Original Article

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A digital scheme of human trials for the evaluation of functional foods

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SUMMARY In this study, we designed a method for conducting a human study by the following process. (1) The host computer stores the subject information. (2) The sample preparer prepares a food sample. (3) The subject (healthy human volunteer) sends the information of an intake of the food sample to the host computer, which creates an event entry for the event. (4) The medical professional (typically a physician) collects and stores the subject's blood sample in a container with the subject's identification (*e.g.*, ID number). (5) The sample analyst analyzes the blood biochemical profiles. (6) The host computer stores the blood biochemical data, and by matching the blood biochemical data with the subject IDs, a final analysis report will automatically be created. In this study, we also run a test case, based on this design, where we obtained a blood biochemical dataset from healthy volunteers. This scheme can reduce the cost of human trials for functional foods and will help acquiring the scientific basis of functional foods.

Keywords human trial, personal devices, functional food, natural food

1. Introduction

As we enter a super-aging society, people's interest in natural foods is increasing. To obtain scientific evidence on the health effects of natural foods, results using laboratory animals are considered. We have proposed invertebrate models to replace mammalian models, and have applied them to a range of research topics such as infectious diseases (*1,2*), immune activation (*1,3-5*), antibiotic discovery (*6,7*), and diabetes (*8-11*). However, the health effects of natural foods must ultimately be verified in humans, which poses challenges due to its cost ($3000-5000 per subject (*12*)) and ethical issues. In conventional human trials to investigate the effects of natural foods on humans, all elements of the trial should be managed by physicians, and ensuring cost effectiveness has been a major issue (*13*). Therefore, in this study, we attempted to propose a method for conducting human trials with the use of personal computing devices, with the aim of facilitating the practical application process of newly developed natural foods and promoting the use of natural foods that may contribute to public health.

In this study, we focused on the fact that, among the processes of human trials for natural foods, only the processes related to invasive medical procedures such as blood sampling needs a direct supervision by physicians, but the other processes do not necessarily need to be performed by physicians. On the other hand, when non-physicians conduct the invasive steps in the human test method for natural foods, it may potentially be difficult to ensure and monitor the intake of test samples by subjects. For this potential hurdle, we thought it possible to overcome it by registering the subject information and trial checkpoints in advance in a computer and setting up a reminder to each event, so that each subject will be tracked throughout the trial *via* a personal computer, smartphone, tablet, *etc.* This method can also visualize the progress and results of the trial to the administrator and will reduce the barriers to the implementation of human trials for natural foods, accelerating the development and use of natural foods based on scientific evidence.

2. Materials and Methods

2.1. Lactic acid bacteria strains and peroral intake

The lactic acid bacteria (LAB) strains used in this study

were *Leuconostoc carnosum* #7-2 (*14*) (LAB strain A) and *Lactococcus lactis* 11/19-B1 (*15*) (LAB strain B). Live cells of each strain were prepared at a dose of 10^{10} cells/day/subject and administered orally to the subjects for 27 days. Three healthy adult volunteers were recruited by Genome Pharmaceuticals Institute Co., Ltd. (Tokyo, Japan).

2.2. Measurement of Natural Killer (NK) Cell Activity

Blood samples were collected by a physician for each subject three times: one day before the start of sample intake (day -1), in the middle of the sample intake period (day 14), and one day after the end of sample intake (day 28). The collected blood samples were refrigerated and transported to the analyst on the day of collection, and the analyst measured the peripheral blood NK cell activity. Specifically, peripheral blood leukocytes (E) and ^{51}Cr-labeled K562 cells (T) were mixed and cultured at E/T = 20 for 3.5 hours, and the %lysis value was determined. All three subjects ingested the samples once a day for 27 days (81 out of 81 times (100%)). Regarding the time of ingestion, 70 out of 81 times (86.4%) were between post-breakfast and 10:30 a.m. ingestion, which was the time period set by the administrator. In addition, a situation occurred twice where one of the subjects took the sample later than the originally scheduled time.

