DNA adduct formation by 2-amino-3-methylimidazo [4,5-*f*] quinoline (IQ) in rat colon

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Abstract: A food-born carcinogen, 2-amino-3-methylimidazo [4,5-*f*] quinoline (IQ) induces cancer in the rat colon. The mechanism for colonic DNA adduct formation leading to cancer by IQ was studied using a colostomized F344 rat model. In this model, the transverse colon of the rat was colostomized, which produced a fecal stream-positive proximal colon and a negative distal colon were produced. When IQ (50 mg/kg) was administered into the distal colon of the colostomized rats (n=5), the ratio of the DNA adduct level of the distal colonic mucosa to the paired muscular layer 24 hr after dosage was 2.02, whereas that was 1.51 and 1.37 when IQ was administered into the stomach (n=6) and the vein (n=5), respectively. This suggested that luminal exposure of IQ induced DNA adduct formation. Since IQ (an amine form) has no reactivity toward DNA, these findings suggested that IQ was immediately activated in the absorbed mucosal cells and reacted with DNA. However, most of the IQ absorbed was metabolically activated in the liver, distributed by blood circulation, and formed DNA adducts in the colonic mucosa and muscular layer. J. Med. Invest. 48 : 102-108, 2001

Keywords **:** *IQ, DNA adduct, colon, ^{32}P-postlabeling, metabolism*

INTRODUCTION

Accumulating evidence demonstrates that a series of genetic alterations are involved in multi-stage colon carcinogenesis (1). In addition, it has been recognized that various dietary carcinogens play an important role in the development of colon cancers (2-4). Our previous study showed that unknown DNA adducts were formed in the human colonic mucosa (5) at a level apparently higher than that of the small intestinal mucosa which shows a much lower incidence of cancer (6). Such DNA adducts may be the cause of critical mutations in cancer-related genes. A heterocyclic amine 2-amino-3-methylimidazo [4,5-*f*] quinoline (IQ) (Fig. 1) is shown to develop a high incidence of tumors in the small and large intestine, liver, Zymbal gland, clitoral gland and skin in F344 rats (7). IQ is suggested to be generated from creatine, free amino acids and hexoses, present in raw meat, via the Maillard reaction during the cooking process (8), and so is recognized as an unavoidable dietary component for humans. IQ forms *N*-(deoxyguanosin-8-*yl*)-2-amino-3-methylimidazo-[4,5-*f*]quinoline (dG-C8-IQ) and 5-(deoxyguanosin-N^2-*yl*)-2-amino-3-methylimidazo[4,5-*f*]

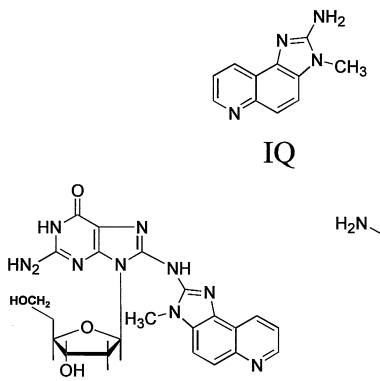

Fig. 1. Structures of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), dG-C 8-IQ and dG-N^2-IQ.

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quinoline (dG-N^2-IQ) *in vitro* and *in vivo* (9-11) (Fig. 1). Although IQ-DNA adduct levels in F 344 rats were highest in the liver, followed, in order, by the lungs, kidneys, stomach, colon, white blood cells and small intestine (12), the carcinogenic targets were only the liver, small intestine and colon. The threshold for the initiation of carcinogenesis by the adducts, repair of the adducts and cell turnover rate in each tissue may be responsible for this discrepancy. Thus, the relationship between the DNA adduct level and tumorigenesis is not a simple matter of cause and effect. However, it has been demonstrated that IQ induced point mutations in *H-ras* and *K-ras* genes in Zymbal gland tumors (13) and in *Apc* and β-catenin genes in colon tumors (14, 15). Thus, the DNA adduct formation and subsequent gene mutations are presumably the major mechanism for the rat colon carcinogenesis by IQ.

There are three theoretical routes by which IQ, administered by gavage, can come into contact with colonic mucosal DNA (16) : (i) Luminal exposure : IQ in the stomach travels through the intestinal lumen and reaches the colonic mucosal cells from the luminal direction, (ii) Circulatory exposure : IQ is absorbed from the gastrointestinal tract, metabolized in the liver, and transferred to the colonic cells through systemic blood circulation, (iii) Biliary exposure : after absorption from the GI-tract, IQ is metabolized in the liver, and excreted into bile, reaching the luminal surface of the colonic mucosa. However, it is not yet thoroughly clarified how these routes work for DNA adduct formation in the colon. In the present study, the colostomized rats having normal proximal colon and fecal stream-excluded distal colon were used to clarify the role of each route.

