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STUDIES ON ECHINOCOCCOSIS XV
SECONDARY MULTILOCULAR ECHINOCOCCOSIS
BY INTRAHEPATIC INOCULATION

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Pertaining to experimental secondary echinococcosis, many investigations have been published. In those investigations, scolices of the echinococcus are inoculated through various routes such as peritoneal, thoracic, subcutaneous, cerebral etc. The present senior authors also have reported results previously obtained from experimental multilocular echinococcosis (YAMASHITA et al., 1957 and 1960). It is described in those reports, how peritoneal inoculations of scolices were given to various uniform strains of the mouse. Generally speaking, some scolices can fully grow up to an echinococcus and foci can be found in the liver at the highest rate. It, however, was proved in those studies that secondary echinococcosis by scolex inoculation resulted in inferior rate of infection in comparison with that of primary one by ingestion of eggs. The authors undertook, for the first time, experiments on secondary echinococcosis for the purpose of preventing probable dangers of infection from passage using dogs. Other purposes were investigations about biological characteristics of the echinococcus, pathological findings of lesions etc.

The authors studied the intrahepatic inoculation of scolices as described below. This experiment was planned on the basis of considerations that the majority of echinococcus foci are found in the liver in cases irrespective of their origin whether due to ingestion of eggs or to peritoneal inoculation of scolices.

MATERIALS AND METHODS

Use was made of 50 mice of 8 uniform strains: 4 mice of strain AKR, 9 of dd, 3 of dba, 5 of CFW, 5 of C57BL/6, 2 of fm, 2 of SM and 20 of gpc.

The inocula, scolices of the Alaskan strain of *Echinococcus multilocularis*, were obtained from the hepatic echinococcus of orally infected cases of dba mouse. Echinococcus tissue was lightly ground in a mortar with added 0.2% trypsin solution for 5~10 minutes; this material was filtered through a gauze and scolices were collected by centrifugation. Physiological saline solution with 200 U/ml of penicillin was added to these scolices.

Experimental animals were each given intrahepatic injection by syringe with 0.05 ml of

inoculum at the right epigastric area. The number of scolices injected was 500~2,000 per animal. Inoculated animals were sacrificed at a desired time and dissected. Macro- and microscopical examination of echinococcus foci was conducted.

RESULTS

Experimental cases were examined macro- and microscopically and were classified into three groups as follows:—

1. Negative No echinococcus foci can be found.

2. Organized Minute foci less than about 1 mm in size are observed. The focus is yellowish grey in color and opaque. Microscopically, the focus is nodular, composed mainly of granulation tissue, its central portion composed of accumulated cells and scolices which have fallen into regressive changes in general. Structure of the focus varies according to length of time after the inoculation and it terminates finally in a fibrous nodule. The focus falls sometimes into calcification.

3. Positive This group manifests circumscribed foci with a cystic structure of various complexity and frequently with typical multilocular structure. In this group, the echinococcus shows progression of development; simple cystic structure, in early stage with accompanied notable tissue reaction, multilocular vesiculation appears with the progress of time and finally formation of brood capsules and scolices is recognizable.

A diagnosis was made of experimental cases in accordance with the above classification (table 1). Findings of positive cases are described below.

Strain AKR Two cases out of 4 were positive, 1 case showed organized hepatic foci and the remaining 1 case was negative.

Case I-4 (14 days after the inoculation) A small focus of 1 mm size in the portal area of the liver; small echinococcus cyst in the central portion of the focus.

Case I-10 (98 days) A multilocular echinococcus focus of 5×6 mm size was found on the diaphragmatic surface of the left lateral lobe of the liver. Almost all the diaphragmatic surface of the right lateral lobe was occupied by multilocular echinococcus tissue. Similar focus was noticed on the visceral surface in an area of 10×8 mm size in contact with the papillary processes. Many masses of the echinococcus were also found in the peritoneal cavity. On microscopical examination, the focus was seen to have been established by fully developed multilocular echinococcus with numerous scolices.

Strain dd Only 1 case out of 9 possessed an echinococcus focus, 3 cases had organized foci in their livers and 5 cases were negative.

