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CORRESPONDENCE/REBUTTAL

Rebuttal to a Comment on Lymphatic Absorption of Quercetin and Rutin in the Rat and Their Pharmacokinetics in Systemic Plasma

We thank Drs. Cermak and Wolffram for their interest in our previous work (*1*). They have presented a comment based on the fact that intact rutin can be detected in blood plasma and lymph of rats when rutin is given by duodenal single dose. They also quoted an earlier work stating that rutin must be hydrolyzed by intestinal microflora for the absorption of its aglycone quercetin (*2*). However, that earlier review did not consider the detectability of intact rutin in blood plasma and lymph; rather, it quoted two citations and stated that the bioavailability of rutin is about 15−20% of that of quercetin 4′-glucoside (*3*, *4*). In the current study, the solvent PEG400/EtOH was used to dissolve the drugs. Because of their low toxicity, low molecular weight PEG and ethanol have been used as emulsifier and adjuvant, respectively, in many pharmaceutical applications (*5*). However, it is possible that these vehicles might affect absorption of the drug.

First, absorption can be generally divided into intestinal capillary and lymphatic absorption pathways. Intestinal capillary absorption passes through the portal vein to enter the liver for biotransformation, but lymphatic absorption bypasses the liver and avoids the first-pass effect. Lymphatic and blood vessels lie side by side, and the ends of lymphatic capillaries are open in spaces between cells at the thoracic duct. The thoracic duct thus receives lymph fluid that then enters the circulation system at the junction of the left jugular and the left subclavian veins (*6*). In our study (*1*), rutin was administered to rats at a dose of 300 mg/kg, much higher than the 30 mg/kg dose of quercetin. The controversy over rutin intestinal absorption might be partly related to the dose relationship, and it also might be the reason for the high clinical dose of rutin (*7*). We do not question that the hydrolysis of rutin by intestinal microflora leads to quercetin through glycuronidase, and we note that both quercetin and rutin can be absorbed through intestinal capillary and lymphatic absorptions, although the flow rates of lymph are much slower than that of blood.

Dr. Cermak's recent study indicated that rutin could be a source of quercetin for dogs, and the absolute bioavailability of quercetin (i.e., the fraction that reaches the systemic circulation) was about 4% in that species (*8*). Some studies have claimed that intestinal microfloral hydrolysis is required for rutin absorption (*9*). It has also been claimed that rutin is absorbed more slowly than quercetin because it must be hydrolyzed by the cecal microflora, whereas quercetin is absorbed by the small intestine (*10*). However, other studies have shown that free rutin could be absorbed through both rat and human intestines (*11*, *12*). An isolated rat small intestine perfusion model has been used to investigate intestinal absorption and metabolism of rutin, and the results confirmed the uptake of rutin in the rat small intestine (*11*). Cermak et al. (*13*) compared the oral bioavailability of quercetin from quercetin aglycone and two different quercetin glycosides in pigs. Those results demonstrated that if the relative bioavailability of quercetin is 100%, the bioavailability of quercetin-3-*O*-glucoside (Q3G) or quercetin-3-*O*-glucorhamnoside (rutin) would be 148 and 23%, respectively, in pigs. However, in the more recent research of Dr. Cermak (*8*) we note that the dose of rutin administered to dogs was 18.33 mg/kg, which is much lower than the dose we administered to rats. It is also possible that there were undetectable rutin levels in the blood if the concentation of analytes was lower than the detection limit. Furthermore, Cermak et al. were discussing rutin as a daily nutrition supplement, whereas we consider rutin as a clinical medicine administered at a higher dose (250−500 mg) (*14*). Thus, it is not surprising that we found different results, due to the large dose differences between the two experiments. Again, it is possible that both quercetin and rutin could be detected in the plasma after rutin was given orally.

Second, Drs. Cermak and Wolffram comment on the analysis of rutin and quercetin by Chen et al. (*1*) without considering that the metabolites in that study were derived from intestinal and hepatic metabolism. There are already numerous papers discussing the metabolism of rutin and quercetin (*15*). Instead, we focus on the routes of intestinal absorption, intestinal capillary and lacteal duct, which would preserve the drug in a more intact form. It would do so because absorption via intestinal capillaries enters the portal vein and liver before circulating throughout the body, and thus the drug would be metabolized before being distributed to target tissues. The lymphatic absorption route avoids the first-pass effect of the liver and provides an alternative route to preserve intact and unmetabolized drug for distribution to target tissues. On the other hand, the lymphatic absorption route also allows lipophilic and macromolecular compounds to enter the whole-body circulation. For example, we have hypothesized that prion is a pathogenic protein of Creutzfeldt−Jacob disease that may be transmitted via a lymphatic absorption route, but not by intestinal capillary absorption in oral exposure because peptides and proteins can be absorbed in the gastrointestinal lymphatic system (*16*, *17*). We are continuing to work on this subject to discover the differences in pharmacokinetic characteristics between lymphatic and intestinal capillary absorption.

Third, the time−concentration courses of compounds in lymph and in plasma were actually determined by samples collected throughout the experimental process, and we compared the two different concentrations in these two body fluids. The total drug amounts in lymph fluid and in plasma were indeed different, but their concentrations in the body fluid is what primarily influences the effect of a drug. We did not use ref *18* to support our pharmacokinetic data, and we mentioned that reference in the introduction simply to indicate the lack of studies on the pharmacokinetics of the lymph system.

Fourth, inspired by Murota and Terao's remarkable work (*19*), we began our study to try to understand the position and effect of the lymph system within the circulation system of the whole body from a pharmacokinetic view. Further studies are clearly still required, and we contiue to conduct additional experiments

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to elucidate pharmacokinetic characteristics of the lymphatic system.

In sum, we appreciate Drs. Cermak and Wolffram's comments on our paper; the absorption pathways of intestinal absorption and lymphatic absorption are issues that we will consider further in future research.

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Tung-Hu Tsai*

Institute of Traditional Medicine, School of Medicine, National Yang-Ming Unviersity, Taipei 112, Taiwan, and Department of Education and Research, Taipei City Hospital, Taipei, Taiwan

I-Lin Chen

Institute of Traditional Medicine, School of Medicine, National Yang-Ming Unviersity, Taipei 112, Taiwan

*E-mail (T.-H.T.) thtsai@ym.edu.tw; (I.-L.C.) hughclaus@yahoo.com.tw).

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