MATERIALS AND METHODS

Chemicals

IQ-HCl was from Dr. Keiji Wakabayashi, National Cancer Center Research Institute, Japan. RNase A, RNase T1, micrococcal nuclease, spleen phosphodiesterase and phosphodiesterase I were purchased from Worthington Biochemical Co. Ltd. (Freehold, NJ). T4 polynucleotide kinase was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Nuclease P1 was purchased from Yamasa Shoyu Co., Ltd. (Choshi, Japan). Proteinase K and apyrase were purchased from Sigma (St. Louis, MO). [α-^{32}P]adenosine-5'-triphosphate (>7,000 Ci/mmol) was obtained from ICN Radiochemical (Irvine, CA). Polyethyleneimine-cellulose sheets (POLYGRAM CELL 300 PEI) were purchased from Machery-Nagel (Duren, Germany).

Animals

Male Fischer 344 rats (4-6 weeks old) were purchased from Nippon SLC Co. (Hamamatsu, Japan) and housed in polycarbonate cages, two to three animals per cage, for one week prior to use in the pathogen-free room of our animal facilities. They were kept under constant conditions of temperature (22 ±2°C) and humidity (55±5%) with a 13 hr light/11 hr dark cycle. They were fed a commercial diet MF (Oriental Yeast, Co., Ltd., Tokyo, Japan) and provided tap water *ad libitum*.

Study design and surgery

Male F344 rats underwent surgery to differentiate the transportation routes in rat body for IQ (Fig. 2). In all the experimental studies, laparotomy was performed under general anesthesia with intraperitoneal administration of pentobarbital sodium (50 mg/kg b.w.). In Study 1, rats (6 weeks old) were colostomized at the middle of the transverse colon, and the divided colonic stumps were separately anchored through the abdominal wall to the exterior (Fig. 3A-C). Using this procedure, the distal side of the colon was completely separated from the fecal stream and naturally became empty within a few days. The distal colon was further cleaned up by enema with 0.9% NaCl solution from the stoma for three days before dosing. A single dose of IQ-HCl (50 mg/kg, 20 mg/ml of water solution) was administered into the stomach (n=6) in Study 1A, into the excluded distal colon (n=5) in Study 1B and intravenously (n=5) in Study 1C two weeks after colostomy. In Study 1B, immediately after administration of IQ into the distal colon via the stoma, the stoma and the anus of the distal colon were stitched to avoid leakage. In Study 2, the bile duct of the rats (8 weeks old) was catheterized under laparotomy (n=5) and bile was drained for 24 hr after intravenous administration of IQ-HCl (50 mg/kg) (Fig. 3D). In all experiments, the rats were sacrificed 24 hr after administration of IQ by carbon dioxide asphyxiation, and the large intestines were collected. The intestines were incised in a longitudinal direction and immediately rinsed free of their contents with 0.9% NaCl solution. The mucosal layer was scraped off, and the separated mucosal layers and muscle layers were stored at -80°C until use.