3. Results

3.1. A scheme for conducting a human study using personal devices

As shown in Figure 1, the host computer stores the subject information so that the administrator can identify the subject in the trial. In the process, the sample preparer first prepares the sample for the subject (for per-oral administration) and fills the sample container. Then, the subject sends the information at the time of intake to the host computer, and the host computer creates an event entry at the time of intake. Next, a medical staff (physician) collects the subject's blood and stores it in a blood storage container (*e.g.*, a

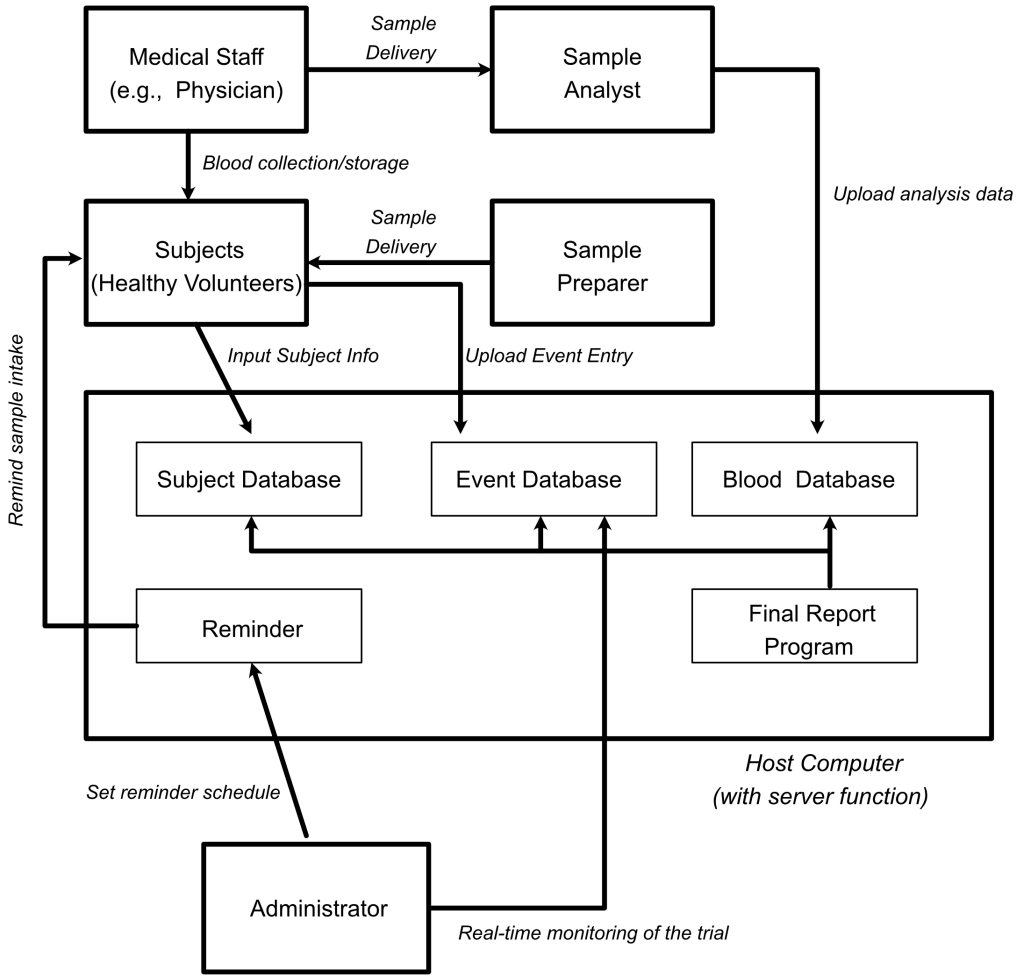

Figure 1. Processing scheme from data acquisition to analysis. This is a schematic diagram of the human trial method proposed in this study. Each of the thick rectangles represents each personnel or the host computer. The objects within the host computer represent a program, function, or storage devices.