Case I-8 (59 days) A focus, 2.5×3 mm in size, on the diaphragmatic surface of the left lateral lobe of the liver; multilocular echinococcus with initial formation of brood capsules.

Strain dba Organized foci alone in the liver of all the 3 cases.

Strain CFW Two cases out of 5 positive, 2 cases with organized foci in the liver and remainder 1 case negative.

Case II-8 (63 days) Multilocular foci on the visceral surface of the left and right median

TABLE 1 *Cases Examined*

STRAINS	CASE NO.	DAYS AFTER INOCUL.	DIAG-NOSIS	STRAINS	CASE NO.	DAYS AFTER INOCUL.	DIAG-NOSIS
AKR	I- 3	7	N	fm	II- 5	39	P
	I- 4	14	P		II-11	63	P
	I- 6	35	O	SM	II- 6	39	O
	I-10	98	P		II-22	146	O
dd	I- 1	7	N	gpc	II- 4	39	O
	I- 2	7	N		II-10	63	P
	I- 5	14	O		III- 1	78	P
	I- 7	35	O		III- 2	78	O
	I- 8	59	P		III- 3	78	O
	I- 9	59	O		III- 4	78	P
	I-11	98	N		III- 5	78	O
	I-12	98	N		III- 6	78	O
	I-13	98	N		III- 7	78	O
	dba	II- 1	39		O	III- 8	78
II- 7		63	O	II-14	114	P	
II-15		146	O	II-20	146	O	
				II-21	146	N	
CFW	II- 2	39	O	III- 9	186	N	
	II- 8	63	P	III-10	272	P	
	II-12	114	P	III-11	272	P	
	II-16	146	O	III-12	272	P	
	II-17	146	N	III-13	272	P	
C57BL/6	II- 3	39	O	III-14	272	P	
	II- 9	63	O	III-15	272	N	
	II-13	114	P				
	II-18	146	P				
	II-19	146	N				

Remarks : I: Exp. 1, 19/V'61~25/VIII'61
 II: Exp. 2, 17/VII'61~10/XII'61
 III: Exp. 3, 23/IX'61~22/VI'62
 P: Positive; O: Organized; N: Negative

lobes; the focus composed of more than 10 small cysts 1 mm in size.

Case II-12 (114 days) A large multilocular echinococcus focus, diameter 11 mm, in the left lateral lobe of the liver and a similar focus of 5 mm size on the diaphragmatic surface of the left median lobe.

Strain C57BL/6 Two cases with positive results, 2 organized and 1 negative.

Case II-13 (114 days) Two pedunculated echinococcus foci at the root of the papillary processes of the liver, the sizes 3×2 mm and 4×2 mm respectively.

Case II-18 (146 days) A multilocular echinococcus focus of 2×1 mm size in the caudate lobe of the liver.

Strain fm Two cases were examined; they were positive.

Case II-5 (39 days) A multilocular echinococcus focus of 5×4 mm size on the diaphragmatic surface of the left lateral lobe of the liver and a similar focus of 6×3 mm in a marginal portion of the same lobe; neither brood capsules nor scolices could be found.

Case II-11 (63 days) An echinococcus focus composed of several minute cysts in contact with the papillary processes on the visceral surface of the liver.

Strain SM Two cases were examined; both cases showed organized foci only.

Strain gpc Nine cases out of 20 were positive, other 7 cases with organized foci in the liver and remainder 4 cases negative.

Case II-10 (63 days) The left papillary process of the liver swollen, 20 mm in length, and changed into a multilocular echinococcus tissue with accompanied scolex formation.

Case III-1 (78 days) An echinococcus focus of 5 mm diameter existing on the diaphragmatic surface of the right lateral lobe of the liver was connected with a pulmonary focus through the diaphragm. As to multilocular echinococcus foci in the thoracic organs: a focus of 4×5 mm in the left lung, 2 foci respectively 4×4 mm and 4×5 mm in the right lung and also 2 foci of 7×2 mm and 7×5 mm in the mediastinum. Microscopically the foci were seen to be without formation of brood capsules.

Case III-4 (78 days) An echinococcus focus, 4 mm in diameter, on the diaphragmatic surface of the right lateral lobe of the liver; no brood capsules. Also several organized foci in the liver.