^{32}P-postlabeling of IQ-DNA adducts

DNA was isolated by the method reported previ-

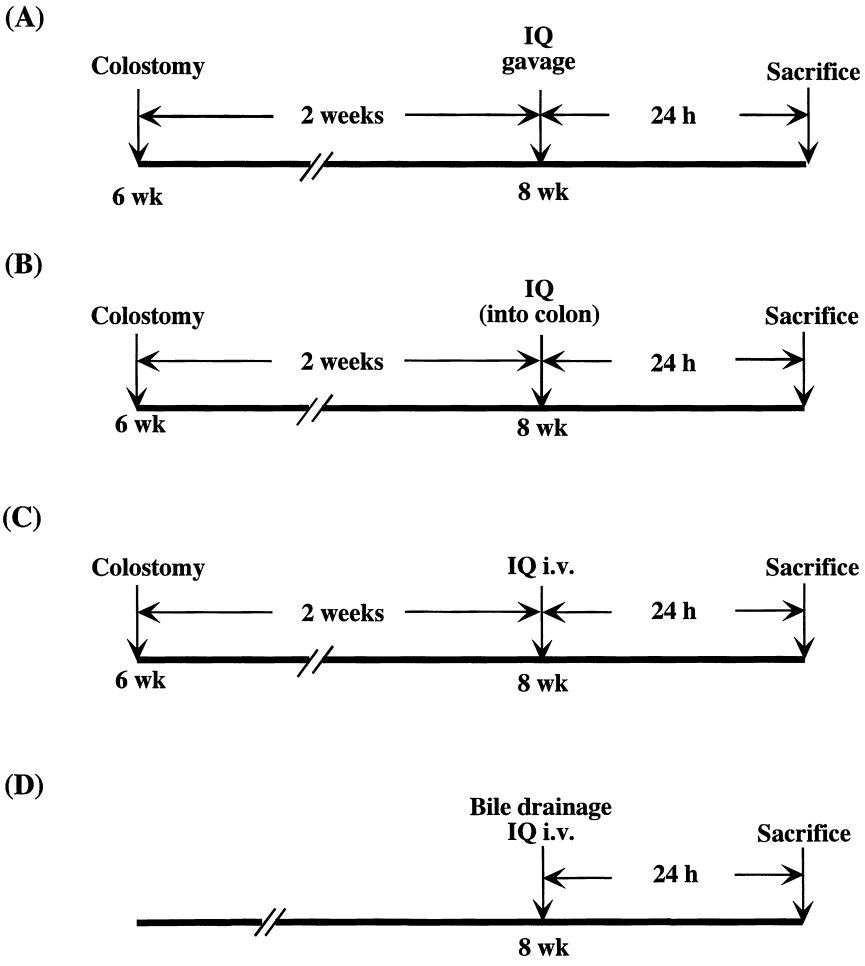

Fig. 2. Study protocols. (A) Study 1A; (B) Study 1B; (C) Study 1C; (D) Study 2.

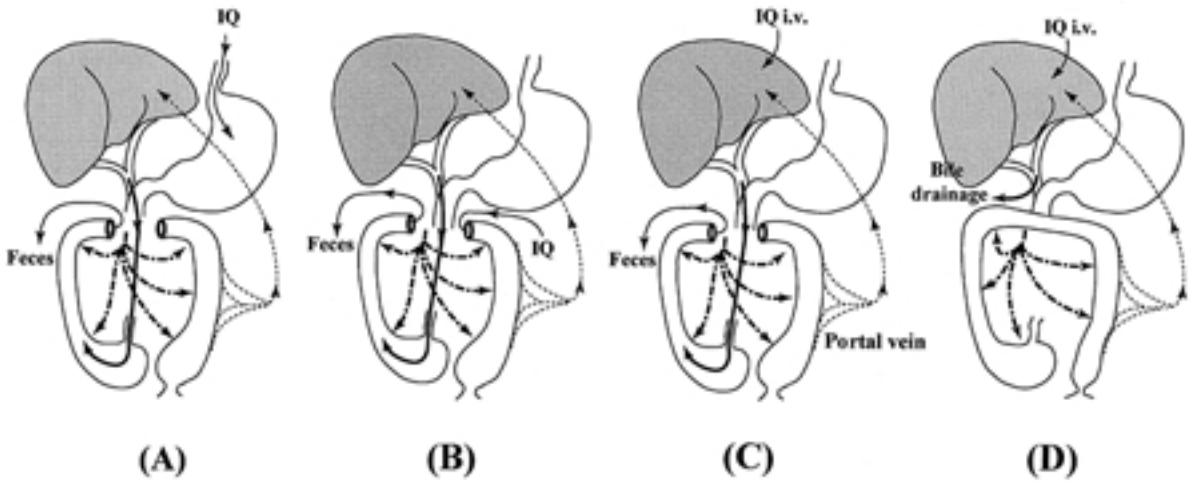

Fig. 3. Rat surgeries and administration of IQ. (A) Study 1A: rats were colostomized, and IQ (50 mg/kg bw) was administered into the stomach. (B) Study 1B: IQ was administered into the excluded distal colon of the colostomized rats. (C) Study 1C: IQ was administered intravenously to the colostomized rats. (D) Study 2: IQ was administered intravenously to the bile-drained rats.

