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Effects of Benzoic Acid and its Analogues on Osmotic Fragility in Guinea Pig Erythrocytes *in Vitro*

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Abstract

In our previous report, benzoic acid and some of its chemical analogues were shown to increase osmotic fragility (OF) in isolated rat red blood cells (RBCs). However, there have been no reports on the effects of benzoic acid and its derivatives on OF in the RBCs of other animals. To clarify these inter-species differences, the effects of those chemicals on OF were examined in guinea pig RBCs. The isolated RBCs were exposed to the chemicals at a concentration of 0.1-100 mM for 1 h. OF was measured by determining the 50% hemolysis of RBCs using a 0.1-0.8% NaCl solution. Benzoic acid and some of its derivatives decreased OF in a dose dependent manner. Replacement of the carboxylic group with another group or the introduction of another element on the benzene ring also affected OF. These effects were dependent on both the type of molecule and the position at which they were introduced into the benzene nucleus. The introduction of chlorine, bromine or iodine tended to increase OF, even at low concentrations. With regard to the action of the tested chemicals, the hydrophobic benzene ring is thought to enter the phospholipid layer while the hydrophilic carboxylic group remains at the membrane surface, perturbing the RBC membrane. Inter-species differences between rat and guinea pig were confirmed in the response of the RBC membrane to benzoic acid and its derivatives. These differences are speculated to be due to differences in the lipid composition of the RBC membrane.

Keywords: Benzoic acid; Carboxylic acid; Erythrocyte; Guinea pig; Membrane; Osmotic fragility; Phospholipid; Structure-activity relationships

Introduction

Benzoic acid is an aromatic hydrocarbon with a carboxyl group bound to a benzene ring. Benzoic acid and sodium benzoate are commonly used as antifungal drugs and food preservatives [1], while carboxylic esters of benzoic acid and its derivatives are used as artificial fragrances in cosmetics and perfumes [2,3]. There are many other chemical analogues of benzoic acid possessing various biological effects, such as salicylic acid, which is used as a painkilling anti-febrile agent [4]. Apart from these widely known physiological and pharmacological activities, benzoic acid and its derivatives demonstrate various biological actions both *in vivo* [5-7] and *in vitro* [8-12]. It has been reported that the derivatives of benzoic acid induce functional and morphological changes in erythrocytes [13,14].

Previous studies have shown that osmotic fragility (OF) in rat red blood cells (RBCs) is a useful indicator for evaluating interactions between mono-carboxylic acids and the cell membrane *in vitro* [15]. In addition, the effects of benzoic acid, one of the mono-carboxylic acids, and its chemical derivatives on the cell membrane have been evaluated by the measurement of OF in isolated rat RBCs *in vitro* [16]. Since various types of benzoic acid analogues are available, these chemical analogues afford a useful set of tools for the detailed examination of the relationships between the chemical structure and their action on the cell membrane structure affecting OF in rat RBCs. A previous study by our lab showed that benzoic acid and some of its chemical analogues decreased resistance to osmotic pressure and increased OF in rat RBCs. It was hypothesized that the benzoic acid and its analogues interact with the lipid bilayer of the cell membrane via their hydrocarbon chains, resulting in an increase in OF in rat RBCs [16].

In the above mentioned series of experiments, rat RBCs were used as a prototypical cellular model in which to examine the chemical-mediated effects on the plasma membrane. However, the biological activities of mono-carboxylic acids, including benzoic acid, may be dependent not only on their chemical structure but also on the characteristics of the erythrocyte membrane, especially the lipid bilayer, and there have been many reports that the composition of phospholipids in erythrocyte membranes differ between species [17-20].

