats. J. Pharm. Pharmacol. 33: 50～51, 1981
3) TOYA, H.; J. RIKU, Y. KATO, H. SUGIMOTO, M. YAMAZAKI & A. KAMADA: Study of rectal absorption of antibiotics. Chemotherapy 31: 634～638, 1983
4) TOYA, H.; T. SHIMMEN, Y. KATO, M. YAMAZAKI & A. KAMADA: Study of rectal absorption of antibiotics II. Chemotherapy in press
5) RUBINSTEIN, A.; E. RUBINSTEIN, E. TOITOU & M. DONBROW: Increase of the intestinal absorption of gentamicin and amikacin by a non-ionic surfactant. Antimicrob. Agents. Chemother. 19: 696～699, 1981
6) MURANISHI, S.; N. MURANUSHI & H. SEZAKI: Improvement of absolute bioavailability of normally poorly absorbed drugs. Inducement of the intestinal absorption of streptomycin and gentamicin by lipid-bile salt mixed micelles in rat and rabbit. Int. J. Pharm. 2: 101～111, 1979
7) MURAKAMI, T.; N. YATA, H. TAMAUCHI, J. NAKAI, M. YAMAZAKI & A. KAMADA: Studies in absorption promoters for rectal delivery preparations I. Promoting efficacy of enamine derivatives of amino acids for the rectal absorption of β-lactam antibiotics in rabbit. Chem. Pharm. Bull. 29: 1998～2004, 1981
8) WHITMORE, D. A.; L. G. BROOKES & K. P. WHEELER: Relative effects of different surfactants on intestinal absorption and the release of proteins and phospholipids from the tissue. J. Pharm. Pharmacol. 31: 277～285, 1979
9) GIBALDI, M & S. FELDMAN: Mechanism of surfactant effects on drug absorption. J. Pharm. Sci. 59: 579～589, 1970
10) FEIWEL, M.: Percutaneous absorption of topical steroids in children. Br. J. Derm. 81: 113～116, 1969
11) RICHARD, L.: M. D. NACHMAN, B. NANCY & M. D. ESTERLY: Increased skin permeability in preterm infants. J. Pediat. 79: 628～632, 1971
12) TAPPEL, A. L.: Free-radical lipid peroxidation damage and its inhibition by vitamin E and selenium. Fed. Proc. 24: 73～78, 1965
13) HARMAN, D.: Atherosclerosis, by pothesis concerning the initiating steps in pathogenesis. J. Gerontol. 16: 199～202, 1957
14) HEGNEN, D & D. PLATT: Effect of essential phospholipids on the properties of ATPase of isolated rat liver plasma membrane of young and old animals. Mech. Ageing. Dev. 4: 191～200, 1975
15) SANTO, Y & D. F. SILBERT: Selective effects of membrane sterol repletion on surface function thymidine and 3-o-methyl-D-glucose transport in a sterol auxotroph. J. Biol. Chem. 245: 1102～1107, 1979
16) URUSHIHARA, H.; H. S. OZAKI & M. TAKAICHI: Immunological detection of cell surface components related with aggregation of chinese hamster and chick embryonic cells. Develop. Biol. 70: 206～216, 1979
17) SHAKESPEARE, V. & J. H. BUCHANAN: Increase degradation rates of protein in aging human fibroblasts and in cells treated with an amino acid analog. Exp. Cell. Res. 100: 1～8, 1976

ゲンタマイシンの直腸吸収に関する研究 III

戸谷治雅[1]・新免友次[1]・加藤敬香[1]・山崎 勝[2]・鎌田 皎[2]
[1] 沢井製薬研究所生物研究室
[2] 大阪大学薬学部薬剤学教室

アミノ配糖体抗生剤であるゲンタマイシン（以下，GM と略す）の直腸吸収について，ビーグル犬を用いて，前報[*1]，前々報[*2]にひきつづき検討した。前報では幼若犬では直腸粘膜の透過性が，成犬や老齢犬に比べ，かなり大であることが示唆された。そこで今回は，界面活性剤などの吸収促進剤を用いず，基剤（油性）中に GM のみを含んだ坐剤を作製し，幼若犬（生後 1～2 か月，3～4 か月），成犬（生後 1～2 年），老齢犬（生後 8～10 年）の 4 つのグループに投与した。GM の投与量は 10，30，50 mg/kg 体重の 3 段階とした。

その結果，成犬，老齢犬のグループではいずれの投与量でも GM の血清中濃度は低く，最高血清中濃度（C_{max} と略す）は，50 mg/kg 投与で 2.6±0.9 μg/ml（成犬），3.0±1.8 μg/ml（老齢犬）であった。これに対し，幼若犬のグループでは，1～2 か月齢で 30 mg/kg の投与で 8.6±2.8 μg/ml，50 mg/kg で 13.6±3.8 μg/ml の C_{max} を得，3～4 か月齢でも 50 mg/kg の投与で C_{max} 8.2±3.6 μg/ml を得た。さらに幼若犬では GM の投与量が増すにつれて C_{max} が一次直線として増加することが観察された。

これらから，幼若犬では吸収促進剤を用いなくとも，GM の投与量の増加や基剤の検討などにより有効な血中濃度（感染菌の MIC 以上の血中濃度）を得ることが可能であることを示すと考える。

本文中では，年齢による GM の吸収の差について 2，3 の考察を加えた。

*1 Toya, H. et al : Chemotherapy in press
*2 Toya, H. et al : Chemotherapy 31 : 634～638, 1983