ously (5, 17). The concentration of DNA was determined by measuring the absorbance at 260 nm (using a value of 20 absorbance units/mg DNA) and adjusted to a final concentration of 2 mg/ml. IQ-DNA adducts were detected by ^{32}P-postlabeling analysis with the intensification method (18). Briefly, 10 μg of DNA was digested to deoxynucleoside 3'-monophosphates with micrococcal nuclease (3 units) and spleen phosphodiesterase (0.03 units) in a total volume of 10 μl of 20 mM sodium succinate, 10 mM $CaCl_2$, pH 6.0 at 37°C for 3.5 hr (19). The DNA digest was diluted 2-fold with water. Then, 10 μl of the digest was taken and incubated

with 5 μl of labeling cocktail containing 1.5 μl of kination buffer (300 mM Tris-HCl, pH 9.5, 100 mM $MgCl_2$, 100 mM dithiothreitol and 10 mM spermidine), 1.0 μl of [α-^{32}P]ATP (150 mCi/ml), 0.5 μl (5 units) of T4 polynucleotide kinase and 2.0 μl of water at 37°C for 1 hr. The labeled digest was further treated with 2 μl potato apyrase (5 units/ml) and 1 μl water for 45 min at 37°C. To purify the IQ-bound nucleotides from normal nucleotides, the ^{32}P-labeled nucleoside bisphosphates were spotted onto PEI-cellulose sheets and developed with 1.7 M sodium phosphate (pH 6.0) at 22°C for 16 hr. The origin was cut out and attached to a new PEI-cellulose sheet using a magnet, and developed with 2.7 M lithium formate/5.1 M urea (pH 3.5) from bottom to top. This was further developed in 0.96 M LiCl/6.4 M urea/0.4 M Tris-HCl (pH 8.0), followed by 1.0 M sodium phosphate (pH 6.0) from left to right, with a 3.5 cm paper wick (Whatman 3MM chromatography paper). The radioactive spots on a thin-layer chromatography (TLC) sheet were visualized and their radioactivity was quantified by BAS-1500 Bio Imaging Analyzer and BAS-IIIs Imaging Plate (Fuji Photo Film Co., Ltd, Tokyo, Japan). For the total nucleotide count, an aliquot of the above DNA digest was labeled, treated with apyrase, spotted on the PEI-cellulose sheet and developed with 0.5 M lithium chloride (17). The relative adduct labeling (RAL) values were calculated according to the formula : RAL = adducts level / (total nucleotides level × dilution factor). All experiments for quantification of the IQ-DNA adduct level by ^{32}P-postlabeling were performed in triplicate. To estimate DNA adduct levels as accurately as possible, all samples in a study group were analyzed at once, and the analysis of samples was performed in a random order. Statistical analysis was performed by ANOVA.

RESULTS

In study-1, the preoperative body weight of the rats (140±2 g) at 6 weeks old slightly decreased to 137±9 g one week after colostomy, but increased again to 162±6 g when IQ was administered at 8 weeks of age. When IQ was administered into the stomach (Study 1A), the IQ-DNA adduct level of the proximal mucosa was significantly higher than that of the distal mucosa ($p<0.05$), and that of the proximal muscular layer was significantly higher than that of the distal muscular layer ($p<0.05$) (Table 1). In the same study, the IQ-DNA adduct level of the proximal mucosa was significantly higher that of the proximal muscular layer ($p<0.05$), and that of the distal mucosa was significantly higher than that of the distal muscular layer ($p<0.05$).

When IQ was administered into the distal colon (Study 1B), the IQ-DNA adduct level of the proximal mucosa was similar to that of the distal mucosa, but that of the proximal muscular layer was significantly higher than that of the distal muscular layer ($p<0.05$) (Table 1). In the same study, the IQ-DNA adduct levels of the proximal and distal mucosa were significantly higher than those of the proximal and distal muscular layer, respectively ($p<0.05$). As a whole, the DNA adduct level of Study 1B dosed in the distal colon was similar to that of Study 1A dosed in the stomach. In contrast, DNA adduct level of Study 1C dosed intravenously was about three-fold higher than that of Study 1A and B.

When IQ was administered intravenously (Study 1C), the IQ-DNA adduct level of the proximal mucosa was significantly higher than that of the distal mucosa ($p<0.05$), but that of the proximal muscular layer was no difference compared with that of the distal muscular layer (Table 1). In the same study, the IQ-DNA adduct level of the proximal mucosa was significantly higher that of the proximal muscular layer ($p<0.05$), and that of the distal mucosa was not significantly higher than that of the distal muscular layer.