Thus, in the present study, we investigated the effects of benzoic acid and its chemical derivatives on OF in erythrocytes isolated from guinea pigs. Guinea pig erythrocytes were first exposed to benzoic acid as the parent of the other derivatives. Secondly, various benzoic acid derivatives were applied to the guinea pig erythrocytes and the changes in OF were assessed. The results of the current study confirmed the effects of mono-carboxylic molecules in altering the erythrocyte membrane lipid bilayer, resulting in changes in OF. The determination also allowed us to answer the problem of inter-species differences, and whether the OF response to benzoic acid and its derivatives reported in rat erythrocytes was similar to that occurring in guinea pig RBCs.

Materials and Methods

Animals

The animals were maintained in accordance with the NIH (National Institute of Health) Guidelines for the Care and Use of Laboratory

Animals. The feeding of animals and sampling of blood were performed at the Institute of Experimental Animals in the New Drug Research Center, Inc. (Eniwa, Hokkaido, Japan).

Male Hartley guinea pigs (Slc: Hartley, 8 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and were housed in stainless-steel metabolic cages individually. The cages were kept in a room with controlled relative humidity (50 ± 20%), temperature (22 ± 3 ℃) and lighting (lights on from 08:00-20:00). The animals were given free access to a pelleted guinea pig diet (Labo G standard, Nosan Co., Yokohama, Japan) and tap water for more than 1 week prior to the start of the experiments. Blood samples were collected from the guinea pigs (540 ± 38 g, n=20) from at 9 to 12 weeks of age (10 ± 1 weeks).

Reagents

Biochemical grade benzoic acid, heptanoic acid, cyclohexanecarboxylic acid, benzenephosphoric acid, benzenesulfonic acid, benzamide, phenol, 2-, 3-, and 4-fluorobenzoic acid, 2-, 3-, and 4-chlorobenzoic acid, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichlorobenzoic acid, 2-, 3-, and 4-bromobenzoic acid, 2-, 3-, and 4-iodobenzoic acid 2-, 3-, and 4-methylbenzoic acid, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dimethylbenzoic acid, and 2-, 3-, and 4-hydroxybenzoic acid, 2-, 3-, and 4-aminobenzoic acid, 2-, 3-, and 4-nitorobenzoic acid were purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan) or Wako Pure Chemical Co., Ltd. (Osaka, Japan). All other reagents used in this study were of analytical grade.

Preparation of guinea pig RBCs

On the day of the experiment, the guinea pigs were anesthetized with pentobarbital sodium (60 mg/kg). Blood (12-15 ml) was collected into a heparinized test tube from the postcava in guinea pigs. The RBCs were separated from plasma by centrifugation at 2000 g for 15 min (Model 2420, Kubota Inc., Tokyo, Japan), and then the plasma and buffy coat were aspirated. The crude RBCs obtained were then washed (3x times) with two volumes of cold 0.9% NaCl solution. The resultant dense-packed cell suspension was thereafter kept in ice-cold water until further treatment.

Experimental procedure

Experimental procedures similar to those described in our previous report [16] were carried out. The dense-packed cell suspension (30 µl) was transferred into 0.6 ml of phosphate-NaCl buffer solution (pH 7.4) that contained benzoic acid or its derivatives at concentrations ranging from 0 to 100 mM in 1.5 ml micro test tubes (Nichiryo Co., Ltd., Tokyo, Japan). Osmolarity was regulated by adjusting the amount of NaCl added to the buffer solution when each substance was applied. For comparison of the effect of benzoic acid (parent chemical) and its derivatives, OF determination was performed on RBCs obtained from the same guinea pig. All RBC suspensions used for treatment with the chemical compounds were incubated by shaking (1 stroke/sec) at 37 ℃ for 1 h (Shaking Bath TBK 202 DA, Advantec Co., Ltd., Tokyo, Japan). After incubation, each RBC suspension was mixed gently using a mixer (Vortex Genie 2, model-G560, Scientific Industry, Inc. NY., USA), and 50 µl of the suspension was then transferred into a 96-deep-well microplate (2 ml volume, Whatman Inc., Piscataway, NJ, USA) containing a 1 ml NaCl solution (0.1 to 0.8%). The deep-well microplate was immediately centrifuged at 1300 g (Plate Spin II, Kubota Inc., Tokyo, Japan) for 10 min at room temperature. The supernatants (200 µl) containing various concentrations of hemolyzed RBC-derived hemoglobin were transferred into another 96-well microplate (300 µl volume, Whatman Inc., Piscataway, NJ, USA). Absorbance at 540 nm was determined using a microplate reader (Microplate reader Model 680, Bio-Rad Laboratories, Tokyo, Japan).