Case II-14 (114 days) A focus, 5 mm in size, in the caudate lobe of the liver; multilocular echinococcus tissue with initial formation of brood capsules.

Case III-10 (272 days) Two foci of respective 4 mm and 2.5 mm size in the left lateral lobe of the liver; the multilocular echinococcus without brood capsule formation.

Case III-11 (272 days) Whole right median lobe of the liver changed into an echinococcus tissue, 13×16×11 mm in size. A mass of echinococcus tissue of 26×17×19 mm in the peritoneal cavity. Neither brood capsules nor scolices could be seen.

Case III-12 (272 days) The left median lobe of the liver swollen as large as 13×11×9.5 mm and replaced by an echinococcus tissue. The right median lobe of the lung also changed into an echinococcus tissue, the size 12×14×10 mm. Initial formation of brood capsules was proved microscopically.

Case III-13 (272 days) The liver was intact. An echinococcus focus of 18×12×11 mm size in the right lung, pressed the left lung and adhered to the diaphragm.

Case III-14 (272 days) A large focus, 33×16×20 mm, in the left lateral lobe of the liver

and an echinococcus focus of 6 mm size in the caudate lobe. Many echinococcus foci, size 1~3 mm, were noted sporadically in the lungs. An echinococcus mass, 19×13×13 mm in size, was found in the peritoneal cavity. Microscopically, the foci were the multilocular echinococcus in type with initial formation of brood capsules.

DISCUSSION

After investigating cases in the present experiment, one can say that positive cases are not so many; both the scolex and brood capsule formation was proved in only 2 cases (AKR I-10 and gpc II-10) while the brood capsule formation alone was found in 3 cases (dd I-8, gpc III-12 and gpc III-14). Eighteen cases, including the above 5 cases, out of 50 were positive in which development of the echinococcus was observed.

The authors have investigated previously the difference of susceptibility of various strains of the mouse to various routes of inoculations; their data of susceptibility of the 8 strains on which the present experiment was conducted are summarized as follows (table 2).

TABLE 2. *Difference of Susceptibility in Mice Inoculated by Various Routes (No. of Cases Positive/Examined)*

ROUTES	STRAINS	AKR	dd	dba	CFW	C57BL/6	fm	SM	gpc
Intrahepatic (Scolices)		2/4	1/9	0/3	2/5	2/5	2/2	0/2	9/20
Peritoneal (Scolices)			1/6	4/9	12/12*	1/6			2/5*
Oral (Eggs)		6/6	1/13	6/6	6/12	15/19	8/8	18/22	15/21

* : Data unpublished.

It is a well-known fact that secondary echinococcosis originates in metamorphosis of the scolex. Regarding to secondary echinococcosis, OHBAYASHI (1960), one of the present authors, investigated the phenomena of daughter cyst formation and metastasis in experimental echinococcosis of cotton rats and he made it clear that these phenomena arise solely from metamorphosis of the scolex. As for the multilocular echinococcus, rather recently studies on experimental secondary echinococcosis have been begun. The authors have tried a passage experiment employing secondary multilocular echinococcosis; the results will be published in the near future. LUBINSKY (1960) succeeded in passage by subcutaneous and peritoneal inoculation. He used cotton rats, gerbils and mice. NORMAN and KAGAN (1961) also successfully conducted the peritoneal passage in cotton rats and gerbils.

The rate of susceptibility of peritoneally inoculated cases, in other words, the rate of echinococcus which can develop, is inferior to that of primary (oral) cases; and these observations were published already by the authors. Strains other than

CFW and dd exhibited this relation as shown in table 2. And comparison between intrahepatic and peritoneal cases show that the former is not superior in incidence to the latter. It, therefore, seems that the susceptibility of oral, peritoneal and intrahepatic cases tends to decline in order. Besides, only 2 cases showed the scolex formation in cases of intrahepatic inoculations as above stated; it can be concluded that the rates of formation of brood capsules and scolices are very low.

SUMMARY AND CONCLUSION

Eight uniform strains of the mouse were investigated on experimental secondary multilocular echinococcosis by the intrahepatic inoculation of scolices. It is concluded that a part of scolices inoculated can develop fully to the multilocular echinococcus, but the susceptibility is not superior to that of peritoneal cases.

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