In Study 2, when IQ was administered intravenously to the bile-drained rats, the IQ-DNA adduct level of the mucosa was about 2-fold higher that of the muscular layer ($p<0.05$) (Table 1). In all studies, approximately 5-6 DNA adduct spots were detected on a TLC sheet by ^{32}P-postlabeling analysis as reported previously (16).

DISCUSSION

Among three theoretical exposure routes of IQ to the colon (luminal, circulatory and biliary exposures), all routes should be potentially active in the colonic mucosa if IQ is administered into the stomach of the non-operated rats. However, the luminal and biliary exposures to the distal colonic mucosa were blocked by the colostomy (Study 1A). When IQ was administered into the distal colon of the colostomized rats, luminal exposure to the proximal mucosa alone was blocked, in contrast, heavy luminal exposure to the distal mucosa occurred (Study 1B). When IQ was administered intravenously to the colostomized rats, luminal exposure to the mucosa was blocked (Study 1C). When IQ was administered intravenously to the bile-drained rats, only circulatory exposure was active (Study 2). Thus, a combination of these experimental

Table 1. IQ-induced DNA adducts in colostomized rat colon

Study	*n*	Sample	Exposure route			DNA adduct level (RAL[a]×10^7)
			Luminal	Circulatory	Biliary	
1A	6	Proximal mucosa	+[b]	+	+	1.92±0.26[de]
		Proximal muscle	–[c]	+	–	1.10±0.05[f]
		Distal mucosa	–	+	–	1.31±0.09[g]
		Distal muscle	–	+	–	0.87±0.05
1B	5	Proximal mucosa	–	+	+	1.83±0.34[d]
		Proximal muscle	–	+	–	1.23±0.24[f]
		Distal mucosa	+	+	–	1.68±0.19[g]
		Distal muscle	–	+	–	0.83±0.05
1C	5	Proximal mucosa	–	+	+	5.50±1.7[de]
		Proximal muscle	–	+	–	3.66±0.85
		Distal mucosa	–	+	–	3.34±0.78
		Distal muscle	–	+	–	2.44±0.95
2	5	Mucosa	–	+	–	2.44±0.56[h]
		Muscle	–	+	–	1.24±0.20

IQ(50 mg/kg) was administered into the stomach of the colostomized rats (Study 1 A, n=6), distal colon of the colostomized rats (Study 1 B, n=5), vein of the colostomized rats (Study 1 C, n=5) and vein of the bile-drained rats (Study 2, n=5), respectively.
[a] Relative adduct labeling.
[b] Active.
[c] Inactive.
[d] $p<0.05$ *versus* proximal muscle of the same rats.
[e] $p<0.05$ *versus* distal mucosa of the same rats.
[f] $p<0.05$ *versus* distal muscle of the same rats.
[g] $p<0.05$ *versus* distal muscle of the same rats.
[h] $p<0.05$ *versus* muscle of the same rats.

models provided important information to clarify the active exposure routes to the colon. The colostomy procedure was an acceptable stress for the rats, and the rats were healthy when IQ was administered. Since the absolute values of ^{32}P-postlabeling have a wide error range in all experiments, the samples to be compared should be analyzed together. However, the number of samples for one time analysis is limited. In the present study, to compare the mucosal DNA adduct levels in the different studies, the paired mucosa-adjacent muscular layer which allows only circulatory exposure was analyzed as a good standard.

The general patterns of the DNA adduct levels in four types of colonic tissues (mucosa/muscular layer of the proximal/distal colon) were similar in Studies 1A, 1B and 1C. However, one difference was that the DNA adduct level of the distal mucosa in Study 1B alone was higher than that in Study 1A or 1C : i.e. the ratio of the DNA adduct level of the distal mucosa to the paired muscular layer was 2.02 in Study 1B, whereas it was 1.51 in Study 1A and 1.37 in Study 1C. This suggests that luminal exposure of IQ induced DNA adduct formation. Since IQ (an amine form) has no reactivity toward DNA, these findings imply that IQ was immediately activated in the absorbed mucosal cell and reacted with DNA. This idea is not recognized in other heterocyclic amines (20). The principal metabolic pathways of heterocyclic amines leading to DNA adducts involve cytochrome P450 (CYP) mediated *N*-oxidation of exocyclic amine nitrogen (phase I) and subsequent esterification by phase II enzymes (21). In hepatic tissue, heterocyclic amines are known to be activated to the *N*-hydroxy form mainly by CYP1A2, but this enzyme activity is absent in the colon (22). However, in the case of IQ, we previously demonstrated, using different rat models, that IQ was partly activated to form DNA adduct in the colonic mucosal cells (16). The present findings support our previous observations. Thus, except CYP1A2, there should be another metabolic activation mechanism such as CYP1B1 (22) and prostaglandin H synthase (23) in the colonic mucosa. However, the major activation was presumably catalyzed by hepatic CYP1A2 as previously recognized, because most DNA adduct was formed via circulatory exposure in the present study.

