Development of Self-emulsifying DHA Formulation

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Abstract

Various physiological functions as essential fatty acids are reported regarding docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The two acids are polyunsaturated fatty acid (PUFAs). Absorption of DHA is often influenced by meal consumption. We prepared a self-emulsifying DHA formulation which achieved stable and high absorption ability without depending on bile acid. Absorption evaluation on rats revealed that bioavailability of this self-emulsifying DHA formulation was three times higher than that of general DHA fish oil. The evaluation also proved that the formulation has high absorption ability without being affected by food consumption. In conclusion, this formulation technology improved DHA absorption by oral intake, leading to more convenient DHA ingestion.

1. Introduction

In 2006, under the new corporate philosophy, "contributing to the advancement of culture, science, technology and industry, as well as improved health and environmental protection in society and thus helping to enhance the quality of life of people worldwide," Fujifilm stepped into a new domain of preventive medicine, launching functional cosmetics and supplements onto the market.

Those healthcare products have been established based on our various technologies and know-how acquired in the field of photography. For example, the main ingredient of photo films is the same collagen as that of the skin, and skin blemishes and aging share the same cause, oxidation, as photograph color fading. Our collagen handling technology and antioxidant technology can thus be applied to healthcare products directly. In addition, to differentiate our products from those of others, we have been developing new technologies to introduce valuable healthcare products only made available by Fujifilm, placing emphasis on an advanced nanotechnology (FTD technology) that allows precise and effective *in vivo* permeation/absorption of functionally combined ingredients and materials.

With the foregoing background, we succeeded in the development of self-emulsifying formulation technology and released "DHA, EPA & Astaxanthin" onto the market in January 2012 (Fig. 1).

Fig. 1 DHA, EPA and Astaxanthin.

2. Development of DHA, EPA & Astaxanthin

2.1 What is DHA?

Among functional materials used for supplements are vitamin C and B groups that are water-soluble; and vitamin E, docosahexaenoic acid (DHA), coenzyme Q10 and astaxanthin that are oil-soluble. The former are tabletized or encapsulated (hard-type) and the latter are formulated into soft capsules.

Similarly to eicosapentaenoic acid (EPA), DHA (Fig. 2) is a kind of n-3 (ω-3) polyunsaturated fatty acid (PUFA) and blueback fish are rich in it. It also exists abundantly in the retina, heart, and central nervous system of mammals and has been reported to have, as an essential fatty acid, various physiological functions such as blood lipid lowering and antithrombotic effects[1].

Fig. 2 Docosahexaenoic acid.

Since the target intake of DHA and EPA (at least 1 g/day) was specified in Dietary Reference Intakes for Japanese

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(2010) as shown in Table 1, n-3 PUFAs including those two have been drawing more and more attention as health maintaining ingredients[2]. In this way, DHA and EPA have established their current popularity as supplements and become widely distributed in society.

Table 1 Recommended daily ingestion amount of EPA and DHA for Japanese.

(g/day)

Age	Male	Female
	Target (minimum)	Target (minimum)
18 to 29	1	1
30 to 49	1	1
50 to 69	1	1
70 or over	1	1

2.2 The effect of feeding and fasting on DHA absorption

As supplements can be taken casually and the timing being usually unspecified, the absorption of useful ingredients may vary depending on when they are taken. The raw ingredients of DHA generally used in supplements exist with other fatty acids in the form of triglyceride. After being micellized by bile and hydrolyzed by lipase within the small intestine, they are absorbed by the intestinal epithelium.

It is known that even in rats, which do not have a gallbladder, the secretion of bile is increased when feeding. DHA absorption may vary greatly, depending on the secretion amount of bile.

To verify the difference in DHA absorbability by intake timing (after feeding or fasting), we conducted experiments with rats. The results are shown in Fig. 3. With this time-blood plasma DHA concentration graph, the maximum blood concentration (Cmax), maximum blood concentration time (Tmax) and area under the blood concentration-time curve (AUC) were derived.

As shown in Table 2, DHA absorbability for fed rats did not differ from that for fasted rats in Tmax but Cmax is about 2.7 times and the AUC0-24 about 2.5 times higher than those fasted.

This reveals that DHA absorbability varies greatly depending on the intake timing.