Statistical analysis

The RBC suspension showed complete hemolysis in the 0.1% NaCl solution, the hemoglobin concentration for which was defined as 100%. No RBC hemolysis was observed in the 0.8% NaCl solution, the hemoglobin concentration for which was defined as 0%. The hemolysis curve was used to calculate the effective concentration of the NaCl solution that induced 50% hemolysis (EC50) of the applied RBCs by using a straight-line equation between the points immediately adjacent to 50%. The EC50 value was used to indicate osmotic fragility (OF) of the erythrocytes. All values are expressed as the mean ± S.D. A one-way ANOVA followed by a Dunnett's post-hoc test was used to assess the differences in OF of RBCs following exposure to varying concentrations of benzoic acid and its derivatives. Excel Tokei for Windows 2012 (SSRI Co., Ltd., Tokyo, Japan) was used for statistical analysis, with differences with a P value<0.05 considered to be statistically significant.

Results

Effect of benzoic acid on OF in guinea RBCs

Typical hemolytic curves are shown for guinea pig RBCs exposed to benzoic acid at 0 (control), 25, 50 or 100 mM (Figure 1A). The 50% hemolysis of the exposed RBCs (the EC50 value) was used as a measure of OF. The EC50 value of guinea pig RBCs was 0.369 ± 0.010 for treatment with benzoic acid at 0 mM, 0.362 ± 0.007 at 25 mM, 0.354 ± 0.017 at 50 mM and 0.326 ± 0.018 at 100 mM.

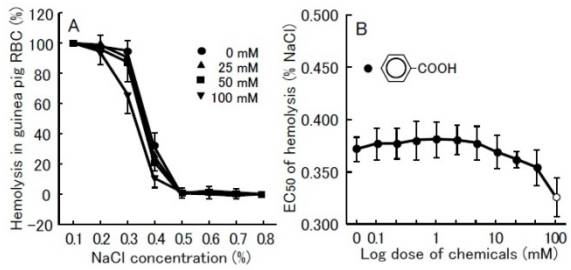

Figure 1: Changes in hemolytic curves (A) and dose-dependent changes in EC50 value of hemolysis (B) for guinea pig RBCs exposed to benzoic acid. Values are means ± S.D. (n=6). Curves were determined for benzoic acid (0, 25, 50 and 100 mM, other concentrations not shown). The EC50 values of hemolysis (concentration in NaCl%) were obtained by using a straight-line equation between the points immediately above and below 50%.

Benzoic acid treatment resulted in a left-shift of the curves in a concentration-dependent manner. Dose-response relationships between the benzoic acid dose and its effects on the EC50 value in guinea pig RBCs are shown in Figure 1B. OF in the RBCs exposed to

benzoic acid was observed to decrease in guinea pig erythrocytes in a dose-dependent manner (P<0.05).

Replacement of the carboxylic group with other elements

The effects of replacing the carboxylic group (COOH) with a phosphoric ($PO(OH)_3$), sulfonic (SO_2OH), amide ($CONH_2$) or hydroxy (OH) group are shown in Figure 2A. The application of benzenephosphoric acid at a concentration above 10 mM increased OF in guinea pig RBCs (P<0.05). Benzenesulfonic acid application at concentrations up to 50 mM did not change OF in the RBCs, whereas OF tended to increase at 100 mM (N.S., Not significant). The change in OF induced by benzamide was comparable to that induced by benzoic acid. Statistically significant decreases in OF were induced by benzamide at 25, 50 and 100 mM (P<0.05). Phenol did not effect OF in the RBCs at concentrations up to 25 mM, and the OF values for 50 and 100 mM phenol could not be determined as the RBCs were hemolyzed in the 0.8% NaCl solution during the determination of the EC50 after the phenol application.