A comparison of DNA adduct levels of the proximal

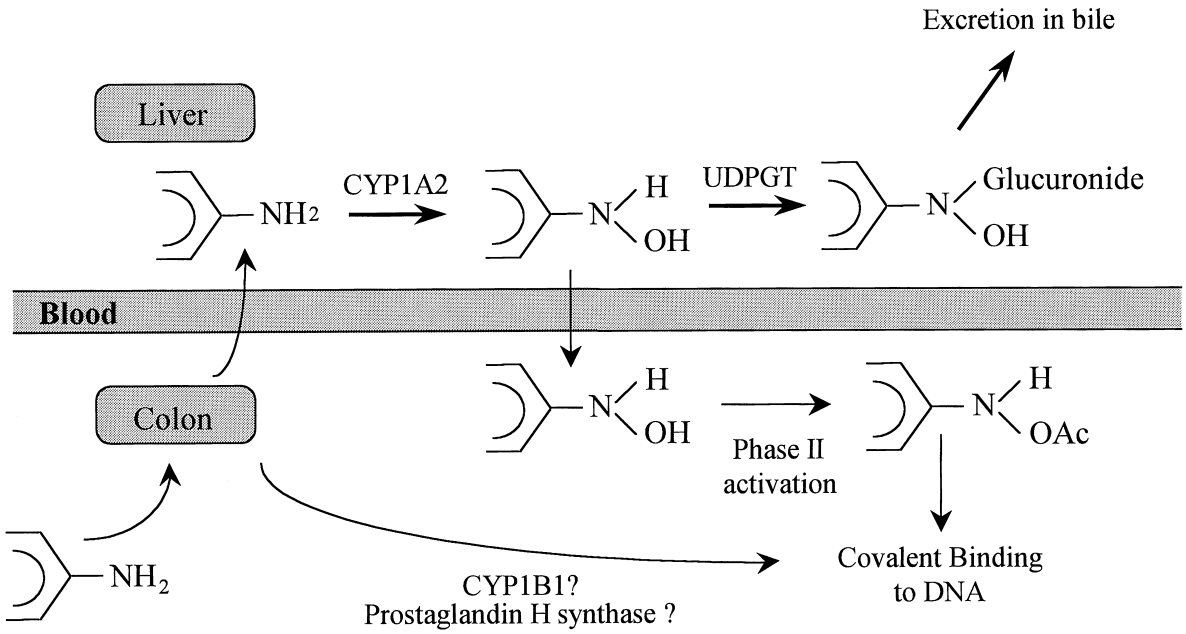

Fig. 4. Proposed metabolism of IQ leading to DNA adduct in the rat colon.

mucosa between Study 2 and Study 1A-C showed the effect of biliary exposure by IQ metabolites on colonic DNA adduct formation. Although the biliary exposure to the proximal mucosa was excluded by bile drainage in Study 2, the DNA adduct level of the proximal mucosa did not decrease compared with that in Study 1. This suggests that the contribution of biliary IQ metabolites to the colonic adduct formation was negligible. Therefore, the finding that the IQ-DNA adduct level of the proximal mucosa was significantly higher than that of the distal mucosa in Study 1C was caused mostly by circulatory exposure. The IQ-DNA adduct level of the proximal muscular layer was also higher than that of the distal colon in all Study 1 experiments. In addition, a preferential DNA adduct formation was shown in the mucosa compared with the paired muscular layer in all studies. The differences in the activities of metabolic activation/inactivation enzymes, the transport of the cell membrane and DNA repair may be the causes of these preferences of DNA adduct formation.

In conclusion, the findings of the present study suggest that most of the part of IQ (an amine form) passes through the colonic mucosal cells without being metabolized when absorbed, is activated to an *N*-hydroxy form in hepatic tissue mainly by CYP1A2, is transported via the systemic circulation to the colon, and forms DNA adducts (Fig. 4). Some of the IQ, however, receives another metabolic activation allowing DNA adduct formation in colonic cells. The role of biliary IQ metabolites in colonic DNA adduct formation is negligible.

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