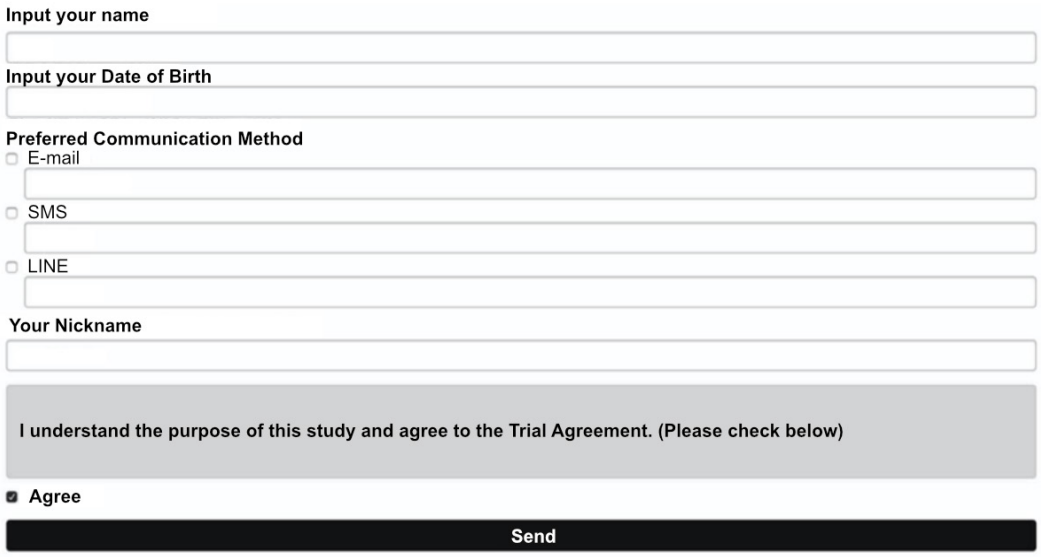

Figure 2. Example of input screen to the host computer. This is an example of the screen when the subject enters the subject data into the host computer.

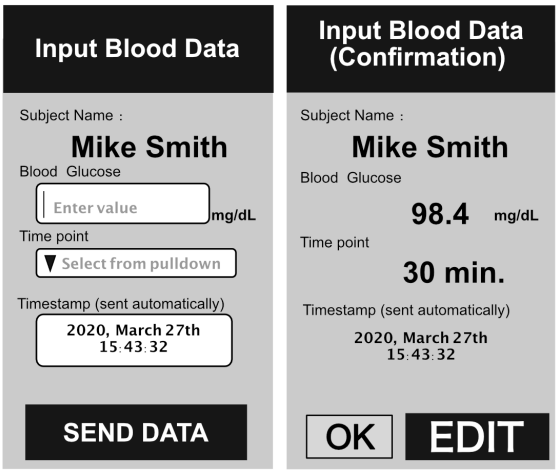

Figure 3. An example screen of subject-host computer communication. When a subject sends intake information (or the blood data in the case shown in the panel) to the host computer, the input screen (left) is displayed first, and then the data will be registered after the confirmation screen for data transmission (right).

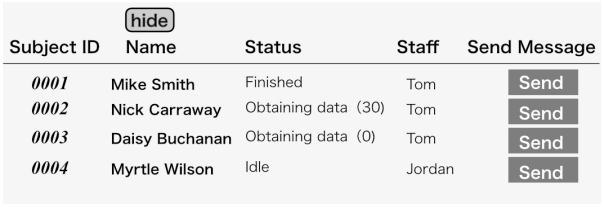

Figure 4. Example of the administrator screen. The administrator collectively manages the scheduled, in-progress, and completed studies. In the example of the operation screen shown in the figure, the entire study can be supervised in real time through the management screen where each subject can be listed.

sterile centrifuge tube) with the subject's identification. In addition, the sample analyst analyzes the blood and obtains the blood (*e.g.*, biochemistry traits or cell counts) analysis data including the corresponding identity of the subject (typically anonymized). Finally, the host computer stores the blood analysis data, and by matching the blood analysis data with the subject data and the intake (event) data, the host computer creates and stores the final report for the effect of the food sample. Figure 1 shows the entire scheme where the host computer integrates the whole processes of a trial.