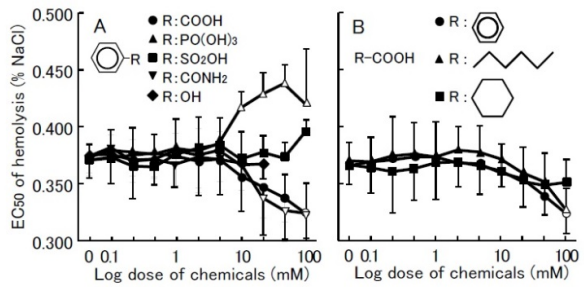

Figure 2: Dose-response changes in OF for benzoic acid and its derivatives in which the carboxylic group was replaced by other groups (A), and benzoic acid, cyclohexanecarboxylic acid and heptanoic acid (B) in guinea pig RBCs. Values are the means ± S.D. (n=6). Open symbols indicate that there was a significant difference between the control (0 mM) and subsequent concentrations (0.1-100 mM) on the basis of Dunnett's test (P<0.05).

Changes in the basic structure of the benzene nucleus

The RBCs were treated with benzoic acid, cyclohexanecarboxylic acid and heptanoic acid, each possessing six hydrocarbons but with different carbon bonds in the moiety (Figure 2B). OF was decreased by treatment with heptanoic acid in a dose-dependent manner (P<0.05), but was not affected by cyclohexanecarboxylic acid.

Introduction of a halogen (F, Cl, Br or I) on the benzene ring

The effects of the substitution of hydrogen for fluorine (F) at three (2-, 3-, and 4-) positions on the benzene ring are shown in Figure 3A. The introduction of F at the 2-position (2-fluorobenzoic acid) did not have any effect on OF in the guinea pig RBCs at any of the tested concentrations. The introduction of F at the 3- or 4-position (3- or 4-fluolobenzoic acid) tended to decrease the OF response compared to that induced by benzoic acid. However, the decreases in OF induced by these two substances were N.S. The effects of the replacement of hydrogen with chlorine (Cl) in the benzene ring are shown in Figure 3B. The introduction of Cl at the 2-position did not have any effect on OF in the RBCs at any of the tested doses; however, 3- and 4-chrolobenzoic acid induced slight changes in OF values compared to that induced by benzoic acid below 25 mM (N.S.). OF values for 3- and 4-chlorobenzoic acid at 50 and 100 mM could not be obtained as RBC hemolysis occurred during the determination of the OF values. The effects of the introduction of bromine (Br) are shown in Figure 3C. The results were comparable to those observed for the substitution with Cl. 2-Bromobenzoic acid did not have any effect on OF in the RBCs at any of the tested doses, whereas 3- and 4-bromobenzoic acid tended to decrease OF up to 25 mM (N.S.). Again the OF values for 3- and 4-bromobenzoic acid at 50 and 100 mM application could not be obtained due to RBC hemolysis. The effects of the introduction of iodine (I) are shown in Figure 3D. 2-Iodobenzoic acid affected OF in almost the same manner as that observed for benzoic acid (N.S.). The application of 25 mM 3-iodobenzoic acid immediately increased OF (P<0.05), but OF could not be determined for treatment with the acid at 50 or 100 mM. Further, OF values at concentrations of 4-iodinebenzoic acid of more than 25 mM could not be determined due to hemolysis.