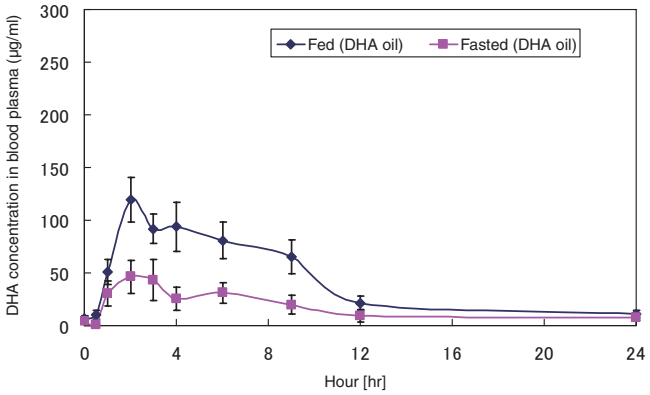


Fig. 3 Plasma concentration profile of DHA.

Table 2 Pharmacokinetic parameters of oral administration of DHA oil to fed rats and fasted rats.

		fed DHA OIL	fasted DHA OIL
C_{max}	μg/ml	119.2±21.2	44.5±15.7
T_{max}	hr	2	2
$AUC_{0\text{-}24h}$	μg・hr/ml	1018.9	405.8*

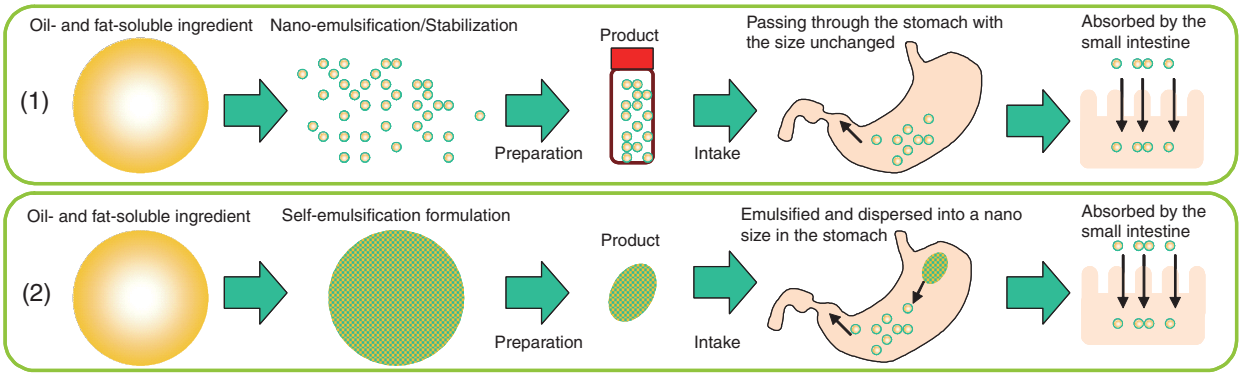
DHA OIL: Common edible fish oil
Cmax: maximum serum concentration
T_{max}: time to reach the C_{max}, AUC: area under the serum concentration-time curve
Values of C_{max} are the mean ± SEM, n=5 * P<0.1

3. Development of self-emulsifying DHA formulation technology

We increased the absorption of oil-soluble, low-absorbability materials, such as astaxanthin, by converting them into nano-emulsions[3]. This technology is effective for oil-soluble materials such as coenzyme Q10 and carotenoids such as astaxanthin whose necessary intake per day is no more than several dozens of milligrams. However, it was difficult to apply the technology to useful ingredients like DHA whose intake per day needs to be several hundreds of milligrams, because that increases the number of capsules to be taken.

Then, aiming to suppress the increase in the number of capsules while keeping high absorbability, we carried out the development of a self-emulsifying DHA formulation, which is expected to reduce the great effect that bile acid has on the absorption of oil-soluble materials while in a fed state and to thereby achieve high absorbability consistently, regardless of feeding or fasting. Fig. 4 shows the difference between nano-emulsions and the self-emulsifying DHA formulation.

Incidentally, a self-emulsifying formulation does not need the shear stress of homomixers, etc., but employs a surface chemistry technique to formulate fine emulsions (particles) spontaneously with water contact as a trigger and to increase the specific surface area, which improves its absorbability. Fig. 5 illustrates a general preparation method for self-emulsifying formulations and their emulsification mechanism[4].



	Characteristics	Suitable materials	Suitable product formulation	Suitable dosage forms
(1) Nano-emulsification	Nanonized and prepared	Materials requiring only a small intake	Mixed with water-based or powder ingredients	Liquid, hard capsules
(2) Self-emulsification	Prepared so that it will be nanonized	Materials requiring a large intake	Mixed with oil-based ingredients	Soft capsules

Fig. 4 Difference in DHA absorption between nano-emulsion and self-emulsification formulations.