In the case of actual operation, the subjects (*i.e.*, healthy volunteers) may first input their data by themselves into the host computer (an example input screen is shown in Figure 2). Then, once the blood data is obtained, each subject will connect to the web server from his or her personal terminal (smartphone, tablet, *etc.*) and upload the measurement data (examples input and confirmation screens are shown in Figure 3). Throughout the study, the administrator should supervise the execution of the study in real time through an integrated manager (an example window is shown in Figure 4).

3.2. A model case

As an example of human trial that the above-mentioned scheme can be used, a study to examine the effect of lactic acid bacteria on human blood Natural Killer cell activity is shown in this paper (Table 1). This experiment involved an administrator (a non-physician faculty of our university), three subjects (three healthy volunteers), a sample preparer (a researcher at our university), a medical worker (a physician faculty member), and an analyst (a staff from a company that owns analytical equipment) were involved in the study.

Table 1. Examples of human studies with repeated intake of lactic acid bacteria

Subject #	NK activity		
	day -1	day 14	day 28
Replicate 1 (LAB strain A)			
1	34	37	31
2	47	36	35
3	57	59	51
mean	46	44	39
SD	12	13	11
Replicate 2 (LAB strain B)			
1	41	39	37
2	23	30	17
3	51	63	40
mean	38	44	31
SD	14	17	13

NK cell activity is shown in the table. SD: standard deviation. Results from three healthy volunteers are shown in the table. See Materials and Methods section for details.

The three subjects orally ingested the sample once a day for 27 consecutive days. The samples were stored in the laboratory freezer within building where the trial was performed, and the sample delivery from the sample preparer to the subjects was smooth.

4. Discussion

In the human trial scheme proposed in this study, the administrator can easily monitor the progress of the trial in real-time. In addition, by developing and using appropriate software, the results of the trial (final report) can be provided in a visualized form (tables, graphs, *etc.*), which greatly reduces the labor required to publish the results of the human trial. This is important in promoting the participation of physicians and others in human trials for natural foods. This scheme can be implemented in a medical university with faculty members with or without medical license as administrators. In this case, it will be easy to recruit staff members, researchers, students, *etc.* at the university (who understand the significance of the trial and are likely to comply with the instructions of the administrator, thus ensuring the reliability of the test results) as volunteer subjects. Furthermore, the preparation of food samples can be done in university laboratories, so that the delivery of it to the subjects will be smooth as demonstrated in this study. This is especially important when handling food samples that may have potential stability issues.

Part of the human trial scheme is carried out by a host computer with server functions. This scheme requires knowledge of the programs and databases used to perform human trials, but both are standard skillsets in today's technology. Furthermore, the scheme can be applied to any human study in which the blood of a subject is collected to directly evaluate and analyze the health effects of food. For example, natural foods, health foods, functional foods, foods for specified health uses, and dietary supplements can be used. The blood collection from subjects is an invasive medical procedure, and the involvement of medical personnel such as physicians is essential in conducting the study. However, for processes other than blood collection, non-physicians can serve as administrators (*i.e.*, the person in charge of conducting the study, who oversees the communication among the parties involved in the human trial, data management, and compilation of results), thereby reducing the burden on researchers in conducting such studies. In conventional human trials, blood collection is usually conducted in hospitals, and medical personnel such as physicians serve as managers, imposing a heavy burden on the trials. In this scheme, non-physicians can act as administrators, and as a result, academia-derived human trials can be conducted at low cost. This scheme is expected to be widely used for human trials of natural foods, health foods, functional foods, food for specified health uses, dietary supplements, *etc.*, and it will enable reliable, smooth, and cost-efficient human trials.

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