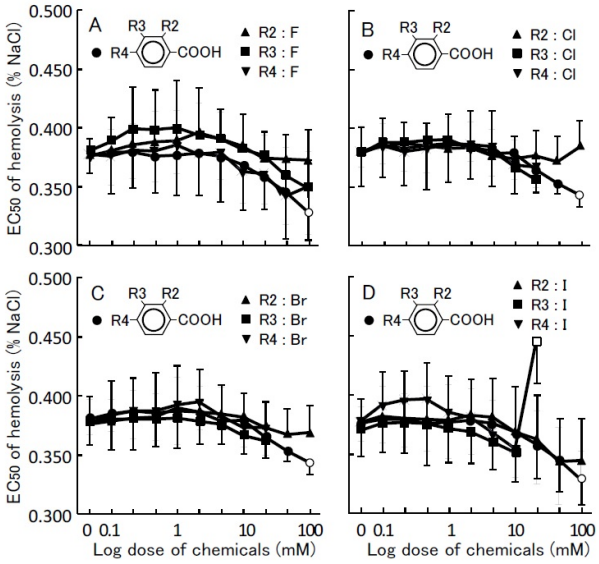

Figure 3: Dose-response changes in OF by benzoic acid and its derivatives in which hydrogen was replaced by F (A), Cl (B), Br (C) or I (D) on the benzene ring in guinea pig RBCs. Values are the means ± S.D. (n=6). Each symbol represents benzoic acid, and 2-, 3- and 4-harogenaized-benzoic acid, respectively. Open symbols indicate that there was a significant difference between the control (0 mM) and subsequent concentrations (0.1-100 mM) on the basis of Dunnett's test (P<0.05).

Introduction of two halogens (Cl) on the benzene ring

The resultant 2,3- and 2,4-dichlorobenzoic acids led to increases in OF (P<0.05) at 100 and 50 mM (Figure 4A); however, no OF value could be obtained for 2,4-dichlorobenzoic acid application at 100 mM due to RBC hemolysis.

Treatment with 2,5-dichlorobenzoic acid tended to induce changes in OF response compared to that induced by benzoic acid up to 50 mM, and to increase OF slightly at 100 mM (N.S.). 2,6-Dichlorobenzoic acid did not have any effect on OF at any of the concentrations treated (Figure 4B). Treatment with the resultant 3,4- and 3,5-dichlorobenzoic acid increased OF at 10 mM ($P<0.05$) and induced hemolysis at concentrations of 25 mM and above.

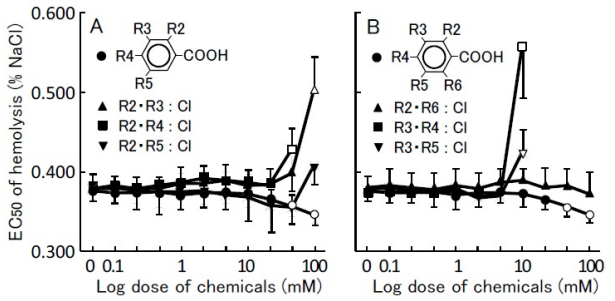

Figure 4: Dose-response changes in OF by benzoic acid and its derivatives in which hydrogen was replaced by chlorine on the benzene ring in guinea pig RBCs. A: Each symbol represents benzoic acid, and 2,3-, 2,4-, and 2,5-chlorobenzoic acid, respectively. B: Each symbol represents benzoic acid, and 2,6-, 3,4-, and 3,5-chlorobenzoic acid, respectively. Values are the means ± S.D. (n=6). Open symbols indicate that there was a significant difference between the control (0 mM) and subsequent concentrations (0.1-100 mM) on the basis of Dunnett's test ($P<0.05$).

Introduction of a methyl group (CH_3) on the benzene ring

Treatment with 2- and 3-methylbenzoic acid at concentrations up to 50 mM tended to effect OF in the same manner as benzoic acid, inducing a slight increase in OF at 100 mM (N.S.) (Figure 5A). 4-Methylbenzoic acid decreased OF at 25 mM ($P<0.05$) and resulted in hemolysis at 50 and 100 mM.

Introduction of a hydroxyl group (OH) on the benzene ring

Treatment with 2-hydroxybenzoic acid at concentrations up to 50 mM tended to decrease OF compared to that induced by benzoic acid (N.S.), but treatment at 100 mM immediately increased the OF value ($P<0.05$). Treatment with 3- and 4-hydroxybenzoic acid did not change OF at any of the concentrations tested (Figure 5B).