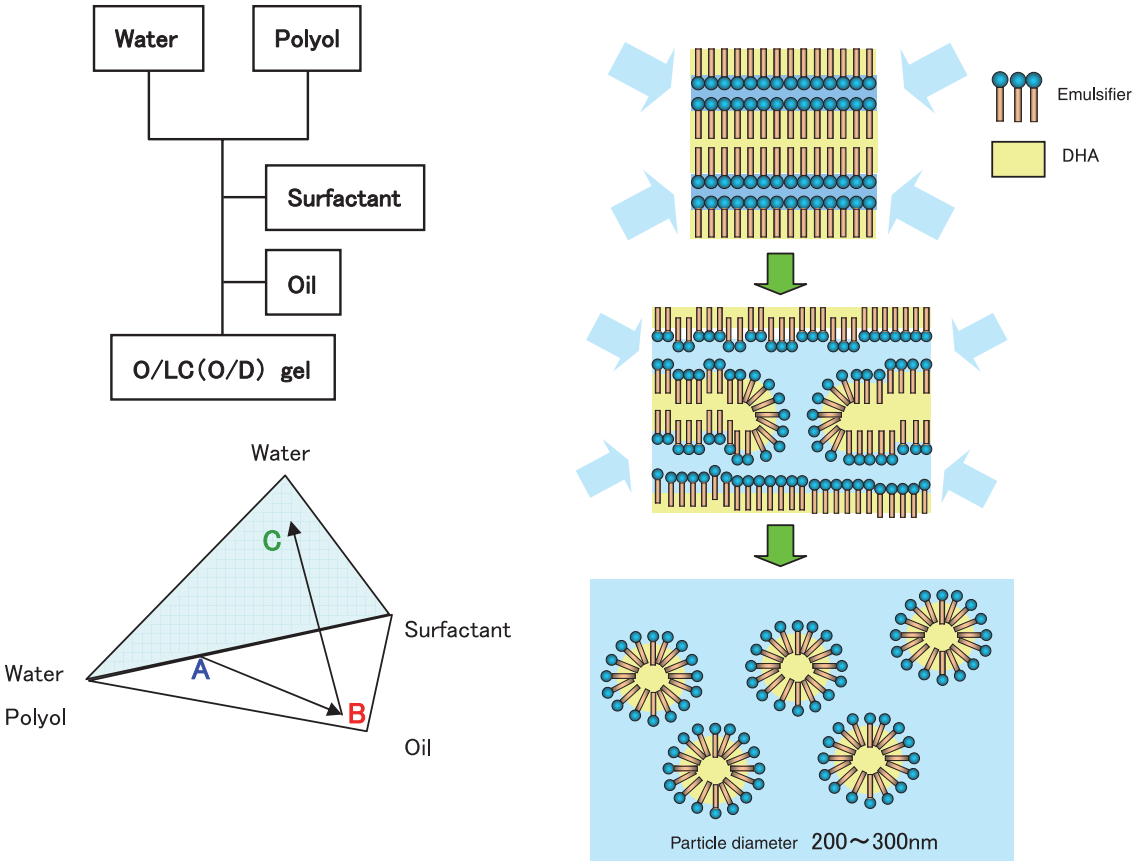


Fig. 5 General self-emulsification preparation and mechanis.

3.1 Assessment of the absorbability of a self-emulsifying DHA formulation (1)

To verify the absorbability of the newly developed self-emulsifying DHA formulation, we first conducted a comparative test with DHA oil commonly used in supplements and self-emulsifying DHA formulation using hungry rats. The results are shown in Fig. 6 and Table 3.

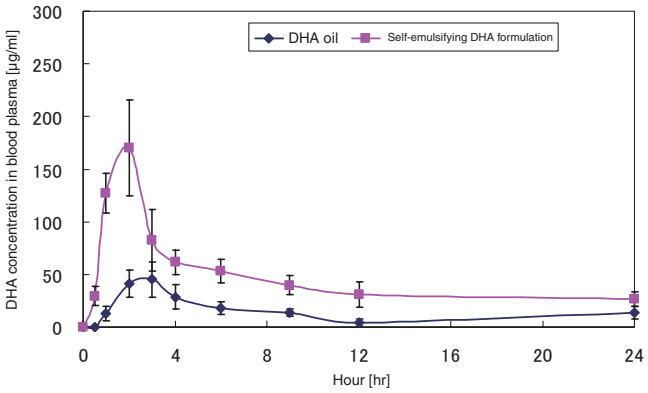


Fig. 6 Plasma concentration profile of DHA.

Table 3 Pharmacokinetic parameters of oral administration of DHA oil and SEDDS-DHA to fasted rats.