Introduction of an amino group (NH_2) on the benzene ring

2-Amonobenzoic acid changed the OF response in parallel with the same concentrations of benzoic acid from 0.1 to 100 mM ($P<0.05$) (Figure 5C). Treatment with 3- and 4-aminobenzoic acid tended to decrease OF, although the changes were N.S.

Introduction of a nitro group (NO_2) on the benzene ring

2-Nitrobenzoic acid did not have any effect on OF at any concentration up to 100 mM. Treatment with 3- and 4-nitrobenzoic acid tended to decrease OF, although the changes were N.S. (Figure 5D).

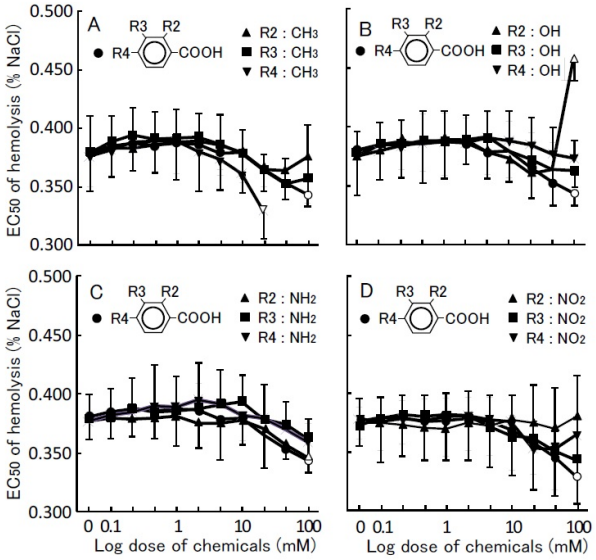

Figure 5: Dose-response changes in OF by benzoic acid and its derivatives in which hydrogen was replaced by CH_3 (A), OH (B), NH_2 (C) or NO_2 (D) on the benzene ring in guinea pig RBCs. Values are the means ± S.D. (n=6). Each symbol represents benzoic acid and its derivatives. Open symbols indicate that there was a significant difference between the control (0 mM) and subsequent concentrations (0.1-100 mM) on the basis of Dunnett's test ($P<0.05$).

Introduction of two methyl groups (CH_3) on the benzene ring

The 2,3- and 2,5-dimethylbenzoic acid decreased OF in the same manner ($P<0.05$) as that observed for benzoic acid (Figure 6A). 2,4-Dimethylbenzoic acid at concentrations up to 50 mM induced changes in OF in comparison with that induced by benzoic acid ($P<0.05$), while treatment with 2,4-dimethylbenzoic acid at 100 mM tended to increase the OF value (N.S.). On the other hand, 2,6-dimethylbenzoic acid tended to decrease OF in a dose-dependent manner (N.S., Figure 6B). Treatment with 3,4- and 3,5-dimethylbenzoic acid at concentrations up to 50 mM decreased OF ($P<0.05$), but induced hemolysis of RBCs at 100 mM.

Discussion

The results of this study have shown that the application of benzoic acid decreased OF in a dose dependent manner in guinea pig RBCs. However, the OF responses to benzoic acid analogues were varied and were found to be dependent on their chemical structure. We have already reported, using the same experimental techniques, that benzoic acid and some of its chemical analogues increase OF in rat RBCs *in vitro* [16]. We hypothesized that benzoic acid and its analogues interact with the lipid bilayer of the RBC membrane, resulting in the observed changes in OF in rat erythrocytes [16]. It was confirmed that, unlike rat RBCs, exposure to benzoic acid increased osmotic

resistance, resulting in a decrease in OF in guinea pig RBCs. In addition, despite treatment with various analogues of benzoic acid inducing changes in OF, most of the changes observed in the guinea pig RBCs differed from those in the rat RBCs [16].

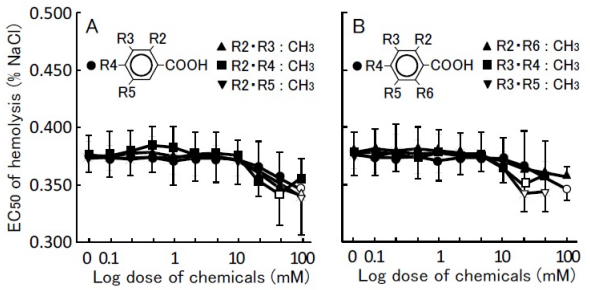

Figure 6: Dose-response changes in OF by benzoic acid and its derivatives in which hydrogen was replaced by a methyl group on the benzene ring in guinea pig RBCs. A: Each symbol represents benzoic acid, and 2,3-, 2,4-, and 2,5-dimethylbenzoic acid, respectively. B: Each symbol represents benzoic acid, and 2,6-, 3,4-, and 3,5-dimethylbenzoic acid, respectively. Values are the means ± S.D. (n=6). Open symbols indicate that there was a significant difference between the control (0 mM) and subsequent concentrations (0.1-100 mM) on the basis of Dunnett's test ($P<0.05$).

With regards to the biological actions of carboxylic acids, it was shown that mono-carboxylic acids induce intracellular Ca^{2+} release via specific receptors and activate leukocytes in humans and mice [21]. G-protein coupled receptors (GPR)-41 and GPR-43, which are activated by carboxylic acids, were identified and characterized [22]. These receptors have been reported to exist in the mucosal cells of the digestive tract [23,24], adipose tissues [25] and pancreatic islets [26], and function as receptor sites for free fatty acids possessing various hydrocarbons of various lengths. At present, however, there have been no reports that these receptors are present in the erythrocytes of any animal species.

On the other hand, there have been many reports that mono-carboxylic acids, including benzoic acid, directly affect the phospholipid bilayer of the cell membrane and induce biological actions in biological tissues and cells. Benzoic acid, its chemical analogues and other mono-carboxylic acids affect the axonal membrane to accelerate procaine absorption into the lipid bilayer both *in vivo* and *in vitro* [5,9]. Salicylic acid derivatives, which are also derivatives of benzoic acid, affect the plasma membrane and change the shape, stiffness and relaxation time of isolated RBCs within 2 minutes [13]. Based on X-ray diffraction and fluorescence spectroscopy studies, it has recently been reported that acetylsalicylic acid and salicylic acid interact with human erythrocytes and perturb the membrane bilayer [14]. These results indicate the possibility that benzoic acid and its derivatives interact with the RBC membrane and change the structure and characteristics of the plasma membrane, thereby inducing various type of action on individual cells.

In order to assess the role of the carboxylic base (COOH), bonded to a benzene nucleus, in inducing changes in OF, the OF responses were compared by replacing the carboxylic base with other chemical groups. The replacement of the carboxylic group with other groups ($PO(OH)_3$, SO_2OH, $CONH_2$ or OH) changed the OF response induced by benzoic acid. In our previous report, the replacement of the carboxylic group with other groups affected benzoic acid-induced OF response in rat RBCs [16]. In terms of its direct effect on the RBC membrane, the hydrophobic benzene ring is expected to enter the RBC membrane while the hydrophilic carboxylic group remains outside, interacting with phospholipids displayed on the outer surface of the cell membrane. Replacement of the carboxylic base with other molecular groups should, therefore, change the amphipathic balance in the moiety and affect the action of the moiety to the RBC membrane.

The effects of benzoic, cyclohexane carboxylic and heptanoic acid, each possessing six carbons with different carbon bonds in the moiety, on OF were compared in guinea pig RBCs. Heptanoic acid, but not cyclohexane carboxylic acid, produces a decrease in OF. These results indicate that the size and shape of the hydrophobic portion of the moiety are also important factors in determining its potential effect on OF. The space occupied by hydrophobic hydrocarbons is speculated to exist in the phospholipid layer in the cell membrane and with certain limitations of the space for accepting hydrophobic elements. In our previous experiment using rat RBCs, both cyclohexane carboxylic and heptanoic acid appeared to increase OF to a much greater extent than that of benzoic acid [16].