		DHA OIL	SEDDS-DHA
C_{max}	μg/ml	45.2±16.8	170.0±45.4
T_{max}	hr	3	2
$AUC_{0\text{-}24h}$	μg·hr/ml	341.3	1100.0**

DHA OIL : Common edible fish oil, SEDDS-DHA : Self-emulsifying DHA formulation
C_{max} : maximum serum concentration, T_{max} : time to reach the C_{max}
AUC : area under the serum concentration-time curve
Values of C_{max} are the mean ± SEM, n=3 ** P<0.05

As shown in the above results, the concentration of DHA in the blood plasma for the self-emulsifying DHA formulation reached its peak (Cmax) two hours after intake and then declined gradually. On the other hand, that for DHA oil commonly used in supplements reached Cmax three hours after intake and then declined gradually. In addition, the AUC for the former is about three times as large as that for the latter. This means that the self-emulsifying DHA formulation had higher absorbability than common DHA oil.

3.2 Assessment of the absorbability of a self-emulsifying DHA formulation (2)

As already described, the DHA absorbability of common DHA oil varies depending on the intake timing. Finally, to verify the DHA absorbability of the self-emulsifying DHA formulation by intake timing, we conducted a comparative test using fed and fasted rats. The test parameters and results are shown in Table 4 and Fig. 7.

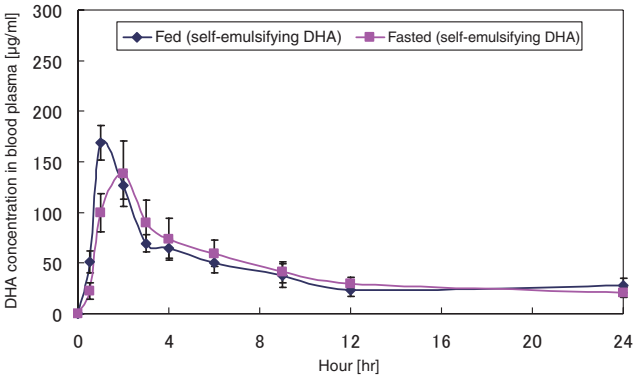


Fig. 7 Plasma concentration profile of DHA.

Table 4 Pharmacokinetic parameters of oral administration of SEDDS-DHA to fed rats and fasted rats.

		fed SEDDS-DHA	fasted SEDDS-DHA
C_{max}	μg/ml	169.1±17.0	138.1±32.2
T_{max}	hr	1	2
$AUC_{0\text{-}24h}$	μg·hr/ml	1028.9	1044.7

SEDDS-DHA: Self-emulsifying DHA formulation
Cmax : maximum serum concentration
T_{max} : time to reach the C_{max}, AUC : area under the serum concentration-time curve
Values of C_{max} are the mean ± SEM, n=5

As shown in the above results, in the case of the fasted group, the concentration of DHA in the blood plasma for the self-emulsifying DHA formulation reached its peak (Cmax) two hours after intake and then declined gradually in the same way as shown in 3.1. On the other hand, in the case of the fed group, the concentration reached Cmax one hour after intake and then declined gradually. No significant difference was observed between the two groups in the AUC. It was apparent that a self-emulsifying DHA formulation can exhibit high absorbability regardless of fasting or feeding.

The one-hour difference between the two groups in the time to reach Cmax is thought to be because the secretion of bile acid of the fed group was larger than that of the fasted group and, consequently, DHA was absorbed faster.

4. Conclusion

For the new product DHA, EPA & Astaxanthin, we developed Aqua-nanosizing DHA that reacts with the water taken together with the supplement and spontaneously nanonizes itself *in vivo*. It is hardly affected by intake timing, either feeding or fasting, and can exhibit high absorbability constantly. In the future, we will further utilize a characteristic technology of ours called FTD to develop valuable functional food products and thereby contribute widely to society, improving the quality of people's life.

References

1) Suzuki, H., et al. *Suisan Shokuhin Eiyo-gaku - Kiso kara Hito he* (Seafood Dietetics - From Basics to Man). Gihodo Shuppan (2004)
2) Dietary Reference Intakes for Japanese (2010). Ministry of Health, Labour and Welfare (2009)
3) Suzuki, K., et al. Development of Astaxanthin Nano-Emulsion with Improved Shelf Life and Enhanced Absorbability. Fujifilm Research & Development No. 52, 27-29 (2007)
4) Binks, B.P. ed. Modern Aspects of Emusion Science. Royal Society of Chemistry. Cambridge (1998).