Various halogens (F, Cl, Br or I) and molecule groups (OH, CH_3, NH_2 or NO_2) introduced into the benzene ring produced changes in OF different from those induced by benzoic acid itself in guinea pig RBCs. The changes in OF were dependent on the substitution position of the molecular groups on the benzene ring. Most of the analogues of benzoic acid produced slight or apparent decreases in OF compared to values induced by benzoic acid. In rat RBCs, most of the derivatives tested in this study produced an increase in OF [16]. In the case of the rat erythrocytes, none of the derivatives tested were observed to decrease OF. These effects on the cell membrane depend on both the kind of elements substituted and the position of the substitution on the benzene ring. Interactions between the derivatives and phospholipids, cholesterols and proteins in the membrane are expected to affect the resistance to osmotic pressure in RBCs.

The effects on OF induced by derivatives of benzoic acid in guinea pig RBCs are also distinct from those in rat RBCs demonstrated in our previous report [16]. We hypothesized that one explanation for the differences in OF observed between guinea pig and rat erythrocytes is the difference in composition of the erythrocyte membrane exposed to the chemicals and not the tested chemicals themselves.

Previous studies have demonstrated that the cholesterol content in the membrane affects osmotic resistance in erythrocytes, or more accurately, the cholesterol: phospholipid ratio is in inverse proportion to OF in human RBCs [27,28]. The ratio of cholesterol to phospholipids in guinea pig was found to be lower than that in rat RBCs [17,18]. There are some reports on the ratio for hydrophilic head type of phospholipids in the rat and guinea pig RBC membrane. The ratios of each head type among the phospholipids in the membrane also differ between guinea pig and rat RBCs [18,19]. Hydrophobic acyl hydrocarbons are derived from fatty acids, which include acyl chains of various lengths, and have both saturated and unsaturated carbon bonds in the moiety. It was demonstrated that, among saturated fatty acids in the cell membrane of erythrocytes, the amount of palmitic and stearic acids vary greatly between rat and guinea pig RBCs [17,20,29]. These reports also demonstrated that, among unsaturated fatty acids, linoleic and arachidonic acids, but not linolenic acid, vary greatly

between the two species. It was reported that OF in rat RBCs is influenced by the distribution of the different types of fatty acids [30].

After hydrophobic hydrocarbons, including benzene ring, in the carboxylic acids enters cell membrane, interaction between these elements and phospholipid layer in the cell membrane should occur under various conditions, resulting in changes to the characteristics of the membrane matrix. The space available to chemical elements in the outer layer of the cell membrane is composed of cholesterols, hydrophobic acyl chains, hydrophilic-headed phospholipids, in addition to protein. A certain chemical element that enters the space in the cell membrane is speculated to affect the nature of erythrocytes, such as their stiffness, permeability, and/or resistance to osmotic pressure. The different OF responses observed on the application of the same compound, as observed in guinea pig and rat erythrocytes, is therefore thought to be related to the composition of the cell membrane, particularly the outer layer of the phospholipid bilayer.

The present study assessed the erythrocyte membrane characteristics in response to benzoic acid and its analogues in guinea pig in RBCs. This experiment also revealed that guinea pig and rat erythrocytes have different responses to benzoic acid and its derivatives. The biological activities of benzoic acid and its derivatives are thought to be dependent not only on their chemical structure but also on the characteristics of the membrane lipid layer. Previous studies have shown that the composition of the cell membrane varies between different tissues within the same species [31], as well as between the same tissues in different species [32]. We, therefore, have to clarify whether the phenomenon observed in this study can be extrapolated to the erythrocytes of other species apart from rats and guinea pigs.

Acknowledgement

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